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BACTERIOLOGY & MYCOLOGY
for 2nd year students

Faculty of Medicine

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Study Guide «BACTERIOLOGY & MYCOLOGY» for self-study and practical training of course «Microbiology, Virology and Immunology» for foreign students of 2nd year Medical Faculty. Meleshko Tamara, Liashyna Kateryna, Bati Viktoriia, Boyko Nadiya.

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Навчальний посібник «БАКТЕРІОЛОГІЯ ТА МІКОЛОГІЯ» для самостійної роботи та практичних занять з курсу «Мікробіологія, вірусологія та імунологія» для іноземних студентів 2 курсу медичного факультету. Мелешко Т., Ляшина К., Баті В., Бойко Н.

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Manual material submitted according to the requirements of typical teaching program of microbiology for students of medical faculties of higher educational institutions of 3-4 accreditation levels for improving the organization and execution of independent study of the topic according to the Bologna Process. Availability of laboratory diagnostics describe, combining theoretical material and implementation of practical problems protocols can greatly intensify the work of students and to minimize the possibility of mechanical performance, and promotes better understanding of covered material and mastering practical skills.

Reviewer:

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Introduction

Medical Microbiology is one of the fundamental disciplines of modern science because its studying is the basis of students' knowledge of biological and medical specialities.

Infectious diseases - are group of diseases caused by different types of infectious agents, characterized by diverse mechanisms of infection transmission and differences in pathogenesis, which must be systematically and efficiently learned during the course of microbiology, virology and immunology for students of medical, stomatological, biological and pharmaceutical specialities at universities of Ukraine and the world.

Over the past decade is marked a sharp increase of infectious diseases incidence caused by bacterial, viral and fungal agents with different ways of transmission. To stop this is one of the primary problems of the world health system.

During the lessons students have opportunity to get acquainted with methods of infectious diseases diagnostics, particularly with the production of ELISA. Attention is paid to the definition of diagnostic markers of bacterial, fungal and other infectious agents.

Knowledge concerning the features of infectious diseases, the ability to choose adequate methods of their laboratory diagnosis and interpret the results are necessary for the formation of students' understanding of viral diseases diagnosing methods.

This English-language textbook includes training material for preparation on 18 topics, clearly and logically structured according to the needs of course «Medical Microbiology» for foreign students of Medical Faculty «UzhNU».

Вступ

Медична мікробіологія є однією з фундаментальних дисциплін сучасного природознавства, тому її вивчення становить основу знань студентів біологічних та медичних спеціальностей.

Інфекційні захворювання - це група захворювань, викликаних різними типами інфекційних агентів, що характеризуються різноманітними механізмами передачі інфекції й відмінностями в патогенезі, які необхідно систематично та якісно вивчати у ході курсів мікробіології, вірусології та імунології для студентів медичних, стоматологічних, біологічних та фармацевтичних спеціальностей у ВНЗ України та світу.

За останні десятиліття відмічається різке зростання захворюваності на інфекційні хвороби, що викликаються бактеріальними, вірусними та грибковими агентами з різними шляхами передачі. Зупинити це і є однією з першорядних проблем світової системи охорони здоров'я.

На заняттях студентам надається можливість ознайомитись з методами діагностики інфекційних захворювань, зокрема, з постановкою ІФА. Приділяється увага визначенню діагностичних маркерів бактеріальних, грибкових та інших інфекційних агентів.

Знання щодо особливостей збудників інфекційних захворювань, вміння обирати адекватні методи їхньої лабораторної діагностики та трактувати отримані результати є необхідним для формування у студентів уявлення про методи діагностики вірусних захворювання.

Даний англомовний навчальний посібник містить навчальний матеріал для підготовки за 18 темами, чітко та логічно структурованими, згідно з потребами до вивчення курсу «Медична мікробіологія» для іноземних студентів медичного факультету УжНУ.

1 Class - Staphylococci. Streptococci. Gonococci. Meningococci

The Staphylococci

Scientific classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Staphylococcaceae
Genus: Staphylococcus
Species: S. aureus, S. epidermidis, S. saprophyticus

Morphology & Identification

A. MORPHOLOGY

Staphylococci are spherical cells about 1 μm in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Staphylococci are nonmotile and do not form spores. Under the influence of drugs like penicillin, staphylococci are lysed. Their colonies can be yellow, red, or orange.

B. STAINING

Typical staphylococci appear as gram positive cocci in clusters in Gram-stained smears of pus or sputum.

C. CULTURE AND GROWTH CHARACTERISTICS

Staphylococci grow readily on most bacteriologic media under aerobic or microaerophilic conditions. They grow most rapidly at 37 °C but form pigment best at room temperature (20-25 °C). Colonies on solid media are round, smooth, raised, and glistening. *S. aureus* usually forms gray to deep golden yellow colonies. *S. epidermidis* colonies usually are gray to white on primary isolation; many colonies develop pigment only upon prolonged incubation. Various degrees of hemolysis are produced by *S. aureus* and occasionally by other species. *Peptostreptococcus* species, which are anaerobic cocci, often resemble staphylococci in morphology.

The staphylococci produce catalase, which differentiates them from the streptococci. Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas. Proteolytic activity varies greatly from one strain to another. Pathogenic staphylococci produce many extracellular substances, which are discussed below. Staphylococci are relatively resistant to drying, heat (they withstand 50 °C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals, eg, 3% hexachlorophene. Staphylococci are variably sensitive to many antimicrobial drugs.

D. VIRULENCE FACTORS

Antigenic Structure

Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure. Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acid or exposure to lysozyme. It is important in the pathogenesis of infection. It elicits production of interleukin-1 (endogenous pyrogen) and opsonic antibodies by monocytes, and it can be a chemoattractant for polymorphonuclear leukocytes, have endotoxin-like activity, and activate complement.

Teichoic acids, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycan and can be antigenic. Antiteichoic acid antibodies detectable by gel diffusion may be found in patients with active endocarditis due to *S. aureus*.

Protein A is a cell wall component of many *S. aureus* strains that binds to the Fc portion of IgG molecules except IgG3. The Fab portion of IgG bound to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology and diagnostic laboratory technology; for example, protein A with attached IgG molecules directed against a specific bacterial antigen will agglutinate bacteria that have that antigen («coagglutination»).

Some *S. aureus* strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present. Most strains of *S. aureus* have coagulase, or clumping factor, on the cell wall surface; coagulase binds nonenzymatically to fibrinogen, yielding aggregation of the bacteria.

Enzymes & Toxins

1) Catalase: staphylococci produce catalase, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative.

2) Coagulase and clumping factor: *S. aureus* produces coagulase, an enzyme-like protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase production is considered synonymous with invasive pathogenic potential.

3) Clumping factor is a surface *S. aureus* compound that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S. aureus* forms clumps. Clumping factor is distinct from coagulase.

4) Oher enzymes: other enzymes produced by staphylococci include a hyaluronidase, or spreading factor; a staphylokinase resulting in fibrinolysis but acting much more slowly than streptokinase; proteinases; lipases; and β-lactamase.

5) Exotoxins: the α-toxin is a heterogeneous protein that acts on a broad spectrum of eukaryotic cell membranes. The α-toxin is a potent hemolysin. The β-toxin degrades sphingomyelin and therefore is toxic for many kinds of cells, including human red blood cells. The δ-toxin is heterogeneous and dissociates into subunits in nonionic detergents. It disrupts biologic membranes and may have a role in *S. aureus* diarrheal diseases. The γ hemolysin refers to three proteins that interact with the two proteins comprising the Panton-Valentine leukocidin to form six potential two-component toxins. All six of these protein toxins are capable of efficiently lysing white blood cells by causing pore formation in the cellular membranes that increase cation permeability.

6) Leukocidin: this toxin of *S. aureus* has two components. It can kill white blood cells of humans and rabbits. The two components act synergistically on the white blood cell membrane as described above for γ toxin. This toxin is an important virulence factor in community associated methicillin resistant *S. aureus* infections.

7) Exfoliative toxins: these epidermolytic toxins of *S. aureus* are two distinct proteins of the same molecular weight. Epidermolytic toxin A is a chromosomal gene product and is heat-stable (resists boiling for 20 minutes). Epidermolytic toxin B is plasmid-mediated and heat-labile. The epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are superantigens.

8) Toxic shock syndrome toxin: most *S. aureus* strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin-1 (TSST-1), which is the same as enterotoxin F. TSST-1 is the prototypical superantigen. TSST-1 binds to MHC class II molecules, yielding T cell stimulation, which promotes the protean manifestations of the toxic shock syndrome. The toxin is associated with fever, shock, and multisystem involvement, including a desquamative skin rash. The gene for TSST-1 is found in about 20% of *S. aureus* isolates.

9) Enterotoxins: there are multiple (A-E, G-I, K-M) enterotoxins. Approximately 50% of *S. aureus* strains can produce one or more of them. Like TSST-1, the enterotoxins are superantigens. The enterotoxins are heat-stable and resistant to the action of gut enzymes. An important cause of food poisoning, enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods. Ingestion of 25 μg of enterotoxin B results in vomiting and diarrhea. The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut.

The exfoliative toxins, TSST-1, and the enterotoxin genes are on a chromosomal element called a pathogenicity island. It interacts with accessory genetic elements - bacteriophages - to produce the toxins.

S. aureus virulence factors	**Biologic Effects**
Structural Components	
Capsule	Inhibits chemotaxis and phagocytosis; inhibits proliferation of mononuclear cells; facilitates adherence to foreign bodies
Peptidoglycan	Provides osmotic stability; stimulates production of endogenous pyrogen (endotoxin-like activity); leukocyte chemoattractant (abscess formation); inhibits phagocytosis
Teichoic acid	Regulates cationic concentration at cell membrane; binds to fibronectin
Protein A	Inhibits antibody-mediated clearance by binding IgG, IgG_2, and IgG_4 Fc receptors; leukocyte chemoattractant; anticomplementary
Cytoplasmic membrane	Osmotic barrier; regulates transport into and out of cell; site of biosynthetic and respiratory enzymes
Toxins	
Cytotoxins (α, β, δ, γ, P-V leukocidin)	Toxic for many cells, including leukocytes, erythrocytes, macrophages, platelets, and fibroblasts
Exfoliative toxins (ETA, ETB)	Serine proteases that split the intercellular bridges in the stratum granulosum epidermis
Enterotoxins (A-E, G-D	Superantigens (stimulates proliferation of T cells and release of cytokines); stimulates release of inflammatory mediators in mast cells, increasing intestinal peristalsis and fluid loss, as well as nausea and vomiting
Toxic Shock Syndrome Toxin-1	Superantigen (stimulates proliferation of T cells and release of cytokines); produces leakage or cellular destruction of endothelial cells
Enzymes	
Coagulase	Converts fibrinogen to fibrin
Catalase	Catalyzes removal of hydrogen peroxide
Hyaluronidase	Hydrolyzes hyaluronic acids in connective tissue, promoting the spread of staphylococci in tissue
Fibrinolysin	Dissolves fibrin clots
Lipases	Hydrolyzes lipids
Nucleases	Hydrolyzes DNA
Penicillinase	Hydrolyzes penicillins

Pathogenesis, Pathology & Clinical Findings

A. DISEASES CAUSED BY STAPHYLOCOCCI

The prototype of a staphylococcal lesion is the furuncle or other localized abscess. Groups of *S. aureus* established in a hair follicle lead to tissue necrosis (dermonecrotic factor). Coagulase is produced and coagulates fibrin around the lesion and within the lymphatics, resulting in formation of a wall that limits the process and is reinforced by the accumulation of inflammatory cells and, later, fibrous tissue. Within the center of the lesion, liquefaction of the necrotic tissue occurs (enhanced by delayed hypersensitivity), and the abscess «points» in the direction of least resistance. Drainage of the liquid center necrotic tissue is followed by slow filling of the cavity with granulation tissue and eventual healing.

Focal suppuration (abscess) is typical of staphylococcal infection. From any one focus, organisms may spread via the lymphatics and bloodstream to other parts of the body. Suppuration within veins, associated with thrombosis, is a common feature of such dissemination. In osteomyelitis, the primary focus of *S. aureus* growth is typically in a terminal blood vessel of the metaphysis of a long bone, leading to necrosis of bone and chronic suppuration. *S. aureus* may cause pneumonia, meningitis, empyema, endocarditis, or sepsis with suppuration in any organ. Staphylococci of low invasiveness are involved in many skin infections (eg, acne, pyoderma, or impetigo). Anaerobic cocci (*Peptostreptococcus*) participate in mixed anaerobic infections.

Staphylococci also cause disease through the elaboration of toxins, without apparent invasive infection.

Bullous exfoliation, the scalded skin syndrome, is caused by the production of exfoliative toxins. Toxic shock syndrome is associated with TSST-1.

Organism	Diseases
Staphylococcus aureus	Toxin-mediated (food poisoning, toxic shock syndrome); cutaneous (impetigo, folliculitis, furuncles, carbuncles, wound infections); other (bacteremia, endocarditis, pneumonia, empyema, osteomyelitis, septic arthritis)
Staphylococcus epidermidis	Bacteremia; endocarditis; surgical wounds; urinary tract infections; opportunistic infections of catheters, shunts, prosthetic devices, and peritoneal dialysates
Staphylococcus saprophytics	Urinary tract infections, opportunistic infections
Staphylococcus capitis	Bacteremia, endocarditis, urinary tract infections, wound infections, pneumonia, bone andjoint infections, opportunistic infections
Staphylococcus haeinolyticus	Bacteremia, endocarditis, urinary tract infections, wound infections, and opportunisticinfections
Micrococcus spp.	Opportunistic infections
Stotnalococcus mucilaginosus	Bacteremia, endocarditis, opportunistic infections
Alloiococcus olitidis	Chronic middle ear infections

B. SOURCE OF AGENTS OF DIFFERENT STAPHYLOCOCCI DISEASES

Staphylococci, particularly *S. epidermidis,* are members of the normal flora of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S. aureus* occurs in 20-50% of humans. Staphylococci are also found regularly on clothing, bed linens, and other fomites in human environments.

C. PATHOGENESIS

The pathogenic capacity of a given strain of *S. aureus* is the combined effect of extracellular factors and toxins together with the invasive properties of the strain. At one end of the disease spectrum is staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin; at the other end are staphylococcal bacteremia and disseminated abscesses in all organs.

Pathogenic, invasive *S. aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic. Nonpathogenic, noninvasive staphylococci such as *S. epidermidis* are coagulase-negative and tend to be nonhemolytic. Such organisms rarely produce suppuration but may infect orthopedic or cardiovascular prostheses or cause disease in immunosuppressed persons. *S. saprophyticus* is typically nonpigmented, novobiocin-resistant, and nonhemolytic; it causes urinary tract infections in young women.

D. CLINICAL FINDINGS OF TYPICAL SYMPTOMS OF STAPHYLOCOCCI INFECTION

A localized staphylococcal infection appears as a «pimple» hair follicle infection, or abscess. There is usually an intense, localized, painful inflammatory reaction that undergoes central suppuration and heals quickly when the pus is drained. The wall of fibrin and cells around the core of the abscess tends to prevent spread of the organisms and should not be broken down by manipulation or trauma.

S. aureus infection can also result from direct contamination of a wound, eg, postoperative staphylococcal wound infection or infection following trauma (chronic osteomyelitis subsequent to an open fracture, meningitis following skull fracture).

If *S. aureus* disseminates and bacteremia ensues, endocarditis, acute hematogenous osteomyelitis, meningitis, or pulmonary infection can result. The clinical presentations resemble those seen with other bloodstream infections. Secondary localization within an organ or system is accompanied by the symptoms and signs of organ dysfunction and intense focal suppuration.

Food poisoning due to staphylococcal enterotoxin is characterized by a short incubation period (1-8 hours); violent nausea, vomiting, and diarrhea; and rapid convalescence. There is no fever.

Toxic shock syndrome is manifested by an abrupt onset of high fever, vomiting, diarrhea, myalgias, a scarlatiniform rash, and hypotension with cardiac and renal failure in the most severe cases. It often occurs within 5 days after the onset of menses in young women who use tampons, but it also occurs in children or in men with staphylococcal wound infections. The syndrome can recur. Toxic shock syndrome-associated *S. aureus* can be found in the vagina, on tampons, in wounds or other localized infections, or in the throat but

virtually never in the bloodstream.

Diagnostic Laboratory Tests

A. SPECIMENS

Surface swab pus, blood, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.

B. SMEARS

Typical staphylococci appear as gram positive cocci in clusters in Gram-stained smears of pus or sputum. It is not possible to distinguish saprophytic *(S. epidermidis)* from pathogenic *(S. aureus)* organisms on smears.

C. CULTURE

Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37 °C, but hemolysis and pigment production may not occur until several days later and are optimal at room temperature. *S. aureus* but not other staphylococci ferment mannitol. Specimens contaminated with a mixed flora can be cultured on media containing 7,5% NaCl; the salt inhibits most other normal flora but not *S. aureus*. Mannitol salt agar or commercially available chromogenic media are used to screen for nasal carriers of *S. aureus* and patients with cystic fibrosis.

D. CATALASE TEST

This test is used to detect the presence of cytochrome oxidase enzymes. A drop of 3% hydrogen peroxide solution is placed on a slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (the release of oxygen) indicates a positive test.

E. COAGULASE TEST

Citrated rabbit (or human) plasma diluted 1:5 is mixed with an equal volume of broth culture or growth from colonies on agar and incubated at 37 °C. A tube of plasma mixed with sterile broth is included as a control. If clots form in 1-4 hours, the test is positive.

Coagulase-positive staphylococci are considered pathogenic for humans; however, coagulase-positive staphylococci of dogs *(Staphylococcus intermedius)* and dolphins *(Staphylococcus delphini)* rarely cause disease in humans. Infections of prosthetic devices can be caused by organisms of the coagulase-negative *S. epidermidis* group.

F. SUSCEPTIBILITY TESTING

Broth microdilution or disk diffusion susceptibility testing should be done routinely on staphylococcal isolates from clinically significant infections. Resistance to penicillin G can be predicted by a positive test for β- lactamase; approximately 90% of *S. aureus* produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% of *S. aureus* and approximately 75% of *S. epidermidis* isolates. Nafcillin resistance correlates with the presence of *mec*A, the gene that codes for a penicillin-binding protein (PBP 2a) not affected by these drugs. The gene can be detected using the polymerase chain reaction. Most clinical laboratories use a phenotypic method such as an oxacillin screening agar plate. Staphylococci that grow on Mueller-Hinton agar containing 4% NaCl and 6 μg/mL of typically are *mec*A-positive and nafcillin-resistant. Alternatively, an assay for the *mecA* gene product, PBP 2a, is commercially available and is much more rapid than PCR for *mecA* or than testing for resistance using growth on oxacillin-containing salt agar.

Serologic and typing tests: serologic tests for diagnosis of *S. aureus* infections have little practical value.

Antibiotic susceptibility patterns are helpful in tracing *S. aureus* infections and in determining if multiple *S. epidermidis* isolates from blood cultures represent bacteremia due to the same strain, seeded by a nidus of infection.

Molecular typing techniques have been used to document the spread of epidemic disease-producing clones of *S. aureus*. Pulsed-field gel electrophoresis and multilocus sequence typing are highly discriminatory.

Treatment

Serious multiple skin infections (acne, furunculosis) occur most often in adolescents. Similar skin infections occur in patients receiving prolonged courses of corticosteroids. In acne, lipases of staphylococci and corynebacteria liberate fatty acids from lipids and thus cause tissue irritation. Tetracyclines are used for long-term treatment.

Abscesses and other closed suppurating lesions are treated by drainage, which is essential, and antimicrobial therapy. Acute hematogenous osteomyelitis responds well to antimicrobial drugs. In chronic and recurrent osteomyelitis, surgical drainage and removal of dead bone is accompanied by long-term administration of appropriate drugs, but eradication of the infecting staphylococci is difficult. Hyperbaric

oxygen and the application of vascularized myocutaneous flaps have aided healing in chronic osteomyelitis.

Bacteremia, endocarditis, pneumonia, and other severe infections due to *S. aureus* require prolonged intravenous therapy with a β-lactamase-resistant penicillin. Vancomycin is often reserved for use with nafcillin-resistant staphylococci. If the infection is found to be due to non-β- lactamase-producing *S. aureus,* penicillin G is the drug of choice, but only a small percentage of *S. aureus* strains are susceptible to penicillin G.

S. epidermidis infections are difficult to cure because they occur in prosthetic devices where the bacteria can sequester themselves in a biofilm. *S. epidermidis* is more often resistant to antimicrobial drugs than is *S. aureus;* approximately 75% of *S. epidermidis* strains are nafcillin- resistant.

Newer antimicrobial agents such as linezolide, daptomycin, and quinupristin/dalfopristin are generally reserved for patients with serious staphylococcal or enterococcal infections that are resistant to the more traditional agents, who are failing clinically or who are highly allergic.

Epidemiology, Prevention & Control

A. EPIDEMIOLOGY

Staphylococci are ubiquitous human parasites. The chief sources of infection are shedding human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin. Contact spread of infection has assumed added importance in hospitals, where a large proportion of the staff and patients carry antibiotic-resistant staphylococci in the nose or on the skin. Although cleanliness, hygiene, and aseptic management of lesions can control the spread of staphylococci from lesions, few methods are available to prevent the wide dissemination of staphylococci from carriers. Aerosols (eg, glycols) and ultraviolet irradiation of air have little effect.

B. PREVENTION

Carrier status prevents complete control.

Proper hygiene, segregation of carrier from highly susceptible individuals.

Good aseptic techniques when handling surgical instruments.

Control of nosocomial infections.

The Streptococci

Scientific classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Streptococcaceae
Genus: Streptococcus
Species: S. agalactiae, S.anginosus, S. bovis, S. mitis

Classification

The classification of streptococci into major categories has been based on a series of observations over many years: (1) colony morphology and hemolytic reactions on blood agar; (2) serologic specificity of the cell wall group-specific substance and other cell wall or capsular antigens; (3) biochemical reactions and resistance to physical and chemical factors; and (4) ecologic features.

A. HEMOLYSIS

Many streptococci are able to hemolyze red blood cells *in vitro* in varying degrees. Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β hemolysis. Incomplete lysis of erythro erythrocytes with reduction of hemoglobin and the formation of green pigment is called ***α***-hemolysis. Other streptococci are non-hemolytic (sometimes called gamma hemolysis).

B. GROUP-SPECIFIC SUBSTANCE (LANCEFIELD CLASSIFICATION)

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into Lancefield groups A-H and K-U. The serologic specificity of the group-specific carbohydrate is determined by an amino sugar. For group A streptococci, this is rhamnose-*N*-acetylglucosamine; for group B, it is rhamnose-glucosamine polysaccharide; for group C, it is rhamnose-*N*-acetylgalactosamine; for group D, it is glycerol teichoic acid containing D-alanine and glucose; and for group F, it is glucopyranosyl-*N*-acetylgalactosamine.

Medically important streptococci**:**

Group A (*Streptococcus pyogenes*)

Group B (*Streptococcus agalactiae*)

Group C and G (viridans streptococci: *S. mutans)*

Group D (*Enterococcus faecalis*)

C. CAPSULAR POLYSACCHARIDES

The antigenic specificity of the capsular polysaccharides is used to classify *S. pneumoniae* into over 90 types and to type the group B streptococci (*S. agalactiae*).

Group A: *Streptococcus pyogenes*

Morphology & Identification

A. MORPHOLOGY

Individual cocci are spherical or ovoid and are arranged in chains. The cocci divide in a plane perpendicular to the long axis of the chain. The members of the chain often have a striking diplococcal appearance, and rod-like forms are occasionally seen. The lengths of the chains vary widely and are conditioned by environmental factors.

Most group A strains produce capsules composed of hyaluronic acid. The capsules are most noticeable in very young cultures. They impede phagocytosis. Capsules of other streptococci (eg, *S. agalactiae* and *S. pneumoniae*) are different. The *S. pyogenes* cell wall contains proteins (M, T, R antigens), carbohydrates (group-specific), and peptidoglycans. Hair-like pili project through the capsule of group A streptococci. The pili consist partly of M protein and are covered with lipoteichoic acid. The latter is important in the attachment of streptococci to epithelial cells. Most streptococci grow in solid media as discoid colonies, usually 1-2 mm in diameter. *S. pyogenes* is β-hemolytic; other species have variable hemolytic characteristics.

B. STAINING

Streptococci are Gram-positive; however, as a culture ages and the bacteria die, they lose their gram-positivity and can appear to be Gram-negative; for some streptococci, this can occur after overnight incubation.

C. CULTURE AND GROWTH CHARACTERISTICS

Energy is obtained principally from the utilization of glucose with lactic acid as the end product. Growth of streptococci tends to be poor on solid media or in broth unless enriched with blood or tissue fluids. Nutritive requirements vary widely among different species. The human pathogens are most exacting, requiring a variety of growth factors. Growth and hemolysis are aided by incubation in 10% CO_2. Most pathogenic hemolytic streptococci grow best at 37 °C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

D. VIRULENCE FACTORS

Antigenic Structure

1. M protein: this substance is a major virulence factor of group A *S. pyogenes.* M protein appears as hair-like projections of the streptococcal cell wall. When M protein is present, the streptococci are virulent, and in the absence of M typespecific antibodies, they are able to resist phagocytosis by polymorphonuclear leukocytes. *S. pyogenes* that lack M protein are not virulent. Immunity to infection with group A streptococci is related to the presence of type-specific antibodies to M protein. Because there are many, perhaps 150, types of M protein, a person can have repeated infections with group A *S. pyogenes* of different M types. Both group C and group G streptococci have genes homologous to the genes for M protein of group A, and M protein has been found on group G streptococci.

2. T substance: this antigen has no relationship to virulence of streptococci. Unlike M protein, T substance is acid-labile and heat-labile. It is obtained from streptococci by proteolytic digestion, which rapidly destroys M proteins. T substance permits differentiation of certain types of streptococci by agglutination with specific antisera, while other types share the same T substance. Yet another surface antigen has been called R protein.

Toxins & Enzymes

1. Streptokinase (fibrinolysin): streptokinase is produced by many strains of group A β- hemolytic streptococci. It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins.

2. Hyaluronidase: hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading

factor). Hyaluronidases are antigenic and specific for each bacterial or tissue source. Following infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.

3. Pyrogenic exotoxins (erythrogenic toxin): pyrogenic exotoxins are elaborated by *S. pyogenes*. There are three antigenically distinct streptococcal pyrogenic exotoxins: A, B, and C. Exotoxin A has been most widely studied. It is produced by group A streptococci that carry a lysogenic phage. The streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever. Streptococcal pyrogenic exotoxin C may also contribute to the syndrome, while the role for streptococcal pyrogenic exotoxin B is unclear. The group A streptococci associated with toxic shock syndrome are primarily of M protein types 1 and 3.

The pyrogenic exotoxins act as superantigens, which stimulate T cells by binding to the class II major histocompatibility complex in the Vβ region of the T cell receptor. The activated T cells release cytokines that mediate shock and tissue injury. The mechanisms of action appear to be similar to those due to staphylococcal toxic syndrome toxin-1 and the staphylococcal enterotoxins.

4. Diphosphopyridine nucleotidase: this enzyme is elaborated into the environment by some streptococci. This substance may be related to the organism's ability to kill leukocytes. Proteinases and amylase are produced by some strains.

5. Hemolysins: the β-hemolytic group A *S. pyogenes* elaborates two hemolysins (streptolysins). Streptolysin O is a protein (MW 60,000) that is hemolytically active in the reduced state (available -SH groups) but rapidly inactivated in the presence of oxygen. Streptolysin O is responsible for some of the hemolysis seen when growth is in cuts deep into the medium in blood agar plates. It combines quantitatively with antistreptolysin O**,** an antibody that appears in humans following infection with any streptococci that produce streptolysin O. This antibody blocks hemolysis by streptolysin O. This phenomenon forms the basis of a quantitative test for the antibody. An antistreptolysin O (ASO) serum titer in excess of 160-200 units is considered abnormally high and suggests either recent infection with *S. pyogenes* or persistently high antibody levels due to an exaggerated immune response to an earlier exposure in a hypersensitive person. Streptolysin S is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates. It is elaborated in the presence of serum - hence the name streptolysin S. It is not antigenic, but it may be inhibited by a nonspecific inhibitor that is frequently present in the sera of humans and animals and is independent of past experience with streptococci.

Pathogenesis, Pathology & Clinical Findings

A. DISEASES ATTRIBUTABLE TO INVASION BY *S. PYOGENES*, B-HEMOLYTIC GROUP A STREPTOCOCCI

The portal of entry determines the principal clinical picture. In each case, however, there is a diffuse and rapidly spreading infection that involves the tissues and extends along lymphatic pathways with only minimal local suppuration. From the lymphatics, the infection can extend to the bloodstream.

1. Erysipelas - if the portal of entry is the skin, erysipelas results, with massive brawny edema and a rapidly advancing margin of infection.

2. Cellulitis - streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. It follows infection associated with mild trauma, burns, wounds, or surgical incisions. Pain, tenderness, swelling, and erythema occur. Cellulitis is differentiated from erysipelas by two clinical findings: in cellulitis, the lesion is not raised, and the line between the involved and uninvolved tissue is indistinct.

3. Necrotizing fasciitis (Streptococcal Gangrene) - this is infection of the subcutaneous tissues and fascia. There is extensive and very rapidly spreading necrosis of the skin and subcutaneous tissues. Bacteria other than *S. pyogenes* can also cause necrotizing fasciitis. The group A streptococci that cause necrotizing fasciitis have sometimes been termed «flesh-eating bacteria».

4. Puerperal fever - if the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially a septicemia originating in the infected wound (endometritis).

5. Bacteremia/Sepsis - infection of traumatic or surgical wounds with streptococci results in bacteremia, which rapidly can be fatal. *S. pyogenes* bacteremia can also follow skin infections, such as cellulitis and rarely pharyngitis.

B. DISEASES ATTRIBUTABLE TO LOCAL INFECTION WITH *S. PYOGENES* AND THEIR BY-PRODUCTS

1. Streptococcal sore throat **-** the most common infection due to β-hemolytic *S. pyogenes* is streptococcal

sore throat or pharyngitis. *S. pyogenes* adhere to the pharyngeal epithelium by means of lipoteichoic acid-covered surface pili. The glycoprotein fibronectin (MW 440,000) on epithelial cells probably serves as lipoteichoic acid ligand. In infants and small children, the sore throat occurs as a subacute nasopharyngitis with a thin serous discharge and little fever but with a tendency of the infection to extend to the middle ear and the mastoid. The cervical lymph nodes are usually enlarged. The illness may persist for weeks. In older children and adults, the disease is more acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness and edema of the mucous membranes, with purulent exudate, enlarged, tender cervical lymph nodes, and (usually) a high fever. Twenty percent of infections are asymptomatic. A similar clinical picture can occur with infectious mononucleosis, diphtheria, gonococcal infection, and adenovirus infection. *S. pyogenes* infection of the upper respiratory tract does not usually involve the lungs. Pneumonia, when it does occur, is rapidly progressive and severe and is most commonly a sequela to viral infections, eg, influenza or measles, which seem to enhance susceptibility greatly.

2. Streptococcal pyoderma - local infection of superficial layers of skin, especially in children, is called impetigo. It consists of superficial vesicles that break down and eroded areas whose denuded surface is covered with pus and later is encrusted. It spreads by continuity and is highly communicable, especially in hot, humid climates. More widespread infection occurs in eczematous or wounded skin or in burns and may progress to cellulitis. Group A streptococcal skin infections are often attributable to M types 49, 57, and 59-61 and may precede glomerulonephritis but do not often lead to rheumatic fever. A clinically identical infection can be caused by *S. aureus* and sometimes both *S. pyogenes* and *S. aureus* are present.

C. INVASIVE GROUP A STREPTOCOCCAL INFECTIONS, STREPTOCOCCAL TOXIC SHOCK SYNDROME, AND SCARLET FEVER

Fulminant, invasive *S. pyogenes* infections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients. The infections tend to follow minor trauma in otherwise healthy persons with several presentations of soft tissue infection. These include necrotizing fasciitis, myositis, and infections at other soft tissue sites; bacteremia occurs frequently. In some patients, particularly those infected with group A streptococci of M types 1 or 3, the disease presents with focal soft tissue infection accompanied by fever and rapidly progressive shock with multiorgan failure. Erythema and desquamation may occur. The *S. pyogenes* of the M types 1 and 3 (and types 12 and 28) that make pyrogenic exotoxin A or B are associated with the severe infections.

Pyrogenic exotoxins A-C also cause scarlet fever in association with *S. pyogenes* pharyngitis or with skin or soft tissue infection. The pharyngitis may be severe. The rash appears on the trunk after 24 hours of illness and spreads to involve the extremities. Streptococcal toxic shock syndrome and scarlet fever are clinically overlapping diseases.

D. POSTSTREPTOCOCCAL DISEASES (RHEUMATIC FEVER, GLOMERULONEPHRITIS)

Following an acute *S. pyogenes* infection, there is a latent period of 1-4 weeks, after which nephritis or rheumatic fever occasionally develops. The latent period suggests that these poststreptococcal diseases are not attributable to the direct effect of disseminated bacteria but represent instead a hypersensitivity response. Nephritis is more commonly preceded by infection of the skin; rheumatic fever is more commonly preceded by infection of the respiratory tract.

1. Acute glomerulonephritis - this sometimes develops 3 weeks after *S. pyogenes* skin infection (pyoderma, impetigo). Some strains are particularly nephritogenic, principally with M types 12, 4, 2, and 49. Other nephritogenic, M types are 59-61. After random streptococcal skin infections, the incidence of nephritis is less than 0,5%.

Glomerulonephritis may be initiated by antigen-antibody complexes on the glomerular basement membrane. The most important antigen is probably in the streptococcal protoplast membrane. In acute nephritis, there is blood and protein in the urine, edema, high blood pressure, and urea nitrogen retention; serum complement levels are also low. A few patients die; some develop chronic glomerulonephritis with ultimate kidney failure; and the majority recover completely.

2. Rheumatic fever - this is the most serious sequela of *S. pyogenes* because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens. Sera from patients with rheumatic fever contain antibodies to these antigens.

The onset of rheumatic fever is often preceded by *S. pyogenes* infection 1-4 weeks earlier, although the infection may be mild and may not be detected. In general, however, patients with more severe streptococcal

sore throats have a greater chance of developing rheumatic fever.

Typical symptoms and signs of rheumatic fever include fever, malaise, a migratory nonsuppurative polyarthritis, and evidence of inflammation of all parts of the heart (endocardium, myocardium, pericardium). The carditis characteristically leads to thickened and deformed valves and to small perivascular granulomas in the myocardium (Aschoff bodies) that are finally replaced by scar tissue. Erythrocyte sedimentation rates, serum transaminase levels, electrocardiograms, and other tests are used to estimate rheumatic activity.

Rheumatic fever has a marked tendency to be reactivated by recurrent streptococcal infections, whereas nephritis does not. The first attack of rheumatic fever usually produces only slight cardiac damage, which, however, increases with each subsequent attack. It is therefore important to protect such patients from recurrent *S. pyogenes* infections by prophylactic penicillin administration.

Diagnostic Laboratory Tests

A. SMEARS

Smears from pus often show single cocci or pairs rather than definite chains. Cocci are sometimes Gram-negative because the organisms are no longer viable and have lost their ability to retain the blue dye (crystal violet) and be Gram-positive. If smears of pus show streptococci but cultures fail to grow, anaerobic organisms must be suspected. Smears of throat swabs are rarely contributory, because viridans streptococci are always present and have the same appearance as group A streptococci on stained smears.

B. CULTURE

Specimens suspected of containing streptococci are cultured on blood agar plates. If anaerobes are suspected, suitable anaerobic media must also be inoculated. Incubation in 10% CO_2 often speeds hemolysis. Slicing the inoculum into the blood agar has a similar effect, because oxygen does not readily diffuse through the medium to the deeply embedded organisms, and it is oxygen that inactivates streptolysin O. Blood cultures will grow hemolytic group A streptococci (eg, in sepsis) within hours or a few days. Certain ***α***-hemolytic streptococci and enterococci may grow slowly, so blood cultures in cases of suspected endocarditis occasionally do not turn positive for a few days. The degree and kind of hemolysis (and colonial appearance) may help place an organism in a definite group. *S. pyogenes* can be identified by rapid tests specific for the presence of the group A-specific antigen and by the PYR test. Streptococci belonging to group A may be presumptively identified by inhibition of growth by bacitracin, but this should be used only when more definitive tests are not available.

C. ANTIGEN DETECTION TESTS

Several commercial kits are available for rapid detection of group A streptococcal antigen from throat swabs. These kits use enzymatic or chemical methods to extract the antigen from the swab, then use EIA or agglutination tests to demonstrate the presence of the antigen. The tests can be completed minutes to hours after the specimen is obtained. They are 60-90% sensitive, depending upon the prevalence of the disease in the population, and 98-99% specific when compared to culture methods.

D. SEROLOGIC TESTS

A rise in the titer of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include antistreptolysin O (ASO), particularly in respiratory disease; anti-DNase and antihyaluronidase, particularly in skin infections; antistreptokinase; anti-M type-specific antibodies; and others. Of these, the anti-ASO titer is most widely used.

Treatment

All *S. pyogenes* are susceptible to penicillin G, and most are susceptible to erythromycin. Some are resistant to tetracyclines. Antimicrobial drugs have no effect on established glomerulonephritis and rheumatic fever. In acute streptococcal infections, however, every effort must be made to rapidly eradicate streptococci from the patient, eliminate the antigenic stimulus (before day 8), and thus prevent poststreptococcal disease. Doses of penicillin or erythromycin that result in effective tissue levels for 10 days usually accomplish this. Antimicrobial drugs are also very useful in preventing reinfection with β-hemolytic group A streptococci in rheumatic fever patients.

Epidemiology, Prevention & Control

A. EPIDEMIOLOGY

The ultimate source of group A streptococci is a person harboring these organisms. The individual may have a clinical or subclinical infection or may be a carrier distributing streptococci directly to other persons via droplets from the respiratory tract or skin. The nasal discharges of a person harboring *S. pyogenes* are the

most dangerous source for spread of these organisms.

Many other streptococci (viridans streptococci, enterococci, etc) are members of the normal flora of the human body. They produce disease only when established in parts of the body.

B. PREVENTION

Control procedures are directed mainly at the human source:

1. Detection and early antimicrobial therapy of respiratory and skin infections with group A streptococci. Prompt eradication of streptococci from early infections can effectively prevent the development of poststreptococcal disease. This requires maintenance of adequate penicillin levels in tissues for 10 days (eg, benzathine penicillin G given once intramuscularly). Erythromycin is an alternative drug, although some *S. pyogenes* are resistant.

2. Antistreptococcal chemoprophylaxis in persons who have suffered an attack of rheumatic fever. This involves giving one injection of benzathine penicillin G intramuscularly, every 3-4 weeks, or daily oral penicillin or oral sulfonamide. The first attack of rheumatic fever infrequently causes major heart damage; however, such persons are particularly susceptible to reinfections with streptococci that precipitate relapses of rheumatic activity and give rise to cardiac damage. Chemoprophylaxis in such individuals, especially children, must be continued for years. Chemoprophylaxis is not used in glomerulonephritis because of the small number of nephritogenic types of streptococci. An exception may be family groups with a high rate of poststreptococcal nephritis.

3. Eradication of *S. pyogenes* from carriers. This is especially important when carriers are in areas such as obstetric delivery rooms, operating rooms, classrooms, or nurseries. Unfortunately, it is often difficult to eradicate ***β***-hemolytic streptococci from permanent carriers, and individuals may occasionally have to be shifted away from «sensitive» areas for some time.

Group B: *Streptococcus agalactiae*

Morphology & Identification

A. MORPHOLOGY

S. agalactiae is a diplococcal (a pair of cocci, circular, pair) Gram-positive, non acid-fast bacterium (~2,0μm) that does not form spores, is not motile, and is catalase-free (catalase is an enzyme that catalyzes the reduction of hydrogen peroxide). It occurs in pairs or short chains and has group B Lancefield antigen present.

B. CULTURE AND GROWTH CHARACTERISTICS

S. agalactiae is a chemoorganotroph that uses glucose as energy source. This bacterium is able to synthesize ATP by oxidative phosphorylation. *S. agalactiae* is also able to ferment different carbon sources to multiple by-products, lactate, acetate, ethanol, formate or acetoin. Growth of streptococci tends to be poor on solid media or in broth unless enriched with blood or tissue fluids. Nutritive requirements vary widely among different species. The human pathogens are most exacting, requiring a variety of growth factors. Growth and hemolysis are aided by incubation in 10% CO_2.

C. VIRULENCE FACTORS

Thock pepidoglycan layer in cell wall permits survival on dry surface. Capsule interferes with phagocytosis. Hydrolytic enzymes may facilitate tissue destruction and systemic spread of the bacteria.

Pathogenesis, Pathology & Clinical Findings

Two forms of neonatal disease: early-onset and late-onset; these diseases are characterized by meningitis, pneumonia and bacteremia. Other infections with group B streptococci are endometritis, urinary tract infection, wound infection and bacteremia.

Diagnostic Laboratory Tests

Most strains of *S. agalactiae* isolated from human sources give a narrow and indistinct zone of beta-hemolysis. Bovine strains are more often nonhemolytic. However, practically all strains, whether hemolytic or not, give a positive CAMP reaction: they produce a diffusible substance that completes the lysis of sheep erythrocytes exposed to a sphingomyelinase C such as staphylococcal b-toxin or the a-toxin of *C. perfringens.*

Purified CAMP factor is lethal to rabbits when injected intravenously. Furthermore, its role as a virulence factor is supported by its ability to bind immunoglobulins G and M of humans and several animal species via the Fc part. It has also been referred to as protein B.

The hemolysin of *S. agalactiae*, which has been cloned and sequenced, is not related to the streptolysins of *S. pyogenes*. It has been considered a virulence factor, but isogenic strains with and without expression of

the hemolysin show no significant difference in virulence in a neonatal rat model.

Hippurase positive.

Treatment

Group B streptococci have never been as exquisitely sensitive to penicillin as group A beta-hemolytic streptococci; therefore, the initial therapy for group B streptococcal infection has always been high-dose parenteral penicillin or ampicillin.

Epidemiology, Prevention & Control

A. EPIDEMIOLOGY

Asymptomatic colonization of the upper respiratory tract and genitourinary tract. Most infections in newborns acquired from mother during pregnancy or at time of birth. Neonates are at higer risk for infection if (1) there is premature rupture of membranes, prolonges labor, preterm birth, or disseminated maternal group B streptococcal disease and (2) mother is without type-specific antibodies and has low complement levels.

Women with genital colonization are at risck for post-partum sepsis. Men and nonpregnant women with diadetes mellitus, cancer, or alcoholism are at increased riack for disease.

B. PREVENTION

Intrapartum antibiotic prophylaxis (IAP) is recommended for:

- women who delivered a previous infant with GBS disease,
- women with GBS bacteriuria in the current pregnancy,
- women with a GBS-positive screening result in the current pregnancy,
- women with unknown GBS status who deliver at less than 37 weeks' gestation, have an intrapartum temperature of 38 °C (100,4 °F) or greater, or have rupture of membranes for 18 hours or longer.

Penicillin is the preferred agent for intrapartum antibiotic prophylaxis, and ampicillin is an acceptable alternative.

Simple anti-septic wipes do not prevent mother-to-child transmission. Up to 90% of early-onset GBS infection would be preventable if intravenous antibiotics were offered in labour to all GBS carriers identified by universal sensitive testing late in pregnancy plus to the mothers of babies in the recognised higher risk situations. Early onset GBS infection is most likely to present with breathing problems and pneumonia; late onset GBS infection is more likely to present with meningitis and septicaemia. Once symptoms are present, the condition can be difficult to treat.

Streptococcus pneumoniae

Morphology & Identification

A. MORPHOLOGY

S. pneumoniae are Gram-positive bacteria in the shape of a slightly pointed cocci. They are usually found in pairs (diplococci), but are also found singly and in short chains. *S. pneumoniae* are alpha hemolytic (a term describing how the cultured bacteria break down red blood cells for the purpose of classification). Individual bacteria are between 0,5 and 1,25 micrometers in diameter. *S pneumoniae* do not form spores and are non-motile, though they sometimes have pili used for adherence.

B. CULTURE AND GROWTH CHARACTERISTICS

S. pneumoniae is a fastidious bacterium, growing best in 5% carbon dioxide. Nearly 20% of fresh clinical isolates require fully anaerobic conditions. In all cases, growth requires a source of catalase (e.g. blood) to neutralize the large amount of hydrogen peroxide produced by the bacteria. In complex media containing blood, at 37 °C the bacterium has a doubling time of 20-30 minutes.

On agar, pneumococci grow as glistening colonies, about 1 mm in diameter. Two serotypes, types 3 and 37, are mucoid. Pneumococci spontaneously undergo a genetically determined, phase variation from opaque to transparent colonies at a rate of 1 in 10^5. The transparent colony type is adapted to colonization of the nasopharynx, whereas the opaque variant is suited for survival in blood. The chemical basis for the difference in colony appearance is not known, but significant difference in surface protein expression between the two types has been shown.

S. pneumoniae is a fermentative aerotolerant anaerobe. It is usually cultured in media that contain blood. On blood agar, colonies characteristically produce a zone of alpha (green) hemolysis, which differentiates *S. pneumoniae* from the group A (beta hemolytic) streptococci, but not from commensal alpha hemolytic (viridans) streptococci which are co-inhabitants of the upper respiratory tract. Special tests such

as inulin fermentation, bile solubility, and optochin (an antibiotic) sensitivity must be routinely employed to differentiate the pneumococcus from *S. viridans*.

S. pneumoniae is a very fragil e bacterium and contains within itself the enzymatic ability to disrupt and to disintegrate the cells. The enzyme responsible is called an autolysin. The physiological role of this autolysin is to cause the culture to undergo a characteristic autolysis that kills the entire culture when grown to stationary phase. Virtually all clinical isolates of pneumococci harbor this autolysin and undergo lysis usually beginning between 18-24 hours after initiation of growth under optimal conditions. Autolysis is consistent with changes in colony morphology. Colonies initially appear with a plateau-type morphology, then start to collapse in the centers when autolysis begins.

C. VIRULENCE FACTORS

The virulence factors of *S. pneumoniae* include a plysaccharide capsule that prevents phagocytosis by the host's immune cells, surface proteins that prevent the activation of complement (part of the immune system that helps clear pathogens from the body), and pili that enable *S. pneumoniae* to attach to epithelial cells in the upper respiratory tract.

The polysaccharide capsule interferes with phagocytosis through its chemical composition, resisting by interfering with binding of complement C3b to the cell's surface. Encapsulated strains of *S. pneumoniae* are found to be 100,000 times more virulent than unencapsulated strains during invasion of mucosal surfaces.

Pili are long, thin extracellular organelles that are able to extend outside of the polysaccharide capsule. They are encoded by the rlrA islet (an area of a genome in which rapid mutation takes place) which is present in only some isolated strains of *S. pneumoniae*. These pili contribute to adherence and virulence, as well as increase the inflammatory response of the host.

Pathogenesis, Pathology & Clinical Findings

A. DISEASES

S. pneumoniae is known to cause bacteremia, otitis media bronchitis, rhinitis, acute sinusitis, conjuncti vitis, meningitis, sepsis, osteomyelitis, endocarditis, peritonitis, pericarditis and brain abscess.

B. SOURCE OF AGENTS

S. pneumoniae is found in the respiratory tracts of mammals. While it is part of the normal flora of this environment, going unnoticed when present in small densities, it acts as a pathogen toward its host when present in large enough densities.

C. PATHOGENESIS

1. Types of pneumococci: in adults, types 1-8 are responsible for about 75% of cases of pneumococcal pneumonia and for more than half of all fatalities in pneumococcal bacteremia; in children, types 6, 14, 19, and 23 are frequent causes.

2. Production of disease: pneumococci produce disease through their ability to multiply in the tissues. They produce no toxins of significance. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytes. A serum that contains antibodies against the type-specific polysaccharide protects against infection. If such a serum is absorbed with the type-specific polysaccharide, it loses its protective power. Animals or humans immunized with a given type of pneumococcal polysaccharide are subsequently immune to that type of pneumococcus and possess precipitating and opsonizing antibodies for that type of polysaccharide.

3. Loss of natural resistance: since 40-70% of humans are at some time carriers of virulent pneumococci, the normal respiratory mucosa must possess great natural resistance to the pneumococcus. Among the factors that probably lower this resistance and thus predispose to pneumococcal infection are the following:

(a) Viral and other respiratory tract infections that damage surface cells; abnormal accumulations of mucus (eg, allergy), which protect pneumococci from phagocytosis; bronchial obstruction (eg, atelectasis); and respiratory tract injury due to irritants disturbing its mucociliary function.

(b) Alcohol or drug intoxication, which depresses phagocytic activity, depresses the cough reflex, and facilitates aspiration of foreign material.

(c) Abnormal circulatory dynamics (eg, pulmonary congestion, heart failure).

(d) Other mechanisms, eg, malnutrition, general debility, sickle cell anemia, hyposplenism, nephrosis, or complement deficiency.

D. CLINICAL FINDINGS

The onset of pneumococcal pneumonia is usually sudden, with fever, chills, and sharp pleural pain. The

sputum is similar to the alveolar exudate, being characteristically bloody or rusty colored. Early in the disease, when the fever is high, bacteremia is present in 10-20% of cases. With antimicrobial therapy, the illness is usually terminated promptly; if drugs are given early, the development of consolidation is interrupted.

Pneumococcal pneumonia must be differentiated from pulmonary infarction, atelectasis, neoplasm, congestive heart failure, and pneumonia caused by many other bacteria. Empyema (pus in the pleural space) is a significant complication and requires aspiration and drainage. From the respiratory tract, pneumococci may reach other sites. The sinuses and middle ear are most frequently involved. Infection sometimes extends from the mastoid to the meninges.

Bacteremia from pneumonia has a triad of severe complications: meningitis, endocarditis, and septic arthritis. With the early use of chemotherapy, acute pneumococcal endocarditis and arthritis have become rare.

Diagnostic Laboratory Tests

Blood is drawn for culture; CSF and sputum are collected for demonstration of pneumococci by smear and culture. Serum antibody tests are impractical. Sputum may be examined in several ways.

A. STAINED SMEARS

A Gram-stained film of rusty-red sputum shows typical organisms, many polymorphonuclear neutrophils, and many red cells.

B. CAPSULE SWELLING TESTS

Fresh emulsified sputum mixed with antiserum causes capsule swelling (the quellung reaction) for identification of pneumococci.

C. CULTURE

The culture is created by sputum cultured on blood agar and incubated in CO_2 or a candle jar. A blood culture is also taken.

Immunity. Vaccination

Immunity to infection with pneumococci is type-specific and depends both on antibodies to capsular polysaccharide and on intact phagocytic function. It is possible to immunize individuals with type-specific polysaccharides. Such vaccines can probably provide 90% protection against bacteremic pneumonia. This vaccine is appropriate for elderly, debilitated, or immunosuppressed individuals. A pneumococcal conjugate vaccine contains capsular polysaccharides conjugated to diphtheria CRM197 protein. This seven-valent vaccine is recommended for all children aged 2-23 months, to help prevent ear infections, and for selected children aged 24-59 months.

Treatment

Since pneumococci are sensitive to many antimicrobial drugs, early treatment usually results in rapid recovery, and antibody response seems to play a much diminished role. Highdose penicillin G with MICs of 0,1-2 μg/mL appears to be effective in treating pneumonia caused by pneumococci but would not be effective in treatment of meningitis due to the same strains. Some penicillin-resistant strains are resistant to cefotaxime. Resistance to tetracycline and erythromycin occurs also. Pneumococci remain susceptible to vancomycin.

Epidemiology

Pneumococcal pneumonia is most common in elderly, debilitated, or immunosuppressed individuals. The disease often sets in after a preceding viral infection damages the respiratory ciliated epithelium; incidence therefore peaks in the winter.

Viridans streptococci

The viridans streptococci include *S. mitis, S. mutans, S. salivarius, S. sanguis,* and others. Typically they are α-hemolytic, but they may be nonhemolytic. Their growth is not inhibited by optochin, and colonies are not soluble in bile (deoxycholate). The viridans streptococci are the most prevalent members of the normal flora of the upper respiratory tract and are important for the healthy state of the mucous membranes there. They may reach the bloodstream as a result of trauma and are a principal cause of endocarditis on abnormal heart valves. Some viridans streptococci (eg, *S. mutans*) synthesize large polysaccharides such as dextrans or levans from sucrose and contribute importantly to the genesis of dental caries.

In the course of bacteremia, viridans streptococci, pneumococci, or enterococci may settle on normal or previously deformed heart valves, producing acute endocarditis. Rapid destruction of the valves frequently leads to fatal cardiac failure in days or weeks unless a prosthesis can be inserted during antimicrobial therapy.

Subacute endocarditis often involves abnormal valves (congenital deformities and rheumatic or

atherosclerotic lesions). Although any organism reaching the bloodstream may establish itself on thrombotic lesions that develop on endothelium injured as a result of circulatory stresses, subacute endocarditis is most frequently due to members of the normal flora of the respiratory or intestinal tract that have accidentally reached the blood. After dental extraction, at least 30% of patients have viridans streptococcal bacteremia. These streptococci, ordinarily the most prevalent members of the upper respiratory flora, are also the most frequent cause of subacute bacterial endocarditis.

The group D streptococci (enterococci and *S. bovis*) also are common causes of subacute endocarditis. About 5-10% of cases are due to enterococci originating in the gut or urinary tract. The lesion is slowly progressive, and a certain amount of healing accompanies the active inflammation; vegetations consist of fibrin, platelets, blood cells, and bacteria adherent to the valve leaflets. The clinical course is gradual, but the disease is invariably fatal in untreated cases. The typical clinical picture includes fever, anemia, weakness, a heart murmur, embolic phenomena, an enlarged spleen, and renal lesions. α-hemolytic streptococci and enterococci vary in their susceptibility to antimicrobial agents. Particularly in bacterial endocarditis, antibiotic susceptibility tests are useful to determine which drugs may be used for optimal therapy. Aminoglycosides often enhance the rate of bactericidal action of penicillin on streptococci, particularly enterococci.

Enterococcus

Scientific classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Entercoccaceae
Genus: Enterococcus
Species: E. faecalis, E. faecium

The enterococci have the group D group-specific substanc and were previously classified as group D streptococci. Because the group D cell wall specific antigen is a teichoic acid, it is not a antigenically good marker enterococci are usually identified by characteristics other than immunologic reaction with group-specific antisera.

Morphology & Identification

A. MORPHOLOGY

Enterococci are Gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci on physical characteristics alone.

B. CULTURE AND GROWTH CHARACTERISTICS

Enterococci are capable of growing at a range of temperatures from 10-45 °C, and can grow in hypotonic, hypertonic, acidic, or alkaline environments. As facultative anaerobes, enterococci can grow under reduced or oxygenated conditions. They are also capable of survival at 60 °C for 30 minutes. *E. faecalis* is able to grow in 6,5% NaCl. Enterococci can also grow in 40% bile salts and over a broad range of pH.

Colonies are usually alpha or gamma hemolytic. Growth on bile-esculin produces a black precipitate derived from esculin; many other bacteria will not grow in the presence of bile. Group D streptococci are divided into those that will grow in 6,5% saline (enterococci) and those that will not (non-enterococci).

C. VIRULENCE FACTORS

1. Colonization factors

Aggregation substance: hairlike protein embedded in cytoplasmic membrane that facilitates lasid exchange and binding to epithelial cells.

Carbohydrate adhesions: present in individual bacterium in multiple types; mediate binding to host cells.

2. Secreted factors

Cytolysin: protein bacteriocin that inhidits of Gram-positive bacteria**.**

Pheromone: chemoattractant for neutrophils that may regulate inflammatory reaction.

Gelatinase: hydrolyzes gelatin, collagen, hemoglobin.

Diseases caused by *Enterococcus*

They are a significant cause of urinary tract infections (but much less common than *E. coli*) and also of

opportunistic infections (including intra-abdominal, septicemia and endocarditis).

Diagnostic Laboratory Tests

Enterococci can be isolated using any blood agar. They can be isolated from Gram-negative bacteria in a sample using bile-esculin azide, phenylethyl alcohol agar, Columbia colistin-nalidixic acid agar, or other media containing azide. Standard laboratory growing conditions for enterococci is a brain heart infusion, or Todd-Hewitt, broth or agar, supplemented with antibiotics when appropriate, at 35-37 °C without aeration.

Esculin hydrolysis.

BEA media.

Treatment

E. faecalis is resistant to many commonly used antimicrobial agents (aminoglycosides, aztreonam, cephalosporins, clindamycin, the semisynthetic penicillins nafcillin and oxacillin, and trimethoprim-sulfamethoxazole). Resistance to vancomycin in *E. faecalis* is becoming more common. Treatment options for vancomycin-resistant *E. faecalis* include nitrofurantoin (in the case of uncomplicated UTIs), linezolid, and daptomycin, although ampicillin is preferred if the bacteria are susceptible. Quinupristin/dalfopristin can be used to treat *E. faecium* but not *E. faecalis*

Epidemiology

They normally inhabit the bowels of animlas, humans included, but they are found in soil, vegetation, and surface water, probably due to contamination by animal excrement.

The enterococci are among the most frequent causes of nosocomial infections, particularly in intensive care units, and are selected by therapy with cephalosporins and other antibiotics to which they are resistant. Enterococci are transmitted from one patient to another primarily on the hands of hospital personnel, some of whom may carry the enterococci in their gastrointestinal tracts. Enterococci occasionally are transmitted on medical devices. In patients, the most common sites of infection are the urinary tract, wounds, biliary tract, and blood. Enterococci may cause meningitis and bacteremia in neonates. In adults, enterococci can cause endocarditis. However, in intra-abdominal, wound, urine, and other infections, enterococci usually are cultured along with other species of bacteria, and it is difficult to define the pathogenic role of the enterococci.

The *Neisseria*

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Beta Proteobacteria
Order: Neisseriales
Family: Neisseriaceae
Genus: Neisseria
Species: N. gonorrhoeae, N. meningitidis

Morphology & Identification

A. MORPHOLOGY

The typical *Neisseria* is a Gram-negative, nonmotile diplococcus, approximately 0,8 μm in diameter. Individual cocci are kidney-shaped; when the organisms occur in pairs, the flat or concave sides are adjacent.

B. CULTURE AND GROWTH CHARACTERISTICS

In 48 hours on enriched media (eg, Mueller-Hinton, modified Thayer-Martin), gonococci and meningococci form convex, glistening, elevated, mucoid colonies 1-5 mm in diameter. Colonies are transparent or opaque, nonpigmented, and nonhemolytic. *Neisseria flavescens, Neisseria subflava,* and *Neisseria lactamica* have yellow pigmentation. *Neisseria sicca* produces opaque, brittle, wrinkled colonies. *M. catarrhalis* produces nonpigmented or pinkish-gray opaque colonies.

The *Neisseria* grow best under aerobic conditions, but some will grow in an anaerobic environment. They have complex growth requirements. Most *Neisseria* ferment carbohydrates, producing acid but not gas, and their carbohydrate fermentation patterns are a means of distinguishing them. The *Neisseria* produce oxidase and give positive oxidase reactions; the oxidase test is a key test for identifying them. When bacteria are spotted on a filter paper soaked with tetramethylparaphenylenediamine hydrochloride (oxidase), the neisseria rapidly turn dark purple.

Meningococci and gonococci grow best on media containing complex organic substances such as

heated blood, hemin, and animal proteins and in an atmosphere containing 5% CO_2 (eg, candle jar). Growth is inhibited by some toxic constituents of the medium, eg, fatty acids or salts. The organisms are rapidly killed by drying, sunlight, moist heat, and many disinfectants. They produce autolytic enzymes that result in rapid swelling and lysis in vitro at 25 °C and at an alkaline pH.

Neisseria gonorrhoeae

Gonococci ferment only glucose and differ antigenically from the other neisseria. Gonococci usually produce smaller colonies than those of the other neisseriae. Gonococci that require arginine, hypoxanthine, and uracil (Arg-, Hyx-, Ura- auxotype) tend to grow most slowly on primary culture. Gonococci isolated from clinical specimens or maintained by selective subculture have typical small colonies containing piliated bacteria. On nonselective subculture, larger colonies containing nonpiliated gonococci are also formed. Opaque and transparent variants of both the small and large colony types also occur; the opaque colonies are associated with the presence of a surface-exposed protein, Opa.

Virulence factors

N. gonorrhoeae is antigenically heterogeneous and capable changing its surface structures *in vitro* - and presumably *in vivo* - to avoid host defenses. Surface structures include the following.

1. Pili (fibriae): pili are the hair-like appendages that extend up to several micrometers from the gonococcal surface. They enhance attachment to host cells and resistance to phagocytosis. They are made up of stacked pilin proteins (MW 17,000-21,000). The amino terminal of the pilin molecule, which contains a high percentage of hydrophobic amino acids, is conserved. The amino acid sequence near the mid portion of the molecule also is conserved; this portion of the molecule serves in attachment to host cells and is less prominent in the immune response. The amino acid sequence near the carboxyl terminal is highly variable; this portion of the molecule is most prominent in the immune response. The pilins of almost all strains of *N. gonorrhoeae* are antigenically different, and a single strain can make many antigenically distinct forms of pilin.

2. Por: por protein extends through the gonococcal cell membrane. It occurs in trimers to form pores in the surface through which some nutrients enter the cell. Por proteins may impact intracellular killing of gonococci within neutrophils by preventing phagosome-lysosome fusion. The molecular weight of Por varies from 34,000 to 37,000. Each strain of gonococcus expresses only one of two types of Por, but the Por of different strains is antigenically different. Serologic typing of Por by agglutination reactions with monoclonal antibodies has distinguished 18 serovars of PorA and 28 serovars of PorB.

3. Opa proteins: these proteins function in adhesion of gonococci within colonies and in attachment of gonococci to host cells, especially cells that express carcinoembryonic antigens (CD66). One portion of the Opa molecule is in the gonococcal outer membrane, and the rest is exposed on the surface. The molecular weight of Opa ranges from 24,000 to 28,000. A strain of gonococcus can express no, one, two, or occasionally three types of Opa, though each strain has eleven or twelve genes for different Opas.

4. Rmp (protein III): this protein (MW about 33,000) is antigenically conserved in all gonococci. It is a reduction-modifiable protein (Rmp) and changes its apparent molecular weight when in a reduced state. It associates with Por in the formation of pores in the cell surface.

5. Lipooligosaccharide (los): in contrast to the enteric Gram-negative rods, gonococcal LPS does not have long O-antigen side chains and is called a lipooligosaccharide. Its molecular weight is 3000-7000. Gonococci can express more than one antigenically different LOS chain simultaneously. Toxicity in gonococcal infections is largely due to the endotoxic effects of LOS. In a form of molecular mimicry, gonococci make LOS molecules that structurally resemble human cell membrane glycosphingolipids. The gonococcal LOS and the human glycosphingolipid of the same structural class react with the same monoclonal antibody, indicating the molecular mimicry. The presence on the gonococcal surface of the same surface structures as human cells helps gonococci evade immune recognition.

6. Other proteins: several antigenically constant proteins of gonococci have poorly defined roles in pathogenesis. Lip (H8) is a surface- exposed protein that is heat-modifiable like Opa. The Fbp (iron-binding protein), similar in molecular weight to Por, is expressed when the available iron supply is limited, eg, in human infection. Gonococci elaborate an IgA1 protease that splits and inactivates IgA1, a major mucosal immunoglobulin of humans. Meningococci, *Haemophilus influenzae,* and *S. pneumoniae* elaborate similar IgA1 proteases.

Pathogenesis, Pathology & Clinical Findings

Gonococci exhibit several morphologic types of colonies, but only piliated bacteria appear to be virulent. Opa protein expression varies depending on the type of infection. Gonococci that form opaque colonies are isolated from men with symptomatic urethritis and from uterine cervical cultures at mid cycle. Gonococci that form transparent colonies are frequently isolated from men with asymptomatic urethral infection, from menstruating women, and from invasive forms of gonorrhea, including salpingitis and disseminated infection. Antigenic variation of surface proteins during infection allows the organism to circumvent host immune response.

Gonococci attack mucous membranes of the genitourinary tract, eye, rectum, and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis. In males, there is usually urethritis, with yellow, creamy pus and painful urination. The process may extend to the epididymis. As suppuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures. Urethral infection in men can be asymptomatic. In females, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. It may then progress to the uterine tubes, causing salpingitis, fibrosis, and obliteration of the tubes. Infertility occurs in 20% of women with gonococcal salpingitis. Chronic gonococcal cervicitis or proctitis is often asymptomatic.

Gonococcal bacteremia leads to skin lesions (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists. Gonococci can be cultured from blood or joint fluid of only 30% of patients with gonococcal arthritis. Gonococcal endocarditis is an uncommon but severe infection. Gonococci sometimes cause meningitis and eye infections in adults; these have manifestations similar to those due to meningococci. Complement deficiency is frequently found in patients with gonoccal bacteremia. Patients with bacteremia, especially if recurrent, should be tested for total hemolytic complement activity.

Gonococcal ophthalmia neonatorum, an infection of the eye of the newborn, is acquired during passage through an infected birth canal. The initial conjunctivitis rapidly progresses and, if untreated, results in blindness. Gonococci that produce localized infection are often serum-sensitive (killed by antibody and complement).

Diagnostic laboratory tests

A. SPECIMENS

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear. Blood culture is necessary in systemic illness, but a special culture system is helpful, since gonococci (and meningococci) may be susceptible to the polyanethol sulfonate present in standard blood culture media.

B. SMEARS

Gram-stained smears of urethral or endocervical exudate reveal many diplococci within pus cells. These give a presumptive diagnosis. Stained smears of the urethral exudate from men have a sensitivity of about 90% and a specificity of 99%. Stained smears of endocervical exudates have a sensitivity of about 50% and a specificity of about 95% when examined by an experienced microscopist. Cultures of urethral exudate from men are not necessary when the stain is positive, but cultures should be done for women. Stained smears of conjunctival exudates can also be diagnostic, but those of specimens from the throat or rectum are generally not helpful.

C. CULTURE

Immediately after collection, pus or mucus is streaked on enriched selective medium (eg, modified Thayer-Martin medium) and incubated in an atmosphere containing 5% CO_2 (candle extinction jar) at 37 °C. To avoid overgrowth by contaminants, the selective medium contains antimicrobial drugs (eg, vancomycin, 3 µg/mL; colistin, 7,5 µg/mL; amphotericin B, 1 µg/mL; and trimethoprim, 3 µg/mL). If immediate incubation is not possible, the specimen should be placed in a CO_2-containing transport-culture system. Forty-eight hours after culture, theorganisms can be quickly identified by their appearance on a Gram-stained smear, by oxidase positivity, and by coagglutination, immunofluorescence staining, or other laboratory tests. The species of subcultured bacteria may be determined by fermentation reactions. The gonococcal isolates from anatomic sites other than the genital tract or from children should be identified as to species using two different confirmatory tests because of the legal and social implications of the isolates.

D. NUCLEIC ACID AMPLIFICATION TESTS

Several Food and Drug Administration-cleared nucleic acid amplification assays are available for direct detection of *N. gonorrhoeae* in genitourinary specimens. In general, these assays have excellent sensitivity and specificity in symptomatic, high-prevalence populations. Advantages include better detection, more rapid results, and the ability to use urine as a specimen source. Disadvantages include poor specificity of some assays due to cross reactivity with nongonococcal *Neisseria* species. These assays are not recommended for use for the diagnosis of extragenital gonococcal infections or for infection in children.

E. SEROLOGY

Serum and genital fluid contain IgG and IgA antibodies against gonococcal pili, outer membrane proteins, and LPS. Some IgM of human sera is bactericidal for gonococci *in vitro*. In infected individuals, antibodies to gonococcal pili and outer membrane proteins can be detected by immunoblotting, radioimmunoassay, and ELISA (enzyme-linked immunosorbent assay) tests. However, these tests are not useful as diagnostic aids for several reasons: gonococcal antigenic heterogeneity; the delay in development of antibodies in acute infection; and a high background level of antibodies in the sexually active population.

Immunity

Repeated gonococcal infections are common. Protective immunity to reinfection does not appear to develop as part of the disease process, because of the antigenic variety of gonococci. While antibodies can be demonstrated, including the IgA and IgG on mucosal surfaces, they either are highly strain-specific or have little protective ability.

Treatment

Since the development and widespread use of penicillin, gonococcal resistance to penicillin has gradually risen, owing to the selection of chromosomal mutants, so that many strains now require high concentrations of penicillin G for inhibition (MIC ≥ 2 μg/mL). Penicillinase-producing *N. gonorrhoeae* (PPNG) also have increased in prevalence. Chromosomally mediated resistance to tetracycline (MIC ≥ 2 μg/mL) is common. High-level resistance to tetracycline (MIC ≥ 32 μg/mL) also occurs. Spectinomycin resistance as well as resistance to fluoroquinolones has been noted. Additional therapy with doxycycline, orally twice a day for 7 days, is recommended for the possible concomitant chlamydial infection; erythromycin base, orally four times a day for 7 days, is substituted for doxycycline in pregnant women. Modifications of these therapies are recommended for other types of *N. gonorrhoeae* infection. Since other sexually transmitted diseases may have been acquired at the same time as gonorrhea, steps must also be taken to diagnose and treat these diseases.

Epidemiology, Prevention & Control

Gonorrhea is worldwide in distribution. Gonorrhea is exclusively transmitted by sexual contact, often by women and men with asymptomatic infections. The infectivity of the organism is such that the chance of acquiring infection from a single exposure to an infected sexual partner is 20-30% for men and even greater for women. The infection rate can be reduced by avoiding multiple sexual partners, rapidly eradicating gonococci from infected individuals by means of early diagnosis and treatment, and finding cases and contacts through education and screening of populations at high risk. Mechanical prophylaxis (condoms) provides partial protection. Chemoprophylaxis is of limited value because of the rise in antibiotic resistance of the gonococcus.

PPNG first appeared in 1976. These totally penicillin-resistant gonococcal strains have appeared in many parts of the world. Areas with a high incidence of PPNG include Singapore, parts of sub-Saharan Africa, and focal areas in the United States. Focal outbreaks of disease due to PPNG have occurred in many areas of the United States and elsewhere, and endemic foci are being established.

Gonococcal ophthalmia neonatorum is prevented by local application of 0,5% erythromycin ophthalmic ointment or 1% tetracycline ointment to the conjunctiva of newborns. Although instillation of silver nitrate solution is also effective and is the classic method for preventing ophthalmia neonatorum, silver nitrate is difficult to store and causes conjunctival irritation; its use has largely been replaced by use of erythromycin or tetracycline ointment.

Neisseria meningitidis

The bacterium *N. meningitidis,* the meningococcus, is identical in its staining and morphological characteristics to *N. gonorrhoeae.* However, at the ultrastructural level, *N. meningitidis* has a prominent antiphagocytic polysaccharide capsule. *N. meningitidis* strains are grouped on the basis of their capsular

polysaccharides, into 12 serogroups, some of which are subdivided according to the presence of outer membrane protein and lipopolysaccharide antigens.

Virulence factors

Antigenic Structure

At least 13 serogroups of meningococci have been identified by immunologic specificity of capsular polysaccharides. The most important serogroups associated with disease in humans are A, B, C, Y, and W-135. The group A polysaccharide is a polymer of *N*-acetylmannosamine phosphate, and that of group C is a polymer of *N*-acetyl-*O*-acetylneuraminic acid. Meningococcal antigens are found in blood and cerebrospinal fluid of patients with active disease. Outbreaks and sporadic cases in the Western Hemisphere in the last decade have been caused mainly by groups B, C, W-135, and Y; outbreaks in southern Finland and Sro Paulo, Brazil, were due to groups A and C; those in Africa were due mainly to group A. Group C and, especially, group A are associated with epidemic disease.

The outer membrane proteins of meningococci have been divided into classes on the basis of molecular weight. All strains have either class 1, class 2, or class 3 proteins; these are analogous to the Por proteins of gonococci and are responsible for the serotype specificity of meningococci. They help form pores in the meningococcal cell wall. As many as 20 serotypes have been defined; serotypes 2 and 15 have been associated with epidemic disease. The Opa (class 5) protein is compara comparable to Opa of the gonococci. Meningococci are piliated, but unlike gonococci, they do not form distinctive colony types indicating piliated bacteria. Meningococcal LPS is responsible for many of the toxic effects found in meningococcal disease.

Pathogenesis, Pathology & Clinical Findings

Humans are the only natural hosts for whom meningococci are pathogenic. The nasopharynx is the portal of entry. There, the organisms attach to epithelial cells with the aid of pili; they may form part of the transient flora without producing symptoms. From the nasopharynx, organisms may reach the bloodstream, producing bacteremia; the symptoms may be like those of an upper respiratory tract infection. Fulminant meningococcemia is more severe, with high fever and hemorrhagic rash; there may be disseminated intravascular coagulation and circulatory collapse (Waterhouse-Friderichsen syndrome).

Meningitis is the most common complication of meningococcemia. It usually begins suddenly, with intense headache, vomiting, and stiff neck, and progresses to coma within a few hours.

During meningococcemia, there is thrombosis of many small blood vessels in many organs, with perivascular infiltration and petechial hemorrhages. There may be interstitial myocarditis, arthritis, and skin lesions. In meningitis, the meninges are acutely inflamed, with thrombosis of blood vessels and exudation of polymorphonuclear leukocytes, so that the surface of the brain is covered with a thick purulent exudate.

It is not known what transforms an asymptomatic infection of the nasopharynx into meningococcemia and meningitis, but this can be prevented by specific bactericidal serum antibodies against the infecting serotype. *Neisseria* bacteremia is favored by the absence of bactericidal antibody (IgM and IgG), inhibition of serum bactericidal action by a blocking IgA antibody, or a complement component deficiency (C5, C6, C7, or C8). Meningococci are readily phagocytosed in the presence of a specific opsonin.

Diagnostic laboratory tests

A. SPECIMENS

Specimens of blood are taken for culture, and specimens of spinal fluid are taken for smear, culture, and chemical determinations. Nasopharyngeal swab cultures are suitable for carrier surveys. Puncture material from petechiae may be taken for smear and culture.

B. SMEARS

Gram-stained smears of the sediment of centrifuged spinal fluid or of petechial aspirate often show typical neisseriae within polymorphonuclear leukocytes or extracellularly.

C. CULTURE

Culture media without sodium polyanethol sulfonate are helpful in culturing blood specimens. Cerebrospinal fluid specimens are plated on «chocolate» agar and incubated at 37 °C in an atmosphere of 5% CO_2 (candle jar). Freshly drawn spinal fluid can be directly incubated at 37 °C if agar culture media are not immediately available. A modified Thayer-Martin medium with antibiotics (vancomycin, colistin, amphotericin) favors the growth of neisseriae, inhibits many other bacteria, and is used for nasopharyngeal cultures. Presumptive colonies of neisseriae on solid media, particularly in mixed culture, can be identified

by Gram stain and the oxidase test. Spinal fluid and blood generally yield pure cultures that can be further identified by carbohydrate fermentation reactions and agglutination with type-specific or polyvalent serum.

D. SEROLOGY

Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests or by their bactericidal activity. These tests are done only in reference laboratories.

Immunity

Immunity to meningococcal infection is associated with the presence of specific, complement-dependent, bactericidal antibodies in the serum. These antibodies develop after subclinical infections with different strains or injection of antigens and are group-specific, type-specific, or both. The immunizing antigens for groups A, C, Y, and W-135 are the capsular polysaccharides. For group B, a specific antigen suitable for use as a vaccine has not been defined; however, group B vaccines with mixtures of antigens have been used in many parts of the world. Currently there are two vaccine types against serogroups A, C, Y, and W-135. A polysaccharide tetravalent vaccine in which each dose consists of four purified bacterial capsular polysaccharides is poorly immunogenic in children under 18 months, does not confer long-lasting immunity, and does not cause a sustainable reduction in nasopharyngeal carriage. A newly approved (2005) tetravalent conjugate vaccine (Encarta, Sanofi Pasteur, Inc.) is licensed for use in persons 11-55 years of age. It contains capsular polysaccharide conjugated to diphtheria toxoid. The advantage of this vaccine is that a T-cell-dependent response to vaccine is induced. This enhances primary response among infants and substantially reduces asymptomatic carriage.

Treatment

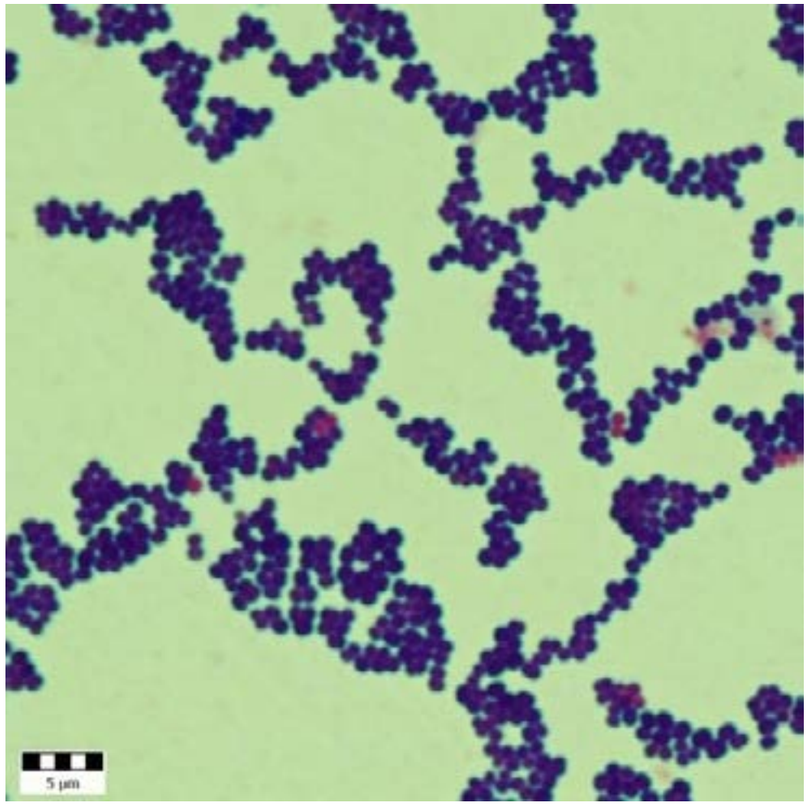
Penicillin G is the drug of choice for treating meningococcal disease. Either chloramphenicol or a third-generation cephalosporin such as cefotaxime or ceftriaxone is used in persons allergic to penicillins.

Epidemiology, Prevention & Control

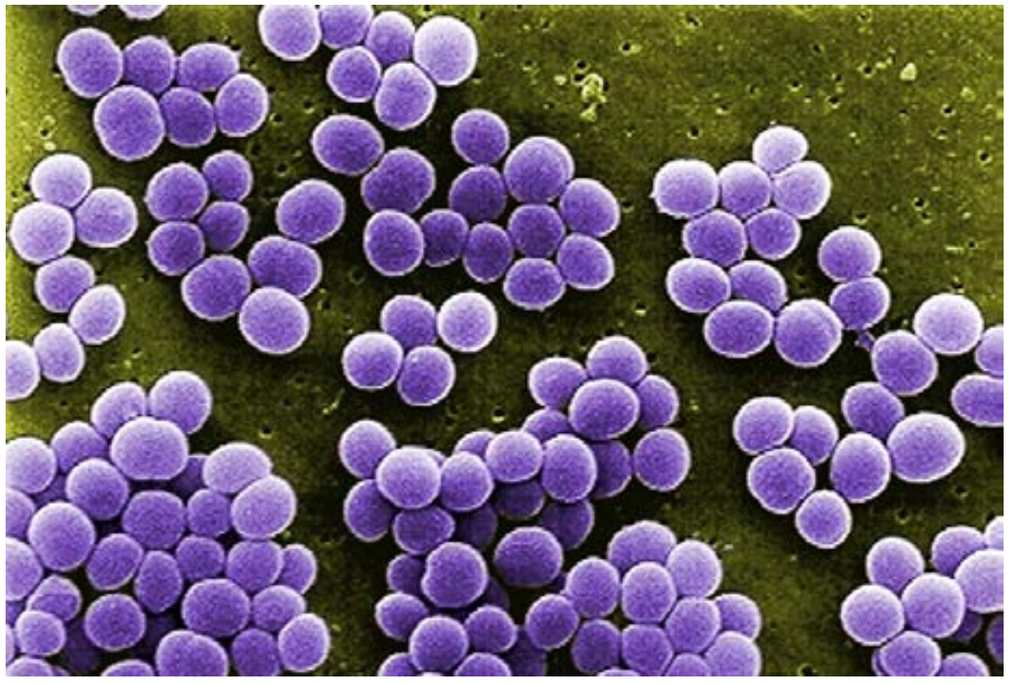
Meningococcal meningitis occurs in epidemic waves (eg, in military encampments, in religious pilgrims, and in sub-Saharan Africa; in Brazil, there were more than 15,000 cases in 1974) and a smaller number of sporadic interepidemic cases. Five to 30% of the normal population may harbor meningococci (often nontypeable isolates) in the nasopharynx during interepidemic periods. During epidemics, the carrier rate goes up to 70-80%. A rise in the number of cases is preceded by an increased number of respiratory carriers. Treatment with oral penicillin does not eradicate the carrier state. Rifampin, 600 mg orally twice daily for 2 days (or minocycline, 100 mg every 12 hours), can often eradicate the carrier state and serve as chemoprophylaxis for household and other close contacts. Since the appearance of many sulfonamideresistant meningococci, chemoprophylaxis with sulfonamides is no longer reliable.

Clinical cases of meningitis present only a negligible source of infection, and isolation therefore has only limited usefulness. More important is the reduction of personal contacts in a population with a high carrier rate. This is accomplished by avoidance of crowding. Specific polysaccharides of groups A, C, Y, and W-135 can stimulate antibody response and protect susceptible persons against infection. Such vaccines are currently used in selected populations (eg, the military and in civilian epidemics).

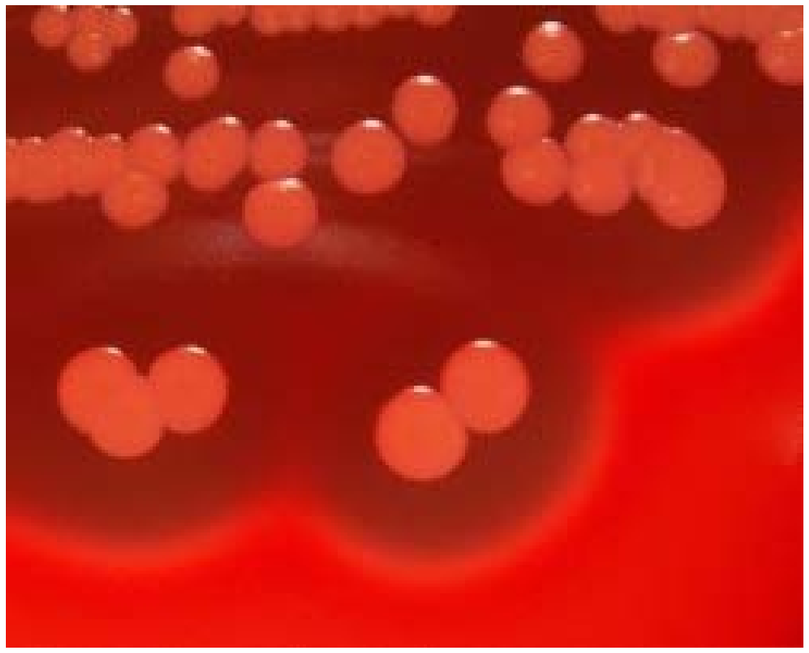
1 Class – Illustrations



Staphylococcus aureus Gram stain



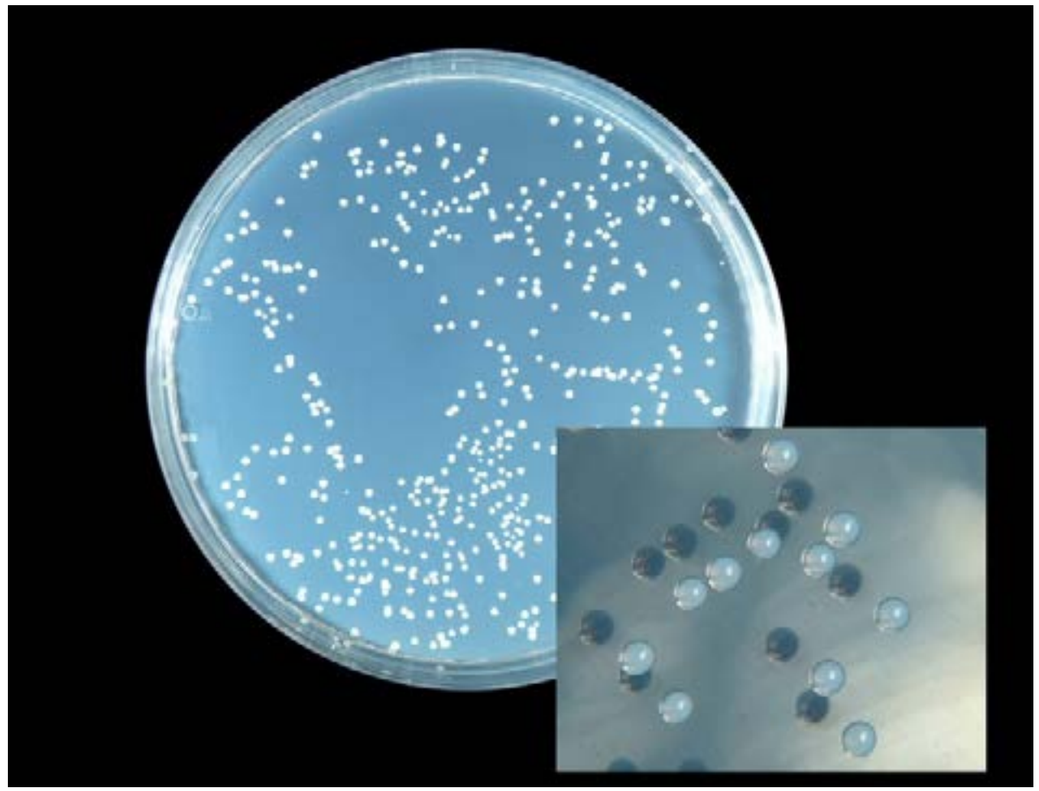
S. aureus (SEM micrograph)



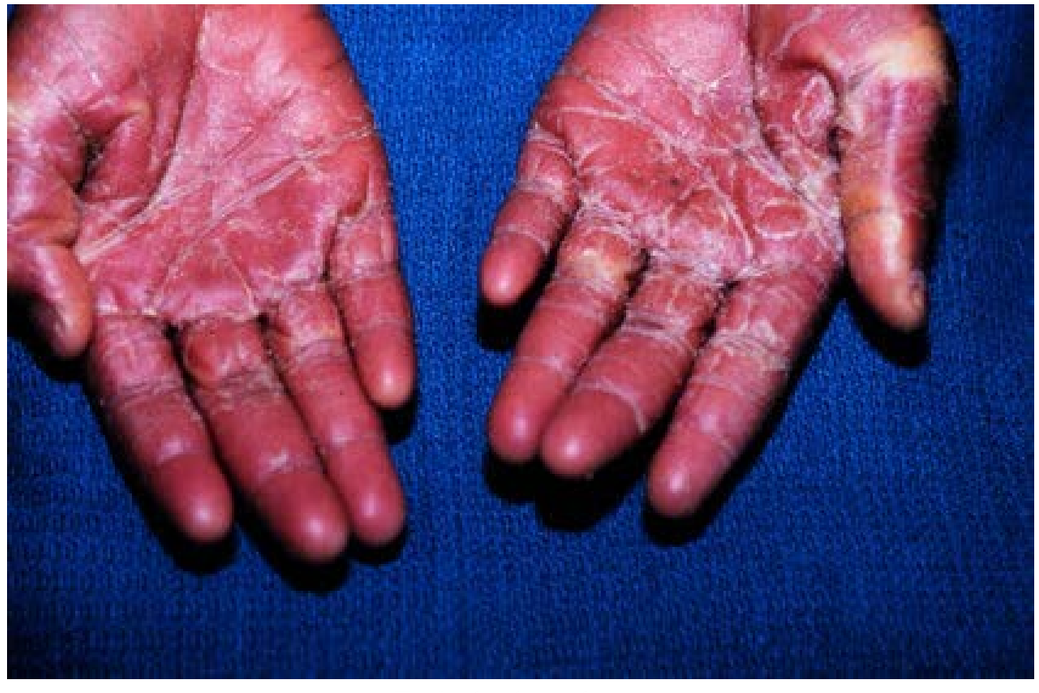
Colonies of *S. aureus* surrounded by wide zones of beta-hemolysis

S. aureus and *S.epidermidis.* Mannitol Salt Agar

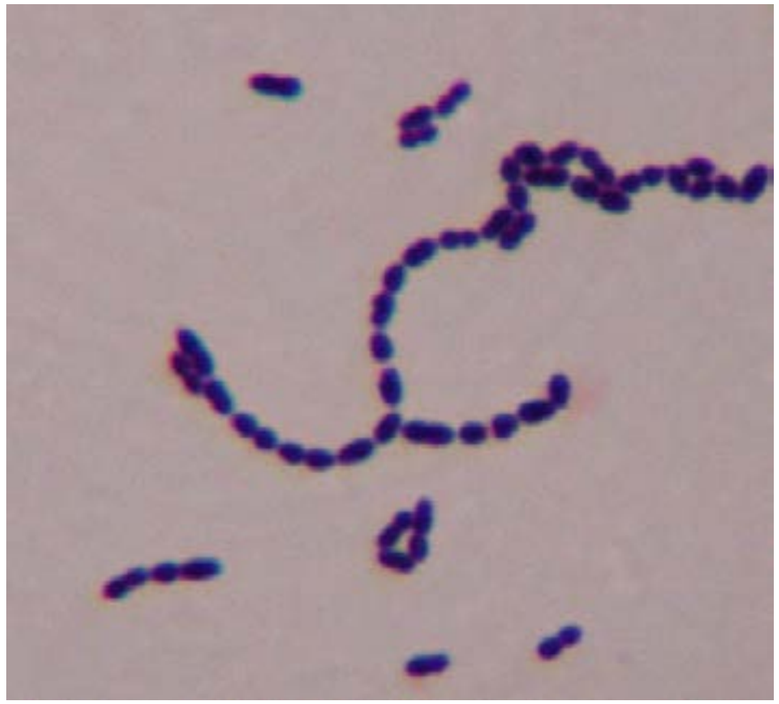
S.



S. epidermidis on Tryptic Soy Agar

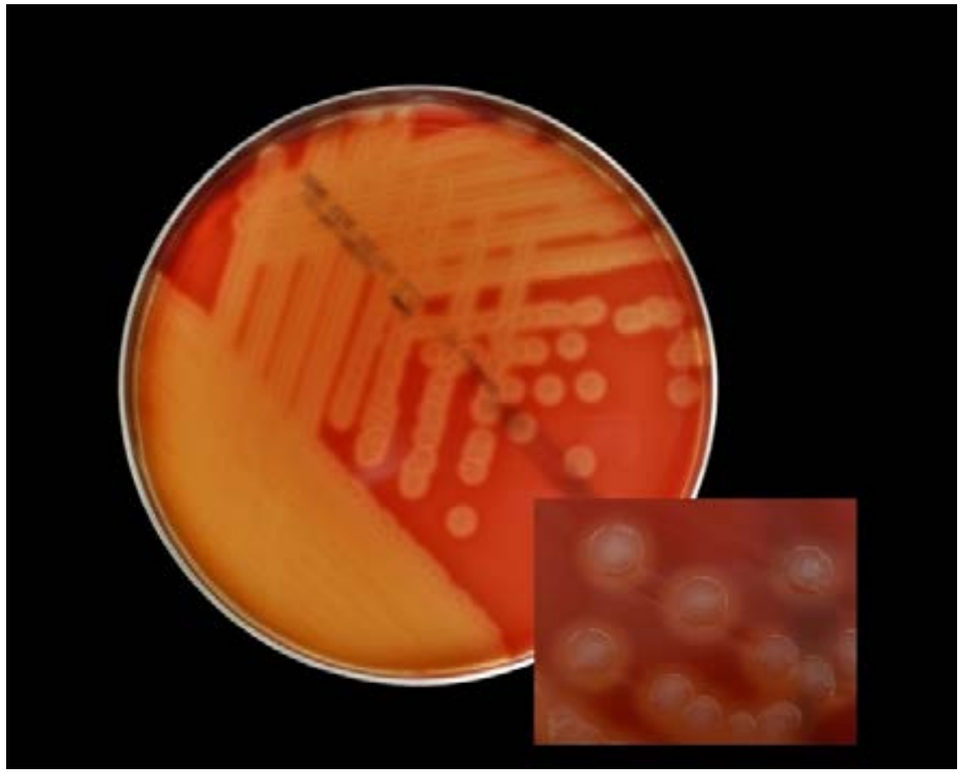


Toxic shock syndrome

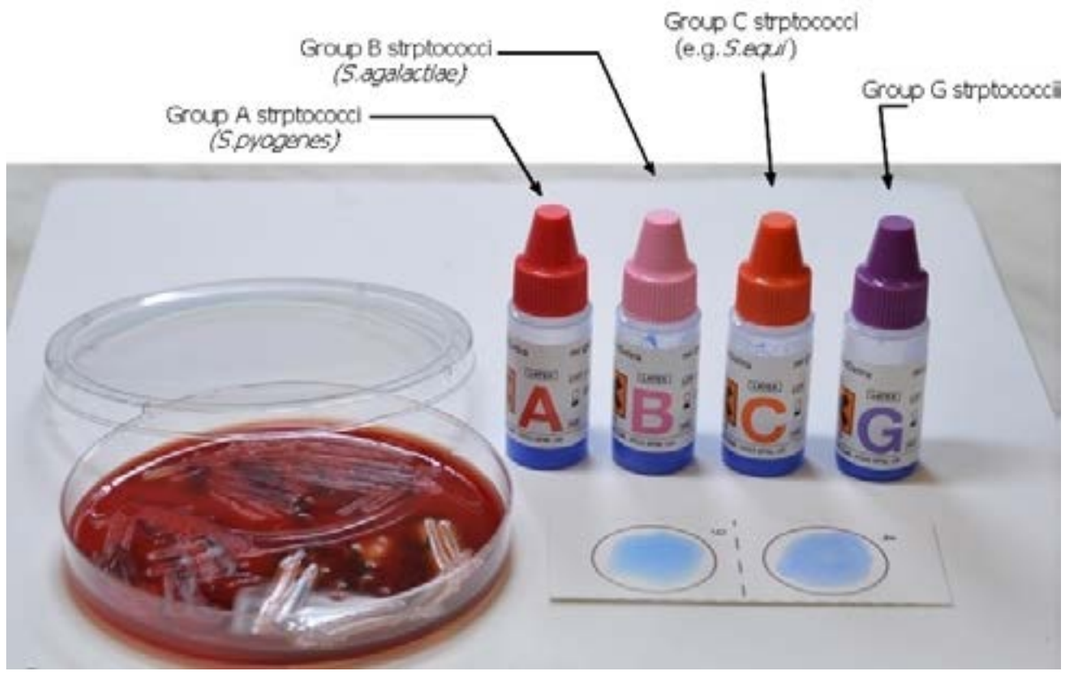


Gram positive stain of *Streptococcus pyogenes*

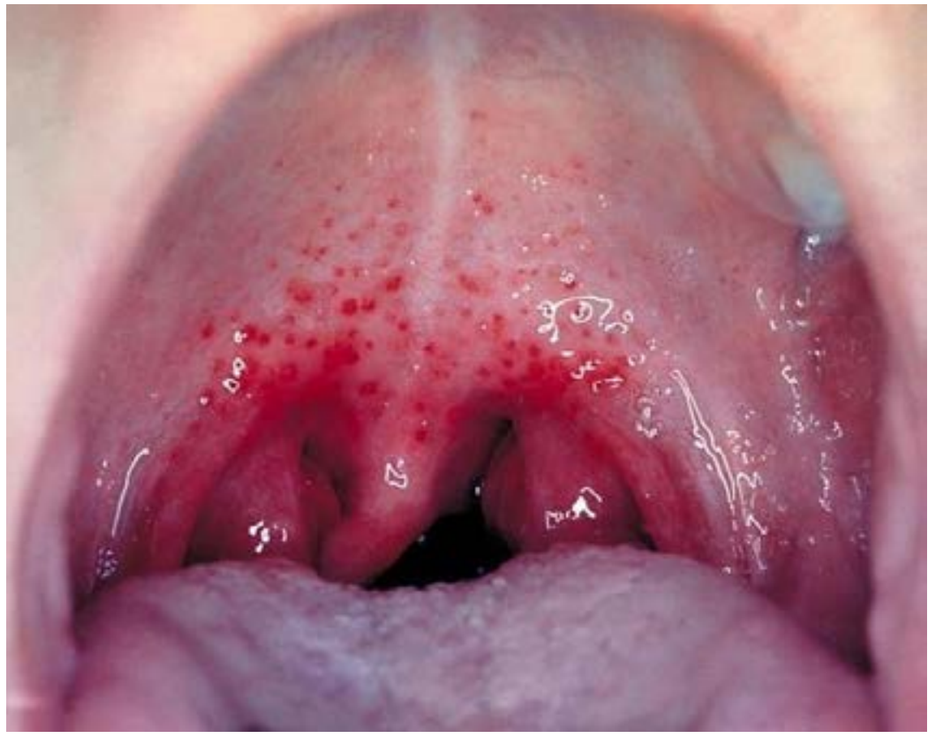
S. pyogenes (SEM)



S. pyogenes on blood agar

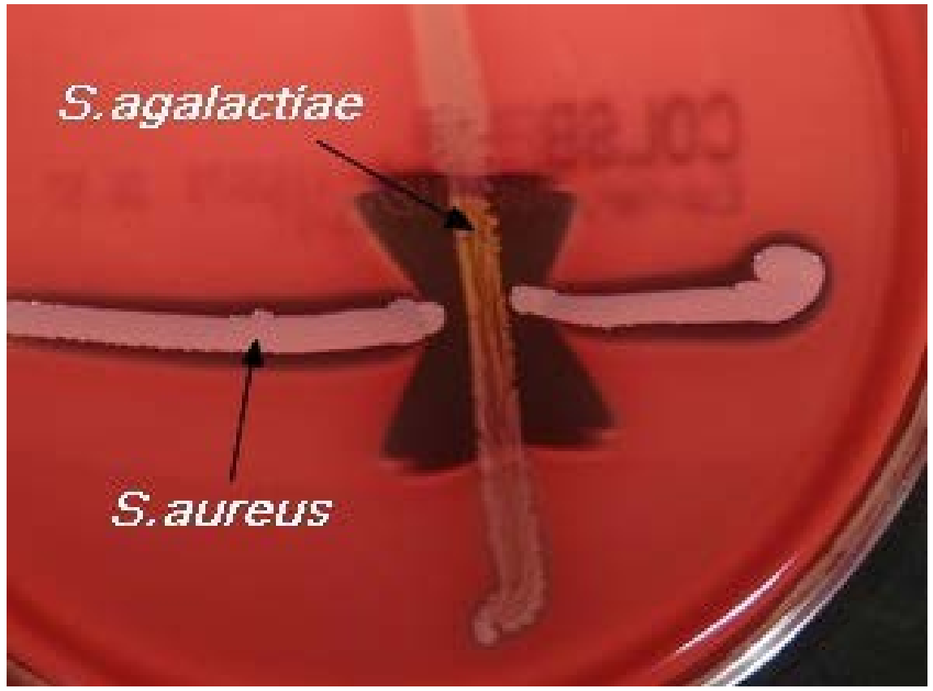


Latex agglutination

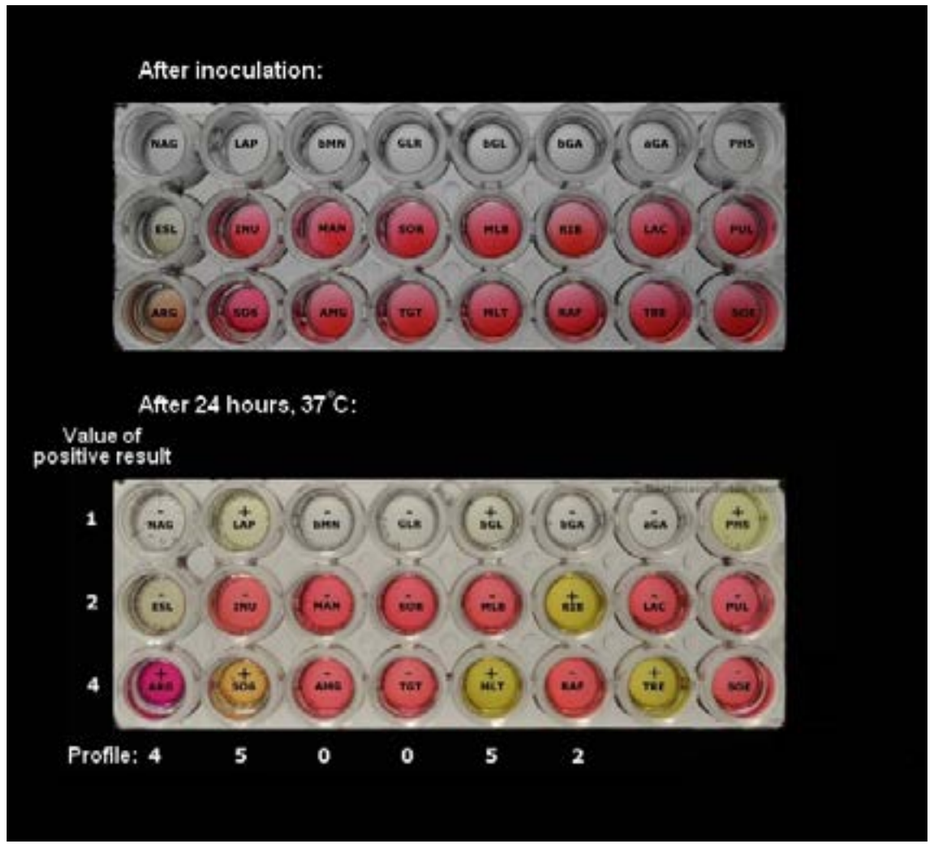


Strep throat

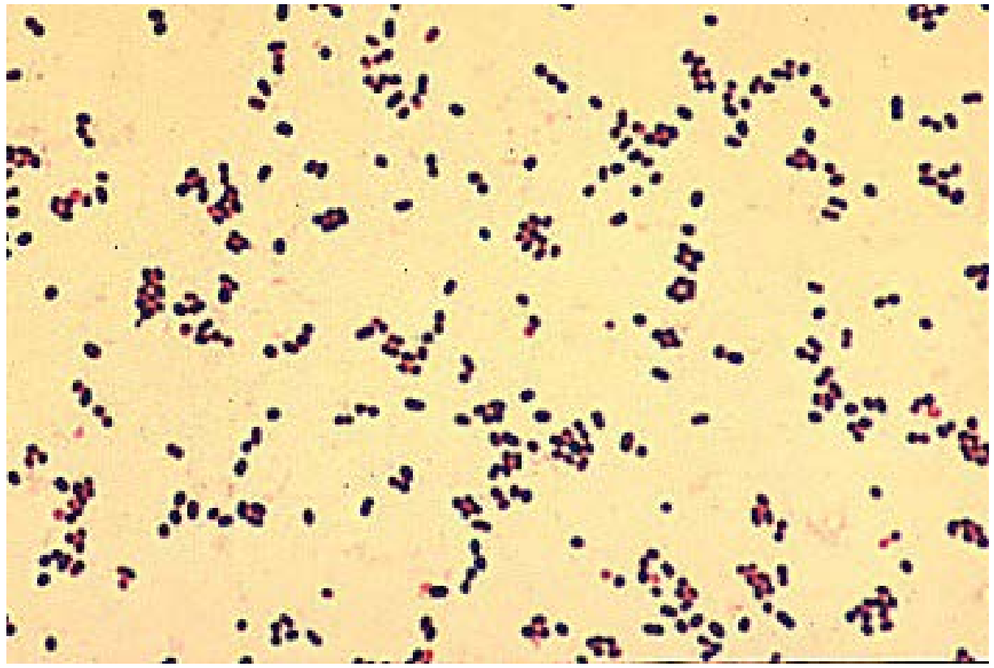
Beta-hemolytic colonies of *S. agalactiae* on sheep blood agar



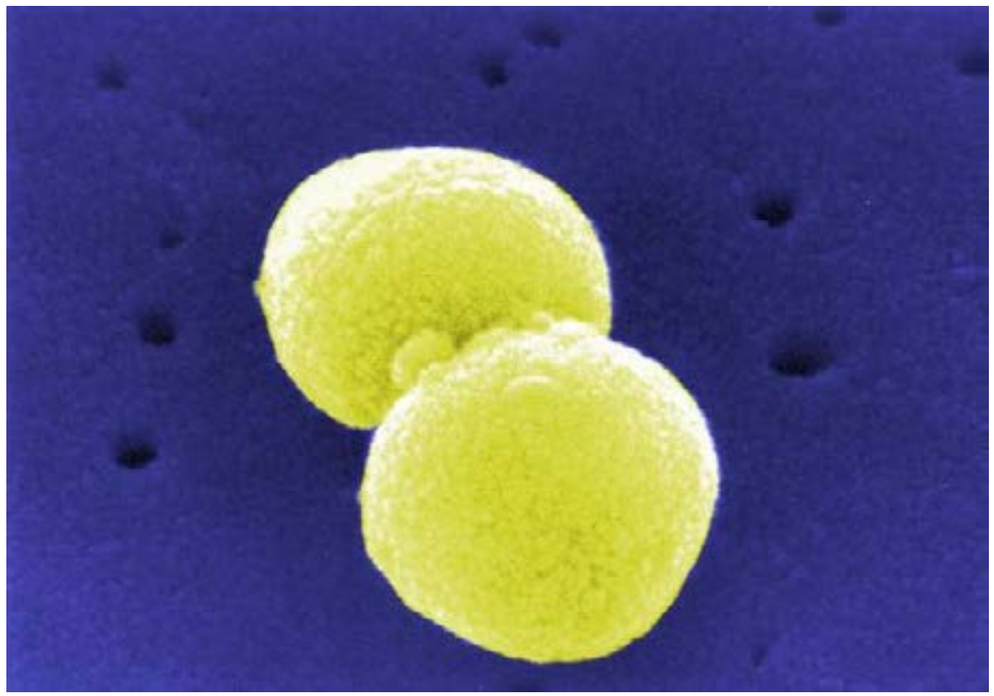
CAMP test



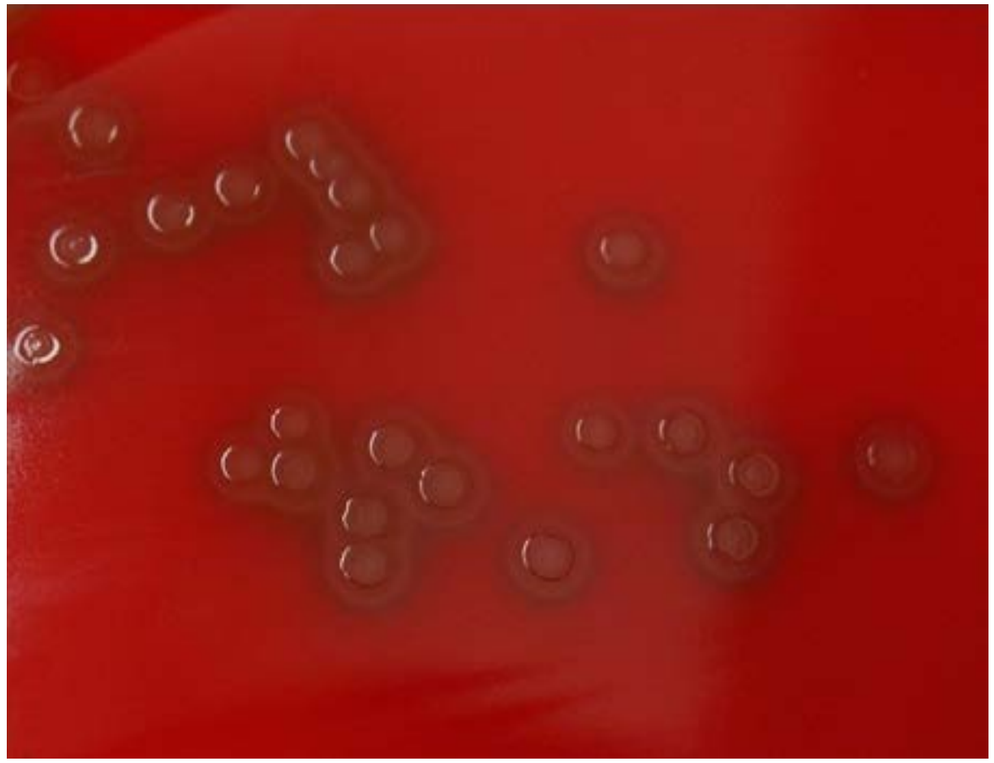
Biochemical identification of *S. agalactiae*



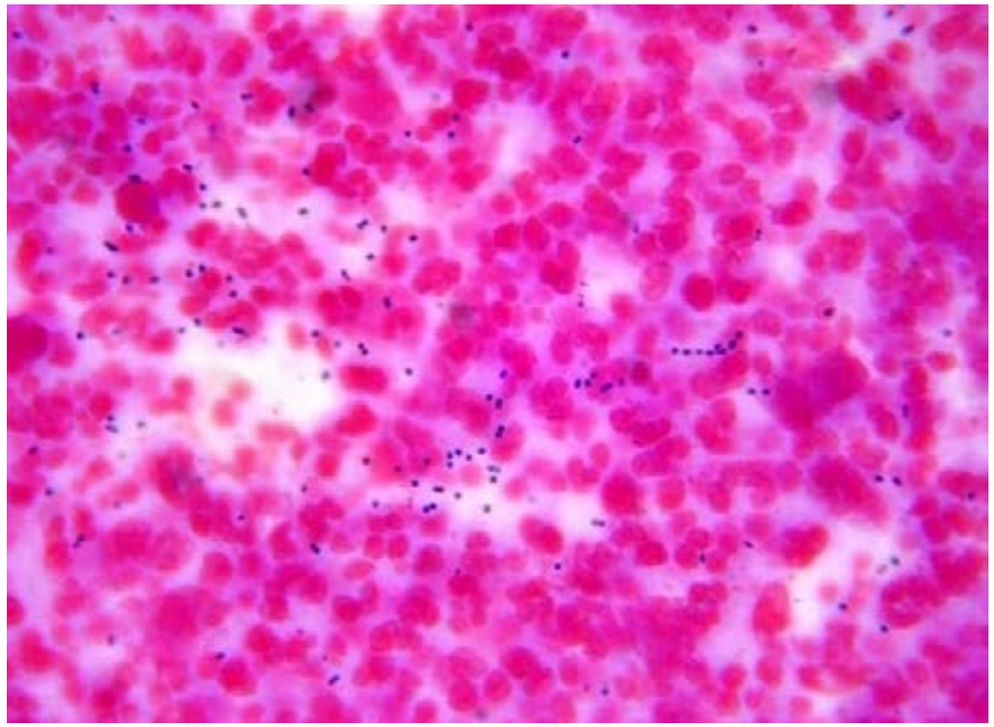
Gram positive stain of *Streptococcus pneumoniae*



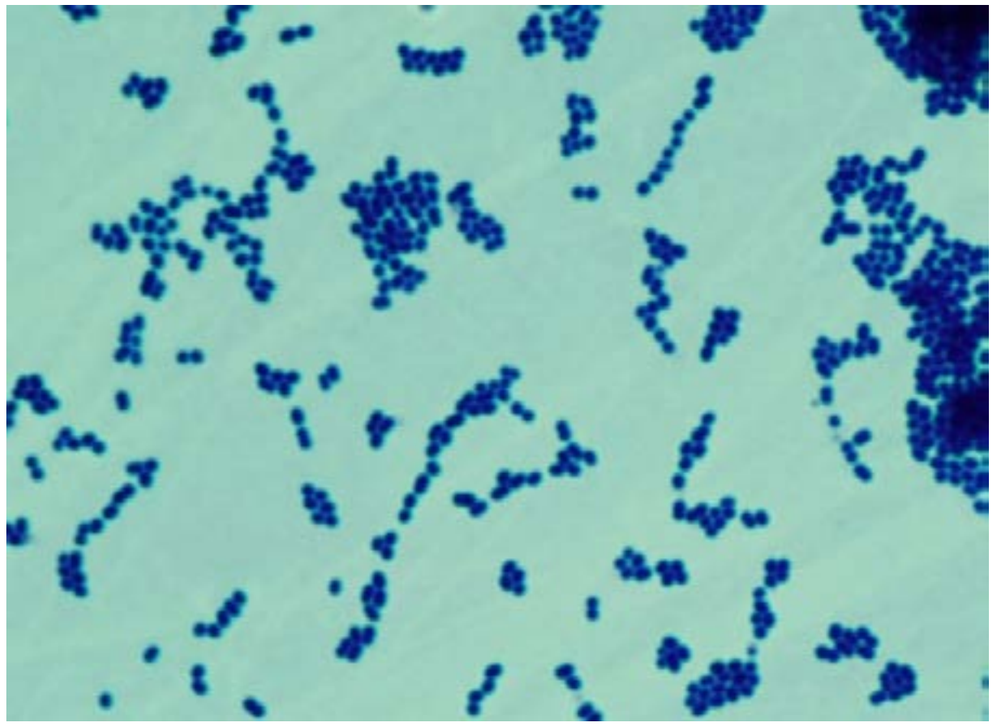
*S. pneumoniae (*SEM)



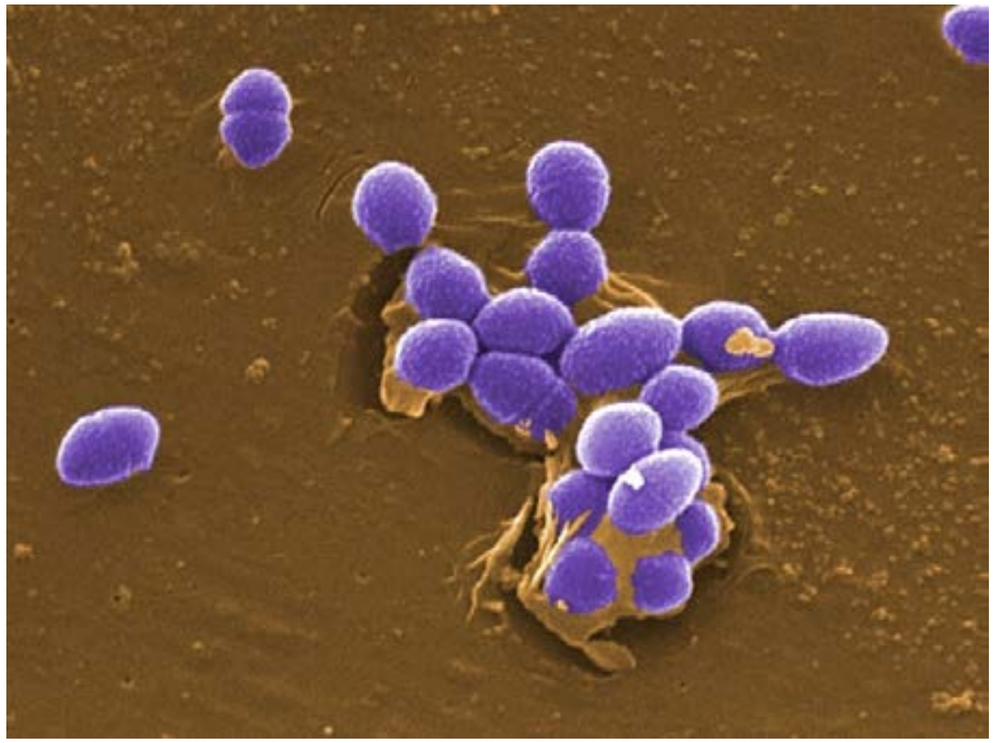
S. pneumoniae Alpha-hemolysis on blood agar



S. pneumoniae in cerebral spinal fluid

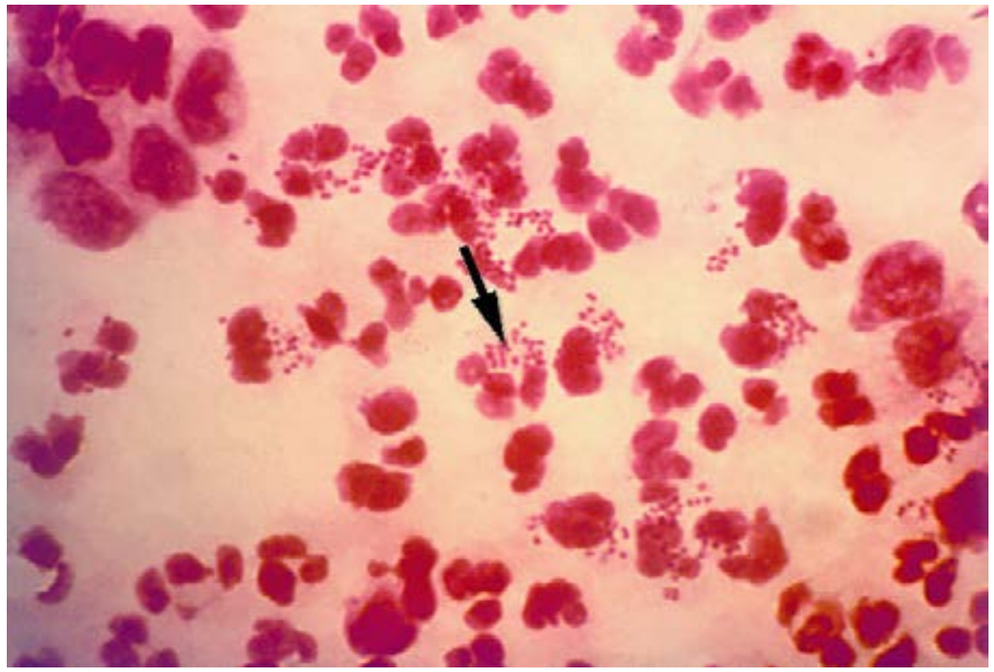


Enterococcus faecalis Gram stain

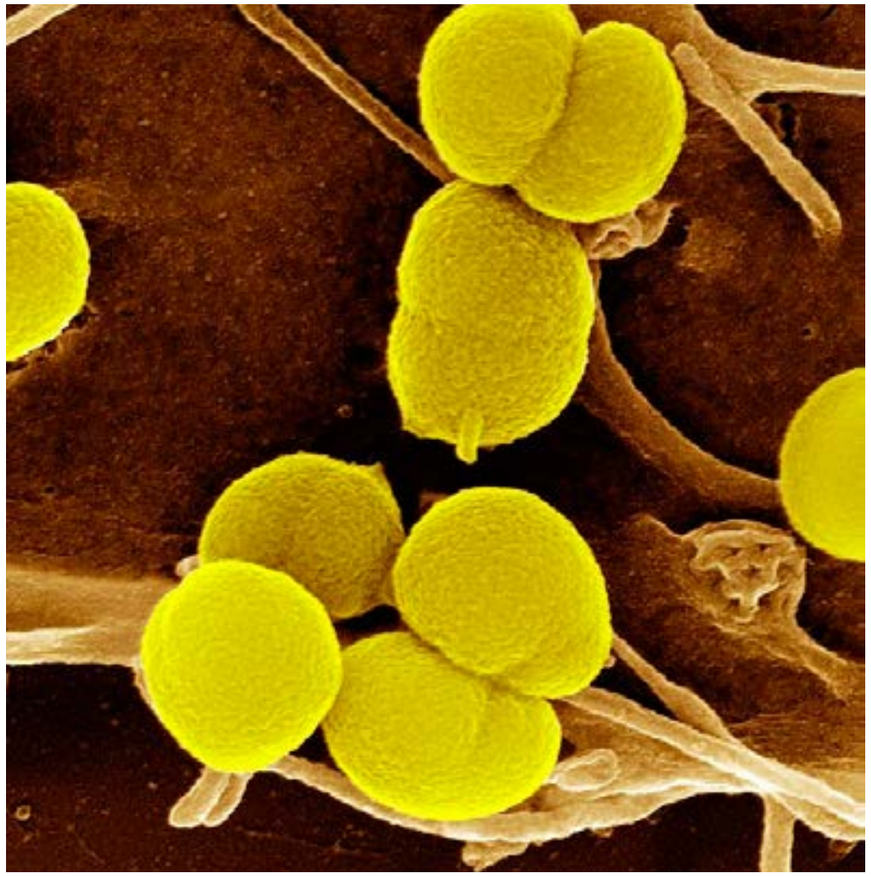


*Enterococcus faecalis (*SEM)

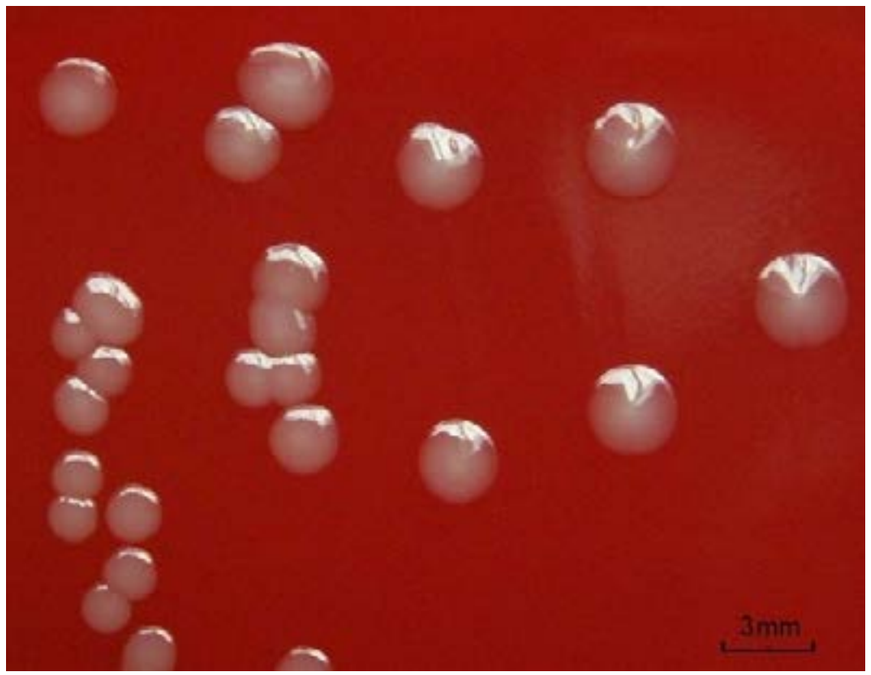
E. faecalis colonies on blood agar



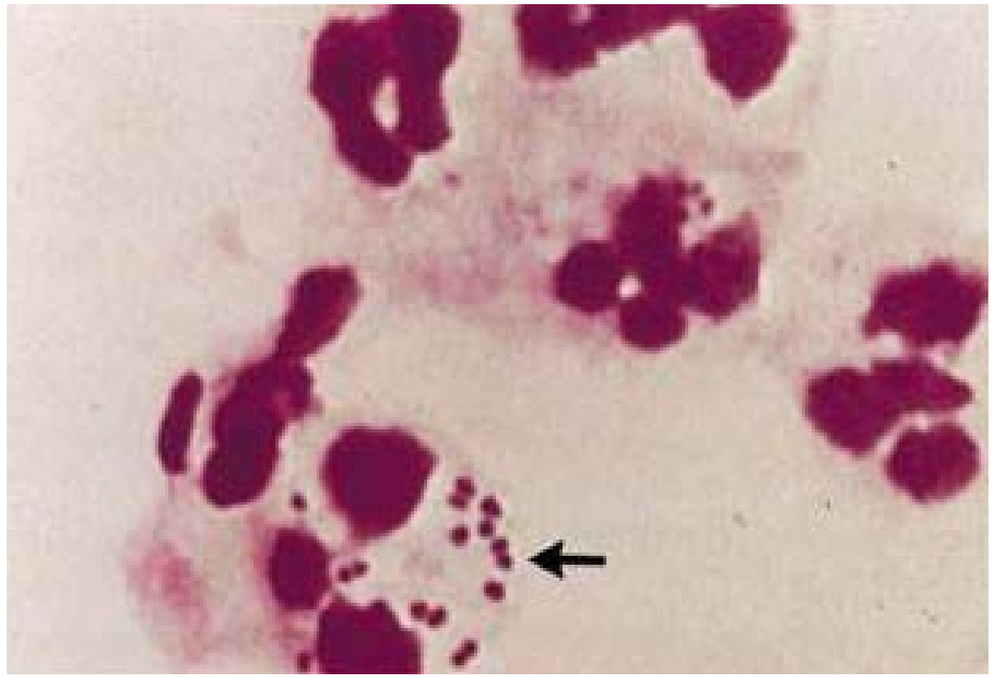
Gram stain of *Neisseria gonorrhoeae*



*Neisseria gonorrhoeae (*SEM)



N. gonorrhoeae on blood agar



Gram stain of *Neisseria meningitidis*

Growth of *Neisseria meningitidis* on a selective medium for pathogenic neisseriae

Neisseria meningitidis colonies on chocolate agar

2 Class - Enterobacteria. *Escherichia. Shigella. Klebsiella*

The Enterobacteriaceae

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus:

Escherichia	*Serratia*
Shigella	*Providensia*
Salmonella	*Edwardsiella*
Yersinia	*Enterobacter*
Klebsiella	*Morganella*
Proteus	*Hafnia*
Citrobacter	*Ervinia*

Classification

The Enterobacteriaceae are the most common group of Gram-negative rods cultured in the clinical laborator and along with staphylococci and streptococci are among the most common bacteria that cause disease. The taxonomy of the Enterobacteriaceae is comple and rapidly changing since the introduction of technique that measure evolutionary distance, such a nucleic acid hybridization and sequencing. More than 25 genera and 110 species or groups have been defined however, the clinically significant Enterobacteriaceae comprise 20-25 species, and other species are encountere infrequently.

Morphology & Identification

A. MORPHOLOGY

Enterobacteriaceae are short Gram-negative rod with rounded ends, 0,5-1,5 lm thick, and 24 lm long. Many have peritrichous flagellation. Species with many flagella (e.g., *Proteus* species) show motility on the agar surface, which phenomenon is known a «swarming». Some Enterobacteriaceae possess a capsule.

B. CULTURE AND GROWTH CHARACTERISTICS

All bacteria in this family can readily be cultured on simple nutrient mediums. They are rapidly growing facultative anaerobes. Their mean generation time in vitro is 20-30 minutes. They show resistance to various chemicals (bile salts, crystal violet), which fact is made use of in selective culturing. Endo agar is an important selective indicator medium; it allows only Gram-negative rod bacteria to grow and indicates lactose breakdown.

C. VIRULENCE FACTORS

1. Antigenic Structure: Enterobacteriaceae have a complex antigenic structure. They are classified by more than 150 different heat-stable somatic O (lipopolysaccharide) antigens, more than 100 heat-labile K (capsular) antigens, and more than 50 H (flagellar) antigens. In *Salmonella typhi,* the capsular antigens are called Vi antigens.

O antigens are the most external part of the cell wall lipopolysaccharide and consist of repeating units of polysaccharide. Some O-specific polysaccharides contain unique sugars. O antigens are resistant to heat and alcohol and usually are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM. While each genus of Enterobacteriaceae is associated with specific O groups, a single organism may carry several O antigens. Thus, most *Shigella* share one or more O antigens with *E. coli. E. coli* may cross-react with some *Providencia, Klebsiella,* and *Salmonella* species. Occasionally, O antigens may be associated with specific human diseases, eg, specific O types of *E. coli* are found in diarrhea and in urinary tract infections.

K antigens are external to O antigens on some but not all Enterobacteriaceae. Some are polysaccharides, including the K antigens of *E. coli;* others are proteins. K antigens may interfere with agglutination by O antisera, and they may be associated with virulence (eg, *E. coli* strains producing K1 antigen are prominent in neonatal meningitis, and K antigens of *E. coli* cause attachment of the bacteria to epithelial cells prior to gastrointestinal or urinary tract invasion).

Klebsiella form large capsules consisting of polysaccharides (K antigens) covering the somatic (O or

H) antigens and can be identified by capsular swelling tests with specific antisera. Human infections of the respiratory tract are caused particularly by capsular types 1 and 2; those of the urinary tract, by types 8, 9, 10, and 24.

H antigens are located on flagella and are denatured or removed by heat or alcohol. They are preserved by treating motile bacterial variants with formalin. Such H antigens agglutinate with anti-H antibodies, mainly IgG. The determinants in H antigens are a function of the amino acid sequence in flagellar protein (flagellin). Within a single serotype, flagellar antigens may be present in either or both of two forms, called phase 1 (conventionally designated by lower-case letters) and phase 2 (conventionally designated by Arabic numerals). The organism tends to change from one phase to the other; this is called phase variation. H antigens on the bacterial surface may interfere with agglutination by anti-O antibody.

There are many examples of overlapping antigenic structures between Enterobacteriaceae and other bacteria. Most Enterobacteriaceae share the O14 antigen of *E. coli.* The type 2 capsular polysaccharide of *Klebsiella* is very similar to the polysaccharide of type 2 pneumococci. Some K antigens cross-react with capsular polysaccharides of *Haemophilus influenzae* or *N. meningitidis*. Thus, *E. coli* O75:K100:H5 can induce antibodies that react with *H. influenzae* type b.

The antigenic classification of Enterobacteriaceae often indicates the presence of each specific antigen. Thus, the antigenic formula of an *E. coli* may be O55:K5:H21.

2. Adhesion factors: attachment fimbriae, attachment pili, colonizing factor antigens (CFAs).

3. Invasive factors: proteins localized in the outer membrane (invasins) that facilitate the invasion of target cells.

4. Exotoxins

- Enterotoxins disturb the normal functioning of enterocytes. Stimulation of adenylate or guanylate cyclase; increased production of cAMP. This results in the loss of large amounts of electrolytes and water.

- Cytotoxins exert a direct toxic effect on cells (enterocytes, endothelial cells).

5. Endotoxin: toxic effect of lipoid A as a component of LPS.

6. Serum resistance: resistance to the membrane attack complex C5b6789 of the complement system.

7. Phagocyte resistance: makes survival in phagocytes possible. Resistance against defensins and/or oxygen radicals.

8. Cumulation of Fe^{2+}: active transport of Fe^{2+} by siderophores in the bacterial cell.

Pathogenesis, Pathology & Clinical Findings

A. URINARY TRACT INFECTION

E. coli is the most common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infections in young women. The symptoms and signs include urinary frequency, dysuria, hematuria, and pyuria. Flank pain is associated with upper tract infection. None of these symptoms or signs is specific for *E. coli* infection. Urinary tract infection can result in bacteremia with clinical signs of sepsis.

Nephropathogenic *E. coli* typically produce a hemolysin. Most of the infections are caused by *E. coli* of a small number of O antigen types. K antigen appears to be important in the pathogenesis of upper tract infection. Pyelonephritis is associated with a specific type of pilus, P pilus, which binds to the P blood group substance.

B. *E. COLI*-ASSOCIATED DIARRHEAL DISEASES

E. coli that cause diarrhea are extremely common worldwide. These *E. coli* are classified by the characteristics of their virulence properties, and each group causes disease by a different mechanism. The small or large bowel epithelial cell adherence properties are encoded by genes on plasmids. Similarly, the toxins often are plasmid-or phage-mediated.

Enteropathogenic *E. coli* (EPEC)

Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhea in infants, especially in developing countries. EPEC previously was associated with outbreaks of diarrhea in nurseries in developed countries. EPEC adhere to the mucosal cells of the small bowel. Chromosomally mediated factors promote tight adherence. There is loss of microvilli (effacement), formation of filamentous actin pedestals or cup-like structures, and, occasionally, entry of the EPEC into the mucosal cells. Characteristic lesions can be seen on electron micrographs of small bowel biopsy lesions. The result of EPEC infection is watery diarrhea, which is usually self-limited but can be chronic. EPEC diarrhea has been associated with multiple specific serotypes of *E. coli;* strains are identified by O antigen and occasionally by H antigen typing. A two-stage infection model

using HEp-2 cells also can be performed. Tests to identify EPEC are performed in reference laboratories. The duration of the EPEC diarrhea can be shortened and the chronic diarrhea cured by antibiotic treatment.

Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* (ETEC) is a common cause of «traveler's diarrhea» and a very important cause of diarrhea in infants in developing countries. ETEC colonization factors specific for humans promote adherence of ETEC to epithelial cells of the small bowel. Some strains of ETEC produce a heat-labile exotoxin (LT) (MW 80,000) that is under the genetic control of a plasmid. Its subunit B attaches to the GM1 ganglioside at the brush border of epithelial cells of the small intestine and facilitates the entry of subunit A (MW 26,000) into the cell, where the latter activates adenylyl cyclase. This markedly increases the local concentration of cyclic adenosine monophosphate (cAMP), which results in intense and prolonged hypersecretion of water and chlorides and inhibits the reabsorption of sodium. The gut lumen is distended with fluid, and hypermotility and diarrhea ensue, lasting for several days. LT is antigenic and cross-reacts with the enterotoxin of *Vibrio cholerae.* LT stimulates the production of neutralizing antibodies in the serum (and perhaps on the gut surface) of persons previously infected with enterotoxigenic *E. coli.* Persons residing in areas where such organisms are highly prevalent (eg, in some developing countries) are likely to possess antibodies and are less prone to develop diarrhea on reexposure to the LT-producing *E. coli*. Assays for LT include the following: (1) fluid accumulation in the intestine of laboratory animals; (2) typical cytologic changes in cultured Chinese hamster ovary cells or other cell lines; (3) stimulation of steroid production in cultured adrenal tumor cells; and (4) binding and immunologic assays with standardized antisera to LT. These assays are done only in reference laboratories.

Some strains of ETEC produce the heat-stable enterotoxin STa (MW 1500-4000), which is under the genetic control of a heterogeneous group of plasmids. STa activates guanylyl cyclase in enteric epithelial cells and stimulates fluid secretion. Many STa-positive strains also produce LT. The strains with both toxins produce a more severe diarrhea. The plasmids carrying the genes for enterotoxins (LT, ST) also may carry genes for the colonization factors that facilitate the attachment of *E. coli* strains to intestinal epithelium. Recognized colonization factors occur with particular frequency in some serotypes. Certain serotypes of ETEC occur worldwide; others have a limited recognized distribution. It is possible that virtually any *E. coli* may acquire a plasmid encoding for enterotoxins. There is no definite association of ETEC with the EPEC strains causing diarrhea in children. Likewise, there is no association between enterotoxigenic strains and those able to invade intestinal epithelial cells.

Enterohemorrhagic *E. coli* (EHEC)

Enterohemorrhagic *E. coli* (EHEC) produces verotoxin, named for its cytotoxic effect on Vero cells, a line of African green monkey kidney cells. There are at least two antigenic forms of the toxin. EHEC has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, a disease resulting in acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. Verotoxin has many properties that are similar to the Shiga toxin produced by some strains of *Shigella dysenteriae* type 1; however, the two toxins are antigenically and genetically distinct. Of the *E. coli* serotypes that produce erotoxin, O157:H7 is the most common and is the one that can be identified in clinical specimens. EHECO157:H7 does not use sorbitol, unlike most other *E. coli,* and is negative on sorbitol MacConkey agar (sorbitol is used instead of lactose); O157:H7 strains also are negative on MUG tests. Specific antisera are used to identify the O157:H7 strains. Assays for verotoxin are done in reference laboratories. Many cases of hemorrhagic colitis and its associated complications can be prevented by thoroughly cooking ground beef.

Enteroinvasive *E. coli* (EIEC)

Enteroinvasive *E. coli* (EIEC) produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries. Like *Shigella*, EIEC strains are nonlactose or late lactose fermenters and are nonmotile. EIEC produce disease by invading intestinal mucosal epithelial cells.

Enteroaggregative *E. coli* (EAEC)

Enteroaggregative *E. coli* (EAEC) causes acute and chronic diarrhea (> 14 days in duration) in persons in developing countries. These organisms also are the cause of food-borne illnesses in industrialized countries. They are characterized by their characteristic pattern of adherence to human cells. EAEC produce ST-like toxin and a hemolysin.

C. SEPSIS

When normal host defenses are inadequate, *E. coli* may reach the bloodstream and cause sepsis. Newborns may be highly susceptible to *E. coli* sepsis because they lack IgM antibodies. Sepsis may occur secondary to urinary tract infection.

D. MENINGITIS

E. coli and group B streptococci are the leading causes of meningitis in infants. Approximately 75% of *E. coli* from meningitis cases have the K1 antigen. This antigen cross-reacts with the group B capsular polysaccharide of *N. meningitidis.* The mechanism of virulence associated with the K1 antigen is not understood.

Diagnostic laboratory tests

E. coli typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. An isolate from urine can be quickly identified as *E. coli* by its hemolysis on blood agar, typical colonial morphology with an iridescent «sheen» on differential media such as EMB agar, and a positive spot indole test. Over 90% of *E. coli* isolates are positive for β-glucuronidase using the substrate 4-methylumbelliferyl-β-glucuronide (MUG). Isolates from anatomic sites other than urine, with characteristic properties (above plus negative oxidase tests) often can be confirmed as *E. coli* with a positive MUG test.

Immunity

Specific antibodies develop in systemic infections, but it is uncertain whether significant immunity to the organisms follows.

Treatment

No single specific therapy is available. The sulfonamides, ampicillin, cephalosporins, fluoroquinolones, and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great, and laboratory tests for antibiotic sensitivity are essential. Multiple drug resistance is common and is under the control of transmissible plasmids.

Certain conditions predisposing to infection by these organisms require surgical correction, eg, relief of urinary tract obstruction, closure of a perforation in an abdominal organ, or resection of a bronchiectatic portion of lung.

Treatment of Gram-negative bacteremia and impending septic shock requires rapid institution of antimicrobial therapy, restoration of fluid and electrolyte balance, and treatment of disseminated intravascular coagulation.

Various means have been proposed for the prevention of traveler's diarrhea, including daily ingestion of bismuth subsalicylate suspension (bismuth subsalicylate can inactivate *E. coli* enterotoxin in vitro) and regular doses of tetracyclines or other antimicrobial drugs for limited periods. Because none of these methods are entirely successful or lacking in adverse effects, it is widely recommended that caution be observed in regard to food and drink in areas where environmental sanitation is poor and that early and brief treatment (eg, with ciprofloxacin or trimethoprim-sulfamethoxazole) be substituted for prophylaxis.

Epidemiology, Prevention & Control

The enteric bacteria establish themselves in the normal intestinal tract within a few days after birth and from then on constitute a main portion of the normal aerobic (facultative anaerobic) microbial flora. *E. coli* is the prototype. Enterics found in water or milk are accepted as proof of fecal contamination from sewage or other sources.

Transmission of intestinal infections is usually indirect via food, drinkingwater, or surfacewater. Fifty percent of travelers' diarrhea cases are caused by *E. coli*, in most cases ETEC.

The most effective preventive measures against intestinal infections, e.g., when travelling in countries with warm climates, is to eat only thoroughly cooked foods and drink only disinfected water. Studies have demonstrated the efficacy of chemoprophylaxis with anti-infective agents in preventing traveler's diarrhea, whereby the agents used must not reduce the normal aerobic intestinal flora (4-quinolones and cotrimoxazole are suitable). This method is hardly practicable, however, in view of the large numbers of travelers.

Shigella

Classification

Shigella species are classified by four serogroups:

Serogroup *A*: *S. dysenteriae* (15 serotypes)

Serogroup *B*: *S. flexneri* (6 serotypes)

Serogroup *C*: *S. boydii* (19 serotypes)

Serogroup *D*: *S. sonnei* (one serotypes)

Morphology & Identification

A. MORPHOLOGY

Shigella are Gram-negative, nonmotile, non-spore forming, rod-shaped bacteria.

B. CULTURE AND GROWTH CHARACTERISTICS

Shigella are facultative anaerobes but grow best aerobically. Convex, circular, transparent colonies with intact edges reach a diameter of about 2 mm in 24 hours.

All *Shigella* ferment glucose. With the exception of *S. sonnei,* they do not ferment lactose. The inability to ferment lactose distinguishes *Shigella* on differential media. *Shigella* form acid from carbohydrates but rarely produce gas. They may also be divided into those that ferment mannitol and those that do not.

C. VIRULENCE FACTORS

1. Antigenic Structure

Shigella have a complex antigenic pattern. There is great overlapping in the serologic behavior of different species, and most of them share O antigens with other enteric bacilli. The somatic O antigens of *Shigella* are lipopolysaccharides. Their serologic specificity depends on the polysaccharide. There are more than 40 serotypes.

2. Toxins

- Endotoxin:

Upon autolysis, all *Shigella* release their toxic lipopolysaccharide. This endotoxin probably contributes to the irritation of the bowel wall.

- *Shigella dysenteriae* exotoxin:

S. dysenteriae type 1 produces a heat-labile exotoxin that affects both the gut and the central nervous system. The exotoxin is a protein that is antigenic (stimulating production of antitoxin) and lethal for experimental animals. Acting as an enterotoxin, it produces diarrhea as does the *E. coli* verotoxin, perhaps by the same mechanism. In humans, the exotoxin also inhibits sugar and amino acid absorption in the small intestine. Acting as a «neurotoxin», this material may contribute to the extreme severity and fatal nature of *S. dysenteriae* infections and to the central nervous system reactions observed in them (ie, meningismus, coma). Patients with *S. flexneri* or *S. sonnei* infections develop antitoxin that neutralizes *S. dysenteriae* exotoxin *in vitro*. The toxic activity is distinct from the invasive property of *Shigella* in dysentery. The two may act in sequence, the toxin producing an early nonbloody, voluminous diarrhea and the invasion of the large intestine resulting in later dysentery with blood and pus in stools.

Pathogenesis, Pathology & Clinical Findings

Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare. *Shigella* are highly communicable; the infective dose is on the order of 10^3 organisms (whereas it usually is 10^5-10^8 for *Salmonella* and vibrios). The essential pathologic process is invasion of the mucosal epithelial cells (eg, M cells) by induced phagocytosis, escape from the phagocytic vacuole, multiplication and spread within the epithelial cell cytoplasm, and passage to adjacent cells. Microabscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of a «pseudomembrane» on the ulcerated area. This consists of fibrin, leukocytes, cell debris, a necrotic mucous membrane, and bacteria. As the process subsides, granulation tissue fills the ulcers and scar tissue forms.

After a short incubation period (1-2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. The diarrhea has been attributed to an exotoxin acting in the small intestine. A day or so later, as the infection involves the ileum and colon, the number of stools increases; they are less liquid but often contain mucus and blood. Each bowel movement is accompanied by straining and tenesmus (rectal spasms), with resulting lower abdominal pain. In more than half of adult cases, fever and diarrhea subside spontaneously in 2-5 days. However, in children and the elderly, loss of water and electrolytes may lead to dehydration, acidosis, and even death. The illness due to *S. dysenteriae* may be particularly severe.

On recovery, most persons shed dysentery bacilli for only a short period, but a few remain chronic intestinal carriers and may have recurrent bouts of the disease. Upon recovery from the infection, most persons develop circulating antibodies to *Shigella*, but these do not protect against reinfection.

Diagnostic laboratory tests

A. SPECIMENS

Specimens include fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically. Serum specimens, if desired, must be taken 10 days apart to demonstrate a rise in titer of agglutinating antibodies.

B. CULTURE

The materials are streaked on differential media (eg, MacConkey's or EMB agar) and on selective media (Hektoen enteric agar or salmonella-shigella agar), which suppress other Enterobacteriaceae and Gram-positive organisms. Colorless (lactose-negative) colonies are inoculated into triple sugar iron agar. Organisms that fail to produce H_2S, that produce acid but not gas in the butt and an alkaline slant in triple sugar iron agar medium, and that are nonmotile should be subjected to slide agglutination by specific S*higella* antisera.

C. SEROLOGY

Normal persons often have agglutinins against several *Shigella* species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is not used to diagnose *Shigella* infections.

Immunity

Infection is followed by a type-specific antibody response. Injection of killed *Shigella* stimulates production of antibodies in serum but fails to protect humans against infection. Ig A antibodies in the gut may be important in limiting reinfection; these may be stimulated by live attenuated strains given orally as experimental vaccines. Serum antibodies to somatic *Shigella* antigens are IgM.

Treatment

Ciprofloxacin, ampicillin, doxycycline, and trimethoprim-sulfamethoxazole are most commonly inhibitory for *Shigella* isolates and can suppress acute clinical attacks of dysentery and shorten the duration of symptoms. They may fail to eradicate the organisms from the intestinal tract. Multiple drug resistance can be transmitted by plasmids, and resistant infections are widespread. Many cases are self-limited. Opioids should be avoided in *Shigella* dysentery.

Epidemiology, Prevention & Control

Shigella are transmitted by «food, fingers, feces, and flies» from person to person. Most cases of *Shigella* infection occur in children under 10 years of age. *S. dysenteriae* can spread widely. Mass chemoprophylaxis for limited periods of time (eg, in military personnel) has been tried, but resistant strains of *Shigella* tend to emerge rapidly. Since humans are the main recognized host of pathogenic *Shigella*, control efforts must be directed at eliminating the organisms from this reservoir by (1) sanitary control of water, food, and milk; sewage disposal; and fly control; (2) isolation of patients and disinfection of excreta; (3) detection of subclinical cases and carriers, particularly food handlers; and (4) antibiotic treatment of infected individuals.

Klebsiella

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Klebsiella
Species: *K. granulomatis, K. oxytoca, K. pneumoniae, K. terrigena, K. planticola*

Morphology & Identification

A. MORPHOLOGY

Klebsiella is a genus of non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms.

B. CULTURE AND GROWTH CHARACTERISTICS

Klebsiella spp. grow readily on ordinary media commonly used to isolate Enterobacteriaceae, e.g., nutrient agar, tryptic casein soy agar, bromocresol purple lactose agar, blood agar, as well as more differential plating media for Enterobacteriaceae, such as Drigalski agar, MacConkey agar, eosin-methylene blue agar

(EMB), and bromo-thymol blue agar (BTB). *K. pneumoniae* and *K. oxytoca* colonies are lactose positive, more or less dome-shaped, 3-4 mm in diameter after overnight incubation at 30 °C or 37 °C, with a mucoid aspect and sometimes stickiness, depending on the strain and the composition of the medium. *Klebsiella planticola* and *K. terrigena* colonies are also lactose positive, 1.5- 2.5 mm in diameter, dome-shaped, with a weakly mucoid aspect. *Enterobacter aerogenes (K. mobilis*) colonies often have the same morphology. *Klebsiella ozaenae*, *K. rhinoscleromatis* and occasionally *K. pneumoniae* K1 grow more slowly on the same media, yielding voluminous, rounded, very mucoid, translucent and confluent colonies in 48 h at 30 °C or 37 °C. Similar colonies indistinguishable from those of *Klebsiella* may be formed by other genera of the Enterobacteriaceae, particularly *E. coli* mucoid varieties with capsular K antigens.

C. VIRULENCE FACTORS

There are three factors that may mediate virulence: cell wall receptors, capsular polysaccharide, and endotoxin. First, the presence of cell wall receptors enables *K. pneumoniae* to attach to the host cell, thereby altering the bacterial surface so that phagocytosis by polymorphonuclear leukocytes and macrophages is impaired and invasion of the non-phagocytic host cell is facilitated. Second, invasion of the host cell is also facilitated by the large polysaccharide capsule surrounding the bacterial cell; in addition this capsule acts as a barrier and protects the bacteria from phagocytosis. Third, *K. pneumoniae* produces an endotoxin that appears to be independent of factors that determine receptors and capsular characteristics. Marked interspecies differences in endotoxin production may correlate with virulence. Although some or all of these factors may ultimately determine virulence, the interaction of these factors *in vivo* has made it difficult to assess the relative contribution of any one of these virulence factors. The pathogenic mechanisms of *K. pneumoniae* that ultimately determine virulence remain unclear and will require further study.

Pathogenesis, Pathology & Clinical Findings

K. pneumoniae is present in the respiratory tract and feces of about 5% of normal individuals. It causes a small proportion (about 1%) of bacterial pneumonias. *K. pneumoniae* can produce extensive hemorrhagic necrotizing consolidation of the lung. It occasionally produces urinary tract infection and bacteremia with focal lesions in debilitated patients. Other enterics also may produce pneumonia. *K. pneumoniae* and *Klebsiella oxytoca* cause hospital-acquired infections. Two other *Klebsiella* are associated with inflammatory conditions of the upper respiratory tract: *Klebsiella ozaenae* has been isolated from the nasal mucosa in ozena, a fetid, progressive atrophy of mucous membranes; and *Klebsiella rhinoscleromatis* from rhinoscleroma, a destructive granuloma of the nose and pharynx.

Diagnostic laboratory tests

Klebsiella species exhibit mucoid growth, large polysaccharide capsules, and lack of motility, and they usually give positive tests for lysine decarboxylase and citrate. Most *Enterobacter* species give positive tests for motility, citrate, and ornithine decarboxylase and produce gas from glucose. *Klebsiella*, *Enterobacter*, and *Serratia* usually give positive Voges-Proskauer reactions.

A. SPECIMENS

Specimens included urine, blood, pus, spinal fluid, sputum, or other material, as indicated by the localization of the disease process.

B. SMEARS

The Enterobacteriaceae resemble each other morphologically. The presence of large capsules is suggestive of *Klebsiella*.

C. CULTURE

Specimens are plated on both blood agar and differential media. With differential media, rapid preliminary identification of Gram-negative enteric bacteria is often possible.

Immunity

Specific antibodies develop in systemic infections, but it is uncertain whether significant immunity to the organisms follows.

Treatment

A. ANTIBIOTIC THERAPY

Unfortunately, *K. pneumoniae* is resistant to a number of antibiotics, deeming treatment options very limited. Choosing an antibiotic treatment for *K. pneumoniae* depends on the organ system that has been targeted. The choice is especially modified for people with confirmed bacteremia. Antibiotics with high intrinsic

activity against *K. pneumoniae* include cephalosporin, carbapenems, aminoglycosides, and quinolones. These treatments are initially used as monotherapy or even as a combination. For patients who are severely ill, an initial course, usually between 48-72 hours of combination aminoglycoside therapy, is suggested. This should then be followed by an extended-spectrum cephalosporin.

B. RESISTANCE

Carbapenem resistance is a emerging issue and is notably due to *K. pneumoniae* carbapenemase beta-lactamase (KPC). With the spread of KPC-producing bacteria, clinicians are dependent on polymyxins and tigecycline for treatment. Polymyxins have been the only agents active against KPC-producing bacteria. However, they were used infrequently due to their association with nephrotoxicity and neurotoxicity. Only a small amount of data support Polymyxin as a quality treatment for KPC. During a Manhattam outbreak caused by KPC-producing *K. peumoniae*, three bloodstream infections were treated with one survivor from the group. This drug is often used in combination with other antimicrobials.

C. SURGERY

Surgey may be needed for patients who experience empyema, lung anscess, pulmonary gangrene, or respiratory tract obstruction following a *Klebsiella* infection. Correction of posterior urethral valves in patients with reoccurring UTIs is a possibility or other abnormalities influenced by infection.

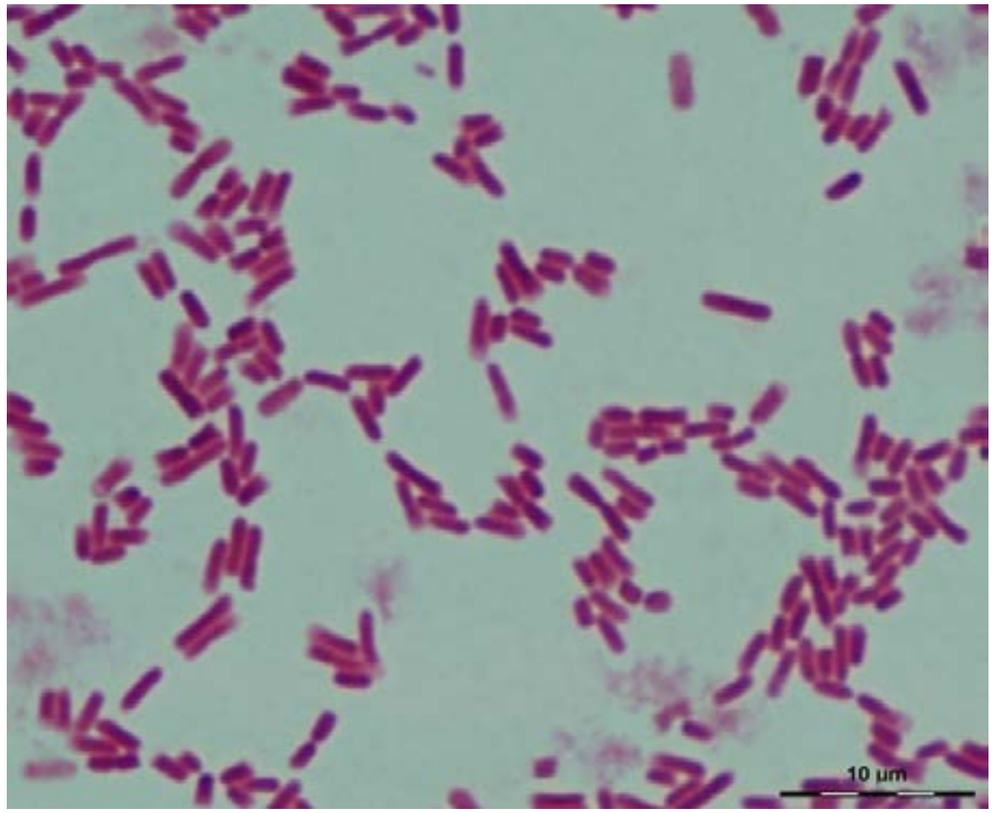
Epidemiology, Prevention & Control

Klebsiella are ubiquitous in nature. In humans, they may colonize the skin, pharynx, or gastrointestinal tract. They may also colonize sterile wounds and urine. Carriage rates vary with different studies. *Klebsiella* may be regarded as normal flora in many parts of the colon and intestinal tract and in the biliary tract. Oropharyngeal carriage has been associated with endotracheal intubation, impaired host defenses, and antimicrobial use.

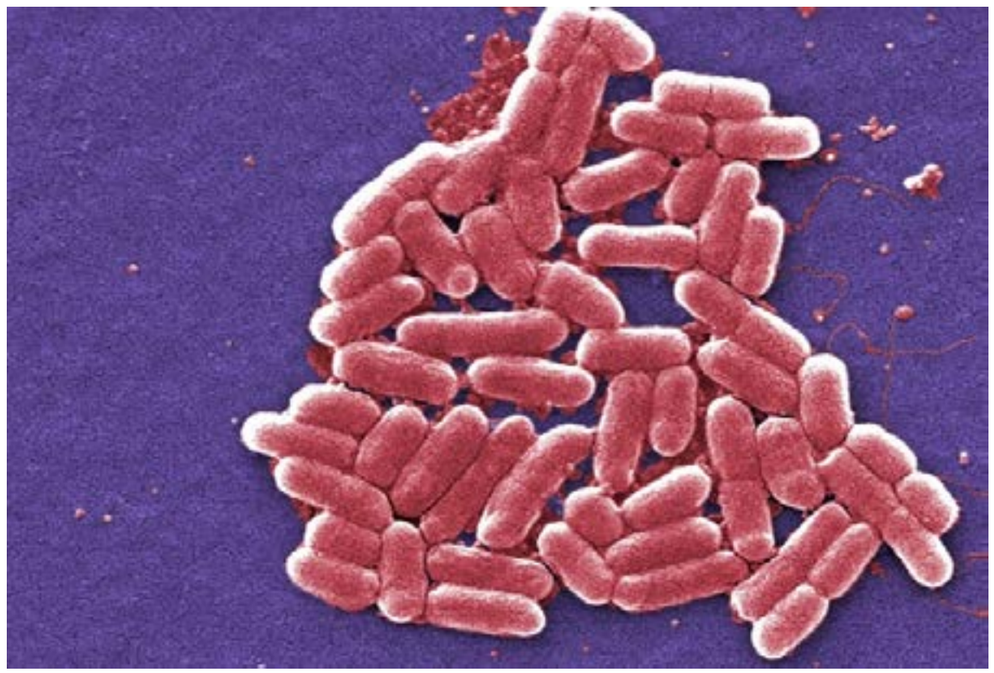
K. pneumoniae and *K. oxytoca* are the 2 members of this genus responsible for most human infections. They are opportunistic pathogens found in the environment and in mammalian mucosal surfaces. The principal pathogenic reservoirs of infection are the gastrointestinal tract of patients and the hands of hospital personnel. Organisms can spread rapidly, often leading to nosocomial outbreaks.

To prevent the spread of infections, patients should remain very cautious of their handwashing habits. Handwashing with soap and water should happen in the following instances: before touching eyes, nose, or mouth, before preparing food, before addressing bandaged or wound areas, after using the restroom, and after using the restroom. It is especially important to be cautious when entering and exiting hospital rooms. Be sure to use proper handwashing techniques after touching doorknobs, bed rails, using hospital restroom, and interacting with sick patients, even if they are loved ones.

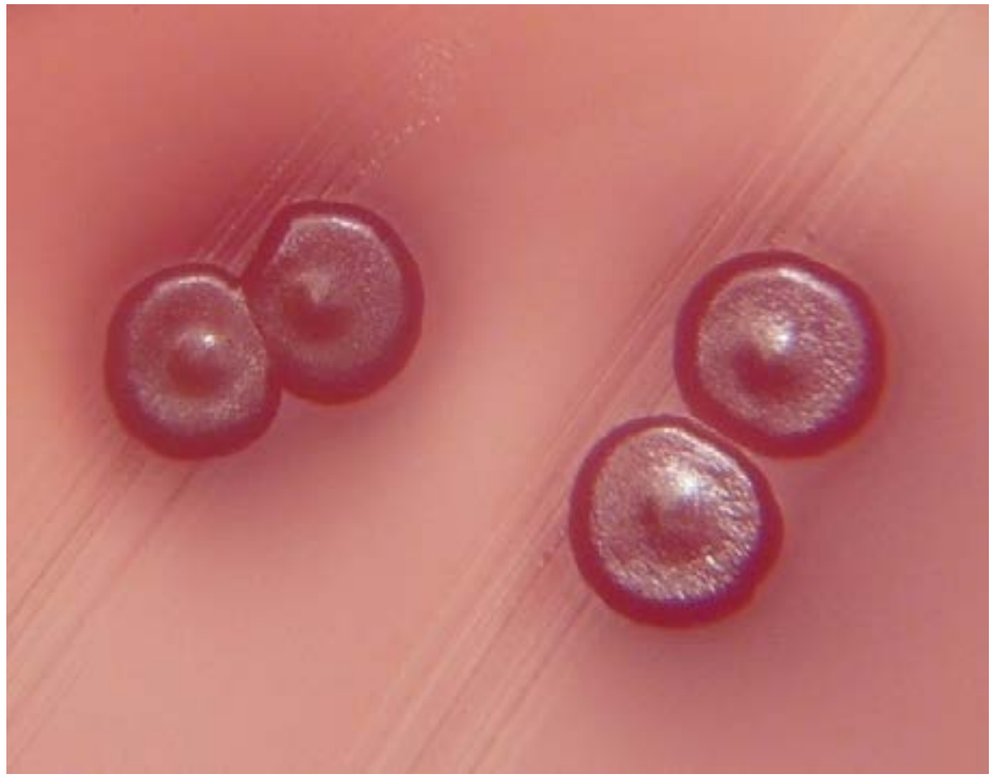
2 Class – Illustrations



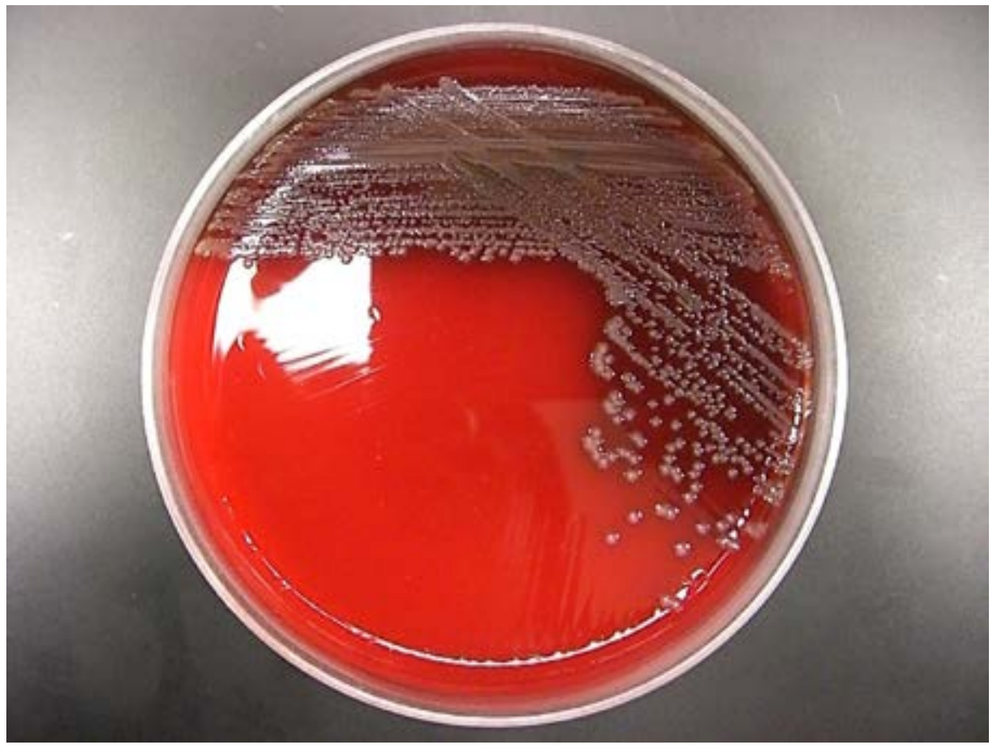
Escherichia coli Gram stain



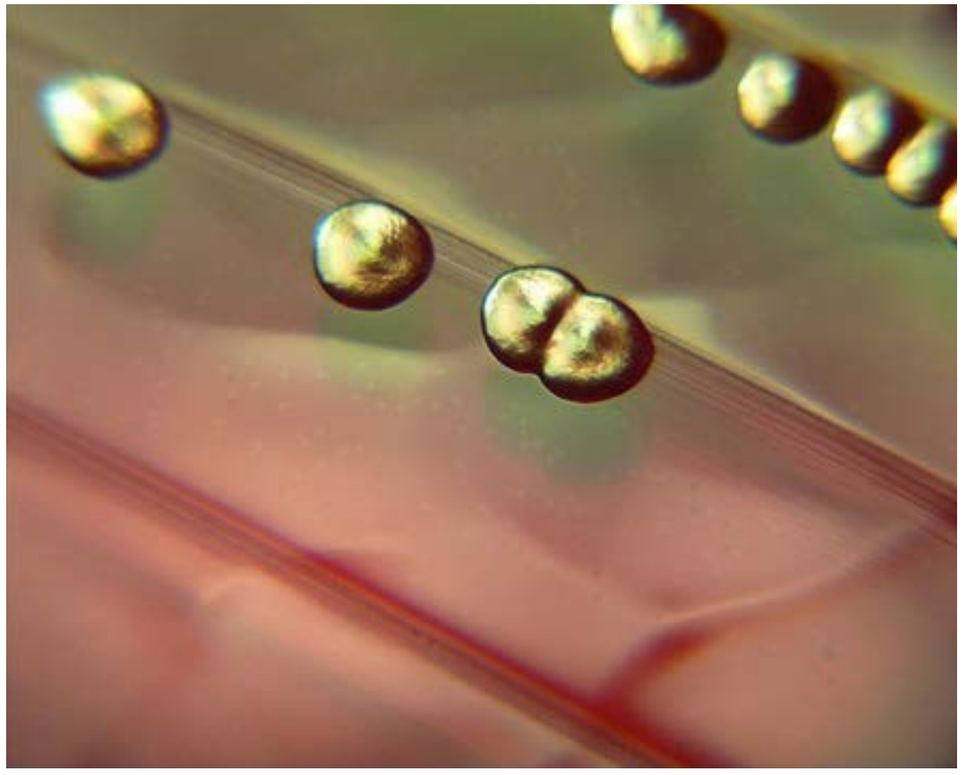
Escherichia coli (scanning electron micrograph)



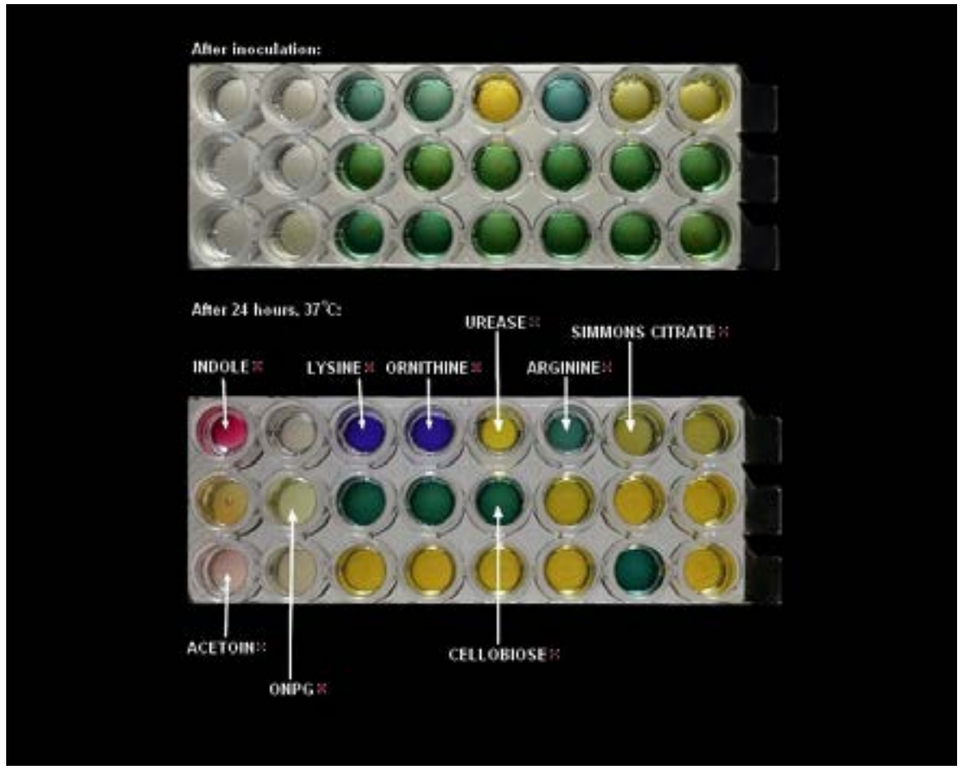
E. coli on MacConkey agar



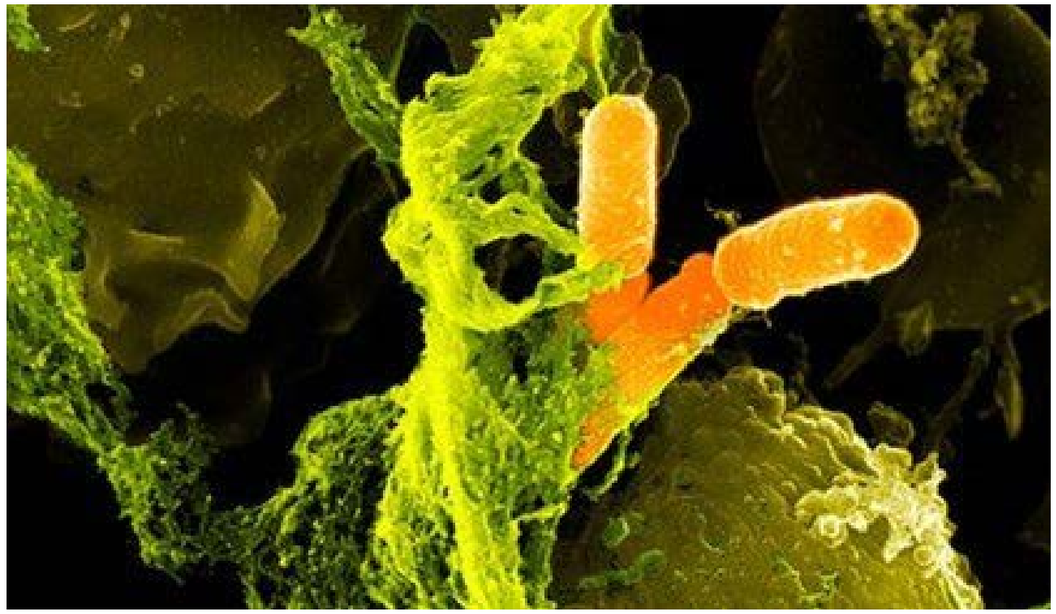
E. coli on blood agar



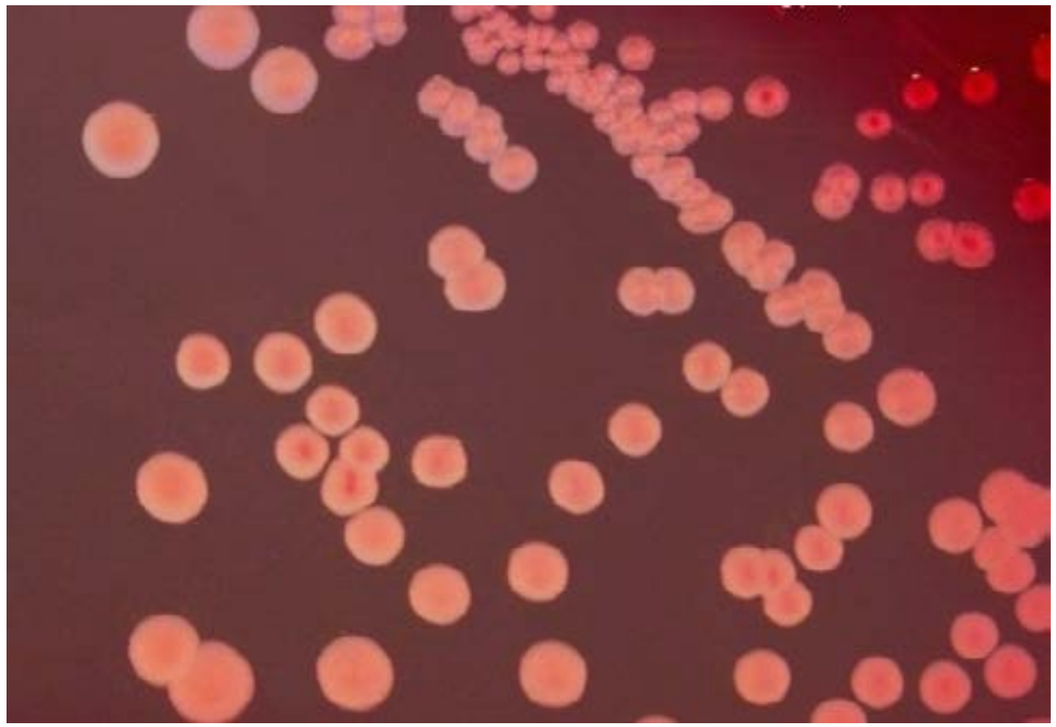
E.coli on Endo agar (lactose positive, metallic sheen)



E. coli identification

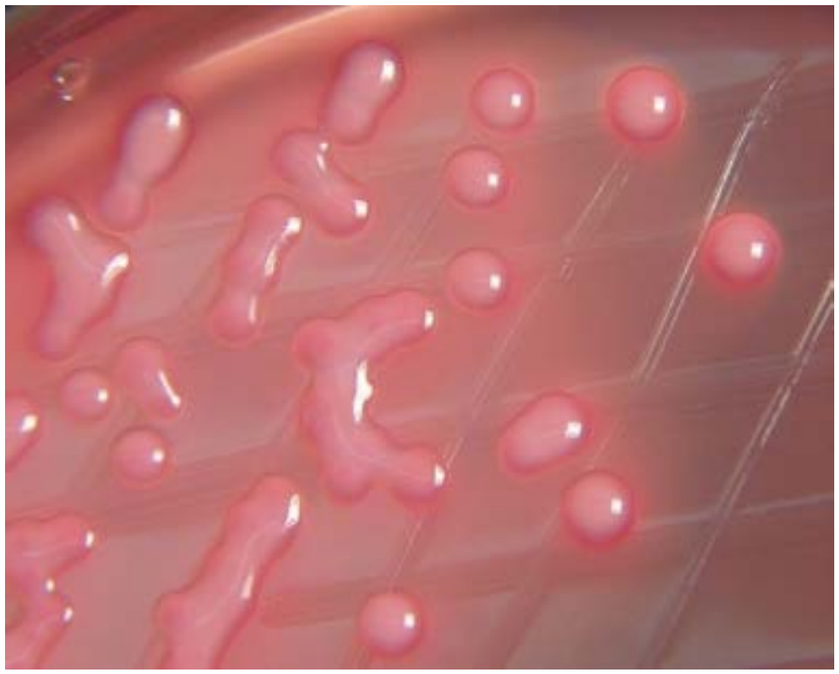


Neutrophils and *Shigella* bacteria, SEM



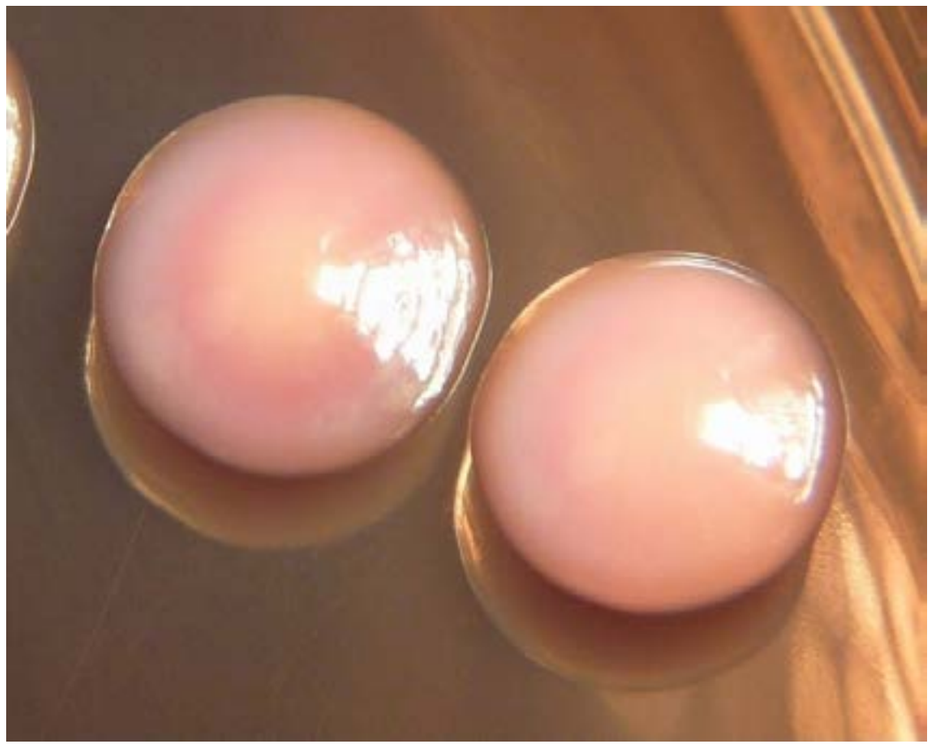
Shigella flexneri on Endo agar

Klebsiella pneumoniae bacteria, SEM

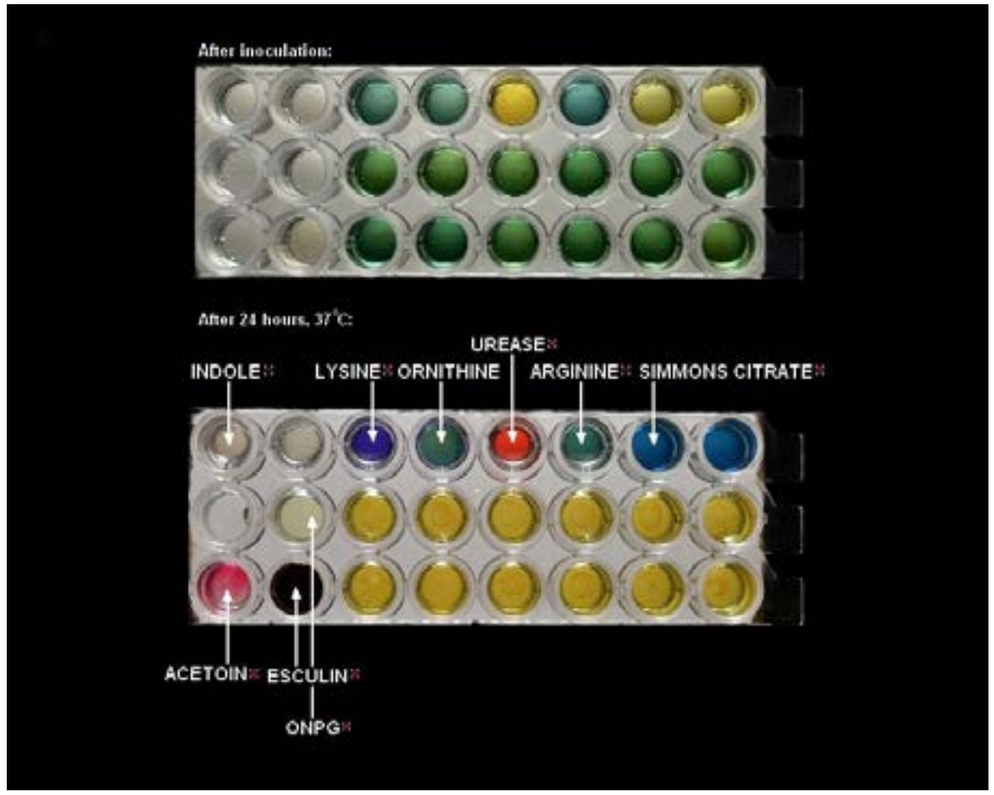


K. pneumoniae on MacConkey agar

K. pneumoniae on Endo agar



K. pneumoniae on desoxycholate-citrate agar



K. pneumoniae identification

3 Class - *Proteus. Salmonella*

Salmonella

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Salmonella

The classification of *Salmonella* is complex because the organisms are a continuum rather than a defined species. The members of the genus *Salmonella* were originally classified on the basis of epidemiology, host range biochemical reactions, and structures of the O, H, and Vi (when present) antigens. The names (eg, *Salmonella typhi, Salmonella typhimurium*) were written as if they were genus and species; this form of the nomenclature remains in widespread but incorrect use. DNA-DNA hybridization studies have demonstrated that there are seven evolutionary groups. Nearly all of the *Salmonella* serotypes that infect humans are in DNA hybridization group I; there are rare human infections with groups IIIa and IIIb. The species name *Salmonella enterica* has been widely accepted, and the organisms in DNA hybridization group I are *S. enterica* subspecies *enterica.* The organisms in the other groups have other subspecies names. It seems probable that the widely accepted nomenclature for classification will be as follows: *S. enterica* subspecies *enterica* serotype Typhimurium, which can be shortened to *Salmonella* Typhimurium with the genus name in italics and the serotype name in roman type. National and international reference laboratories may use the antigenic formulas following the subspecies name because they impart more precise information about the isolates.

There are more than 2500 serotypes of *Salmonella*, including more than 1400 in DNA hybridization group I that can infect humans. Four serotypes of *Salmonella* that cause enteric fever can be identified in the clinical laboratory by biochemical and serologic tests. These serotypes should be routinely identified because of their clinical significance. They are as follows: *Salmonella* Paratyphi A (serogroup A), *Salmonella* Paratyphi B (serogroup B), *Salmonella* Choleraesuis (serogroup C1), and *Salmonella* Typhi (serogroup D). The more than 1400 other *Salmonella* that are isolated in clinical laboratories are serogrouped by their O antigens as A, B, C1, C2, D, and E; some are nontypeable with this set of antisera. The isolates are then sent to reference laboratories for definitive serologic identification. This allows public health officials to monitor and assess the epidemiology of *Salmonella* infections on a statewide and nationwide basis.

Morphology & Identification

A. MORPHOLOGY

Salmonella are non-spore-forming, predominantly motile enterobacteria with diameters around 0,7 to 1,5 µm, lengths from 2 to 5 µm, and peritrichous flagella.

B. CULTURE AND GROWTH CHARACTERISTICS

Colonies are 1-3 mm diameter, S-type, but sometimes R-type may appear. Motile, with the exception of Gallinarum and Pullorum serovars. Facultatively anaerobic, growth temperature 37 °C. Grow easily on various media: Nutrient Agar or Nutrient Broth, Trypticase Soy Agar + 5% sheep blood, Mac Conkey Agar-white/colourless colonies, SS Agar - colourless colonies, Rambach Agar - red colonies, XLD Agar - red colonies, some with black center, Muller-Hinton Agar.

Positive results for catalase, nitrate reduction, methyl red, acid production from: glucose, mannitol, D-mannose. Negative results for indole production, urea hydrolysis, phenylalanine deaminase, gelatin hydrolysis, esculin hydrolysis, oxidase, DN-ase, lipase, acid production from: adonitol, sucrose, cellobiose, raffinose.

C. VIRULENCE FACTORS

Salmonella possess a number of virulence factors that contribute to its pathogenesis. *Salmonella* invades the epithelial cells of the human intestines using a type three secretion system (TTSS) to inject its outer proteins into the host's intestinal cells. The TTSS is a needle-like structure that helps the bacteria find the host and then insert effector proteins. As soon as the needle comes into contact with the host cell it starts secreting these effector molecules. The host cell then engulfs the bacteria, consequently allowing the bacteria to reproduce

and cause infection. The effectors that are inserted into the host cell signal the host to take-up the bacteria. This happens by the effectors taking control of the actin polymerization process. The actin polymerizes to the point where it causes the host cell wall to begin to raise on either side of the bacteria. Once the bacteria is surrounded on all sides by the cell wall it is engulfed into the host and encased in a vesicle. *Salmonella* mature within the cell inside of «*Salmonella* containing vacuoles» (SCVs) thereby evading the host's immune response. Additionally, *Salmonella* have at least 16 adhesion factors which allow them to better adhere to the intestinal epithelium. *Salmonella* has acquired at least 5 SPI's (*Salmonella* Pathogenicity Islands) via horizontal gene transfer which contribute to its virulence. Specifically, SPI-1 and SPI-2 are the main contributors to *Salmonella's* virulence. In addition to its pathogenicity islands, *Salmonella,* like all Gram-negative bacteria, are surrounded by an outer membrane containing lipopolysaccharide (LPS). *Salmonella* lipopolysaccharide contains the lipid A endotoxin which, upon release, can cause shock in the host. Another virulence factor of *Salmonella* its ability to synthesize enterobactin. Enterobactin, the strongest siderophore known, secreted by *Salmonella* allows it to commandeer iron from the hosts body which it can use for growth within the host.

Pathogenesis, Pathology & Clinical Findings

Salmonella Typhi, *Salmonella* Choleraesuis, and perhaps *Salmonella* Paratyphi A, and *Salmonella* Paratyphi B are primarily infective for humans, and infection with these organisms implies acquisition from a human source. The vast majority of *Salmonella*, however, are chiefly pathogenic in animals that constitute the reservoir for human infection: poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others. The organisms almost always enter via the oral route, usually with contaminated food or drink. The mean infective dose to produce clinical or subclinical infection in humans is 10^5-10^8 *Salmonella* (but perhaps as few as 10^3 *Salmonella* Typhi organisms). Among the host factors that contribute to resistance to *Salmonella* infection are gastric acidity, normal intestinal microbial flora, and local intestinal immunity. *Salmonella* produce three main types of disease in humans, but mixed forms are frequent.

A. THE «ENTERIC FEVERS» (TYPHOID FEVER)

This syndrome is produced by only a few of the *Salmonella*, of which *Salmonella* Typhi (typhoid fever) is the most important. The ingested *Salmonella* reach the small intestine, from which they enter the lymphatics and then the bloodstream. They are carried by the blood to many organs, including the intestine. The organisms multiply in intestinal lymphoid tissue and are excreted in stools. After an incubation period of 10-14 days, fever, malaise, headache, constipation, bradycardia, and myalgia occur. The fever rises to a high plateau, and the spleen and liver become enlarged. Rose spots, usually on the skin of the abdomen or chest, are seen briefly in rare cases. The white blood cell count is normal or low. In the preantibiotic era, the chief complications of enteric fever were intestinal hemorrhage and perforation, and the mortality rate was 10-15%. Treatment with antibiotics has reduced the mortality rate to less than 1%. The principal lesions are hyperplasia and necrosis of lymphoid tissue (eg, Peyer's patches), hepatitis, focal necrosis of the liver, and inflammation of the gallbladder, periosteum, lungs, and other organs.

B. BACTEREMIA WITH FOCAL LESIONS

This is associated commonly with *S. choleraesuis* but may be caused by any *Salmonella* serotype. Following oral infection, there is early invasion of the bloodstream (with possible focal lesions in lungs, bones, meninges, etc), but intestinal manifestations are often absent. Blood cultures are positive.

C. ENTEROCOLITIS

This is the most common manifestation of *Salmonella* infection. Eight to 48 hours after ingestion of *Salmonella*, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stools. Low-grade fever is common, but the episode usually resolves in 2-3 days. Inflammatory lesions of the small and large intestine are present. Bacteremia is rare (2-4%) except in immunodeficient persons. Blood cultures are usually negative, but stool cultures are positive for *Salmonella* and may remain positive for several weeks after clinical recovery.

Diagnostic laboratory tests

A. SPECIMENS

Blood for culture must be taken repeatedly. In enteric fevers and septicemias, blood cultures are often positive in the first week of the disease. Bone marrow cultures may be useful. Urine cultures may be positive after the second week. Stool specimens also must be taken repeatedly. In enteric fevers, the stools yield positive results from the second or third week on; in enterocolitis, during the first week. A positive culture of duodenal drainage establishes the presence of salmonella in the biliary tract in carriers.

B. BACTERIOLOGIC METHODS FOR ISOLATION OF *SALMONELLA*

1. Differential Medium Cultures

EMB, MacConkey's, or deoxycholate medium permits rapid detection of lactose nonfermenters (not only *Salmonella* and *Shigella* but also *Proteus, Serratia, Pseudomonas*, etc). Gram-positive organisms are somewhat inhibited. Bismuth sulfite medium permits rapid detection of *Salmonella* which form black colonies because of H_2S production. Many *Salmonella* produce H_2S.

2. Selective Medium Cultures

The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, XLD, or deoxycholate-citrate agar, which favor growth of *Salmonella* and *Shigella* over other Enterobacteriaceae.

3. Enrichment Cultures

The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of *Salmonella.* After incubation for 1-2 days, this is plated on differential and selective media.

4. Final Identification

Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.

C. SEROLOGIC METHODS

Serologic techniques are used to identify unknown cultures with known sera and may also be used to determine antibody titers in patients with unknown illness, although the latter is not very useful in diagnosis of *Salmonella* infections.

1. Agglutination Test

In this test, known sera and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes. This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup *Salmonella* by their O antigens: A, B, C_1, C_2, D, and E.

2. Tube Dilution Agglutination Test (Widal Test)

Serum agglutinins rise sharply during the second and third weeks of *Salmonella* Typhi infection. The Widal test to detect these antibodies against the O and H antigens has been in use for decades. At least two serum specimens, obtained at intervals of 7-10 days, are needed to prove a rise in antibody titer. Serial dilutions of unknown sera are tested against antigens from representative *Salmonella*. False-positive and false-negative results occur. The interpretive criteria when single serum specimens are tested vary, but a titer against the O antigen of > 1:320 and against the H antigen of > 1:640 is considered positive. High titer of antibody to the Vi antigen occurs in some carriers. Results of serologic tests for *Salmonella* infection must be interpreted cautiously because the possible presence of cross-reactive antibodies limits the use of serology. The test is not useful in diagnosis of enteric fevers caused by *Salmonella* other than *Salmonella* Typhi.

Immunity

Infections with *Salmonella* Typhi or *Salmonella* Paratyphi usually confer a certain degree of immunity. Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and Vi are related to resistance to infection and disease. However, relapses may occur in 2-3 weeks after recovery in spite of antibodies. Secretory IgA antibodies may prevent attachment of *Salmonella* to intestinal epithelium. Persons with S/S hemoglobin (sickle cell disease) are exceedingly susceptible to *Salmonella* infections, particularly osteomyelitis. Persons with A/S hemoglobin (sickle cell trait) may be more susceptible than normal individuals (those with A/A hemoglobin).

Treatment

While enteric fevers and bacteremias with focal lesions require antimicrobial treatment, the vast majority of cases of enterocolitis do not. Antimicrobial treatment of *Salmonella enteritis* in neonates is important. In enterocolitis, clinical symptoms and excretion of the *Salmonella* may be prolonged by antimicrobial therapy. In severe diarrhea, replacement of fluids and electrolytes is essential.

Antimicrobial therapy of invasive *Salmonella* infections is with ampicillin, trimethoprim-sulfamethoxazole, or a third-generation cephalosporin. Multiple drug resistance transmitted genetically by plasmids among enteric bacteria is a problem in *Salmonella* infections. Susceptibility testing is an important adjunct to selecting a proper antibiotic.

In most carriers, the organisms persist in the gallbladder (particularly if gallstones are present) and in the

biliary tract. Some chronic carriers have been cured by ampicillin alone, but in most cases cholecystectomy must be combined with drug treatment.

Epidemiology, Prevention & Control

The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, eg, when carriers working as food handlers are «shedding» organisms. Many animals, including cattle, rodents, and fowl, are naturally infected with a variety of *Salmonella* and have the bacteria in their tissues (meat), excreta, or eggs. The high incidence of *Salmonella* in commercially prepared chickens has been widely publicized. The problem probably is aggravated by the widespread use of animal feeds containing antimicrobial drugs that favor the proliferation of drug-resistant *Salmonella* and their potential transmission to humans.

A. Carriers: after manifest or subclinical infection, some individuals continue to harbor *Salmonella* in their tissues for variable lengths of time (convalescent carriers or healthy permanent carriers). Three percent of survivors of typhoid become permanent carriers, harboring the organisms in the gallbladder, biliary tract, or, rarely, the intestine or urinary tract.

B. Sources of infection: the sources of infection are food and drink that have been contaminated with *Salmonella.* The following sources are important:

1. Water - contamination with feces often results inexplosive epidemics.
2. Milk and Other Dairy Products (Ice Cream, Cheese, Custard) - contamination with feces and inadequate pasteurization or improper handling. Some outbreaks are traceable to the source of supply.
3. Shellfish - from contaminated water.
4. Dried or Frozen Eggs - from infected fowl or contaminated during processing.
5. Meats and Meat Products - from infected animals (poultry) or contamination with feces by rodents or humans.
6. «Recreational» Drugs - marijuana and other drugs.
7. Animal Dyes - dyes (eg, carmine) used in drugs, foods, and cosmetics.
8. Household Pets - turtles, dogs, cats, etc.

Sanitary measures must be taken to prevent contamination of food and water by rodents or other animals that excrete *Salmonella*. Infected poultry, meats, and eggs must be thoroughly cooked. Carriers must not be allowed to work as food handlers and should observe strict hygienic precautions.

Two injections of acetone-killed bacterial suspensions of *Salmonella* Typhi, followed by a booster injection some months later, give partial resistance to small infectious inocula of typhoid bacilli but not to large ones. Oral administration of a live avirulent mutant strain of *Salmonella* Typhi has given significant protection in areas of high endemicity. Vaccines against other *Salmonella* give less protection and are not recommended.

Proteus

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Proteus
Species: P. hauseri, P. mirabilis, P. myxofaciens, P. penneri, P. vulgaris

Morphology & Identification

A. MORPHOLOGY

Proteus can display two different morphological and physiological forms; one is known as the swimmer cells and the other as swarmer cells. In aqueous suspension *P. mirabilis* is found in the swimmer state, which is a small rod-like cells1 to 2 μm in length. They contain 8 to 10 flagella that aid in their swimming motility. On contact with a surface, *P. mirabilis* changes to the swarmer state where the cell considerably increases in length to form highly flagellated filaments that are 20 to 80 μm in length. These cells line up in parallel to form rafts that are able to move rapidly over surfaces en masse. On semi-solid surfaces such as an agar surface, they form concentric rings of growth. This pattern is caused by the coordinated burst of swarming activity

interspeded with a consolidation to the swimmer state.

An important feature of *P. mirabilis* is their swarming motility, facilitated by peritrichous flagella, which is a rapid and coordinated translocation of a bacterial population across solid or semi-solid surfaces. This ability aids them in food acquisition, reproduction and growth. It also aids the bacteria in expressing its virulence factors and invading hosts' urothelial cells.

B. CULTURE AND GROWTH CHARACTERISTICS

Facultative anaerobic, growth temperature 37 °C. It is motile, alternating between vegetative swimmers and hyper-flagellated swarmer cells. It also makes a variety of fimbriae. The endotoxins of its LPS membrane elicit an inflammatory response from the host.

P. mirabilis produces urease, an enzyme that converts urea into ammonia by the following process: $(NH_2)_2CO \rightarrow 2NH_3 + CO_2$. Infection by *P. mirabilis* can therefore be detected by an alkaline urine sample (pH 8 and up) with large amounts of ammonia.

C. VIRULENCE FACTORS

The invaders *(P. mirabilis, P. vulgaris, and P. penneri)* have numerous factors including fimbriae, flagella, outer membrane proteins, lipopolysaccharide, capsule antigen, urease, immunoglobulin A proteases, hemolysins, amino acid deaminases, and, finally, the most characteristic attribute of *Proteus,* swarming growth.

The flagellum of *P. mirabilis* is crucial to its motility, a characteristic that helps the organism colonize. The flagellum has also been linked to the ability of *P. mirabilis* to form biofilms, aiding in the bacteria's resistance to defenses of the host and select antibiotics. *P. mirabilis* also relies on its pili for adhesion to avoid being flushed out of the urinary tract system.

Important to *P. mirabilis* is urease, responsible for raising the pH and consequently making it easier to thrive. Increased pH allows stone formation to take place. On occasion the stones fill the entire renal pelvis.

Also present are endotoxins, responsible for induction of the inflammatory response system and pore-forming hemolysins.

Pathogenesis, Pathology & Clinical Findings

The most common infection involving *P. mirabilis* occurs when the bacteria moves to the urethra and urinary bladder. Although *P. mirabilis* mostly known to cause urinary tract infections, the majority of urinary tract infections are due to *E. coli*. One-hundred thousand cfus per milliliter in the urine are usually indicative of a urinary tract infection. Urinary tract infections caused by *P. mirabilis* occur usually in patients under long-term catherization. The bacteria have been found to move and create encrustations on the urinary catheters. The encrustations cause the catheter to block.

Symptoms for urethritis are mild including frequency of urination and pyuria (presence of white blob cells in the urine). Cystitis (bladder infection) symptoms are easier to distinguish and include back pain, concentrated appearance, urgency, hematuria (presence of red blood cells in the urine), and suprapubic pain as well as increased frequency of urination and pyuria.

Pyelonephritis (kidney infection) can occur when the bacteria migrates from the lower urinary tract. Although it is seen as a furtherance of infections, not all patients have the symptoms associated with urethritis and cystitis. Pyelonephritis is marked by nausea and vomiting.

P. mirabilis can enter the bloodstream through wounds. This happens with contact between the wound and an infected surface. The bacteria induce inflammatory response that can cause sepsis and systemic inflammatory response syndrome (SIRS). SIRS has a mortality rate between 20 and 50 percent.

P. mirabilis can also, though less common, colonize the lungs. This is the result of infected hospital breathing equipment and causes pneumonia. Symptoms for pneumonia include fever, chills, chest pain, rales, and cough.

Prostatitis can occur as a result of *P. mirabilis* infection, causing fever, chills, and tender prostate in men.

Diagnostic laboratory tests

The members of this group deaminate phenylalanine, are motile, grow on potassium cyanide medium (KCN), and ferment xylose. *Proteus* species move very actively by means of peritrichous flagella, resulting in «swarming» on solid media unless the swarming is inhibited by chemicals, eg, phenylethyl alcohol or CLED (cystinelactose- electrolyte-deficient) medium. *P. mirabilis* is more susceptible to antimicrobial drugs, including penicillins, than other members of the group.

The microorganism tests:

Indole-negative and nitrate reductase-positive (no gas bubbles produced)

Methyl red-positive and Voges-Proskauer negative (Can be both MR- and V-P-positive)

Catalase positive and cytochrome oxidase-negative

Phenylalanine deaminase-positive

Tryptophan test-negative

Urea test- positive

Casein test-negative

Starch test- negative

Hydrogen sulfide test-positive

Citrate agar test-negative

Ornithine decarboxylase-positive

Lysine decarboxylase-negative

Immunity

Specific antibodies develop in systemic infections, but it is uncertain whether significant immunity to the organisms follows.

Treatment

P. mirabilis infections can be treated with broad-spectrum penicillins or cephalosporins except in severe cases. It is not susceptible to nitrofurantoin or tetracycline and has experienced increasing drug resistance of ampicillin, trimethoprim, and ciprofloxin. In cases with severe stone formation, surgery is necessary to remove the blockage.

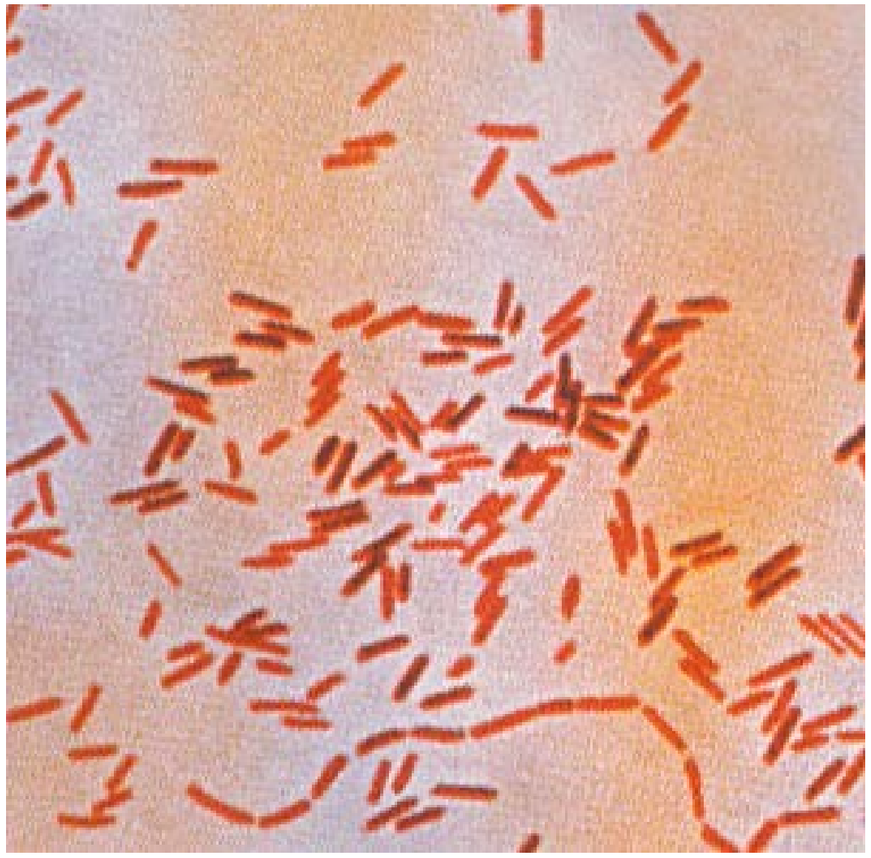
Epidemiology, Prevention & Control

Infections caused by *P. mirabilis* are seen most often in nursing home patients. These infections are commonly caused by infected medical equipment including catheters, nebulizers (responsible for inhalation), and examination gloves (responsible for wound infections). The length of catherization is directly related to incidence of infection. Each day of catherization gives an infection rate of 3-5%.

Urinary tract infections caused by *P. mirabilis* also occur commonly in sexually active women and men, especially those engaging in unprotected intercourse. Younger women are at greater risk than younger men; however, older men are at greater risk than older women due to the occurrence of prostate disease.

Consequently, prevention includes good sanitation and hygiene, including proper sterilization of medical equipment. It is also suggested that patients not requiring catheterization should not receive catheterization, despite its convenience for the caretaker.

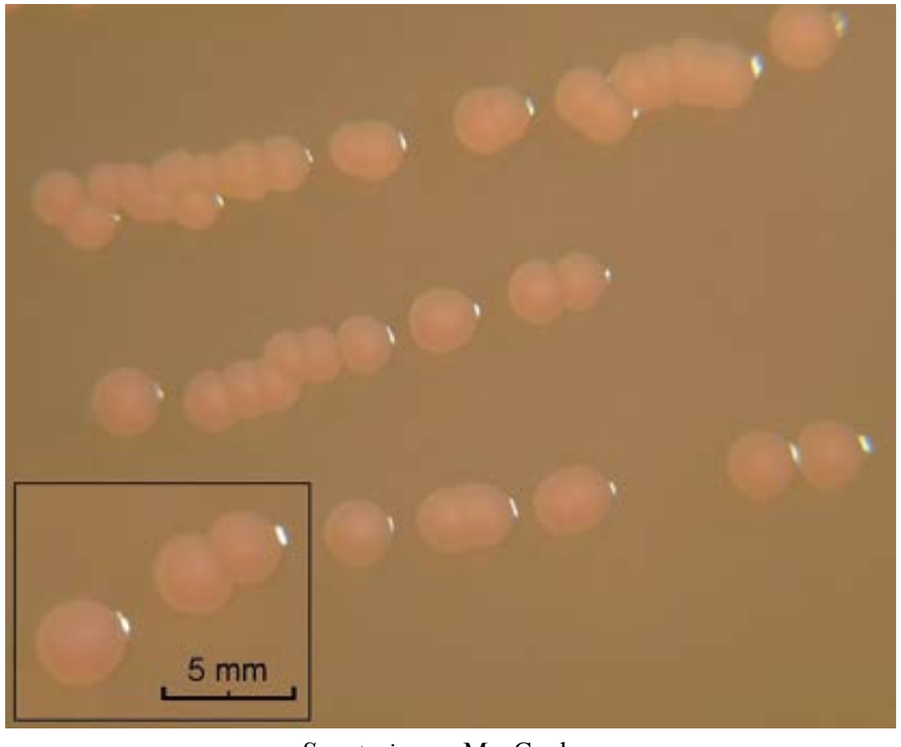
3 Class – Illustrations



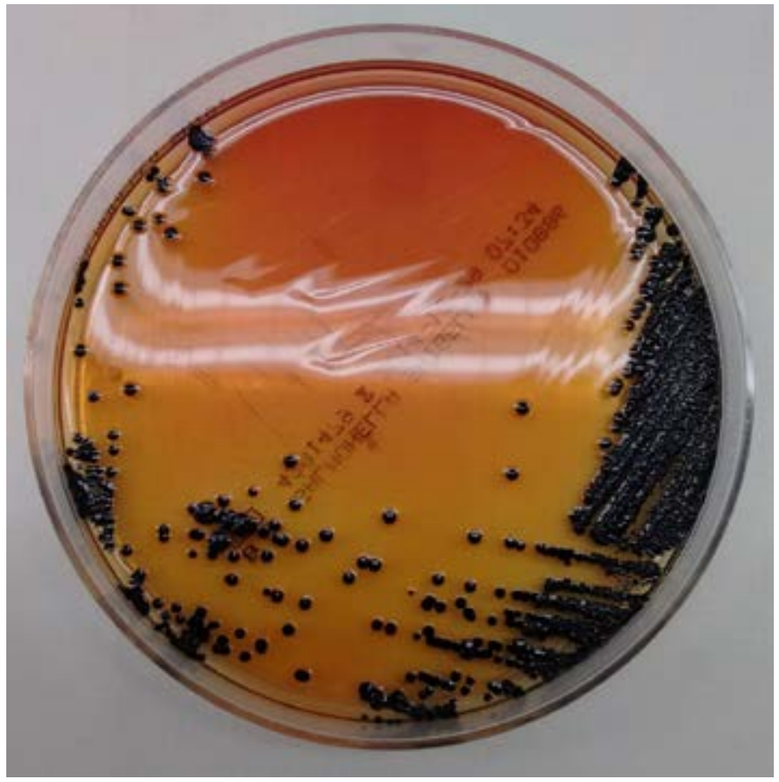
Salmonella typhi. Gram stain

Salmonella enterica (SEM)

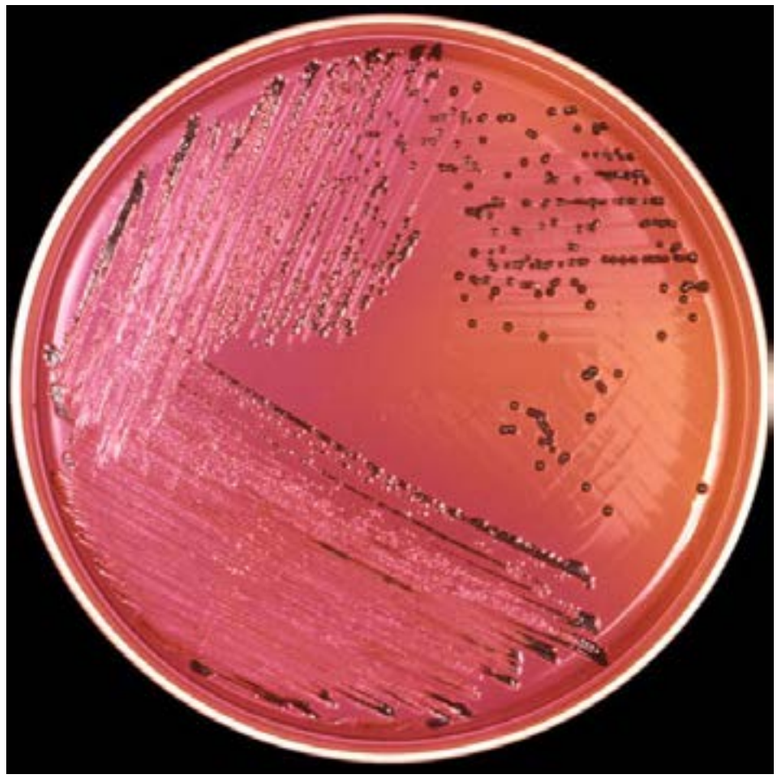
Lactose negative colonies of *S. enterica* on Endo agar



S. enterica on MacConkey

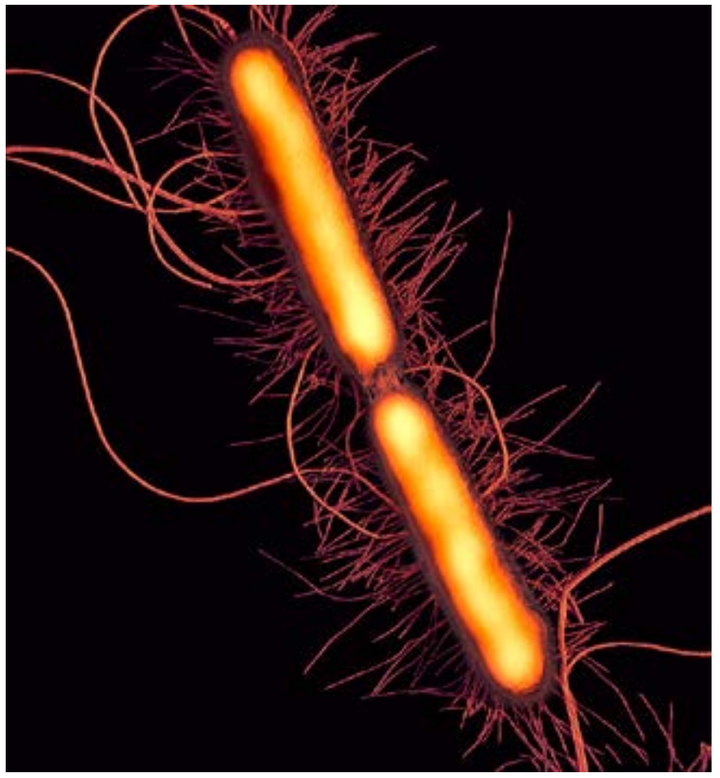


Salmonella colonies on SS agar

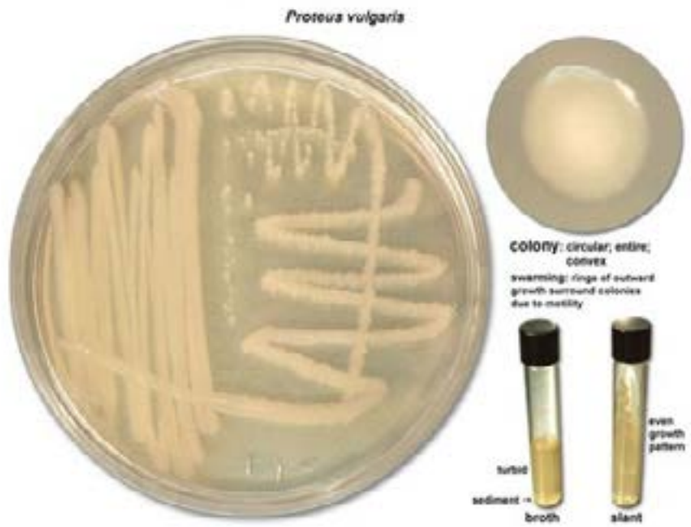


Salmonella colonies on XLD agar

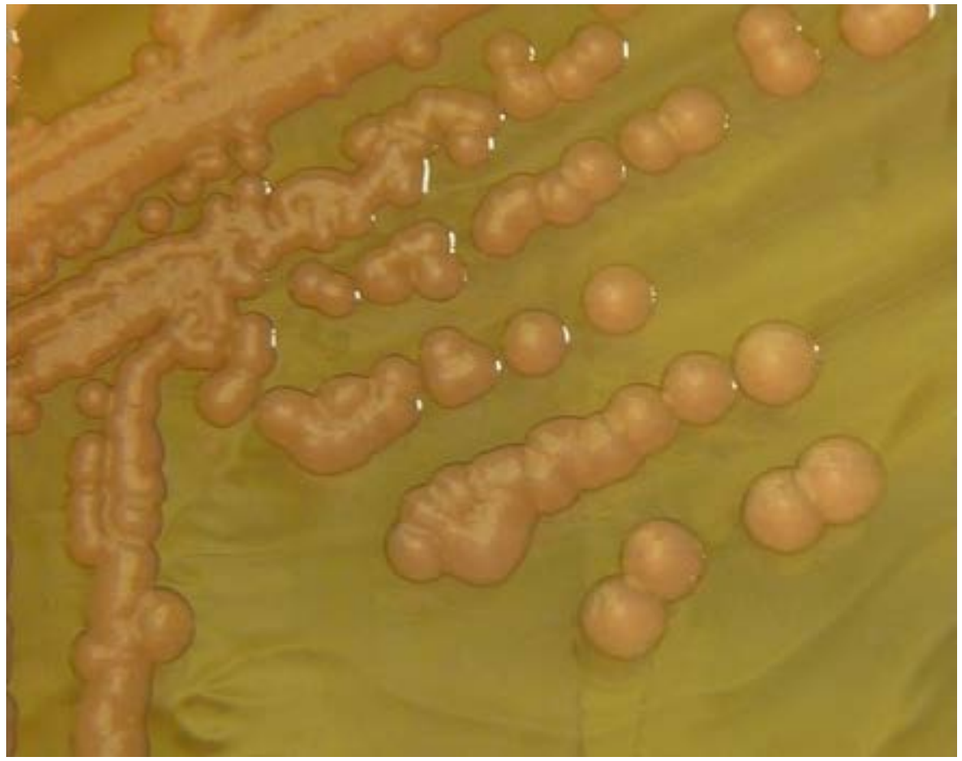
Widal Test



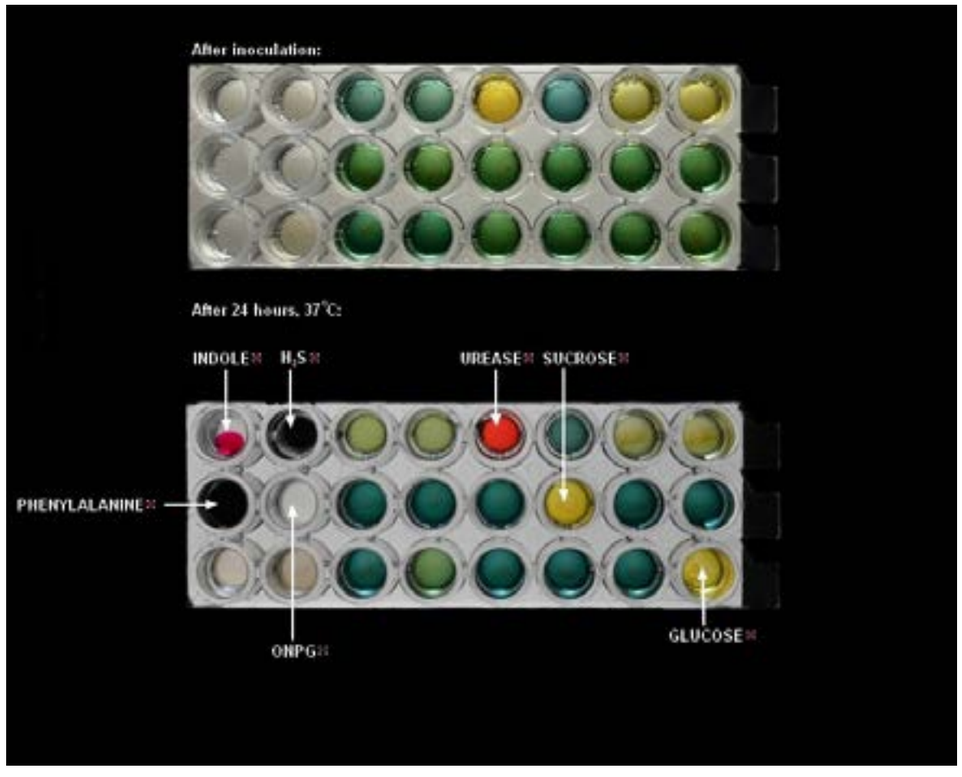
Proteus vulgaris under Scanning Electron Microscope



Proteus mirabilis on Blood Agar



Proteus on deoxycholate citrate agar



Proteus vulgaris identification

4 Class - Opportunistic microorganisms. Nosocomial (intra-hospital) Infections. Clinical Microbiology

An opportunistic infection is an infection caused by bacterial, viral, fungal, or protozoan pathogens that take advantage of a host with a weakened immune system. Many of these pathogens do not cause disease in a healthy host that has a normal immune system. A compromised immune system, however, presents an «opportunity» for the pathogen to infect.

Causes

Immunodeficiency or immunosuppression can be caused by:

Malnutrition

Fatigue

Recurrent infections

Immunosuppressing agents for organ transplant recipients

Advanced HIV infection

Chemotherapy for cancer

Genetic predisposition

Skin damage

Antibiotic treatment leading to disruption of the physiological microbiome, thus allowing some microorganisms to outcompete others and become pathogenic (e.g. disruption of intestinal flora may lead to *Clostridium difficile* infection

Medical procedures

Pregnancy

Further information: Susceptibility and severity of infections in pregnancy

Ageing

Leukopenia (i.e. neutropenia and lymphocytopenia)

The lack of or the disruption of normal vaginal flora allows the proliferation of opportunistic microorganisms and will cause the opportunistic infection - bacterial vaginosis.

Opportunistic microorganism - a bacterium, virus, protozoan or fungus that takes an advantage of certain opportunities to cause disease. Those opportunities are called opportunistic conditions. These microorganisms are often ones that can lie dormant in body tissues for many years, such as the human herpes viruses, or that are extremely common but usually cause no symptoms of illness. When the immune system cannot raise an adequate response, these microorganisms are activated, begin to multiply, and soon overwhelm the body's weakened defences.

The most common opportunistic infections include the following:

(1) Protozoa: *Toxoplasma gondii*, *Isospora belli*, *Cryptosporidium* species.

(2) Fungi: *Candida albicans*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Pneumocystis jiroveci*.

(3) Bacteria: *Mycobacterium avium*-intracellulare, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Nocardia asteroides*, *Salmonella* species, *Streptococcus* species.

(4) Viruses: Cytomegalovirus, herpes simplex virus, varicella-zoster virus, adenovirus, polyomavirus, JC virus, hepatitis B virus, hepatitis C virus.

Herpesvirus infections are frequently detected being shed in saliva. Cytomegalovirus retinitis is the most common severe ocular complication.

Prophylaxis (Prevention)

Since opportunistic infections can cause severe disease, much emphasis is placed on measures to prevent infection. Such a strategy usually includes restoration of the immune system as soon as possible, avoiding exposures to infectious agents, and using antimicrobial medications («prophylactic medications») directed against specific infections.

Restoration of Immune System

In patients with HIV, starting antiretroviral therapy is especially important for restoration of the immune system and reduces the incidence of all opportunistic infections.

In patients undergoing chemotherapy, completion of and recovery from treatment is the primary method

for immune system restoration. In a select subset of high risk patients, granulocyte colony stimulating factors (G-CSF) can be used to aid immune system recovery.

Infectious Exposures to Avoid

Cat feces (e.g. cat litter): source of *Toxoplasma gondii*, *Bartonella* spp.

Eating undercooked meat or eggs, unpasteurized dairy products or juices.

Potential sources of tuberculosis (high risk healthcare facilities, regions with high rates of tuberculosis, patients with known tuberculosis).

Contact with farm animals, especially those with diarrhea: source of *Toxoplasma gondii*, *Cryptosporidium parvum.*

Soil/dust in areas where there is known histoplasmosis, coccidiomycosis.

Reptiles, chicks, ducklings: source of *Salmonella* spp.

Unprotected sexual intercourse with individuals with known sexually transmitted infections. Any sex practice that might result in oral exposure to feces.

Prophylactic Medications

Individuals at higher risk are often prescribed prophylactic medication to prevent an infection from occurring. A patient's risk level for developing an opportunistic infection is approximated using the patient's CD4 T-cell count and sometimes other markers of susceptibility. Common prophylaxis treatments include the following:

Infection	When to Give Prophylaxis	Agent
Pneumocystis jirovecii	CD4 < 200 cells/mm3 or oropharyngeal candidasis (thrush)	TMP-SMX
Toxoplasma gondii	CD4 < 100 cells/mm3 and positive *Toxoplasma gondii* Ig G immunoassay	TMP-SMX
Mycobacterium avium complex	CD4 < 50	Azithromycin

Treatment

Treatment depends on the type of opportunistic infection, but usually involves different antibiotics.

Hospital-acquired infection (HAI) - also known as nosocomial infection - is an infection whose development is favored by a hospital environment, such as one acquired by a patient during a hospital visit or one developing among hospital staff.

In the United States, the Centers for Disease Control and Prevention estimated roughly 1.7 million hospital-associated infections, from all types of microorganisms, including bacteria, combined, cause or contribute to 99,000 deaths each year. In Europe, where hospital surveys have been conducted, the category of Gram-negative infections are estimated to account for two-thirds of the 25,000 deaths each year. Nosocomial infections can cause severe pneumonia and infections of the urinary tract, bloodstream and other parts of the body. Many types are difficult to attack with antibiotics, and antibiotic resistance is spreading to Gram-negative bacteria that can infect people outside the hospital.

Hospital-acquired infections are an important category of hospital-acquired conditions. HAI is sometimes expanded as healthcare-associated infection to emphasize that infections can be correlated with health care in various settings (not just hospitals), which is also true of hospital-acquired conditions generally.

Types

Ventilator-associated pneumonia

Staphylococcus aureus

Methicillin resistant *Staphylococcus aureus*

Candida albicans

Pseudomonas aeruginosa

Acinetobacter baumannii

Stenotrophomonas maltophilia

Clostridium difficile

Tuberculosis

Urinary tract infection
Hospital-acquired pneumonia
Gastroenteritis
Vancomycin-resistant *Enterococcus*
Legionnaires' disease
Puerperal fever

Cause

Nosocomial infections are commonly transmitted when health care providers become complacent and do not practice correct hygiene regularly. Also, increased use of outpatient treatment in recent decades means that a greater percentage of people who are hospitalized today are likely to be seriously ill with more weakened immune systems than in the past. Moreover, some medical procedures bypass the body's natural protective barriers. Since medical staff move from patient to patient, the staff themselves serve as a means for spreading pathogens. Essentially, the staff act as vectors.

Transmission

The drug-resistant Gram-negative bacteria, for the most part, threaten only hospitalized patients whose immune systems are weak. They can survive for a long time on surfaces in the hospital and enter the body through wounds, catheters, and ventilators.

Contact transmission is divided into two subgroups: direct-contact transmission and indirect-contact transmission.

Prevention

The most effective technique for controlling nosocomial infection is to strategically implement QA/QC measures to the health care sectors, and evidence-based management can be a feasible approach. For those with ventilator-associated or hospital-acquired pneumonia, controlling and monitoring hospital indoor air quality needs to be on agenda in management, whereas for nosocomial rotavirus infection, a hand hygiene protocol has to be enforced. Other areas needing management include ambulance transport.

Hospitals have sanitation protocols regarding uniforms, equipment sterilization, washing, and other preventive measures. Thorough hand washing and/or use of alcohol rubs by all medical personnel before and after each patient contact is one of the most effective ways to combat nosocomial infections. More careful use of antimicrobial agents, such as antibiotics, is also considered vital.

Despite sanitation protocol, patients cannot be entirely isolated from infectious agents. Furthermore, patients are often prescribed antibiotics and other antimicrobial drugs to help treat illness; this may increase the selection pressure for the emergence of resistant strains.

Sterilization

Sterilization goes further than just sanitizing. It kills all microorganisms on equipment and surfaces through exposure to chemicals, ionizing radiation, dry heat, or steam under pressure.

Isolation

Isolation is the implementation of isolating precautions designed to prevent transmission of microorganisms by common routes in hospitals. (See Universal precautions and Transmission-based precautions.) Because agent and host factors are more difficult to control, interruption of transfer of microorganisms is directed primarily at transmission for example isolation of infectious cases in special hospitals and isolation of patient with infected wounds in special rooms also isolation of joint transplantation patients on specific rooms.

Handwashing

Handwashing frequently is called the single most important measure to reduce the risks of transmitting skin microorganisms from one person to another or from one site to another on the same patient. Washing hands as promptly and thoroughly as possible between patient contacts and after contact with blood, body fluids, secretions, excretions, and equipment or articles contaminated by them is an important component of infection control and isolation precautions. The spread of nosocomial infections, among immunocompromised patients is connected with health care workers' hand contamination in almost 40% of cases, and is a challenging problem in the modern hospitals. The best way for workers to overcome this problem is conducting correct hand-hygiene procedures.

Two categories of microorganisms can be present on health care workers' hands: transient flora and resident flora. The first is represented by the microorganisms taken by workers from the environment, and the

bacteria in it are capable of surviving on the human skin and sometimes to grow. The second group is represented by the permanent microorganisms living on the skin surface (on the stratum corneum or immediately under it). They are capable of surviving on the human skin and to grow freely on it.

They have low pathogenicity and infection rate, and they create a kind of protection from the colonization from other more pathogenic bacteria. The skin of workers is colonized by $3{,}9 \times 10^4 – 4.6 \times 10^6$ CFU/cm^2. The microbes comprising the resident flora are: *Staphylococcus epidermidis*, *S. hominis*, and *Micrococcus*, *Propionibacterium*, *Corynebacterium*, *Dermobacterium*, and *Pitosporum* spp., while in the transitional could be found *S. aureus*, and *Klebsiella pneumoniae*, and *Acinetobacter*, *Enterobacter* and *Candida* spp. The goal of hand hygiene is to eliminate the transient flora with a careful and proper performance of hand washing, using diferent kinds of soap, (normal and antiseptic), and alcohol-based gels.

The main problems found in the practice of hand hygiene is connected with the lack of available sinks and time-consuming performance of hand washing. An easy way to resolve this problem could be the use of alcohol-based hand rubs, because of faster application compared to correct handwashing.

All visitors must follow the same procedures as hospital staff to adequately control the spread of infections.

Visitors and healthcare personnel are equally to blame in transmitting infections. Moreover, multi-drug-resistant infections can leave the hospital and become part of the community flora if steps are not taken to stop this transmission. As of 2014, it is unclear whether or not nail polish or rings affected surgical wound infection rates.

Gloves

In addition to handwashing, gloves play an important role in reducing the risks of transmission of microorganisms. Gloves are worn for three important reasons in hospitals. First, they are worn to provide a protective barrier for personnel, preventing gross contamination of the hands when touching blood, body fluids, secretions, excretions, mucous membranes, and nonintact skin. Second, gloves are worn to reduce the likelihood that microorganisms present on the hands of personnel will be transmitted to patients during invasive or other patient-care procedures that involve touching a patient's mucous membranes and nonintact skin. Third, they are worn to reduce the likelihood that the hands of personnel contaminated with microorganisms from a patient or a fomite can transmit those micro-organisms to another patient.

In this situation, gloves must be changed between patient contacts, and hands should be washed after gloves are removed.

Wearing gloves does not replace the need for handwashing, because gloves may have small, inapparent defects or may be torn during use, and hands can become contaminated during removal of gloves. Failure to change gloves between patient contacts is an infection control hazard.

Surface sanitation

Sanitizing surfaces is an often overlooked, yet crucial, component of breaking the cycle of infection in health care environments. Modern sanitizing methods such as NAV-CO^2 have been effective against gastroenteritis, MRSA, and influenza agents. Use of hydrogen peroxide vapor has been clinically proven to reduce infection rates and risk of acquisition. Hydrogen peroxide is effective against endospore-forming bacteria, such as *Clostridium difficile*, where alcohol has been shown to be ineffective. Ultraviolet cleaning devices may also be used to disinfect the rooms of patients infected with *Clostridium difficile* after discharge.

Antimicrobial surfaces

Microorganisms are known to survive on inanimate «touch» surfaces for extended periods of time. This can be especially troublesome in hospital environments where patients with immunodeficiencies are at enhanced risk for contracting nosocomial infections.

Touch surfaces commonly found in hospital rooms, such as bed rails, call buttons, touch plates, chairs, door handles, light switches, grab rails, intravenous poles, dispensers (alcohol gel, paper towel, soap), dressing trolleys, and counter and table tops are known to be contaminated with *Staphylococcus*, MRSA (one of the most virulent strains of antibiotic-resistant bacteria) and vancomycin-resistant *Enterococcus* (VRE). Objects in closest proximity to patients have the highest levels of MRSA and VRE. This is why touch surfaces in hospital rooms can serve as sources, or reservoirs, for the spread of bacteria from the hands of healthcare workers and visitors to patients.

A number of compounds can decrease the risk of bacteria growing on surfaces including: copper, silver, and germicides.

Aprons

Wearing an apron during patient care reduces the risk of infection. The apron should either be disposable or be used only when caring for a specific patient.

Treatment

Among the categories of bacteria most known to infect patients are the category MRSA (resistant strain of *S. aureus*), member of Gram-positive bacteria and *Acinetobacter* (*A. baumannii*), which is Gram-negative.

While antibiotic drugs to treat diseases caused by Gram-positive MRSA are available, few effective drugs are available for *Acinetobacter*. *Acinetobacter* bacteria are evolving and becoming immune to existing antibiotics, so in many cases, polymyxin-type antibacterials need to be used.

Another growing disease is the drug-resistant, Gram-negative *Klebsiella pneumoniae*. An estimated more than 20% of the *Klebsiella* infections in hospitals are now resistant to virtually all modern antibiotics, and those supergerms are now spreading worldwide.

The bacteria, classified as Gram-negative because of their reaction to the Gram stain test, can cause severe pneumonia and infections of the urinary tract, bloodstream, and other parts of the body. Their cell structures make them more difficult to attack with antibiotics than Gram-positive organisms like MRSA. In some cases, antibiotic resistance is spreading to Gram-negative bacteria that can infect people outside the hospital.

One-third of nosocomial infections are considered preventable. The most common nosocomial infections are of the urinary tract, surgical site and various pneumonias.

Epidemiology

The methods used differ from country to country (definitions used, type of nosocomial infections covered, health units surveyed, inclusion or exclusion of imported infections, etc.), so the international comparisons of nosocomial infection rates should be made with the utmost care.

Medical, or clinical, microbiology is a branch of medicine concerned with the prevention, diagnosis and treatment of infectious diseases. In addition, this field of science studies various clinical applications of microbes for the improvement of health. There are four kinds of microorganisms that cause infectious disease: bacteria, fungi, parasites and viruses and one type of infectious protein called a prion.

A medical microbiologist studies the characteristics of pathogens, their modes of transmission, mechanisms of infection and growth. Using this information a treatment can be devised. Medical microbiologists often serve as consultants for physicians, providing identification of pathogens and suggesting treatment options. Other tasks may include the identification of potential health risks to the community or monitoring the evolution of potentially virulent or resistant strains of microbes, educating the community and assisting in the design of health practices. They may also assist in preventing or controlling epidemics and outbreaks of disease. Not all medical microbiologists study microbial pathology; some study common, non-pathogenic species to determine whether their properties can be used to develop antibiotics or other treatment methods.

Whilst epidemiology is the study of the patterns, causes, and effects of health and disease conditions in populations, medical microbiology primarily focuses on the presence and growth of microbial infections in individuals, their effects on the human body and the methods of treating those infections.

5 Class - Anthrax. Antracoides. *Yersinia*

Bacillus anthracis

Scientific classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: Bacillus
Species: *Bacillus anthracis*

Morphology & Identification

A. MORPHOLOGY

B. anthracis, a large Gram positive, aerobic, spore bearing bacillus, 1-1,5 × 3-10 μm in size, is the only obligate pathogen within the genus *Bacillus*.

B. STAINING

Gram stain - Gram positive;
Polychrome Methylene blue by McFadyean method - blue bacilli with irregular purple capsule;
Gimsa stain - purpule bacilli with red capsule;
Unstained spaces in bacilli - spores.

C. CULTURE AND GROWTH CHARACTERISTICS

1. CULTURE

Colonies of *B. anthracis* are round and have a «cut glass» appearance in transmitted light. Hemolysis is uncommon with *B. anthracis* but common with the saprophytic bacilli. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree.

2. GROWTH CHARACTERISTICS

The saprophytic bacilli utilize simple sources of nitrogen and carbon for energy and growth. The spores are resistant to environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods, and persist for years in dry earth. Animal products contaminated with anthrax spores (eg, hides, bristles, hair, wool, bone) can be sterilized by autoclaving.

D. VIRULANCE FACTORS

B. anthracis clearly owes its pathogenicity to two major determinants of virulence: the formation of a poly-D-glutamyl capsule, which mediates the invasive stage of the infection, and the production of the multicomponent anthrax toxin which mediates the toxigenic stage.

Anthrax toxin is made up of three proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF). PA binds to specific cell receptors, and following proteolytic activation it forms a membrane channel that mediates entry of EF and LF into the cell. EF is an adenylyl cyclase; with PA it forms a toxin known as edema toxin. LF plus PA form lethal toxin, which is a major virulence factor and cause of death in infected animals. When injected into laboratory animals (eg, rats) the lethal toxin can quickly kill the animals. The anthrax toxin genes are on another plasmid.

Pathogenesis, Pathology & Clinical Findings

ANTHRAX

Anthrax is primarily a disease of domesticated and wild animals, particularly herbivorous animals, such as cattle, sheep, horses, mules and goats. Humans become infected incidentally when brought into contact with diseased animals, which includes their flesh, bones, hides, hair and excrement.

1. Cutaneous

Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands, followed in frequency by the face and neck. A pruritic papule develops 1-7 days after entry of the organisms or spores through a scratch. Initially it resembles an insect bite. The papule rapidly changes into a vesicle or small ring of vesicles that coalesce, and a necrotic ulcer develops. The lesions typically are 1-3 cm in diameter and have a characteristic central black eschar. Marked edema occurs. Lymphangitis and lymphadenopathy and systemic signs and symptoms of fever, malaise, and headache may occur. After 7-10 days the eschar is fully developed.

Eventually it dries, loosens, and separates; healing is by granulation and leaves a scar. It may take many weeks for the lesion to heal and the edema to subside. Antibiotic therapy does not appear to change the natural progression of the disease. In as many as 20% of patients, cutaneous anthrax can lead to sepsis, the consequences of systemic infection - including meningitis - and death.

The incubation period in inhalation anthrax may be as long as 6 weeks. The early clinical manifestations are associated with marked hemorrhagic necrosis and edema of the mediastinum. Substernal pain may be prominent, and there is pronounced mediastinal widening visible on x-ray chest films. Hemorrhagic pleural effusions follow involvement of the pleura; cough is secondary to the effects on the trachea. Sepsis occurs, and there may be hematogenous spread to the gastrointestinal tract, causing bowel ulceration, or to the meninges, causing hemorrhagic meningitis. The fatality rate in inhalation anthrax is high in the setting of known exposure; it is higher when the diagnosis is not initially suspected.

Animals acquire anthrax through ingestion of spores and spread of the organisms from the intestinal tract. This is rare in humans, and gastrointestinal anthrax is extremely uncommon. Abdominal pain, vomiting, and bloody diarrhea are clinical signs.

2. Pulmonary

Respiratory infection in humans is relatively rare and initially presents with cold or flu-like symptoms for several days, followed by pneumonia and severe (and often fatal) respiratory collapse. Distinguishing pulmonary anthrax from more common causes of respiratory illness is essential to avoiding delays in diagnosis and thereby improving outcomes. An algorithm for this purpose has been developed.

A lethal infection is reported to result from inhalation of about 10,000-20,000 spores, though this dose varies among host species. As with all diseases, a wide variation in susceptibility is presumed, with evidence indicating some people may die from much lower exposures; little documented evidence is available to verify the exact or average number of spores needed for infection. Inhalational anthrax is also known as woolsorters' or ragpickers' disease. These professions were more susceptible to the disease due to their exposure to infected animal products. Other practices associated with exposure include the slicing up of animal horns for the manufacture of buttons, the handling of hair bristles used for the manufacturing of brushes, and the handling of animal skins.

3. Gastrointestinal

Gastrointestinal (GI) infection in humans is most often caused by consuming anthrax-infected meat and is characterized by serious GI difficulty, vomiting of blood, severe diarrhea, acute inflammation of the intestinal tract, and loss of appetite. Lesions have been found in the intestines and in the mouth and throat. After the bacterium invades the bowel system, it spreads through the bloodstream throughout the body, while also continuing to make toxins. GI infections can be treated, but usually result in fatality rates of 25% to 60%, depending upon how soon treatment commences. This form of anthrax is the rarest form.

Diagnostic laboratory tests

Specimens to be examined are fluid or pus from a local lesion, blood, and sputum. Stained smears from the local lesion or of blood from dead animals often show chains of large Gram-positive rods. Anthrax can be identified in dried smears by immunofluorescence staining techniques.

All *Bacillus* species grow well on 5% sheep blood agar and other routine culture media. Polymyxin-lysozyme-EDTA-thallous acetate can be used to isolate *B. anthracis* from contaminated specimens, and bicarbonate agar is used as an identification method to induce capsule formation. *Bacillus spp.* usually grow within 24 hours of incubation at 35 °C, in ambient air (room temperature) or in 5% CO_2. If bicarbonate agar is used for identification, then the medium must be incubated in 5% CO_2. *B. anthracis* colonies are medium-large, gray, flat, and irregular with swirling projections, often referred to as having a «medusa head» appearance, and are not hemolytic on 5% sheep blood agar. The bacteria are not motile, susceptible to penicillin, and produce a wide zone of lecithinase on egg yolk agar.

Virulent anthrax cultures kill mice or guinea pigs upon intraperitoneal injection. Demonstration of capsule requires growth on bicarbonate-containing medium in 5 - 7% carbon dioxide. Lysis by a specific anthrax γ-bacteriophage may be helpful in identifying the organism.

An enzyme-linked immunoassay (ELISA) has been developed to measure antibodies against edema and lethal toxins, but the test has not been extensively studied. Acute and convalescent sera obtained 4 weeks apart should be tested. A positive result is a fourfold change or a single titer of greater than 1:32.

Immunity

Vaccines composed of killed bacilli and/or capsular antigens produce no significant immunity. A nonencapsulated toxigenic strain has been used effectively in livestock. The Sterne Strain of *B. anthracis* produces sublethal amounts of the toxin that induces formation of protective antibody.

The anthrax vaccine for humans, is a preparation of the protective antigen recovered from the culture filtrate of an avirulent, nonencapsulated strain of *B. anthracis* that produces PA during active growth. Anthrax immunization consists of three subcutaneous injections given two weeks apart followed by three additional subcutaneous injections given at 6, 12, and 18 months. Annual booster injections of the vaccine are required to maintain a protective level of immunity.

The vaccine is indicated for individuals who come in contact in the workplace with imported animal hides, furs, bone, meat, wool, animal hair (especially goat hair) and bristles; and for individuals engaged in diagnostic or investigational activities which may bring them into contact with anthrax spores. Otherwise, it has been indicated for the military during the current era of biological warfare.

The vaccine should only be administered to healthy individuals from 18 to 65 years of age, since investigations to date have been conducted exclusively in that population. It is not known whether the anthrax vaccine can cause fetal harm, and pregnant women should not be vaccinated.

Crystal, et al envision a possible scenario wherein both the passive and active vaccine might be given. Passive vaccines lose their effectiveness fairly rapidly over time, whereas active vaccines do not. The passive vaccine could provide protection that would last a couple of weeks, but that would provide a safety margin for development of more active, long-term immunity stimulated by the active vaccine.

Passive immunotherapy with such adenovirus-based vectors expressing anti-PA antibody, either alone or in combination with antibiotics, may be a rapid, convenient, and highly effective strategy to protect against or treat anthrax in a bioterrorism attack.

Also, in cases of anthrax, coadministration of the passive vaccine with antibiotics may maximize the utility of antibiotic therapy. Coadministration would counter the effects of lethal toxin, and likely prolong the time frame for effective antibiotic treatment and/or reduce the amount of antibiotic therapy required.

Treatment

Many antibiotics are effective against anthrax in humans, but treatment must be started early. Ciprofloxacin is recommended for treatment; penicillin G, along with gentamicin or streptomycin, has previously been used to treat anthrax.

In the setting of potential exposure to *B. anthracis* as an agent of biologic warfare, prophylaxis with ciprofloxacin or doxycycline should be continued for 4 weeks while three doses of vaccine are being given, or for 8 weeks if no vaccine is administered.

Some other Gram-positive bacilli, such as *B. cereus,* are resistant to penicillin by virtue of β-lactamase production. Doxycycline, erythromycin, or ciprofloxacin may be effective alternatives to penicillin.

Epidemiology, Prevention & Control

Soil is contaminated with anthrax spores from the carcasses of dead animals. These spores remain viable for decades. Perhaps spores can germinate in soil at pH 6,5 at proper temperature. Grazing animals infected through injured mucous membranes serve to perpetuate the chain of infection. Contact with infected animals or with their hides, hair, and bristles is the source of infection in humans. Control measures include (1) disposal of animal carcasses by burning or by deep burial in lime pits, (2) decontamination (usually by autoclaving) of animal products, (3) protective clothing and gloves for handling potentially infected materials, and (4) active immunization of domestic animals with live attenuated vaccines. Persons with high occupational risk should be immunized.

Yersinia

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Yersinia
Species: Y. pestis, Y. pseudotuberculosis, Y. enterocolitica

Yersinia pestis

Morphology & Identification

A. MORPHOLOGY

Gram-negative bacillus.

Grows at 35-37 °C, faster at room temperature.

Catalase positive.

Non-motile (37° C and room temperature). Note: *Y. pestis* is the only species of *Yersinia* that is non-motile at room temperature.

Oxidase negative.

Biochemical characteristics: Included in the database of most enteric identification systems, but an identification of *Y. pestis* must be considered presumptive until confirmed by a reference laboratory.

B. CULTURE AND GROWTH CHARACTERISTICS

Grows well on most non-selective standard laboratory media (ie. sheep blood, chocolate and tryptic soy agars). Pinpoint, gray-white, non-hemolytic at 24 hrs, by 48 hrs, colonies resemble typical enteric bacteria. After 48-72 hrs, gray-white to slightly yellow opague raised, irregular «fried egg» appearance; alternatively colonies may have a «hammered copper» shiny surface.

Grows more slowly than other Enterobacteriaceae at 35-37 °C, but faster than most at room temperature.

Grows on MacConkey agar appearing as small non-lactose-fermenting colonies.

In BHI or other enriched broth, undisturbed growth is flocculent, producing structures resembling stalactites and clumps at the side and bottom of tubes.

Hardy Diagnostics CIN Agar is recommended for use in the selective and differential isolation of *Yersinia* and *Aeromonas* species from clinical specimens, environmental samples, and food sources.

A virulent inoculum, derived from infected tissue, produces gray and viscous colonies, but after passage in the laboratory the colonies become irregular and rough. The organism has little biochemical activity, and this is somewhat variable.

C. VIRULENCE FACTORS

All *Yersinia* possess lipopolysaccharides that have endotoxic activity when released. The three pathogenic species produce antigens and toxins that act as virulence factors. They have type III secretion systems that consist of a membrane-spanning complex that allows the bacteria to inject proteins directly into cytoplasm of the host cells. The virulent yersiniae produce V and W antigens, which are encoded by genes on a plasmid of approximately 70 kb. This is essential for virulence; the V and W antigens yield the requirement for calcium for growth at 37 °C. In *Y. pestis* there is a capsular protein (fraction I) that is produced mainly at 37 °C and confers antiphagocytic properties; the gene for this protein is on a plasmid of approximately 110 kb. *Y. pestis* has a 9.6-kb plasmid that yields a plasminogen-activating protease and temperature-dependent coagulase activity (20-28 °C, the temperature of the flea) and fibrinolytic activity (35-37 °C, the temperature of the host).

The three pathogenic yersiniae have a pathogenecity island (PAI) that encodes for an iron-scavenging siderophore.

Among several exotoxins produced, one is lethal for mice in amounts of 1 μg. This homogeneous protein (MW 74,000) produces beta-adrenergic blockade and is cardiotoxic in animals. Its role in human infection is unknown.

Pathogenesis, Pathology & Clinical Findings

Plague is an infection of wild rodents, transmitted from one rodent to another and occasionally from rodents to humans by the bites of fleas. Serious infection often results, which in previous centuries produced pandemics of «black death» with millions of fatalities.

A. BUBONIC PLAGUE

When a flea bites a human and contaminates the wound with regurgitated blood, the plague carrying bacteria are passed into the tissue. *Y. pestis* can reproduce inside cells, so even if phagocytosed, they can still survive. Once in the body, the bacteria can enter the lymphatic system, which drains interstitial fluid. Plague bacteria secrete several toxins, one of which is known to cause dangerous beta-adrenergic blockade.

Y. pestis spreads through the lymphatics of the infected human until it reaches a lymph node, where it stimulates severe haemorrhagic inflammation that causes the lymph nodes to expand. The expansion of lymph nodes is the cause of the characteristic «bubo» associated with the disease.

If the lymph node is overwhelmed, the infection can pass into the bloodstream, causing secondary septicemic plague and if the lungs are seeded, it can cause secondary pneumonic plague.

B. SEPTICEMIC PLAGUE

Lymphatics ultimately drain into the bloodstream, so the plague bacteria may enter the blood and travel to almost any part of the body. In septicemic plague, bacterial endotoxins cause disseminated intravascular coagulation (DIC), causing tiny clots throughout the body and possibly ischaemic necrosis (tissue death due to lack of circulation/perfusion to that tissue) from the clots. DIC results in depletion of the body's clotting resources, so that it can no longer control bleeding. Consequently, there is bleeding into the skin and other organs, which can cause red and/or black patchy rash and hemoptysis/hematemesis (coughing up/ vomiting of blood). There are bumps on the skin that look somewhat like insect bites; these are usually red, and sometimes white in the center. Untreated, septicemic plague is usually fatal. Early treatment with antibiotics reduces the mortality rate to between 4 and 15 percent. People who die from this form of plague often die on the same day symptoms first appear.

C. PNEUMONIC PLAGUE

The pneumonic form of plague arises from infection of the lungs. It causes coughing and sneezing and thereby produces airborne droplets that contain bacterial cells and are likely to infect anyone inhaling them. The incubation period for pneumonic plague is short, usually two to four days, but sometimes just a few hours. The initial signs are indistinguishable from several other respiratory illnesses; they include headache, weakness, and hemoptysis or hematemesis (spitting or vomiting of blood). The course of the disease is rapid; unless diagnosed and treated soon enough, typically within a few hours, death may follow in one to six days; in untreated cases mortality is nearly 100%.

D. PHARYNGEAL PLAGUE

This is an uncommon form of plague that resembles tonsillitis found in cases of close contact of patients with other forms of plague.

E. MENINGEAL PLAGUE

This form of plague occurs when bacteria cross the blood-brain barrier, leading to infectious meningitis.

F. OTHER CLINICAL FORMS

There are a few other rare manifestations of plague, including asymptomatic plague and abortive plague. Cellulocutaneous plague sometimes results in infection of the skin and soft tissue, often around the bite site of a flea.

Diagnostic laboratory tests

Plague should be suspected in febrile patients who have been exposed to rodents in known endemic areas. Rapid recognition and laboratory confirmation of the disease are essential in order to institute lifesaving therapy.

A. SPECIMENS

Blood is taken for culture and aspirates of enlarged lymph nodes for smear and culture. Acute and convalescent sera may be examined for antibody levels. In pneumonia, sputum is cultured; in possible meningitis, cerebrospinal fluid is taken for smear and culture.

B. SMEARS

Material from needle aspiration is examined after staining with Giemsa's stain and with specific immunofluorescent stains. With Wayson's stain, *Y. pestis* may show a striking bipolar appearance. Spinal fluid and sputum smears should also be stained.

C. CULTURE

All materials are cultured on blood agar and MacConkey's agar plates and in infusion broth. Growth on solid media may be slow, but blood cultures are often positive in 24 hours. Cultures can be tentatively identified by biochemical reactions. Definite identification of cultures is best done by immunofluorescence. All cultures are highly infectious and must be handled with extreme caution.

D. SEROLOGY

In patients who have not been previously vaccinated, a convalescent serum antibody titer of 1:16 or greater is presumptive evidence of *Y. pestis* infection. A titer rise in two sequential specimens confirms the serologic diagnosis.

Immunity

A plague vaccine is used for an induction of active specific immunity in a susceptible organism to plague by means of administration an antigenic material (a vaccine) via a variety of routes to people at risk

of contracting any clinical form of plague. This method is known as plague immunization. There is strong evidence for the efficacy of administration of some plague vaccines in preventing or ameliorating the effects of a variety of clinical forms of infection by *Y. pestis.* Plague immunization also encompasses incurring state of passive specific immunity to plague in a susceptible organism after administration of a plague serum or plague immunoglobulin in people with an immediate risk of developing the disease.

Treatment

Unless promptly treated, plague may have a mortality rate of nearly 50%; pneumonic plague, nearly 100%. The drug of choice is streptomycin. Tetracycline is an alternative drug and is sometimes given in combination with streptomycin. Drug resistance has been noted in *Y. pestis.*

Epidemiology, Prevention & Control

Plague is an infection of wild rodents (field mice, gerbils, moles, skunks, and other animals) that occurs in many parts of the world. The chief enzootic areas are India, Southeast Asia (especially Vietnam), Africa, and North and South America. Epizootics with high mortality rates occur intermittently; at such times, the infection can spread to domestic rodents (eg, rats) and other animals (eg, cats), and humans can be infected by flea bites or by contact. The commonest vector of plague is the rat flea *(Xenopsylla cheopis),* but other fleas may also transmit the infection.

Prevention:

Reduce rodent habitat around your home, work place, and recreational areas. Remove brush rock piles, junk, cluttered firewood, and possible rodent food supplies, such as pet and wild animal food. Make your home and outbuildings rodent-proof.

Wear gloves if you are handling or skinning potentially infected animals to prevent contact between your skin and the plague bacteria. Contact your local health department if you have questions about disposal of dead animals.

Use repellent if you think you could be exposed to rodent fleas during activities such as camping, hiking, or working outdoors. Products containing DEET can be applied to the skin as well as clothing and products containing permethrin can be applied to clothing (always follow instructions on the label).

Keep fleas off of your pets by applying flea control products. Animals that roam freely are more likely to come in contact with plague infected animals or fleas and could bring them into homes. If your pet becomes sick, seek care from a veterinarian as soon as possible.

Do not allow dogs or cats that roam free in endemic areas to sleep on your bed.

Yersinia enterocolitica

Classification

Y. enterocolitica is classified according to various distinct biochemical and serologic reactions. Based on biochemical characteristics, 6 biotypes of the bacterium have been described. Biotypes 2, 3, and 4 are most common in humans. The serotyping is based on O and H antigens. More than 60 serotypes of *Y. enterocolitica* have been described. The serotypes most clearly pathogenic to humans include O:3, O:5,27, O:8, O:9, and O:13.

Morphology & Identification

A. MORPHOLOGY

Yersinia it is a group of ovoid- or rod-shaped bacteria of the family Enterobacteriaceae. *Yersinia* are Gram-negative bacteria and are described as facultative anaerobes, which means that they are capable of surviving in both aerobic and anaerobic environments. Though several species are motile below 37 °C, all *Yersinia* organisms are rendered nonmotile at this temperature and above.

B. CULTURE AND GROWTH CHARACTERISTICS

Y. enterocolitica is non-lactose-fermenting, glucose-fermenting, and oxidase-negative facultative anaerobe that is motile at 25 °C and nonmotile at 37°C. Most, but not all, *Y. enterocolitica* isolates reduce nitrates. The presence of bile salts in the medium prevents the organism from fermenting lactose. Colonies of *Y. enterocolitica* do not produce hydrogen sulfide in triple sugar iron medium, but the organism is urease positive.

C. VIRULENCE FACTORS

There are 3 invasive proteins - Ail, YadA and invasin that are produced by *Y. entercolitica*. These proteins promote adherence and invasion of microfold cells, which are found in the follicle-associated epithelium of

Peyer's patches. Microfold cells, or M cells, are a type of antigen-sampling intestinal epithelial cells.

The toxin Yst, included on the genes ystA and ystB, is a membrane-acting virulence factor. It is a heat-stable enterotoxin that is important in causing diarrhea in the host, however, it is only present in virulent strains of *Y. enterocolitica.* It stimulates the cGMP synthesis in the intestinal lining, which leads to an overall effect of fluid loss due to a lack of fluid absorption. The enterotoxin produced by *Y. enterocolitica* is similar to that of *E. coli*. Nevertheless, the enterotoxin only plays a minor role in pathogenesis, because diarrheal symptoms have been observed in the absence of the enterotoxin. The outer membrane antigens are associated with bacterial resistance to opsonization and phagocytosis by neutrophils

One unique property of *Y. enterocolitica* is its inability to chelate iron, which is an essential growth factor for most bacteria and is obtained through the production of chelators known as siderophores. *Y. enterocolitica* does not produce siderophores but can utilize siderophores produced by other bacteria (eg, desferrioxamine E produced by *Streptomyces pilosus*).

Iron overload substantially increases the pathogenicity of *Y. enterocolitica*, perhaps through attenuation of the bactericidal activity of the serum. Researchers observe differences in the iron requirements of different serotypes of the organism; such differences may explain, in part, the varying degrees of virulence among serotypes.

Pathogenesis, Pathology & Clinical Findings

Y. enterocolitica typically causes diarrhea in all manifestations of the infection, with occasional more severe cases containing blood in the stools. The disease may present differently when comparing adult and adolescent cases. Pediatric cases are characterized by fever, abdominal pain, and more commonly present with bloody diarrhea than in adults. The most common presentation of *Y. enterocolitica* in children is enterocolitis. The symptoms include watery and mucoid diarrhea, fever, colicky abdominal pain, and stools containing a high number of white blood cells. Adults characterize their infection symptoms as being diarrhea, localized lower right quadrant abdominal pain, vomiting, and low-grade fever. Often the infection will clear without the aid of antibiotics, but those that do not display more severe side effects which includes red or purple raised lesions, known as erythema nodosum and joint pain commonly in the knees, wrists, or ankles. Erythema nodosum lesions appear 2-20 days after the onset of the infection and usually resolve without medical treatment in about a month. Lesions are more commonly seen in females and present on the patient's trunk and legs. Joint pain may manifest about a month after the onset of symptoms and cease after 1 to 6 months. Septicemia, or bacteria in the blood, can occur in adults with predisposing conditions, such as diabetes mellitus, immune defect, alcoholism, thalassemia, sickle cell disease, or hemochromatosis. Septicemia can also cause metastatic infections including abscesses in the liver, spleen, kidneys, and lungs.

Diagnostic laboratory tests

A. SPECIMENS

Specimens may be stool, blood, or material obtained at surgical exploration. Stained smears are not contributory.

B. CULTURE

The number of *Yersinia* in stool may be small and can be increased by «cold enrichment»: a small amount of feces or a rectal swab is placed in buffered saline, pH 7,6 and kept at 4 °C for 2-4 weeks; many fecal organisms do not survive, but *Y. enterocolitica* will multiply. Subcultures made at intervals on MacConkey agar may yield *Yersinia*.

Isolation of *Yersinia* and pathogenic *Yersinia* (pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*) can be done using Cefsulodin-Irgasan-Novobiocin agar (CIN agar, Difco, Oxoid) and CIN agar containing 0,1% esculin and 0,05% ferric citrate (modified virulent *Y. enterocolitica* agar (mVYE agar)).

CIN agar is useful to expedite the recovery of *Y. enterocolitica* and mVYE agar to differentiate virulent from avirulent isolates. The characteristic deep red center («bull's eye») with a transparent margin and diameter 2-4 mm appearance of *Yersinia* colonies on CIN incubated at 30 °C for 24 hr is important for identification and is due to the presence of mannitol. *Yersinia* ferments the mannitol in the medium, producing an acid pH which gives the colonies their red color and the «bull's eye» appearance.

C. SEROLOGY

In paired serum specimens taken 2 or more weeks apart, a rise in agglutinating antibodies can be shown; however, cross reactions between *Yersinia* and other organisms (*Vibrios, Salmonella, Brucella*) may confuse the results.

Immunity

Host Immune Response

During the later stages of *Yersinia* infection, there are many clinical and histological signs of host response. The host's innate immune response activates macrophages against the infection. Macrophage activation is dependent upon the number of CFUs in the host and the duration of the infection. The immune response will produce multiple TLR ligands, which activate the macrophages. The release of the aforementioned ligands by macrophages will contribute to inflammation of the small intestine. Induction of inflammation is a typical host immune response to enteric pathogens.

Treatment

Treatment of *Y. enterocolitica* infection is usually supportive and directed at maintaining euvolemia. Antibiotics may be used in some cases. Septicemia carries a high mortality rate and should therefore be treated with antibiotics.

Y. enterocolitica is usually susceptible in vitro to aminoglycosides, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole (TMP-SMZ), piperacillin, ciprofloxacin, and third-generation cephalosporins. Isolates are often resistant to penicillin, ampicillin, and first-generation cephalosporins, as the organism often produces beta-lactamase. Clinical failure with cefotaxime has been reported. Resistance to macrolides and fluoroquinolones is also sporadically reported.

Clinically, *Y. enterocolitica* infection responds well to aminoglycosides, TMP-SMZ, ciprofloxacin, and doxycycline.

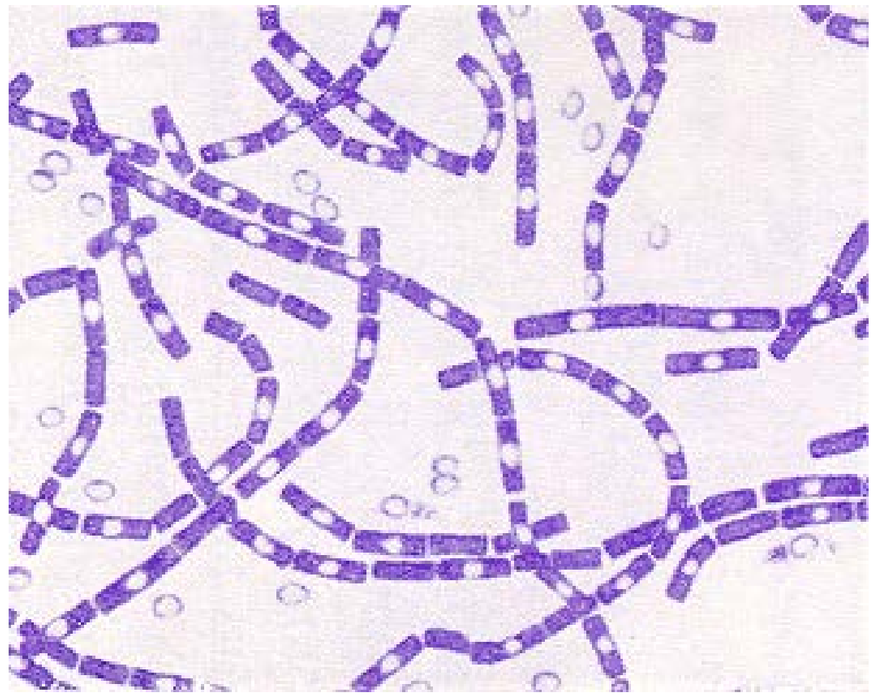
Antimotility agents are contraindicated in the treatment of *Y. enterocolitica* infection because of the increased risk of invasion.

Epidemiology, Prevention & Control

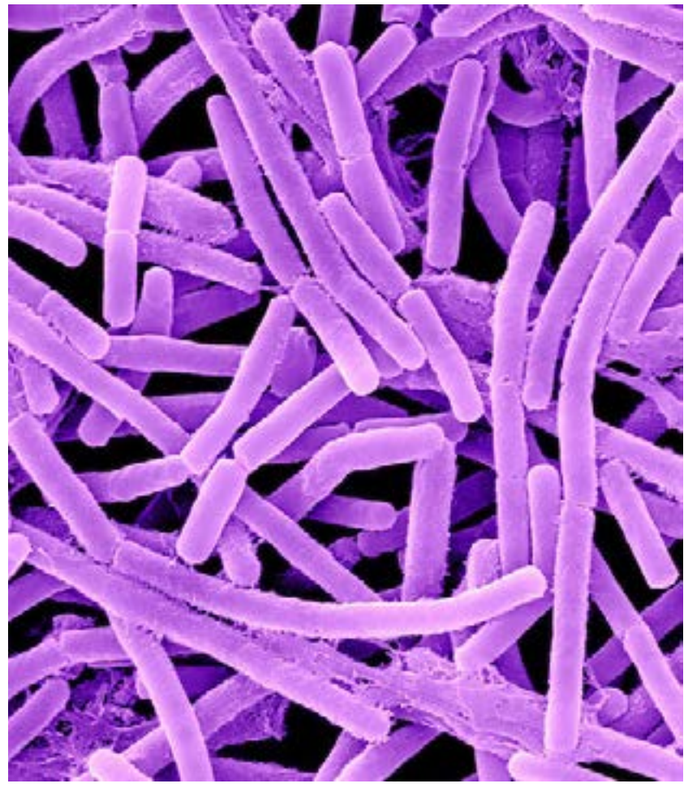
Y. enterocolitica has been isolated from rodents and domestic animals (eg, sheep, cattle, swine, dogs, and cats) and waters contaminated by them. Transmission to humans probably occurs by contamination of food, drink, or fomites.

Prevention of *Y. enterocolitica* infection is a matter of avoidance of certain foods. The most common mode of transmission is via poultry and livestock. Thus, avoid raw or undercooked meats. Milk products must be pasteurized before consumption. If raw meat is handled, clean hands thoroughly to avoid cross-contamination. Beware of cross-contamination in the kitchen by cleaning all surfaces and appliances with soap and hot water. Animal feces may also be contaminated, so dispose of all animal waste properly.

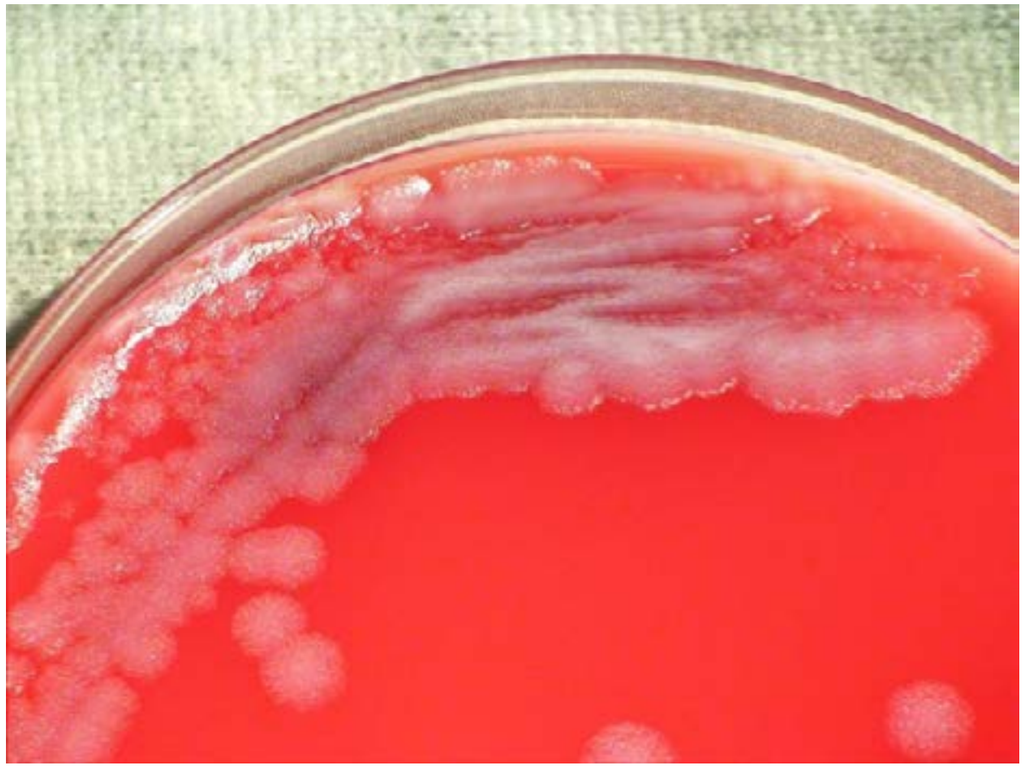
5 Class – Illustrations



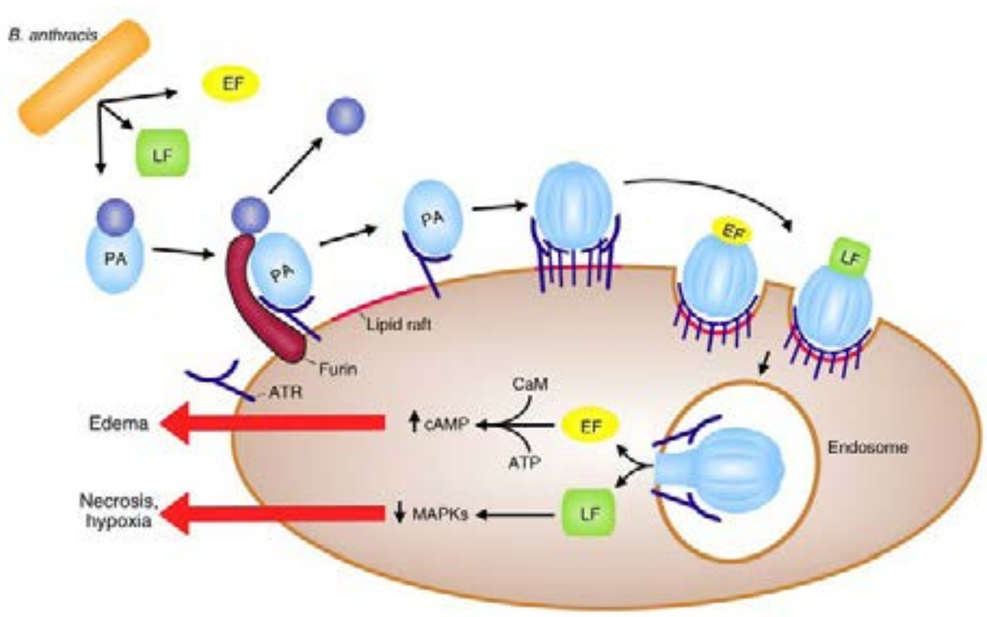
Bacillus anthracis. Gram stain



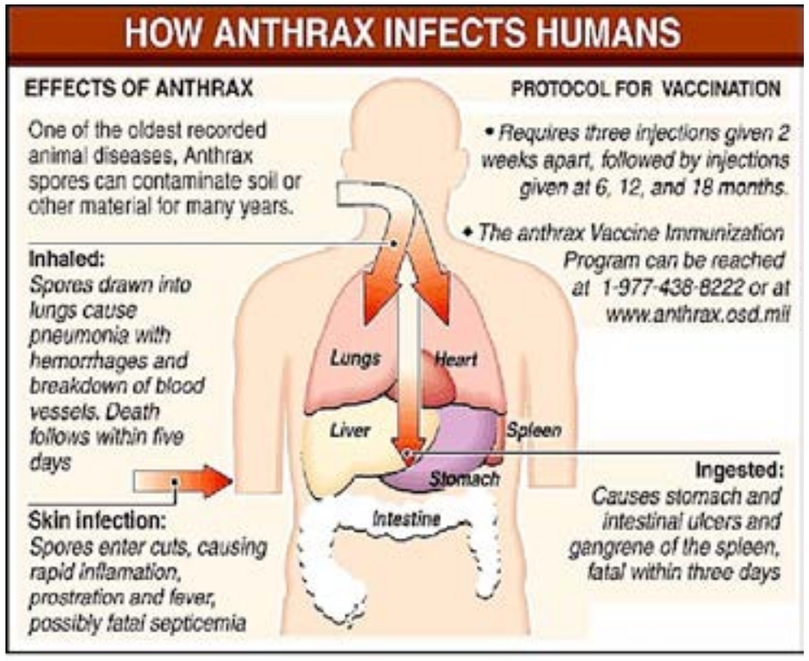
Bacillus anthracis

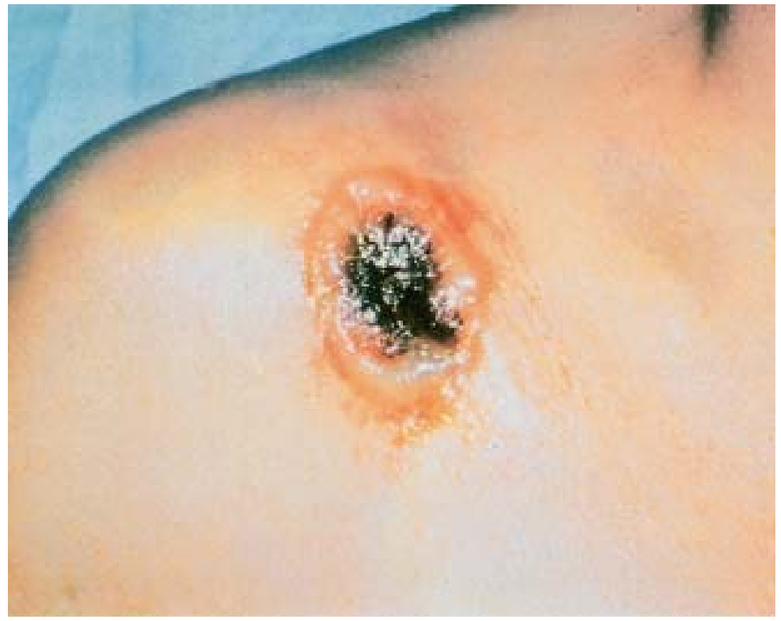


B. anthracis - a closer view



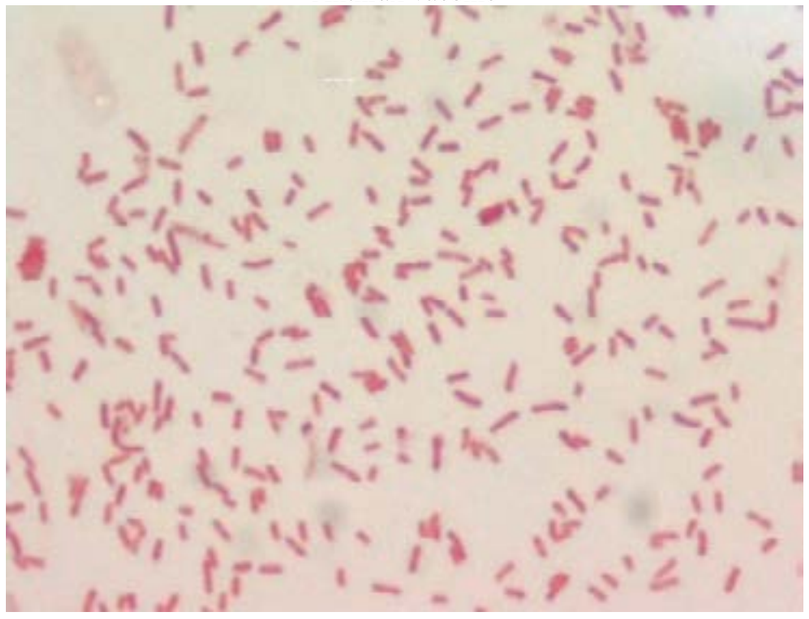
Mechanism of Anthrax Toxins





Anthrax

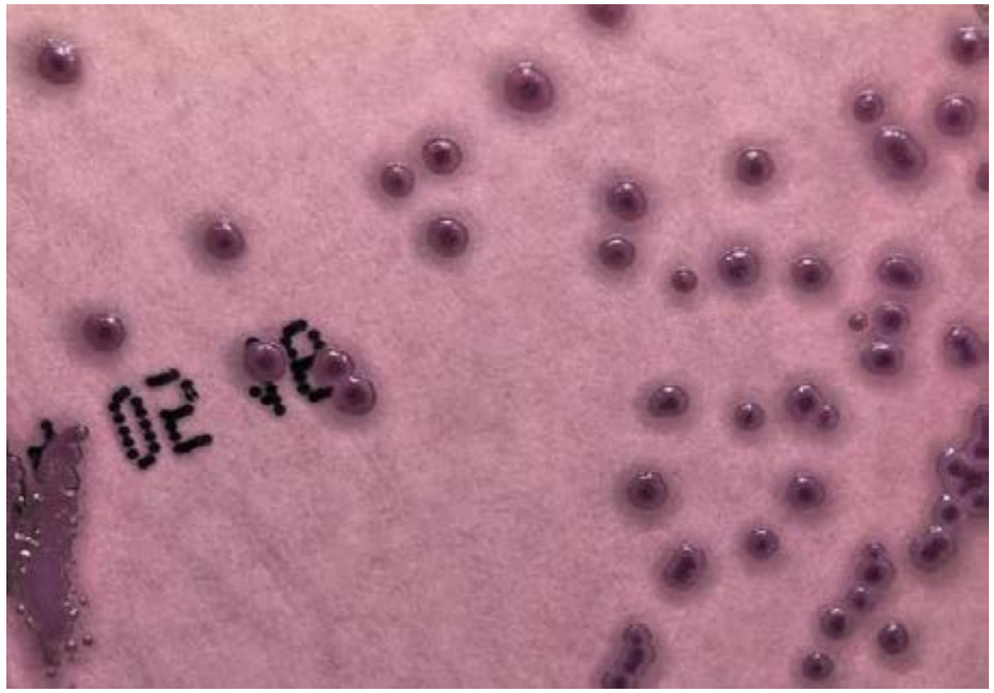
Anthrax vaccine



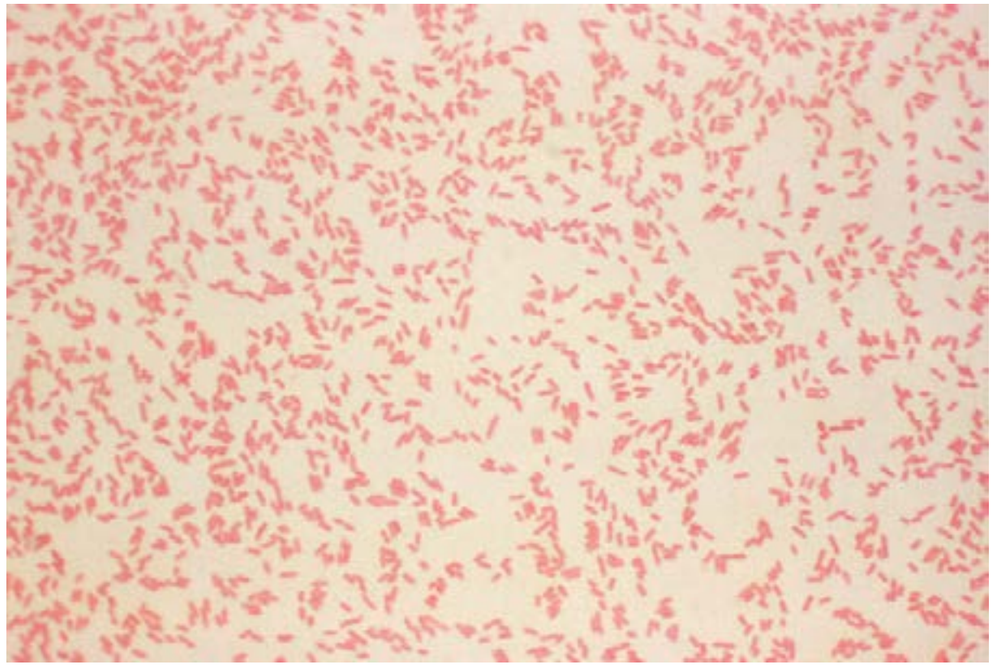
Yersinia pestis. Gram stain

Y. pestis (SEM micrograph)

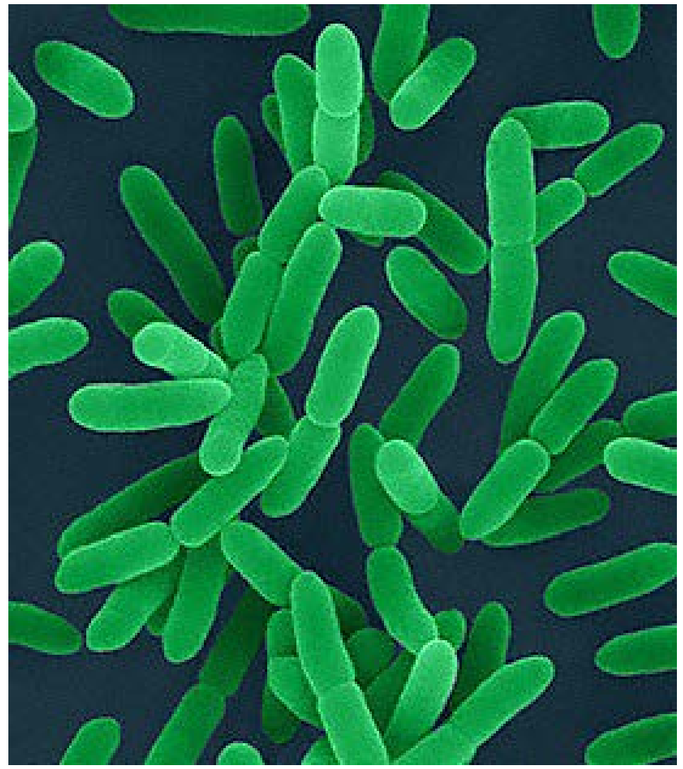
Y. pestis on blood agar



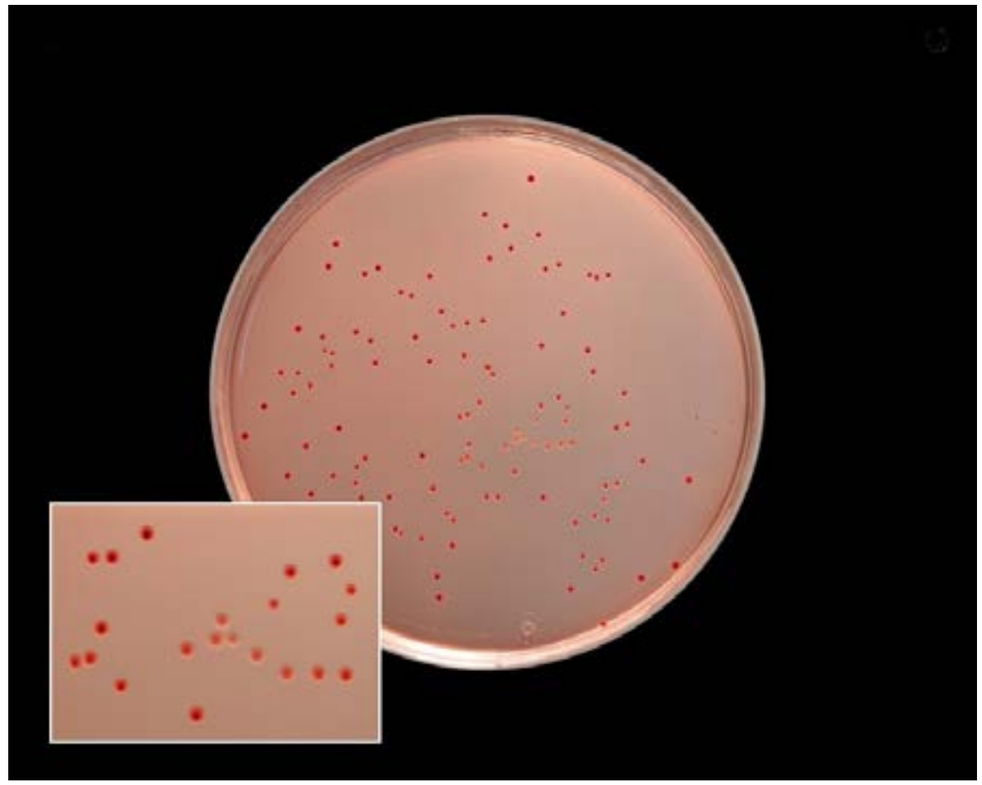
Y. pestis (CIN agar)



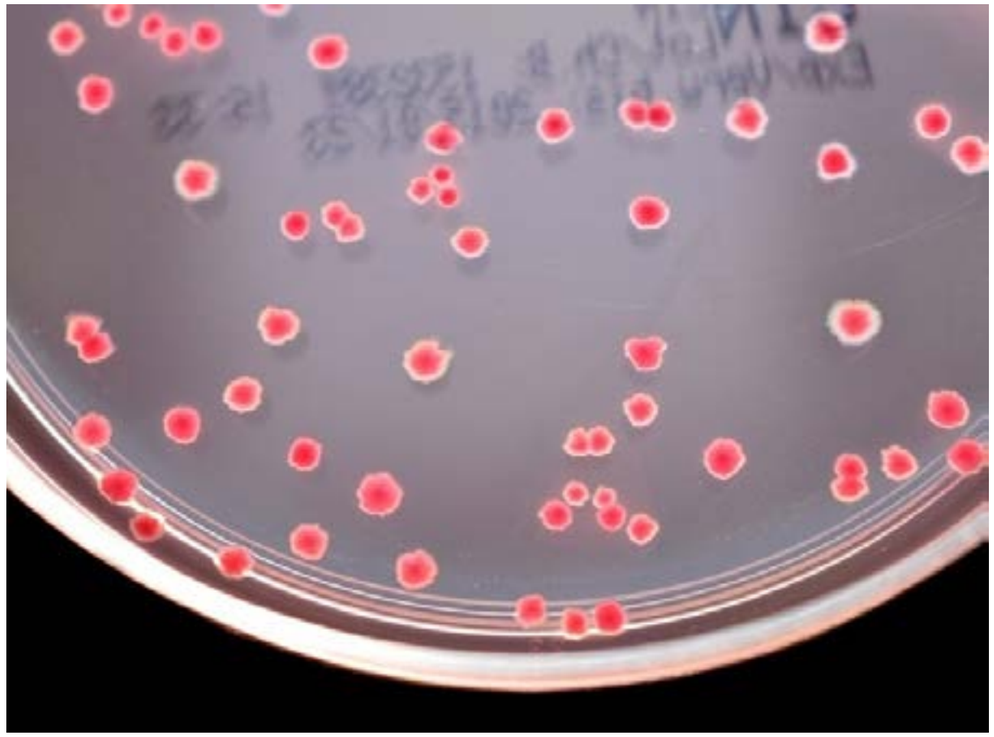
A photomicrograph of *Yersinia enterocolitica* using gram stain technique



Scanning electron microscope image of *Y. enterocolitica*



Y. enterocolitica colonies on MacConkey agar



Y. enterocolitica colonies on CIN agar

6 Class - *Vibrio cholera*. Tularemia. Brucellosis

Vibrio cholerae

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Vibrionales
Family: Vibrionaceae
Genus: Vibrio
Species: Vibrio cholerae

Morphology & Identification

A. MORPHOLOGY

Upon first isolation, is a Gram-negative, comma-shaped, curve rod 2-4 μm long. It is actively motile by means of a polar flagellum. On prolonged cultivation vibrios may become straight rods that resemble the Gram-negative enteric bacteria.

B. CULTURE AND GROWTH CHARACTERISTICS

V. cholerae produces convex, smooth, round colonies that are opaque and granular in transmitted light. *V. cholerae* and most other vibrios grow well at 37 °C on many kinds of media, including defined media containing mineral salts and asparagine as sources of carbon and nitrogen. *V. cholerae* grows well on thiosulfate-citrate-bile-sucrose (TCBS) agar, on which it produces yellow colonies that are readily visible against the dark-green background of the agar. *Vibrio* are oxidase-positive, which differentiates them from enteric Gram-negative bacteria. Characteristically, vibrios grow at a very high pH (8,5-9,5) and are rapidly killed by acid. Cultures containing fermentable carbohydrates therefore quickly become sterile.

In areas where cholera is endemic, direct cultures of stool on selective media such as TCBS, and enrichment cultures in alkaline peptone water are appropriate. However, routine stool cultures on special media such as TCBS generally are not necessary or cost-effective in areas where cholera is rare.

V. cholerae regularly ferments sucrose and mannose but not arabinose. A positive oxidase test is a key step in the preliminary identification of *V. cholerae* and other *Vibrio*. *Vibrio* species are susceptible to the compound O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate), which differentiates them from *Aeromonas* species, which are resistant to O/129. Most *Vibrio* species are halotolerant, and NaCl often stimulates their growth. Some *Vibrio* are halophilic, requiring the presence of NaCl to grow. Another difference between *Vibrio* and *Aeromonas* is that *Vibrio* grow on media containing 6% NaCl, whereas *Aeromonas* does not.

C. VIRULANCE FACTORS

Many *Vibrio* share a single heat-labile flagellar H antigen. Antibodies to the H antigen are probably not involved in the protection of susceptible hosts.

V. cholerae has O lipopolysaccharides that confer serologic specificity. There are at least 139 O antigen groups. *V. cholerae* strains of O group 1 and O group 139 cause classic cholera; occasionally, non-O1/non-O139 *V. cholerae* causes cholera-like disease. Antibodies to the O antigens tend to protect laboratory animals against infections with *V. cholerae.*

The *V. cholerae* serogroup O1 antigen has determinants that make possible further typing; the serotypes are Ogawa, Inaba, and Hikojima. Two biotypes of epidemic *V. cholerae* have been defined, classic and El Tor. The El Tor biotype produces a hemolysin, gives positive results on the Voges-Proskauer test, and is resistant to polymyxin B.

V. cholerae O139 is very similar to *V. cholerae* O1 El Tor biotype. *V. cholerae* O139 does not produce the O1 lipopolysaccharide and does not have all the genes necessary to make this antigen. *V. cholerae* O139 makes a polysaccharide capsule like other non-O1 *V. cholerae* strains, while *V. cholerae* O1 does not make a capsule.

V. cholerae enterotoxin: *V. cholerae* produce a heat-labile enterotoxin with a molecular weight of about 84,000, consisting of subunits A (MW 28,000) and B. Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell. Activation of subunit A1 yields increased levels of intracellular cAMP and results in prolonged hypersecretion of water and electrolytes. There is increased

sodium-dependent chloride secretion, and absorption of sodium and chloride is inhibited. Diarrhea occurs - as much as 20-30 L/d - with resulting dehydration, shock, acidosis, and death. The genes for *V. cholerae* enterotoxin are on the bacterial chromosome. Cholera enterotoxin is antigenically related to LT of *E. coli* and can stimulate the production of neutralizing antibodies.

Pathogenesis, Pathology & Clinical Findings

Under natural conditions, *V. cholerae* is pathogenic only for humans. Cholera is not an invasive infection. The organisms do not reach the bloodstream but remain within the intestinal tract. Virulent *V. cholerae* organisms attach to the microvilli of the brush border of epithelial cells. There they multiply and liberate cholera toxin and perhaps mucinases and endotoxin**.**

About 60% of infections with classic *V. cholerae* are asymptomatic, as are about 75% of infections with the El Tor biotype. The incubation period is 1-4 days for persons who develop symptoms, depending largely upon the size of the inoculum ingested. There is a sudden onset of nausea and vomiting and profuse diarrhea with abdominal cramps. Stools, which resemble «ricew ater», contain mucus, epithelial cells, and large numbers of *Vibrio*. There is rapid loss of fluid and electrolytes, which leads to profound dehydration, circulatory collapse, and anuria. The mortality rate without treatment is between 25% and 50%. The diagnosis of a fullblown case of cholera presents no problem in the presence of an epidemic. However, sporadic or mild cases are not readily differentiated from other diarrheal diseases. The El Tor biotype tends to cause milder disease than the classic biotype.

Diagnostic laboratory tests

A. SPECIMENS

Specimens for culture consist of mucus flecks from stools.

B. SMEARS

The microscopic appearance of smears made from stool samples is not distinctive. Dark-field or phase contrast microscopy may show the rapidly motile *Vibrio*.

C. CULTURE

Fresh stool can be directly plated on a non-selective medium like MacConkey agar and a selective medium such as Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar.

However, in case of rectal swab, the swab stick should be dipped in 10 ml of alkaline peptone water (APW) and incubate for 6-8 hours. After incubation, inoculation on solid media should be done only from the pellicle formed at the upper layer of the broth.

After 18-24 hrs of incubation at 37 °C colonies on MacConkey agar appear pale, non lactose fermenting, 1-2 mm in diameter, flat with a serrated margin .

Gulf Coast and the Australia clones of *V. cholerae* O1, which are strongly hemolytic when assayed by either the plate or tube hemolysis assay. For this reason, hemolysis continues to be a useful phenotypic characteristic for differentiating the Gulf Coast and Australia clones of *V. cholerae* O1 from El Tor strains from the rest of the world, including Latin America.

TCBS is the medium of choice for the isolation of *V. cholerae* and is widely used worldwide. TCBS agar is green when prepared. Sodium citrate, sodium thiosulfate, sodium cholate, and Oxbile are selective agents, providing an alkaline pH to inhibit Gram-positive organisms and suppress coliforms. An increased pH is used to enhance growth of *V. cholerae*, because this organism is sensitive to acid environments. Overnight growth (18 to 24 hours) of *V. cholerae* will produce large (2 to 4 mm in diameter), slightly flattened, yellow colonies with opaque centers and translucent peripheries. The yellow color is caused by the fermentation of sucrose in the medium. Sucrose nonfermenting organisms, such as *V. parahaemolyticus*, produce green to blue-green colonies.

D. BIOCHEMICAL REACTIONS

Catalase/Oxidase - both positive
Citrate- positive or negative
Indole- Positive
Urea hydrolysis-Negative
Motility- Motile
Methyl Red-Positive
Vogues Proskauer- Negative
TSI- Alkali/Acid (R/Y) or Acid /Acid (Y/Y) without gas and H_2S.

The string test may be performed on a glass microscope slide or plastic petri dish by suspending 18- to 24-hour growth from HIA or other noninhibitory medium in a drop of 0,5% aqueous solution of sodium deoxycholate. If the result is positive, the bacterial cells will be lysed by the sodium deoxycholate, the suspension will lose turbidity, and DNA will be released from the lysed cells causing the mixture to become viscous. A mucoid «string» is formed when an inoculating loop is drawn slowly away from the suspension. Most *Vibrio* are positive, whereas *Aeromona* strains are usually negative.

E. SPECIFIC TESTS

V. cholerae organisms are further identified by slide agglutination tests using anti-O group 1 or group 139 antisera and by biochemical reaction patterns.

Immunity

Gastric acid provides some protection against *V. cholerae*. An attack of cholera is followed by immunity to reinfection, but the duration and degree of immunity are not known. In experimental animals, specific IgA antibodies occur in the lumen of the intestine. Similar antibodies in serum develop after infection but last only a few months. Vibriocidal antibodies in serum (titer $\geq$ 1:20) have been associated with protection against colonization and disease. The presence of antitoxin antibodies has not been associated with protection.

Treatment

The most important part of therapy consists of water and electrolyte replacement to correct the severe dehydration and salt depletion. Many antimicrobial agents are effective against *V. cholerae*. Oral tetracycline tends to reduce stool output in cholera and shortens the period of excretion of *Vibrio*. In some endemic areas, tetracycline resistance of *V. cholerae* has emerged; the genes are carried by transmissible plasmids.

Epidemiology, Prevention & Control

Cholera is endemic in India and Southeast Asia. From these centers, it is carried along shipping lanes, trade routes, and pilgrim migration routes. The disease is spread by contact involving individuals with mild or early illness and by water, food, and flies. In many instances, only 1 - 5% of exposed susceptible persons develop disease. The carrier state seldom exceeds 3-4 weeks, and the importance of carriers in transmission is unclear. *Vibrio* survive in water for up to 3 weeks.

V. cholerae lives in aquatic environments. And such environments are the *Vibrio* natural reservoir. *V. cholerae* lives attached to algae, copepods, and crustacean shells. It can survive for years and grow, but when conditions are not suitable for growth it can become dormant.

Control rests on education and on improvement of sanitation, particularly of food and water. Patients should be isolated, their excreta disinfected, and contacts followed up. Chemoprophylaxis with antimicrobial drugs may have a place. Repeated injection of a vaccine containing either lipopolysaccharides extracted from *Vibrio* or dense *Vibrio* suspensions can confer limited protection to heavily exposed persons (eg, family contacts) but is not effective as an epidemic control measure.

Francisella tularensis

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Thiotrichales
Family: Francisellaceae
Genus: Francisella
Species: F. tularensis

Four subspecies (biovars) of *F. tularensis* have been classified:

The subspecies *F. t. tularensis* (or type A) is found predominantly in North America, is the most virulent of the four known subspecies, and is associated with lethal pulmonary infections. This includes the primary type A laboratory strain, SCHUS4.

Subspecies *F. t. holarctica* (also known as biovar *F. t. palearctica* or type B) is found predominantly in Europe and Asia, but rarely leads to fatal disease. An attenuated livevaccine strain of subspecies *F. t. holarctica* has been described, though it is not yet fully licensed by the FDA as a vaccine. This subspecies lacks the citrulline ureidase activity and ability to produce acid from glucose of biovar *F. t. palearctica*.

Subspecies *F. t. novicida* (previously classified as *F. novicida*) was characterized as a relatively

nonvirulent strain; only two tularemia cases in North America have been attributed to *F. t. novicida* and these were only in severely immunocompromised individuals.

Subspecies *F. t. mediasiatica*, is found primarily in central Asia; little is currently known about this subspecies or its ability to infect humans.

Morphology & Identification

A. MORPHOLOGY

F. tularensis is a pathogenic species of Gram-negative, rod-shaped coccobacillus, an aerobe bacterium. It is non-spore forming, non-motile.

B. CULTURE AND GROWTH CHARACTERISTICS

Growth requires enriched media containing cysteine. In the past, glucose-cysteine blood agar was preferred, but *F. tularensis* will grow on commercially available hemin containing media such as chocolate agar, modified Thayer-Martin agar, and buffered charcoal yeast extract (BCYE) agar used to grow *Legionella* species. Media should be incubated in CO_2 at 35-37 °C for 2-5 days.

C. VIRULANCE FACTORS

The virulence mechanisms for *F. tularensis* have not been well characterized. Like other intracellular bacteria that break out of phagosomal compartments to replicate in the cytosol, *F. tularensis* strains produce different hemolytic agents, which may facilitate degradation of the phagosome. A hemolysin activity, named NlyA, with immunological reactivity to *E. coli* anti-HlyA antibody, was identified in biovar *F. t. novicida*. Acid phosphatase AcpA has been found in other bacteria to act as a hemolysin, whereas in *Francisella*, its role as a virulence factor is under vigorous debate.

While *F. tularensis* does not contain virulence secretion systems typical of some better-characterized pathogenic bacteria, it does contain a number of ATP-binding cassette (ABC) proteins that may be linked to the secretion of virulence factors. *F. tularensis* uses type IV pili to bind to the exterior of a host cell and thus become phagocytosed. Mutant strains lacking pili show severely attenuated pathogenicity.

The expression of a 23-kD protein known as IglC is required for *F. tularensis* phagosomal breakout and intracellular replication; in its absence, mutant *F. tularensis* cells die and are degraded by the macrophage. This protein is located in a putative pathogenicity island regulated by the transcription factor MglA.

Pathogenesis, Pathology & Clinical Findings

Tularemia

Ulceroglandular tularemia - includes painful regional lymphadenopathy and an ulcerated skin lesion.

Glandular tularemia - tender lymphadenopathy without evidence of local cutaneous lesions.

Oculoglandular tularemia - unilateral conjunctivitis, corneal ulceration, lymphadenopathy, photophobia, lacrimation, lid edema, vision loss (rare).

Oropharyngeal tularemia - stomatitis and exudative pharyngitis or tonsillitis; abdominal pain, nausea, and vomiting; cervical lymphadenopathy; diarrhea; gastrointestinal bleeding.

Pneumonic tularemia - dry cough, dyspnea, and pleuritic-type chest pain.

Typhoidal tularemia - fever, chills, myalgias, malaise, and weight loss.

F. tularensis is highly infectious: penetration of the skin or mucous membranes or inhalation of 50 organisms can result in infection. Most commonly, organisms enter through skin abrasions. In 2-6 days, an inflammatory, ulcerating papule develops. Regional lymph nodes enlarge and may become necrotic, sometimes draining for weeks (ulceroglandular tularemia). Inhalation of an infective aerosol results in peribronchial inflammation and localized pneumonitis (pneumonic tularemia). Oculoglandular tularemia can develop when an infected finger or droplet touches the conjunctiva. Yellowish granulomatous lesions on the lids may be accompanied by preauricular adenopathy. The other forms of the disease are glandular tularemia (lymphadenopathy but no ulcers), oropharyngeal tularemia, and typhoidal tularemia (septicemia). In all cases there is fever, malaise, headache, and pain in the involved region and regional lymph nodes.

Because of the highly infectious nature of *F. tularensis,* this organism is a potential agent of bioterrorism and is currently classified on the select agent list as a category A agent. Laboratories that recover a suspected *F. tularensis* should notify public health officials and should send the isolate to a reference laboratory capable of performing definitive identification.

Diagnostic laboratory tests

A. SEROLOGY

The diagnosis of tularemia is usually based on serology results. Tests vary from antibody detection (using latex agglutination or enzyme-linked immunosorbent assay (ELISA) testing) to the examination of a range of polymerase chain reaction (PCR) assay products.

An agglutination titer greater than 1:160 is considered presumptively positive, and treatment may be started if this result is obtained. A second titer, demonstrating a 4-fold increase after 2 weeks, confirms the diagnosis.

B. INDIRECT FLUORESCENT ANTIBODY TESTING

Indirect fluorescent antibody testing of suppurative material is rapid and specific. Microscopic examination of tissue and smear specimens is possible using fluorescently labeled antibodies at reference laboratories, possibly providing rapid confirmation of disease.

C. HISTOLOGIC STUDIES

Early tularemic lesions may demonstrate areas of focal necrosis surrounded by neutrophils and macrophages. Later, the necrotic areas become surrounded by epithelioid cells and lymphocytes. Caseating granulomata with or without multinucleated giant cells develops in some lesions.

D. BACTERIAL CULTURING

Although *F. tularensis* has been cultured from sputum, pleural fluid, wounds, blood, lymph node biopsy samples, and gastric washings, the yield is extremely low and culturing poses a danger to laboratory personnel.

Immunity

IgM and Ig G agglutinating antibodies persist indefinitely, probably not protective.

Cellular immunity major factor in acquired resistance.

Treatment

Streptomycin or gentamicin therapy for 10 days almost always produces rapid improvement. Tetracycline may be equally effective, but relapses occur more frequently. *F. tularensis* is resistant to all β-lactam antibiotics.

Epidemiology, Prevention & Control

The disease is endemic in North America, and parts of Europe and Asia. The most common mode of transmission is via arthropod vectors. Ticks involved include Amblyomma, Dermacentor, Haemaphysalis, and Ixodes. Rodents, rabbits, and hares often serve as reservoir hosts, but waterborne infection accounts for 5 to 10% of all tularemia in the US. Tularemia can also be transmitted by biting flies, particularly the deer fly *Chrysops discalis*. Individual flies can remain infective for 14 days and ticks for over two years. Tularemia may also be spread by direct contact with contaminated animals or material, by ingestion of poorly cooked flesh of infected animals or contaminated water, or by inhalation.

Humans acquire tularemia from handling infected rabbits or muskrats or from bites by an infected tick or deerfly. Less often, the source is contaminated water or food or contact with a dog or cat that has caught an infected wild animal. Avoidance is the key to prevention. The infection in wild animals cannot be controlled.

Persons at exceedingly high risk - particularly research laboratory personnel - may be immunized by the administration of a live attenuated strain of *F. tularensis.* The vaccine is administered by multiple punctures through the skin and provides only partial immunity.

Brucella

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rhizobiales
Family: Brucellaceae
Genus: Brucella
Species: *B. suis, B. abortus, B. melitensis*

Morphology & Identification

A. MORPHOLOGY

The appearance in young cultures varies from cocci to rods 1,2 μm in length, with short coccobacillary forms predominating. They are Gram-negative but often stain irregularly, and they are aerobic, nonmotile,

and non-spore-forming.

B. CULTURE AND GROWTH CHARACTERISTICS

1. CULTURE

Small, convex, smooth colonies appear on enriched media in 2-5 days.

2. GROWTH CHARACTERISTICS

Brucella are adapted to an intracellular habitat, and their nutritional requirements are complex. Some strains have been cultivated on defined media containing amino acids, vitamins, salts, and glucose. Fresh specimens from animal or human sources are usually inoculated on trypticase- soy agar or blood culture media. *B. abortus* requires 5-10% CO_2 for growth, whereas the other three species grow in air.

Brucella utilize carbohydrates but produce neither acid nor gas in amounts sufficient for classification. Catalase and oxidase are produced by the four species that infect humans. Hydrogen sulfide is produced by many strains, and nitrates are reduced to nitrites. *Brucella* are moderately sensitive to heat and acidity. They are killed in milk by pasteurization.

C. VIRULANCE FACTORS

The typical virulent organism forms a smooth, transparent colony; upon culture, it tends to change to the rough form, which is avirulent. The serum of susceptible animals contains a globulin and a lipoprotein that suppress growth of nonsmooth, avirulent types and favor the growth of virulent types. Resistant animal species lack these factors, so that rapid mutation to avirulence can occur. D-Alanine has a similar effect *in vitro*.

Antigenic Structure

Differentiation among *Brucella* species or biovars is made possible by their characteristic sensitivity to dyes and their production of H_2S. Few laboratories have maintained the procedures for these tests, and the *Brucella* are seldom placed into the traditional species. Because *Brucella* are hazardous in the laboratory, tests to classify them should be performed only in reference public health laboratories using biologic safety cabinets.

Pathogenesis, Pathology & Clinical Findings

Although each species of brucella has a preferred host, all can infect a wide range of animals, including humans. The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurized goats' milk is a particularly common vehicle. The organisms progress from the portal of entry, via lymphatic channels and regional lymph nodes, to the thoracic duct and the bloodstream, which distributes them to the parenchymatous organs. Granulomatous nodules that may develop into abscesses form in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system. In such lesions, the *Brucella* are principally intracellular. Osteomyelitis, meningitis, or cholecystitis also occasionally occurs. The main histologic re tion in brucellosis consists of proliferation of mononuclear cells, exudation of fibrin, coagulation necrosis, and fibrosis. The granulomas consist of epithelioid and giant cells, with central necrosis and peripheral fibrosis. The *Brucella* that infect humans have apparent differences in pathogenicity. *B. abortus* usually causes mild disease without suppurative complications; noncaseating granulomas of the reticuloendothelial system are found. *B. canis* also causes mild disease. *B. suis* infection tends to be chronic with suppurative lesions; caseating granulomas may be present. *B. melitensis* infection is more acute and severe.

Persons with active brucellosis react more markedly (fever, myalgia) than normal persons to injected *Brucella* endotoxin. Sensitivity to endotoxin thus may play a role in pathogenesis.

Placentas and fetal membranes of cattle, swine, sheep, and goats contain erythritol, a growth factor for *Brucella*. The proliferation of organisms in pregnant animals leads to placentitis and abortion in these species. There is no erythritol in human placentas, and abortion is not part of brucella infection of humans.

The incubation period is 1-6 weeks. The onset is insidious, with malaise, fever, weakness, aches, and sweats. The fever usually rises in the afternoon; its fall during the night is accompanied by drenching sweat. There may be gastrointestinal and nervous symptoms. Lymph nodes enlarge, and the spleen becomes palpable. Hepatitis may be accompanied by jaundice. Deep pain and disturbances of motion, particularly in vertebral bodies, suggest osteomyelitis. These symptoms of generalized *Brucella* infection generally subside in weeks or months, although localized lesions and symptoms may continue.

Following the initial infection, a chronic stage may develop, characterized by weakness, aches and pains, low-grade fever, nervousness, and other nonspecific manifestations compatible with psychoneurotic symptoms. *Brucella* cannot be isolated from the patient at this stage, but the agglutinin titer may be high. The

diagnosis of «chronic brucellosis» is difficult to establish with certainty unless local lesions are present.

Diagnostic laboratory tests

A. SPECIMENS

Blood should be taken for culture, biopsy material for culture (lymph nodes, bone, etc), and serum for serologic tests.

B. CULTURE

Brucella agar was specifically designed to culture *Brucella* species. The medium is highly enriched and - in reduced form - is used primarily in cultures for anaerobic bacteria. In oxygenated form, the medium grows *Brucella* species very well. However, infection with *Brucella* species is often not suspected when cultures of a patient's specimens are set up, and brucella agar incubated aerobically is seldom used. The *Brucella* species will grow on commonly used media, including trypticase soy medium with or without 5% sheep blood, brain heart infusion medium, and chocolate agar. Blood culture media readily grow *Brucella* species. Liquid medium used to culture *Mycobacterium tuberculosis* also supports the growth of at least some strains. All cultures should be incubated in 8-10% CO_2 at 35-37 °C and should be observed for 3 weeks before being discarded as negative; liquid media cultures should be blindly subcultured during this time. Bone marrow and blood are the specimens from which *Brucella* are most often isolated. The method of choice for bone marrow is to use pediatric Isolator tubes, which do not require centrifugation, with inoculation of the entire contents of the tube onto solid media. Media used in semiautomated and automated blood culture systems readily grow *Brucella*, usually within 1 week; however, holding the cultures for 3 weeks is recommended. Negative cultures for *Brucella* do not exclude the disease because *Brucella* can be cultivated from patients only during the acute phase of the illness or during recurrence of activity.

After a few days of incubation on agar media, the *Brucella* form colonies in the primary streak that are < 1 mm in diameter. They are nonhemolytic. The observation of tiny Gram-negative coccobacilli that are catalase-positive and oxidase-positive suggests *Brucella* species. All further work on such a culture should be done in a biologic safety cabinet. A Christensen urea slant should be inoculated and observed frequently. A positive urease test is characteristic of *Brucella* species. *B. suis* and some strains of *B. melitensis* can yield a positive test less than 5 minutes after inoculating the slant; other strains will take a few hours to 24 hours. Bacteria that meet these criteria should be quickly submitted to a reference public health laboratory for presumptive identification. *Brucella* species are category B select agents. Molecular methods have been developed to rapidly differentiate among the various biovars.

C. SEROLOGY

IgM antibody levels rise during the first week of acute illness, peak at 3 months, and may persist during chronic disease. Even with appropriate antibiotic therapy, high IgM levels may persist for up to 2 years in a small percentage of patients. Ig G antibody levels rise about 3 weeks after onset of acute disease, peak at 6-8 weeks, and remain high during chronic disease. IgA levels parallel the Ig G levels. The usual serologic tests may fail to detect infection with *B. canis.*

1. Agglutination test

To be reliable, serum agglutination tests must be performed with standardized heatkilled, phenolized, smooth *Brucella* antigens. IgG agglutinin titers above 1:80 indicate active infection. Individuals injected with cholera vaccine may develop agglutination titers to *Brucella*. If the serum agglutination test is negative in patients with strong clinical evidence of *Brucella* infection, tests must be made for the presence of «blocking» antibodies. These can be detected by adding antihuman globulin to the antigen-serum mixture. Brucellosis agglutinins are cross-reactive with tularemia agglutinins, and tests for both diseases should be done on positive sera; usually, the titer for one disease will be much higher than that for the other.

2. Blocking antibodies

These are IgA antibodies that interfere with agglutination by IgG and IgM and cause a serologic test to be negative in low serum dilutions (prozone) although positive in higher dilutions. These antibodies appear during the subacute stage of infection, tend to persist for many years independently of activity of infection, and are detected by the Coombs antiglobulin method.

3. ELISA

IgG, IgA, and IgM antibodies may be detected using ELISA assays, which use cytoplasmic proteins as antigens. These assays tend to be more sensitive and specific than the agglutination test.

Immunity

An antibody response occurs with infection, and it is probable that some resistance to subsequent attacks is produced. Immunogenic fractions from *Brucella* cell walls have a high phospholipid content; lysine predominates among eight amino acids; and there is no heptose (thus distinguishing the fractions from endotoxin).

Treatment

Brucella may be susceptible to tetracyclines or ampicillin. Symptomatic relief may occur within a few days after treatment with these drugs is begun. However, because of their intracellular location, the organisms are not readily eradicated completely from the host. For best results, treatment must be prolonged. Combined treatment with a tetracycline (such as doxycycline) and either streptomycin for 2-3 weeks or rifampin for 6 weeks is recommended.

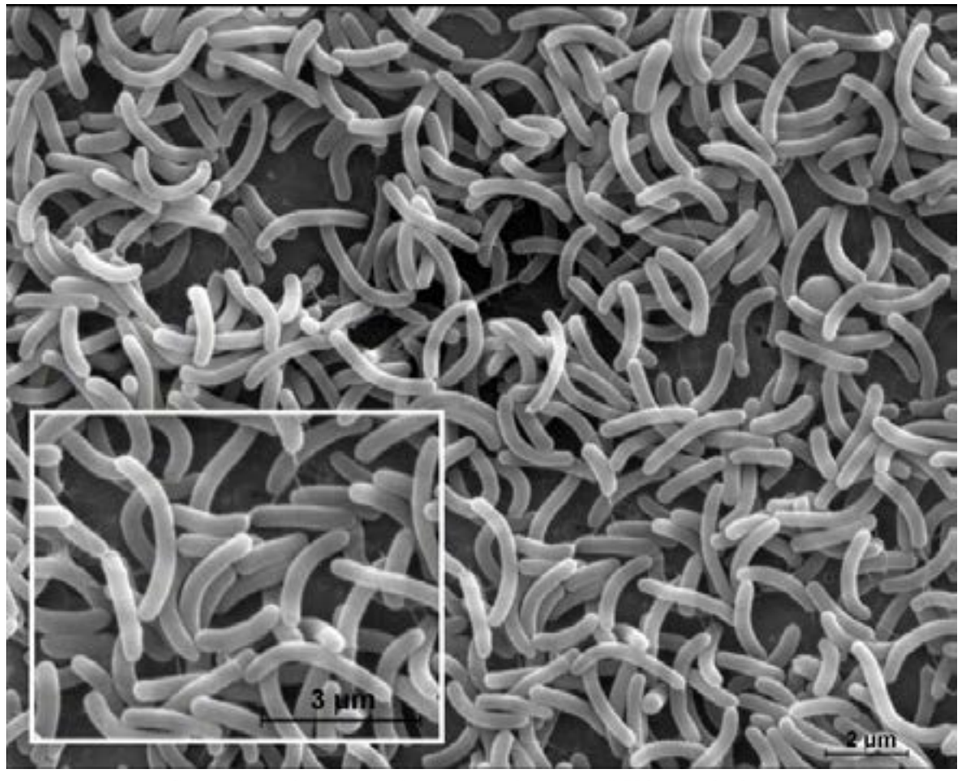
Epidemiology, Prevention & Control

Brucella are animal pathogens transmitted to humans by accidental contact with infected animal feces, urine, milk, and tissues. The common sources of infection for humans are unpasteurized milk, milk products, and cheese, and occupational contact (eg, farmers, veterinarians, slaughterhouse workers) with infected animals.

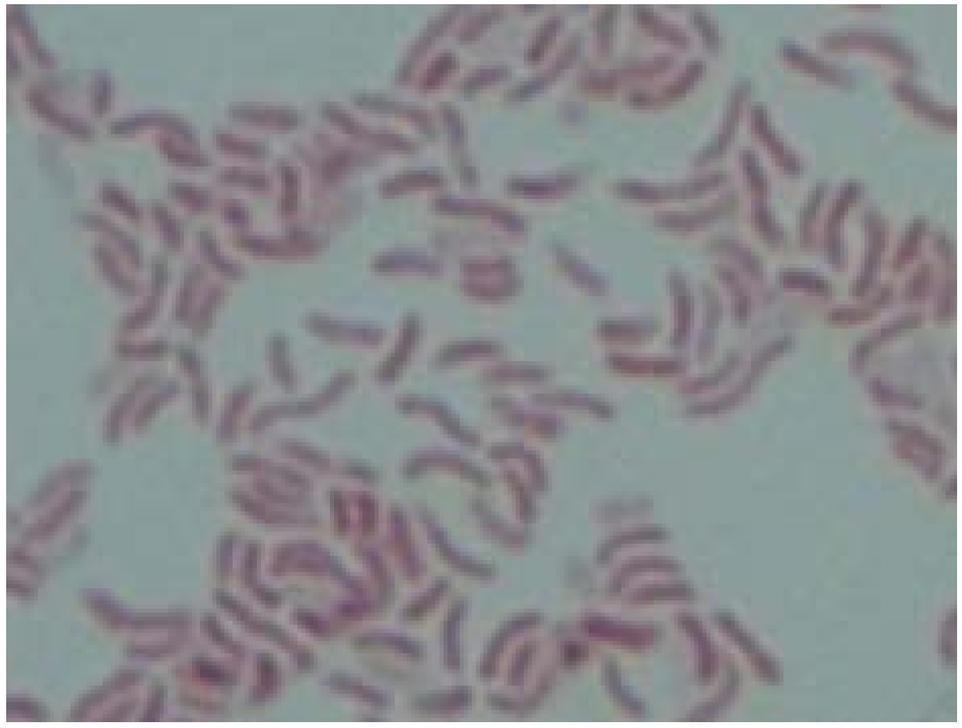
Cheese made from unpasteurized goat's milk is a particularly common vehicle for transmission of brucellosis. Occasionally the airborne route may be important. Because of occupational contact, *Brucella* infection is much more frequent in men. The majority of infections remain asymptomatic (latent). Infection rates vary greatly with different animals and in different countries. Outside the United States, infection is more prevalent. Eradication of brucellosis in cattle can be attempted by test and slaughter, active immunization of heifers with avirulent live strain 19, or combined testing, segregation, and immunization. Cattle are examined by means of agglutination tests.

Active immunization of humans against brucella infection is experimental. Control rests on limitation of spread and possible eradication of animal infection, pasteurization of milk and milk products, and reduction of occupational hazards wherever possible.

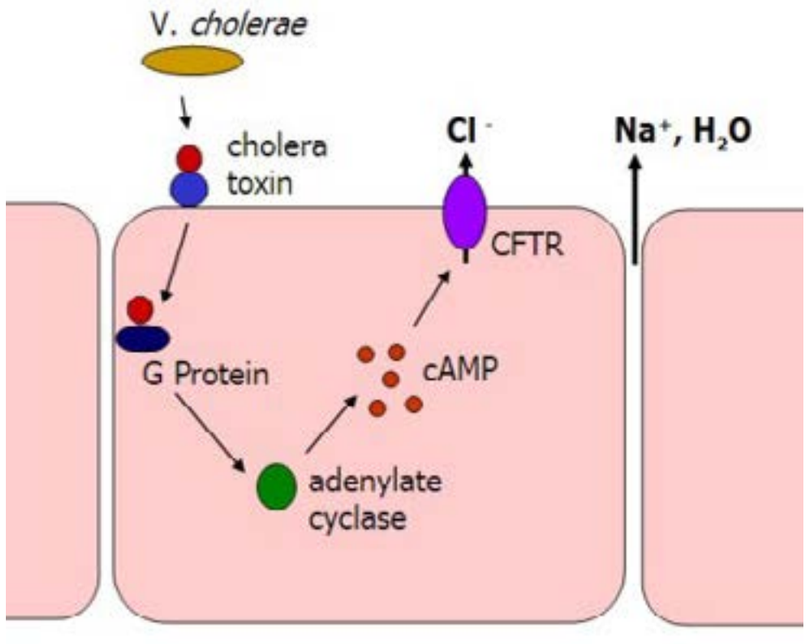
6 Class – Illustrations



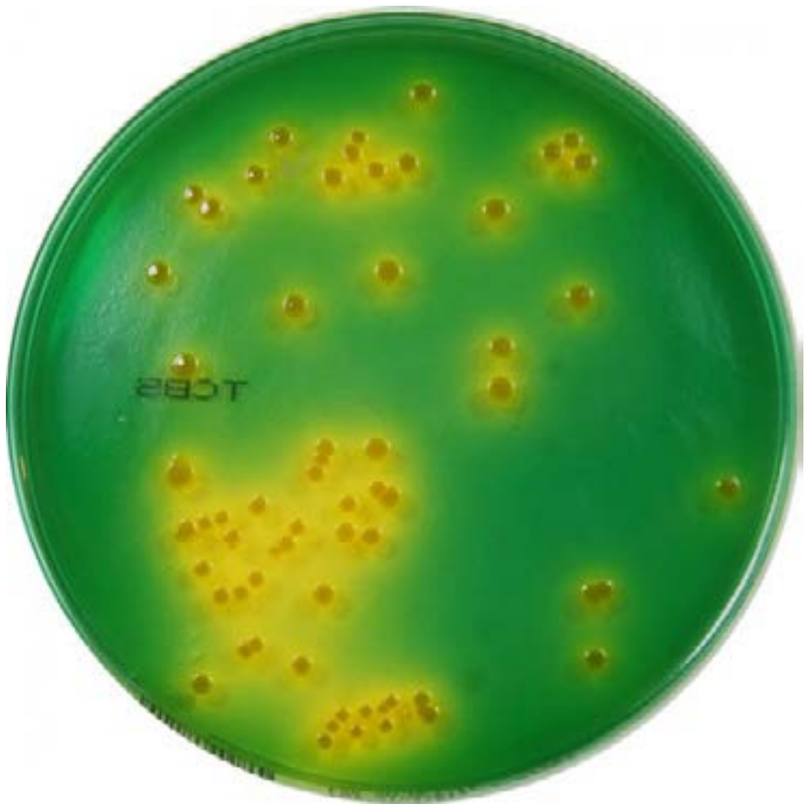
Scanning electron microscope image of *Vibrio cholerae*



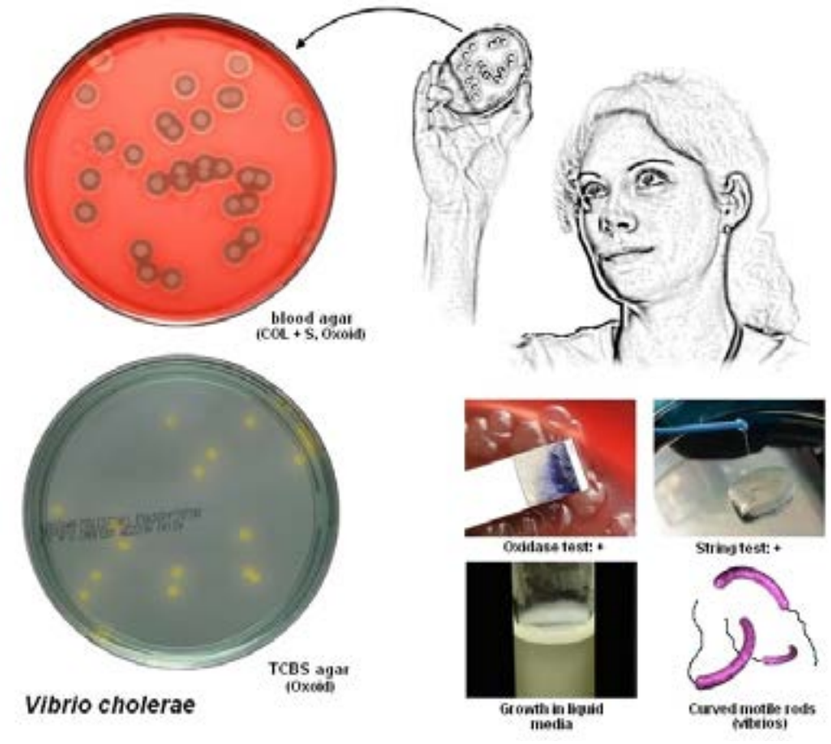
V. cholerae Gram-stain



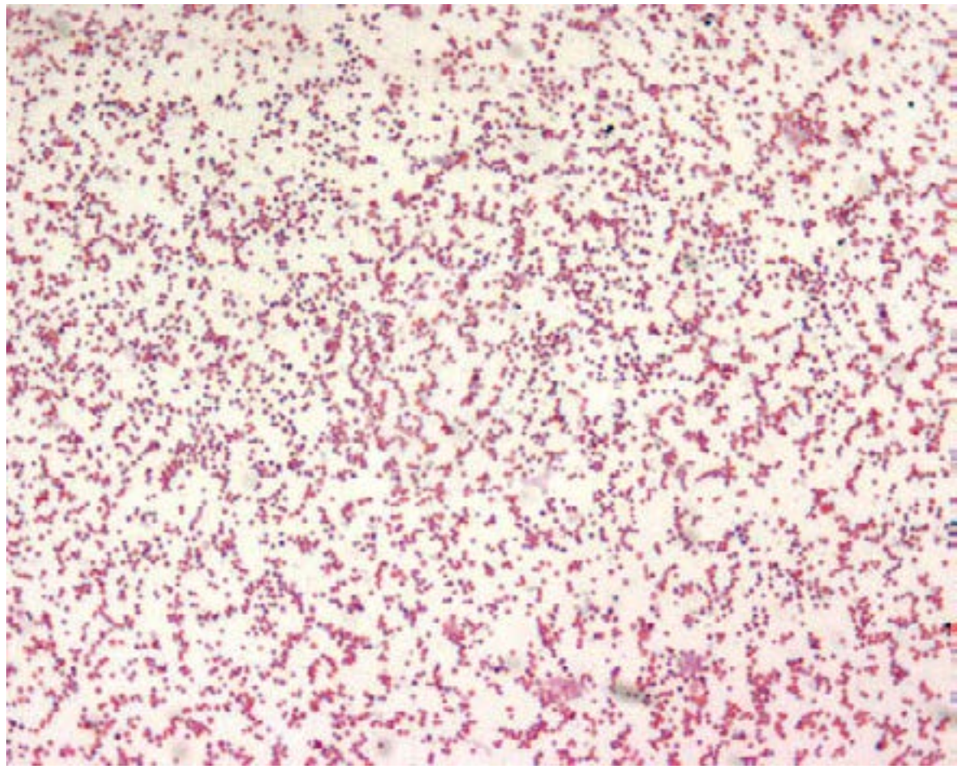
Mechanism of action of Cholera Toxin



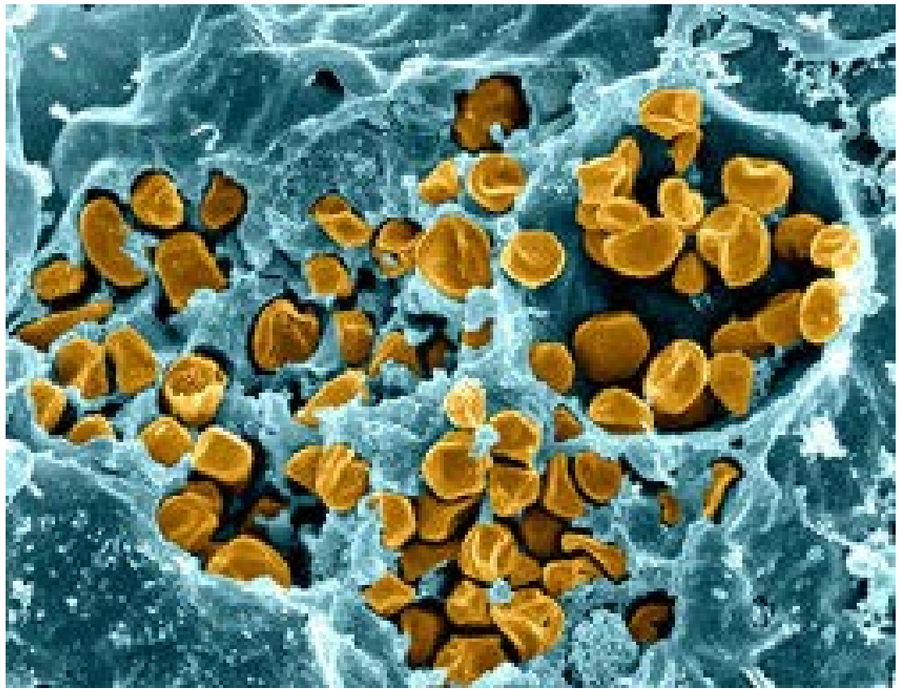
Yellow coloured (sucrose fermenting) colonies of *V. cholerae* on TCBS agar



Tests for identification of *V. cholerae*

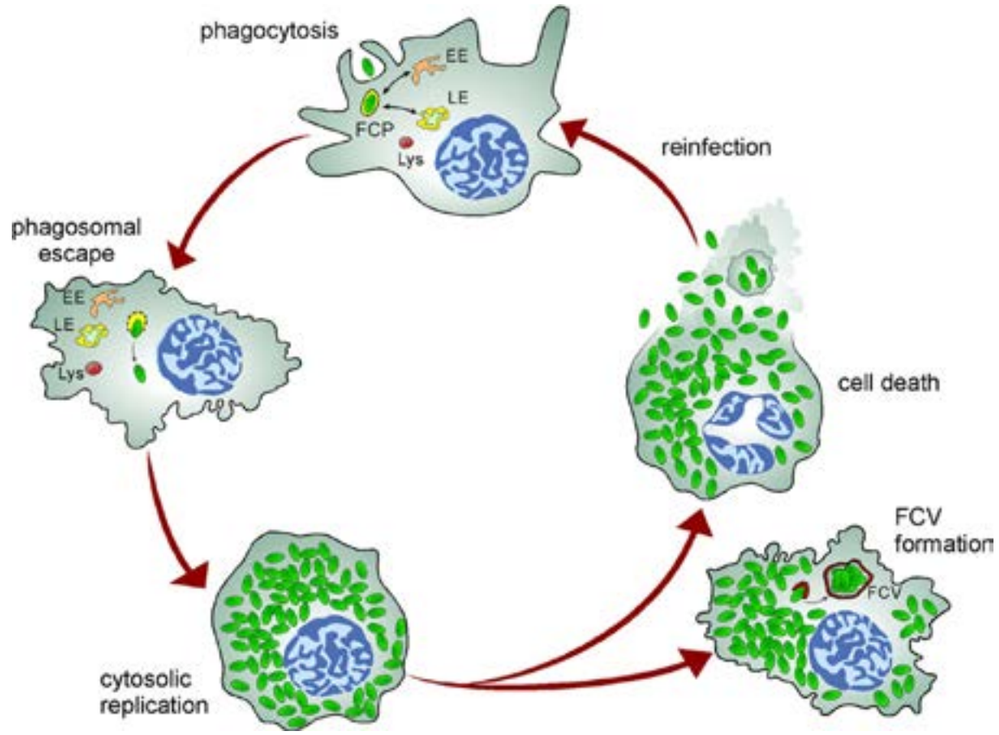


Francisella tularensis Gram-stain

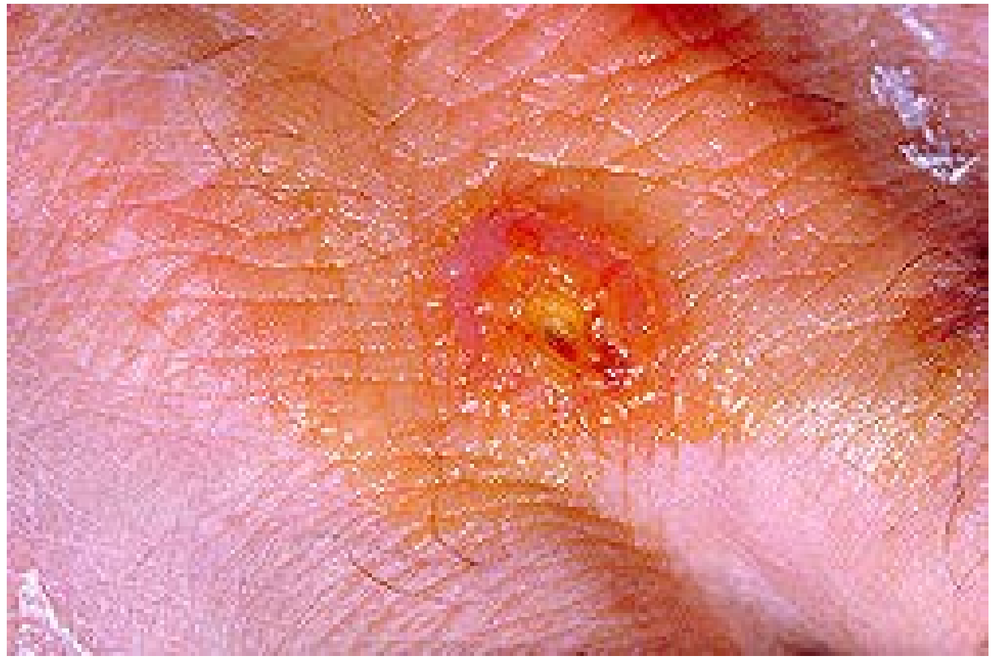


Microscopic image of *F. tularensis*

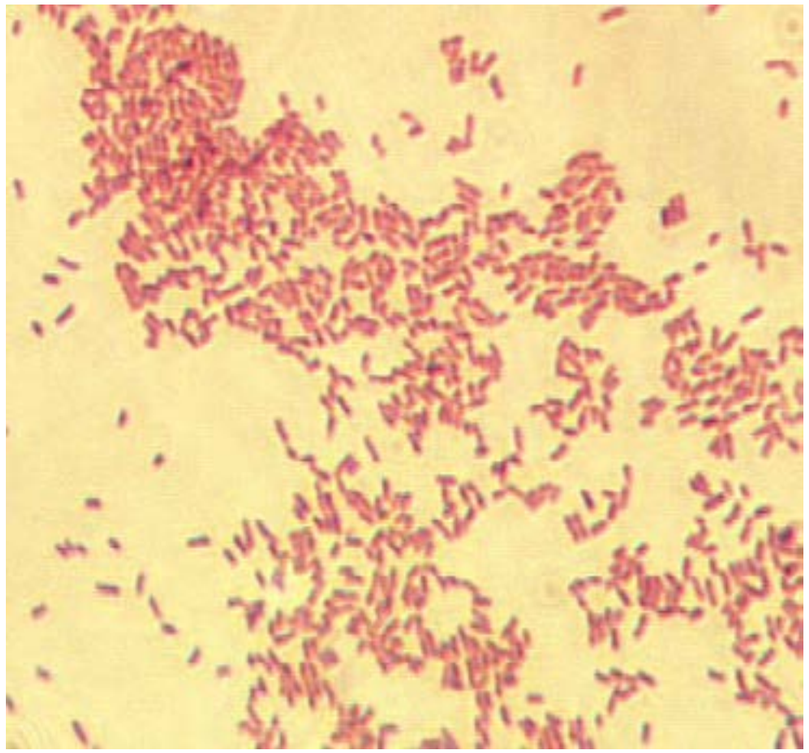
Chocolate agar showing *F. tularensis* colonies



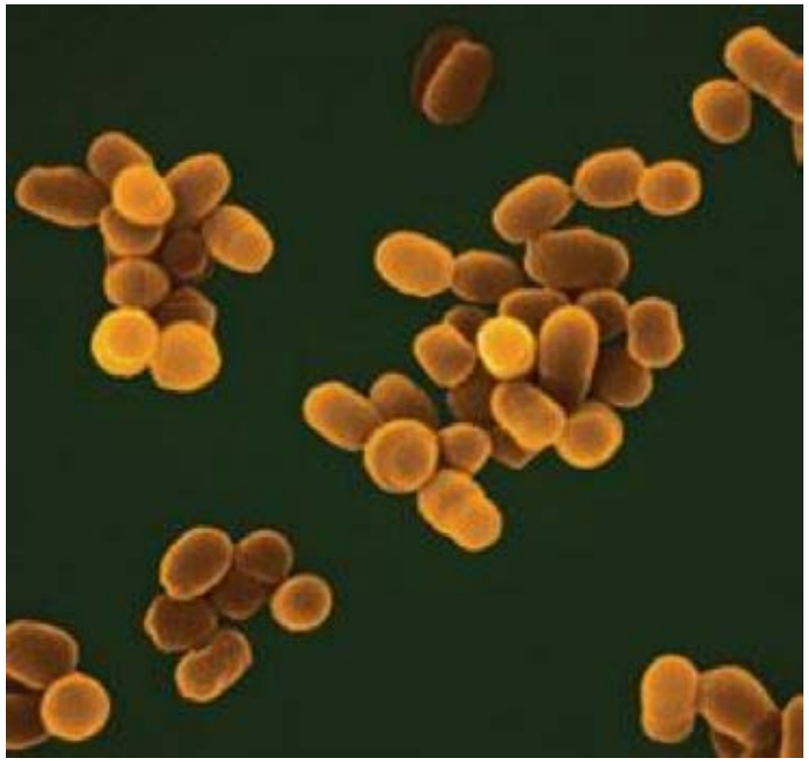
Model of the *Francicella intracellular* cycle in macrophages



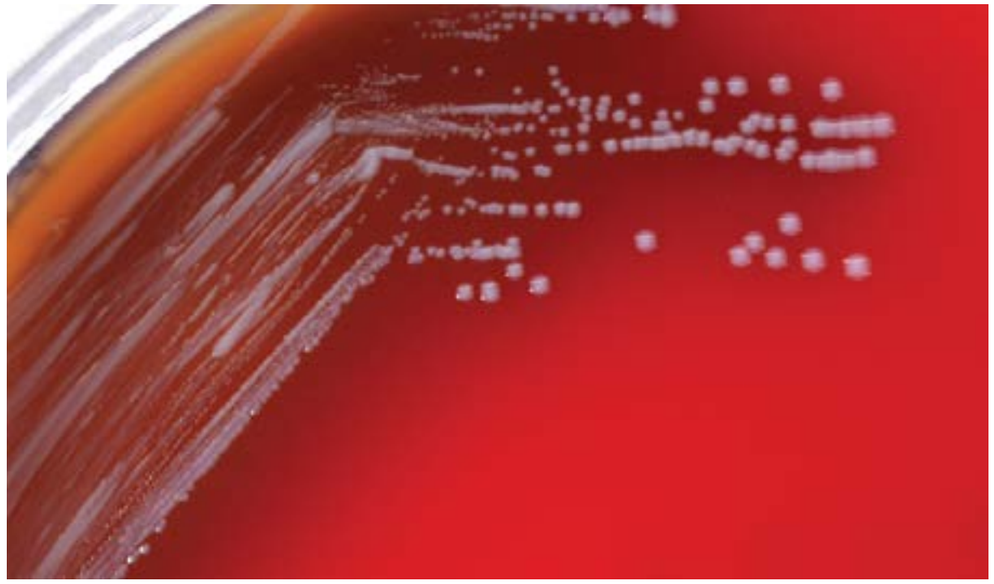
A Tularemia lesion on the dorsal skin of right hand



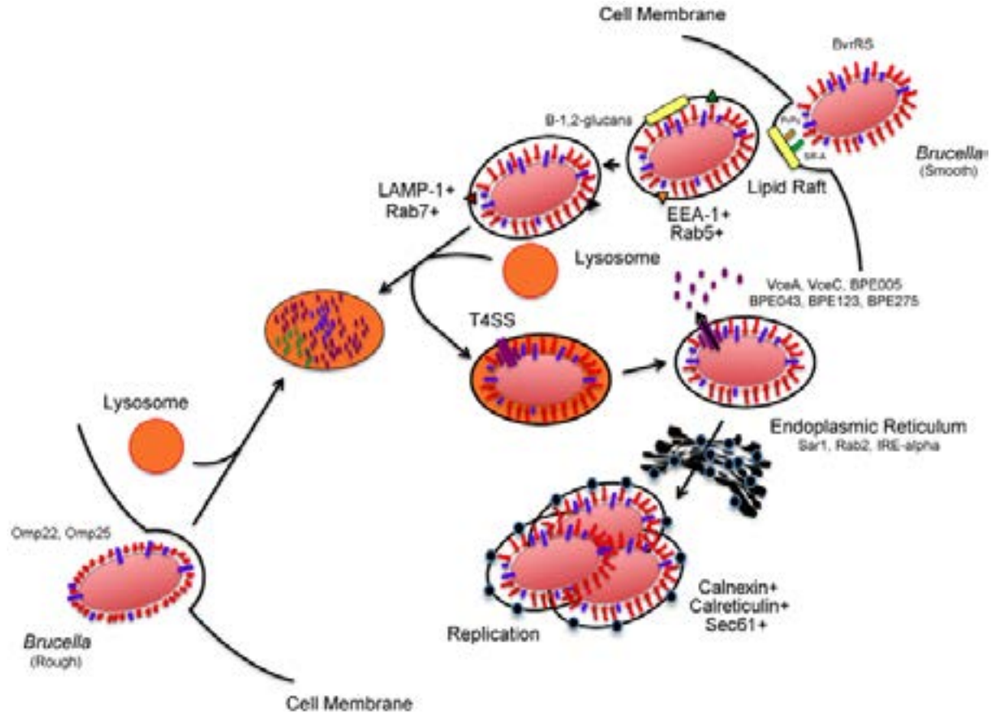
Brucella Gram-stain



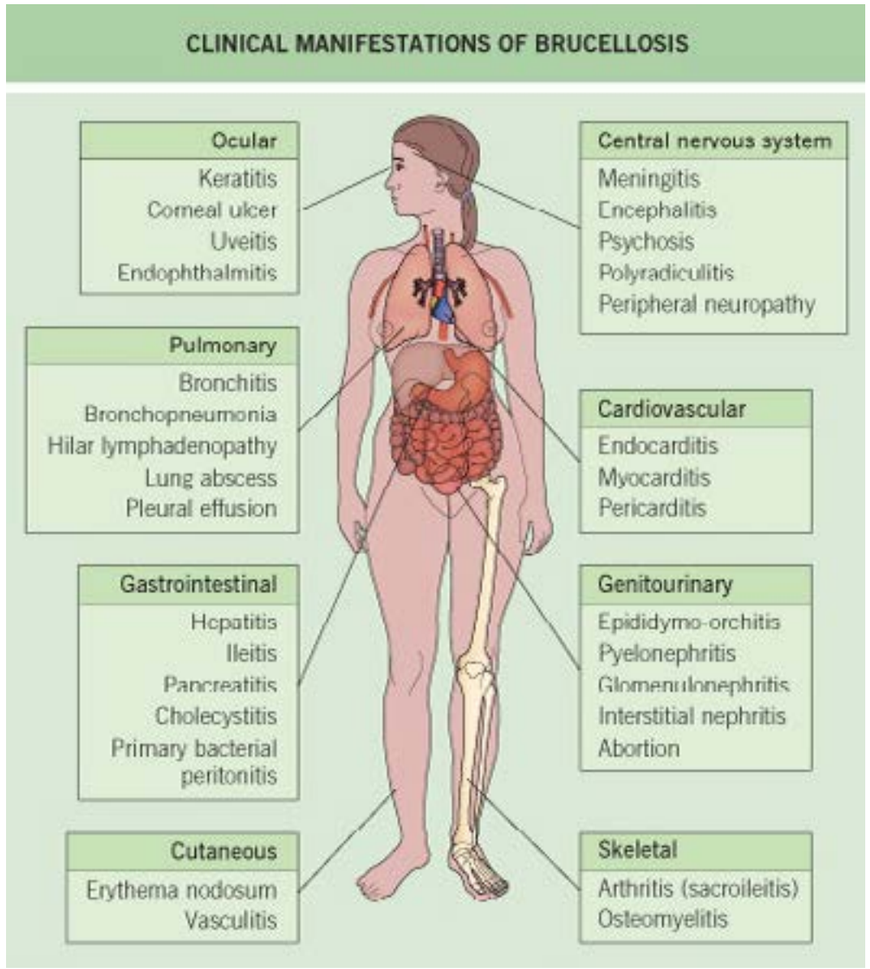
Brucella abortus - scan microscopy



Brucella on brucella blood agar



Brucella invasion and intracellular trafficking in host mammalian cells



Clinical manifestation of brucellosis

Brucella abortus vaccine

Brucella ELISA kit

7 Class - The repetition of examined material. Submodule-1

1. General characteristics of pyogenic cocci.

2. *Staphylococcus, Staphylococcus* species, differentiation signs of staphylococci. Factor of pathogenicity and toxins. Characteristics of the diseases that they cause and pathogenesis. Laboratory diagnosis, prevention and treatment.

3. Streptococci. General characteristics of the properties and classification of the streptococci. Hemolytic streptococci group A and their role in human pathology. Laboratory diagnosis and prevention.

4. *Streptococcus pneumonia.* Serological groups. Their properties and role in human pathology. Laboratory diagnosis. Prevention.

5. The role of streptococci in the pathogenesis of scarlet fever. Pathogenesis of the disease. Laboratory diagnosis, treatment and prevention. Schick phenomenon; Phenomenon Arystovskyy-Fanconi.

6. The role of *Streptococcus* in pathogenesis of rheumatic fever. Characteristic of rheumatism cycles. Laboratory diagnosis, prevention and treatment.

7. Gonococcus. General characteristics of gonococci, Asha forms. Disease pathogenesis. Laboratory diagnosis of acute and chronic gonorrhea forms. Gonococci vaccine. Peculiarities of phagocytic reaction with gonorrhea. Treatment.

8. Meningococcus. Characteristic properties of meningococcus. Pathogenesis of the disease. Symptoms. Laboratory diagnostics. Prevention.

9. *Pseudomonas aeruginosa*. Properties. Pathogenicity to humans. Methods of laboratory diagnostic.

10. The causative agent of glanders - general characteristics and pathogenesis of disease. Laboratory diagnostics and treatment.

11. The causative agent of melioidosis. General characteristics of properties, the pathogenesis of the disease. Laboratory diagnostics. Prevention and treatment. The Strauss phenomenon.

12. Enterobacteriaceae and their evolution. Classification. The values in human pathology.

13. *Escherichia*, their properties, pathogenic *Escherichia* serovars their differentiation. Laboratory diagnostics, prevention and treatment. Immunity.

14. *Salmonella*. General description. Pathogens of the typhoid and paratyphoid. Pathogenesis of typhoid fever. *Salmonella* serological classification. Laboratory diagnostics. Prevention and treatment.

15. *Salmonella* the causative agents of acute gastroenteritis. Pathogenesis of the disease. Laboratory diagnostics. Prevention and treatment.

16. *Shigella*. General description, classification. The role of toxins and pathogen enzymes in the dysentery pathogenesis. Clinical symptoms, laboratory diagnosis. Prevention and treatment.

17. *Klebsiella*, general characteristics, classification. Characteristic properties and antigenic structure. The pathogenesis of diseases that they cause. Specific laboratory diagnosis.

18. *Proteus. Proteus* properties. Types of *Proteus*. Etiological role in purulent, mixed and internal hospital infections. The role of *Proteus* in food toxic infections causing. Laboratory diagnostics. Prevention and treatment.

19. *Helicobacter. Campylobacter*. General description. Etiological role in peptic ulcer. Laboratory diagnostics. Prevention.

20. *Yersinia.* The causative agent of plague disease. General description of the plague causative agent, pathogenesis, clinical features, laboratory diagnosis. Prevention and treatment.

21. *Yersinia pseudotuberculosis* and *Y. enterocolitis*. Morphological and physiological properties. Pathogenicity to humans and animals. Pathogenesis of the disease. Laboratory diagnostics. Treatment and immunity.

22. Cholera. General description *Vibrio parahaemolyticus*. The role of *Vibrio parahaemolyticus* in causing of the food toxic infections. Laboratory diagnostics.

23. *Vibrio cholera*. Morphological, cultural, biochemical, antigenic properties. Factor of pathogenicity. The toxins. The cholera pathogenesis and immunity. Laboratory diagnostics. Prevention. Treatment.

24. The causative agent of tularemia. General description. Pathogenesis of the disease. Clinical forms of tularaemia. Laboratory diagnostics. Treatment and prevention.

25. The causative agent of brucellosis. General description morphological, cultural and biochemical properties of *Brucella*. Laboratory diagnostics. Pathogenesis of the disease. Peculiarities of immunity.

Treatment and prevention.

26. *Haemophilus* bacteria. *Bordetella* - causative agents of whooping cough. General characteristic of their properties. Disease pathogenesis. Laboratory diagnosis. Treatment, prevention.

27. Causative agents of the influenza and chancroid. General characteristic of their properties. The pathogenesis of disease. Laboratory diagnosis, prevention.

8 Class - Pathogenic anaerobes. Tetanus. *Clostridium botulinum*

Clostridium species

Scientific classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Clostridia
Order: Clostridiales
Family: Clostridiaceae
Genus: Clostridium
Species: C. perfringens, C. tetani, C. botulinum

Morphology & Identification

A. MORPHOLOGY

Clostridium is a Gram-positive, rod-shaped, anaerobic, spore-forming, motile bacterium. Spores of clostridia are usually wider than the diameter of the rods in which they are formed. In the various species, the spore is placed centrally, subterminally, or terminally. Most species of clostridia are motile and possess peritrichous flagella.

B. CULTURE AND GROWTH CHARACTERISTICS

Clostridia are anaerobes and grow under anaerobic conditions; a few species are aerotolerant and will also grow in ambient air. In general, the *Clostridium* grow wel on the blood-enriched media used to grow anaerobes an on other media used to culture anaerobes as well.

Some clostridia produce large raised colonies (eg, *C. perfringens*); others produce smaller colonies (eg, *C. tetani*). Some clostridia form colonies that spread on the agar surface. Many clostridia produce a zone of hemolysis on blood agar. *C. perfringens* typically produces multiple zones of hemolysis around colonies.

Clostridia can ferment a variety of sugars; many can digest proteins. Milk is turned acid by some and digested by others and undergoes «stormy fermentation» (ie, clot torn by gas) with a third group (eg, *C. perfringens*). Various enzymes are produced by different species.

Clostridium botulinum

C. VIRULANCE FACTORS

During the growth of *C. botulinum* and during autolysis of the bacteria, toxin is liberated into the environment. Seven antigenic varieties of toxin (A-G) are known. Types A, B, and E (and occasionally F) are the principal causes of human illness. Types A and B have been associated with a variety of foods and type E predominantly with fish products. Type C produces limberneck in birds; type D causes botulism in mammals. The toxin is a 150,000-MW protein that is cleaved into 100,000-MW and 50,000-MW proteins linked by a disulfide bond. Botulinum toxin is absorbed from the gut and binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves. Proteolysis - by the light chain of botulinum toxin - of the target SNARE proteins in the neurons inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis. The SNARE proteins are synaptobrevin, SNAP 25, and syntaxin. The toxins of *C. botulinum* types A and E cleave the 25,000-MW SNAP-25. Type B toxin cleaves synaptobrevin. *C. botulinum* toxins are among the most toxic substances known: the lethal dose for a human is probably about 1-2 µg. The toxins are destroyed by heating for 20 minutes at 100 °C.

Pathogenesis, Pathology & Clinical Findings

Although *C. botulinum* types A and B have been implicated in cases of wound infection and botulism, most often the illness is not an infection. Rather, it is an intoxication resulting from the ingestion of food in which *C. botulinum* has grown and produced toxin. The most common offenders are spiced, smoked, vacuum-packed, or canned alkaline foods that are eaten without cooking. In such foods, spores of *C. botulinum* germinate; under anaerobic conditions, vegetative forms grow and produce toxin. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions. Flaccid paralysis results. The electromyogram and edrophonium strength tests are typical.

Symptoms begin 18-24 hours after ingestion of the toxic food, with visual disturbances (incoordination of eye muscles, double vision), inability to swallow, and speech difficulty; signs of bulbar paralysis are progressive, and death occurs from respiratory paralysis or cardiac arrest. Gastrointestinal symptoms are not

regularly prominent. There is no fever. The patient remains fully conscious until shortly before death. The mortality rate is high. Patients who recover do not develop antitoxin in the blood.

Infant botulism is as common as or more common than the classic form of paralytic botulism associated with the ingestion of toxin-contaminated food. The infants in the first months of life develop poor feeding, weakness, and signs of paralysis («floppy baby»).

Infant botulism may be one of the causes of sudden infant death syndrome. *C. botulinum* and botulinum toxin are found in feces but not in serum. It is assumed that *C. botulinum* spores are in the babies' food, yielding toxin production in the gut. Honey has been implicated as a possible vehicle for the spores.

Diagnostic laboratory tests

Toxin can often be demonstrated in serum from the patient, and toxin may be found in leftover food. Mice injected intraperitoneally die rapidly. The antigenic type of toxin is identified by neutralization with specific antitoxin in mice. *C. botulinum* may be grown from food remains and tested for toxin production, but this is rarely done and is of questionable significance. In infant botulism, *C. botulinum* and toxin can be demonstrated in bowel contents but not in serum. Toxin may be demonstrated by passive hemagglutination or radioimmunoassay.

Immunity

When the botulism toxin is produced, antibodies bind and recognize a foreign substance in the host bodies. Most of the antibodies that distinguish the neurotoxin consists of IgG and IgM. Both of these antibodies initiate opsonization which coats the pathogen for phagocytosis by a leukocyte. Once the toxin has penetrated a nerve cell, the adaptive immune system begins to target intracellular antigens.

Treatment

Potent antitoxins to three types of botulinum toxins have been prepared in horses. Since the type responsible for an individual case is usually not known, trivalent (A, B, E) antitoxin must be promptly administered intravenously with customary precautions. Adequate ventilation must be maintained by mechanical respirator, if necessary. These measures have reduced the mortality rate from 65% to below 25%. Although most infants with botulism recover with supportive care alone, antitoxin therapy is recommended.

Epidemiology, Prevention & Control

Since spores of *C. botulinum* are widely distributed in soil, they often contaminate vegetables, fruits, and other materials. A large restaurant-based outbreak was associated with sauteed onions. When such foods are canned or otherwise preserved, they either must be sufficiently heated to ensure destruction of spores or must be boiled for 20 minutes before consumption. Strict regulation of commercial canning has largely overcome the danger of widespread outbreaks, but commercially prepared foods have caused deaths. A chief risk factor for botulism lies in home-canned foods, particularly string beans, corn, peppers, olives, peas, and smoked fish or vacuum-packed fresh fish in plastic bags. Toxic foods may be spoiled and rancid, and cans may «swell», or the appearance may be innocuous. The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption. Toxoids are used for active immunization of cattle in South Africa. Botulinum toxin is considered to be a major agent for bioterrorism and biologic warfare.

Clostridium tetani

C. tetani, which causes tetanus, is worldwide in distribution in the soil and in the feces of horses and other animals. Several types of *C. tetani* can be distinguished by specific flagellar antigens. All share a common O (somatic) antigen, which may be masked, and all produce the same antigenic type of neurotoxin, tetanospasmin.

C. VIRULANCE FACTORS

C. tetani spores germinate in proper anaerobic conditions, and one ideal medium are wounds with dead cells. When the conditions are right, they will germinate and produce two toxins: tetanolysin and tatanospasmin. Tetanolysin is a hemolysin with no recognized pathogenic ability while tetanospasmin is the peptide responsible for tetanus.

Tetanospasmin is a 150kD peptide made out of a heavy chain (B) and a light chain (A) joined by a disulfide bond. The heavy chain specifically binds to neuronal cells (disialogangliosides). The light chain, a zinc endopeptidase, attacks the vessicle associated membrane protein and blocks the release of inhibitory neurotransmitters.

Inhibitory nerutransmitter produce inhibitors that bind to receptors on excited neurons. The binding of

the inhibitor blocks the neuron from releasing the acetylcholine that is responsible for muscle contraction. As a result, the muscle relaxes. If these neurotransmitter are blocked (as the case when these toxins are present), there is nothing stopping the release of acetycholine from the excited neuron. As a result, the muscle will stay contracted.

Tetanospasmin is used to create the toxoid used in immunization vaccine. Tetanus toxoid was first produced in 1924 and consists of a formaldehyde-treated toxin.

Pathogenesis, Pathology & Clinical Findings

Tetanus

C. tetani is not an invasive organism. The infection remains strictly localized in the area of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) into which the spores have been introduced. The volume of infected tissue is small, and the disease is almost entirely a toxemia. Germination of the spore and development of vegetative organisms that produce toxin are aided by (1) necrotic tissue, (2) calcium salts, and (3) associated pyogenic infections, all of which aid establishment of low oxidationreduction potential.

The toxin released from vegetative cells reaches the central nervous system and rapidly becomes fixed to receptors in the spinal cord and brain stem and exerts the actions described above.

C. tetani causes tetanus and there are 4 clinical types. Incubation period ranges from 3-21 days, with an average of about a week. It has a fatality rate of 30%.

A. GENERALIZED - (80%) MOST COMMON TYPE

Toxins get distributed via lymphatic and vascular system and spread more widely and affect more nerves. First symptom is the characteristic lock jaw. It spreads and begins to affect the rest of your muscle, starting with the neck and moving to your back. This generalized muscle rigidity comes with reflex spasms as your body tries to respond to various stimuli. These spasms can cause fractures, tendon rupture and respiratory failure. Death from tetanus results from respiratory failure and cardiovascular instability. Other symptoms caused by autonomic dysfunction may include fever, sweating and high blood pressure. Recovery can take months but is usually complete unless complications occur.

B. LOCALIZED

Very uncommon. Patients with this clinical type experience muscle rigidity close to the site of injury. These contractions can persist for many weeks before disappearing 1% fatality rate.

C. CEPHALIC

Form of localized disease that affects cranial nerves. It can happen after ear infections or head injuries. It affects cranial nerves so it can affect the muscles in your face (eyelid, tongue, lips, etc).

D. NEONATAL

Form of generalized tetanus that occurs in newborn infants. Usually happens when the umbilical cord is cut with an unsterile instrument. There are some cultures where it is ritualistic to apply cow dung to the already cut umbilical cords of newborn infants. 90% fatality rate. Very common in developing countries.

Diagnostic laboratory tests

The diagnosis rests on the clinical picture and a history of injury, although only 50% of patients with tetanus have an injury for which they seek medical attention. The primary differential diagnosis of tetanus is strychnine poisoning. Anaerobic culture of tissues from contaminated wounds may yield *C. tetani,* but neither preventive nor therapeutic use of antitoxin should ever be withheld pending such demonstration. Proof of isolation of *C. tetani* must rest on production of toxin and its neutralization by specific antitoxin.

There are currently no blood tests for diagnosing tetanus. The diagnosis is based on the presentation of tetanus symptoms and does not depend upon isolation of the bacterium, which is recovered from the wound in only 30% of cases and can be isolated from patients without tetanus. Laboratory identification of *C. tetani* can be demonstrated only by production of tetanospasmin in mice.

The «spatula test» is a clinical test for tetanus that involves touching the posterior pharyngeal wall with a soft-tipped instrument and observing the effect. A positive test result is the involuntary contraction of the jaw (biting down on the «spatula») and a negative test result would normally be a gag reflex attempting to expel the foreign object.

Epidemiology, prevention and treatment

C. tetani is found mostly in warm, damp areas, especially in manure treated soil, but can also be found in the intestines or feces of many animals, such as horse, sheep, and dogs. In its vegetative state, *C. tetani* is

heat sensitive and cannot survive in the presence of oxygen. However its spores are resistant to heat and some antiseptics, but oxygen rich areas are also toxic to them. When in soil, they can last for months or even years in the proper conditions. The spores can germinate through the dead cells of the body, thus spreading toxins.

Prevention of tetanus depends upon (1) active immunization with toxoids; (2) proper care of wounds contaminated with soil, etc; (3) prophylactic use of antitoxin; and (4) administration of penicillin.

Universal active immunization with tetanus toxoid should be mandatory. Tetanus toxoid is produced by detoxifying the toxin with formalin and then concentrating it. Aluminum-saltadsorbed toxoids are employed. Three injections comprise the initial course of immunization, followed by another dose about 1 year later. Initial immunization should be carried out in all children during the first year of life. A «booster» injection of toxoid is given upon entry into school. Thereafter, «boosters» can be spaced 10 years apart to maintain serum levels of more than 0,01 unit antitoxin per milliliter. In young children, tetanus toxoid is often combined with diphtheria toxoid and pertussis vaccine.

The intramuscular administration of 250-500 units of human antitoxin (tetanus immune globulin) gives adequate systemic protection (0,01 unit or more per milliliter of serum) for 2-4 weeks. It neutralizes the toxin that has not been fixed to nervous tissue. Active immunization with tetanus toxoid should accompany antitoxin prophylaxis. Patients who develop symptoms of tetanus should receive muscle relaxants, sedation, and assisted ventilation. Sometimes they are given very large doses of antitoxin (3000-10,000 units of tetanus immune globulin) intravenously in an effort to neutralize toxin that has not yet been bound to nervous tissue. However, the efficacy of antitoxin for treatment is doubtful except in neonatal tetanus, where it may be lifesaving. Surgical debridement is vitally important because it removes the necrotic tissue that is essential for proliferation of the organisms. Hyperbaric oxygen has no proved effect. Penicillin strongly inhibits the growth of *C. tetani* and stops further toxin production. Antibiotics may also control associated pyogenic infection. When a previously immunized individual sustains a potentially dangerous wound, an additional dose of toxoid should be injected to restimulate antitoxin production. This «recall» injection of toxoid may be accompanied by a dose of antitoxin if the patient has not had current immunization or boosters or if the history of immunization is unknown.

Clostridia that produce invasive infections

Many different toxin-producing clostridia (*C. perfringens* and related clostridia) can produce invasive infection (including myonecrosis and gas gangrene) if introduced into damaged tissue.

About 30 species of clostridia may produce such an effect, but the most common in invasive disease is *C. perfringens* (90%). An enterotoxin of *C. perfringens* is a common cause of food poisoning.

C. VIRULANCE FACTORS

The invasive clostridia produce a large variety of toxins and enzymes that result in a spreading infection. Many of these toxins have lethal, necrotizing, and hemolytic properties. In some cases, these are different properties of a single substance; in other instances, they are due to different chemical entities. The alpha toxin of *C. perfringens* type A is a lecithinase, and its lethal action is proportionate to the rate at which it splits lecithin (an important constituent of cell membranes) to phosphorylcholine and diglyceride. The theta toxin has similar hemolytic and necrotizing effects but is not a lecithinase. DNase and hyaluronidase, a collagenase that digests collagen of subcutaneous tissue and muscle, are also produced.

Some strains of *C. perfringens* produce a powerful enterotoxin, especially when grown in meat dishes. When more than 10^8 vegetative cells are ingested and sporulate in the gut, enterotoxin is formed. The enterotoxin is a protein (MW 35,000) that may be a nones-sential component of the spore coat; it is distinct from other clostridial toxins. It induces intense diarrhea in 6-18 hours. The action of *C. perfringens* enterotoxin involves marked hypersecretion in the jejunum and ileum, with loss of fluids and electrolytes in diarrhea. Much less frequent symptoms include nausea, vomiting, and fever. This illness is similar to that produced by *B. cereus* and tends to be self-limited.

Pathogenesis, Pathology & Clinical Findings

In invasive clostridial infections, spores reach tissue either by contamination of traumatized areas (soil, feces) or from the intestinal tract. The spores germinate at low oxidation-reduction potential; vegetative cells multiply, ferment carbohydrates present in tissue, and produce gas. The distention of tissue and interference with blood supply, together with the secretion of necrotizing toxin and hyaluronidase, favor the spread of infection. Tissue necrosis extends, providing an opportunity for increased bacterial growth, hemolytic anemia, and, ultimately, severe toxemia and death.

In gas gangrene (clostridial myonecrosis), a mixed infection is the rule. In addition to the toxigenic clostridia, proteolytic clostridia and various cocci and Gram-negative organisms are also usually present. *C. perfringens* occurs in the genital tract of 5% of women. Clostridial uterine infections followed instrumental abortions. *Clostridium sordellii* has many of the properties of *C. perfringens*. *C. sordellii* has been reported to cause a toxic shock syndrome after medical abortion with mifepristone and intravaginal misoprostol. Endometrial infection with *C. sordellii* is implicated. Clostridial bacteremia is a frequent occurrence in patients with neoplasms. In New Guinea, *C. perfringens* type C produces a necrotizing enteritis (pigbel) that can be highly fatal in children. Immunization with type C toxoid appears to have preventive value.

From a contaminated wound (eg, a compound fracture, postpartum uterus), the infection spreads in 1-3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death. Treatment is with early surgery (amputation) and antibiotic administration. Until the advent of specific therapy, early amputation was the only treatment. At times, the infection results only in anaerobic fasciitis or cellulitis.

C. perfringens food poisoning usually follows the ingestion of large numbers of clostridia that have grown in warmed meat dishes. The toxin forms when the organisms sporulate in the gut, with the onset of diarrhea - usually without vomiting or fever - in 6-18 hours. The illness lasts only 1-2 days.

Diagnostic laboratory tests

Specimens consist of material from wounds, pus, and tissue. The presence of large Gram-positive rods in Gram-stained smears suggests gas gangrene clostridia; spores are not regularly present.

Material is inoculated into chopped meat-glucose medium and thioglycolate medium and onto blood agar plates incubated anaerobically. The growth from one of the media is transferred into milk. A clot torn by gas in 24 hours is suggestive of *C. perfringens.* Once pure cultures have been obtained by selecting colonies from anaerobically incubated blood plates, they are identified by biochemical reactions (various sugars in thioglycolate, action on milk), hemolysis, and colony form. Lecithinase activity is evaluated by the precipitate formed around colonies on egg yolk media. Final identification rests on toxin production and neutralization by specific antitoxin. *C. perfringens* rarely produces spores when cultured on agar in the laboratory.

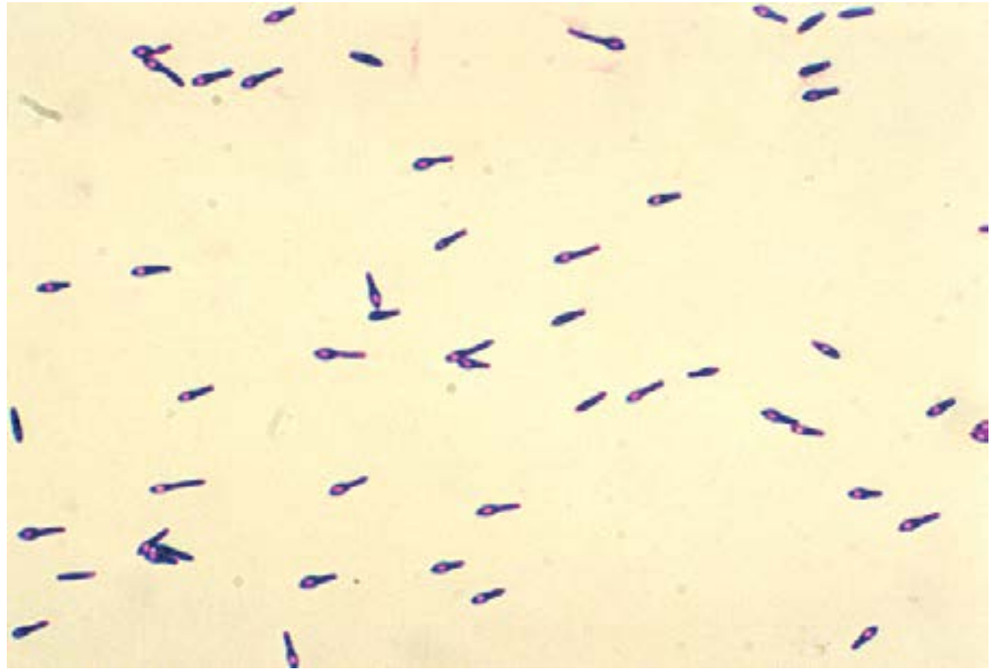
Epidemiology, prevention and treatment

The most important aspect of treatment is prompt and extensive surgical debridement of the involved area and excision of all devitalized tissue, in which the organisms are prone to grow. Administration of antimicrobial drugs, particularly penicillin, is begun at the same time. Hyperbaric oxygen may be of help in the medical management of clostridial tissue infections. It is said to «detoxify» patients rapidly.

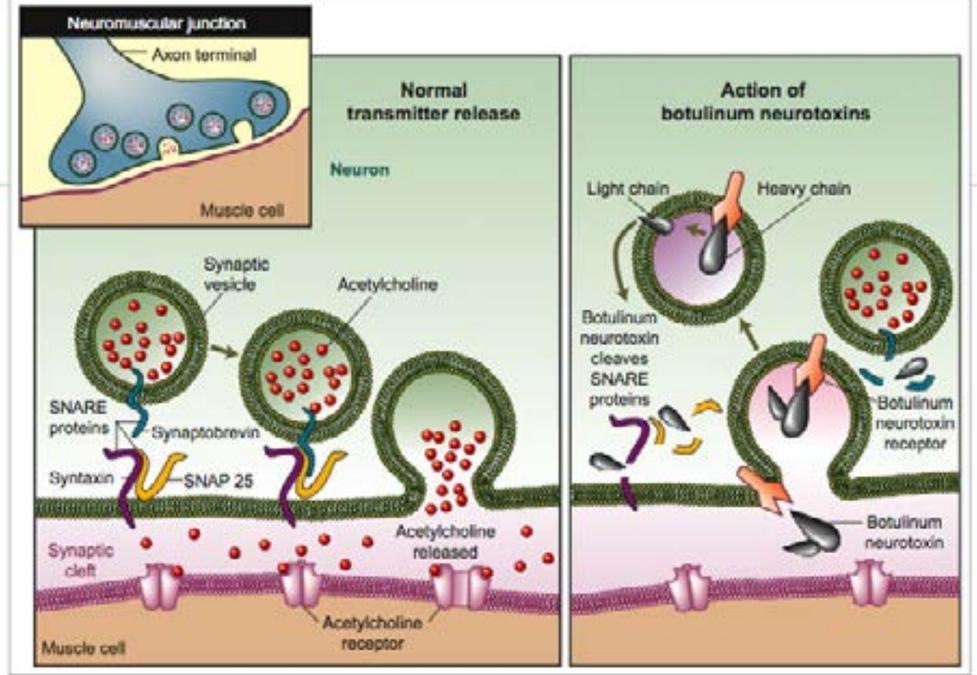
Antitoxins are available against the toxins of *C. perfringens, Clostridium novyi, Clostridium histolyticum,* and *Clostridium septicum,* usually in the form of concentrated immune globulins. Polyvalent antitoxin (containing antibodies to several toxins) has been used. Although such antitoxin is sometimes administered to individuals with contaminated wounds containing much devitalized tissue, there is no evidence for its efficacy. Food poisoning due to *C. perfringens* enterotoxin usually requires only symptomatic care.

Early and adequate cleansing of contaminated wounds and surgical debridement, together with the administration of antimicrobial drugs directed against clostridia (eg, penicillin), are the best available preventive measures. Antitoxins should not be relied on. Although toxoids for active immunization have been prepared, they have not come into practical use.

8 Class – Illustrations

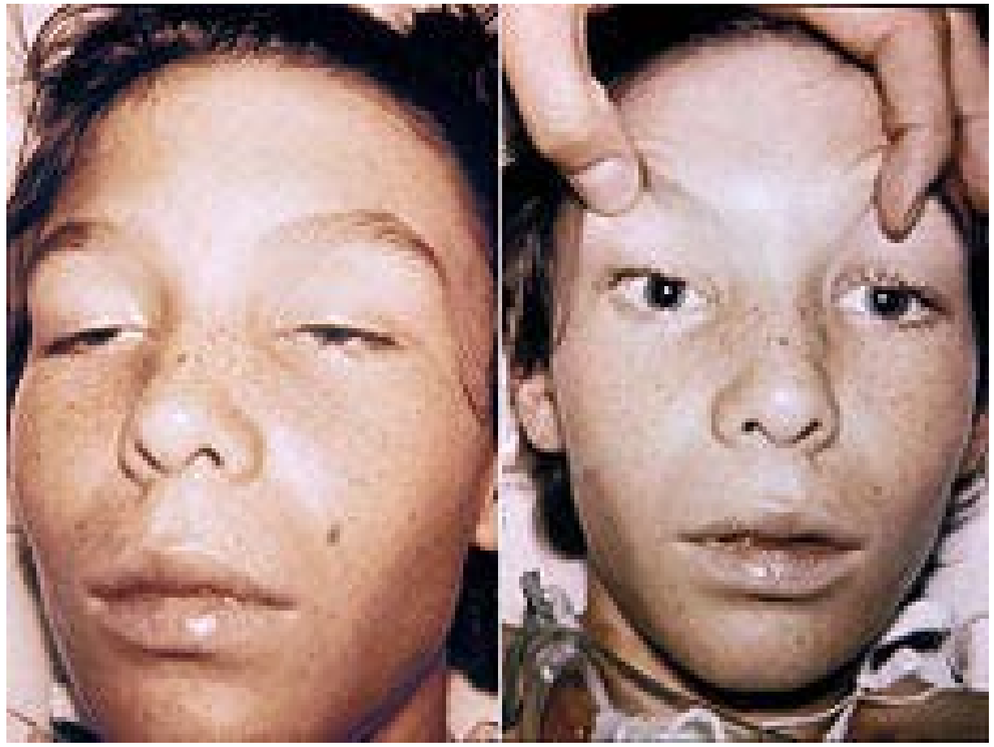


Gram positive stain of *Clostridium botulinum*



Mechanism of Action of Botulinum Toxin

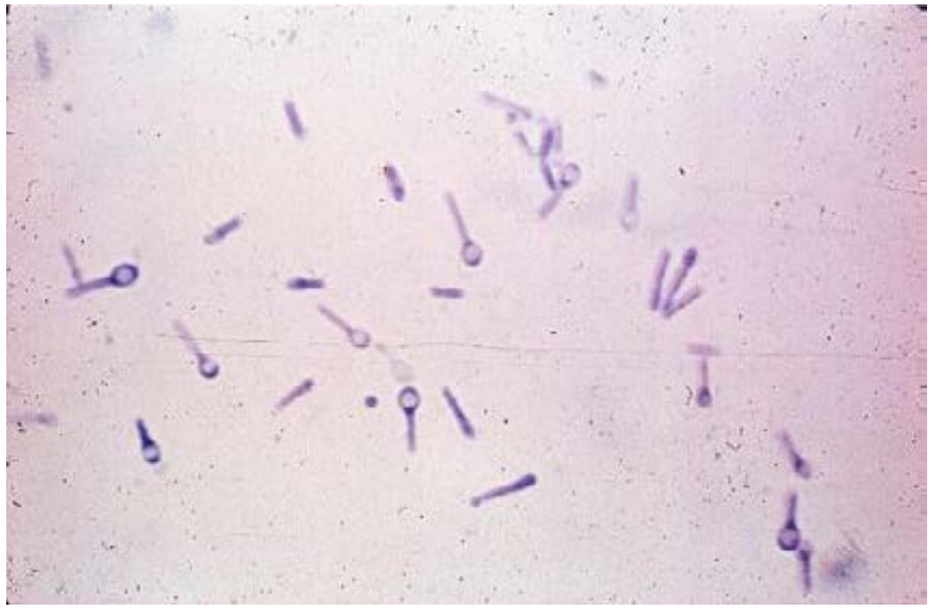
C. botulinum on cultural media



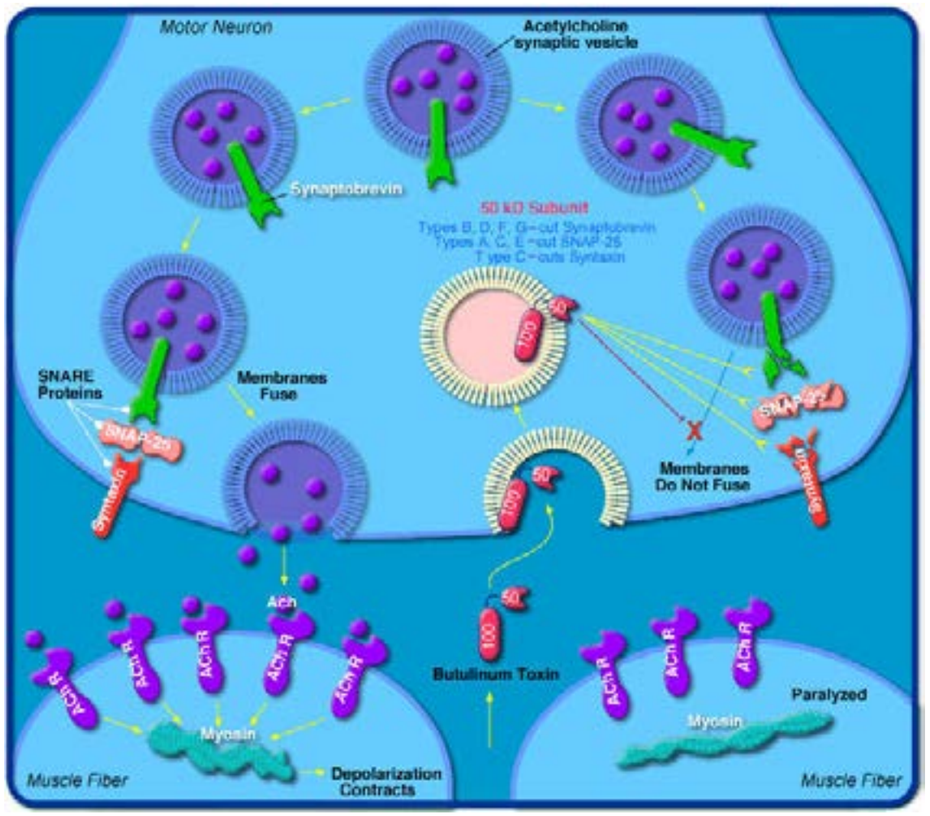
A 14-year-old with botulism

Mouse injected with botulinum

Antitoxins to botulinum toxins



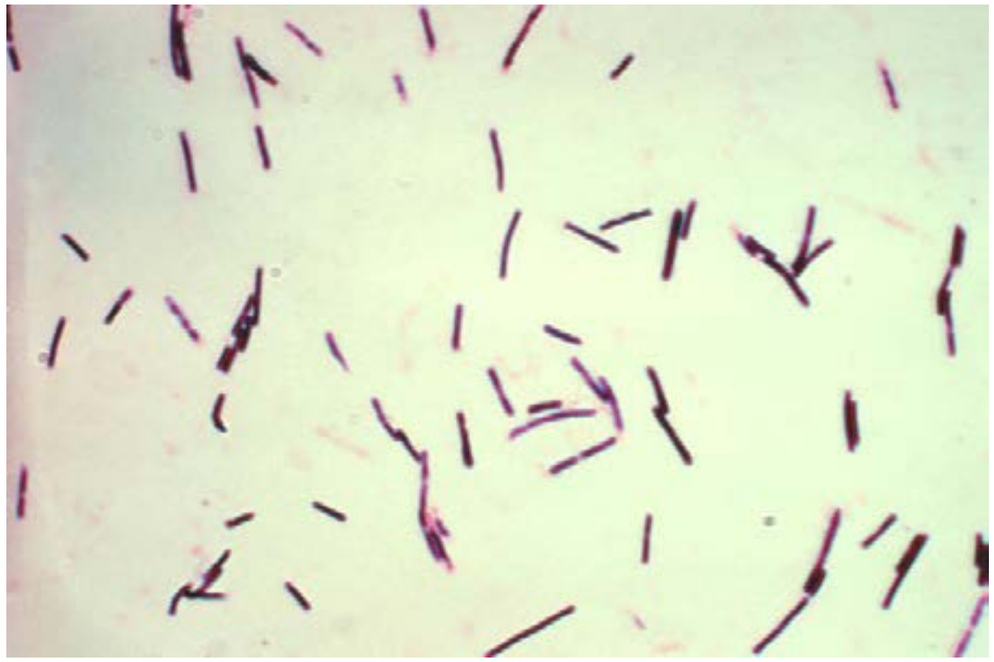
Clostridium tetani - Gram stain



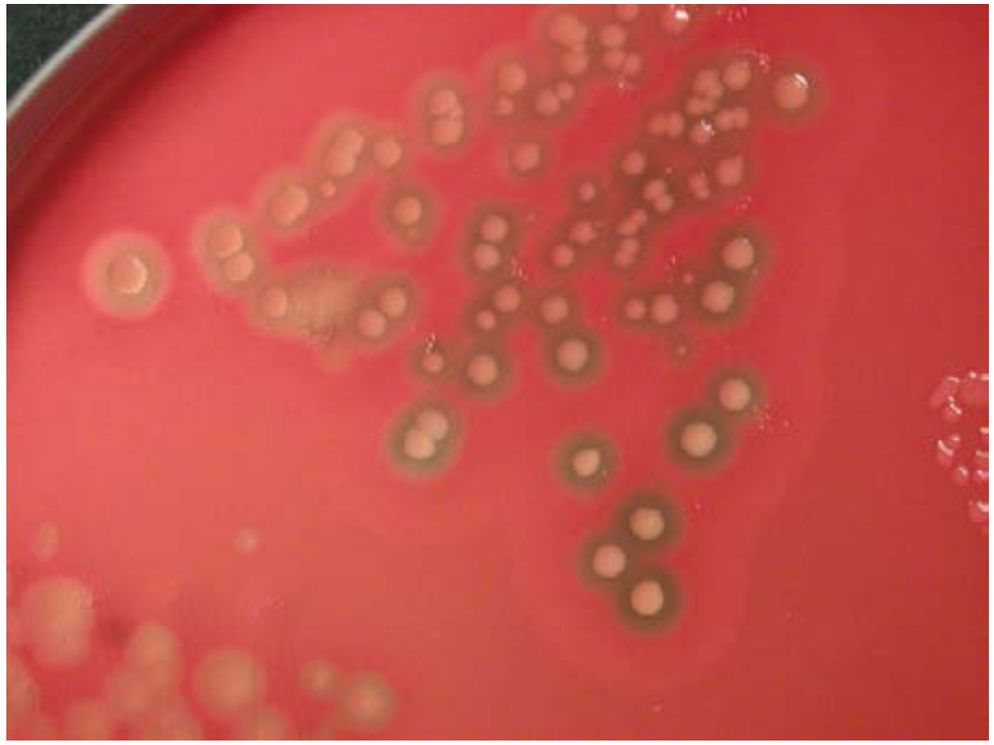
Mode of action of botulinum toxin

Tetanus

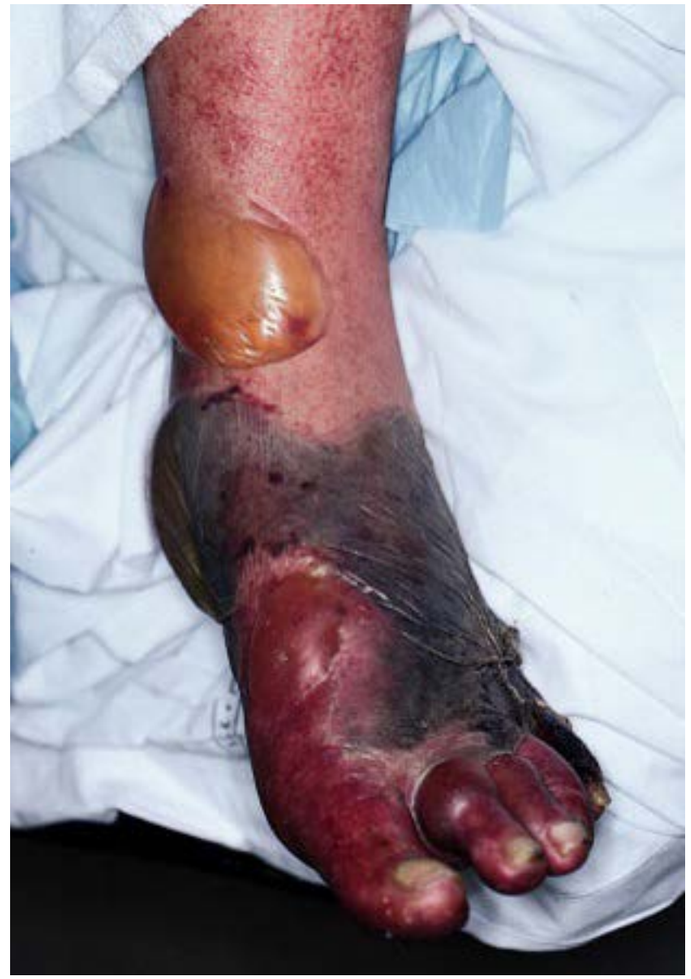
Mouse with tetanus signs



Clostridium perfringens - Gram stain



C. perfringens on blood agar



Gas gangrene

9 Class - *Corynebacterium. Listeria*. Pathogenic mycobacteria

Corynebacterium diphtheriae

Scientific classification

Kingdom: Bacteria
Phylum: Actinetobacteria
Class: Actinomycetales
Order: Corynebacterineae
Family: Corynebacteriaceae
Genus: Corynebacterium
Species: C. diphtheriae

Four subspecies are recognized*: C. d. mitis, C. d. intermedius, C. d. gravis*, and *C. d. belfanti*. The four subspecies differ slightly in their colonial morphology and biochemical properties, such as the ability to metabolize certain nutrients, but all may be toxigenic (and therefore cause diphtheria) or not toxigenic.

Morphology & Identification

A. MORPHOLOGY

Corynebacterium are Gram-positive, aerobic, nonmotile, rod-shaped bacteria. They are 0,5-1 μm in diameter and several micrometers long. Characteristically, they possess irregular swellings at one end that give them the «club-shaped» appearance. Irregularly distributed within the rod (often near the poles) are granules staining deeply with aniline dyes (metachromatic granules) that give the rod a beaded appearance. Individual *Corynebacterium* in stained smears tend to lie parallel or at acute angles to on another. True branching is rarely observed in cultures.

B. CULTURE AND GROWTH CHARACTERISTICS

On blood agar, the *C. diphtheriae* colonies are small, granular, and gray, with irregular edges, and may have small zones of hemolysis. On agar containing potassium tellurite, the colonies are brown to black with a brown-black halo because the tellurite is reduced intracellularly (staphylococci and streptococci can also produce black colonies). Four biotypes of *C. diphtheriae* have been widely recognized: gravis, mitis, intermedius, and belfanti. These variants have been classified on the basis of growth characteristics such as colony morphology, biochemical reactions, and, severity of disease produced by infection. Very few reference laboratories provide the biotype characterization; the incidence of diphtheria has greatly decreased and the association of severity of disease with biovar is not important to clinical or public health management of cases or outbreaks. If necessary in the setting of an outbreak, immunochemical and molecular methods can be used to type the *C. diphtheriae* isolates. *C. diphtheriae* and other corynebacteria grow aerobically on most ordinary laboratory media. On Loeffler's serum medium, corynebacteria grow much more readily than other respiratory organisms, and the morphology of organisms is typical in smears.

C. VIRULANCE FACTORS

Diphtheria toxin is a heat-labile polypeptide (MW 62,000) that can be lethal in a dose of 0,1 μg/kg. If disulfide bonds are broken, the molecule can be split into two fragments. Fragment B (MW = 38,000) has no independent activity but is required for the transport of fragment A into the cell. Fragment A inhibits polypeptide chain elongation - provided nicotinamide adenine dinucleotide (NAD) is present - by inactivating the elongation factor EF-2. This factor is required for translocation of polypeptidyl-transfer RNA from the acceptor to the donor site on the eukaryotic ribosome. Toxin fragment A inactivates EF-2 by catalyzing a reaction that yields free nicotinamide plus an inactive adenosine diphosphate-ribose-EF-2 complex. It is assumed that the abrupt arrest of protein synthesis is responsible for the necrotizing and neurotoxic effects of diphtheria toxin.

In vitro production of this toxin depends largely on the concentration of iron. Toxin production is optimal at 0,14 μg of iron per milliliter of medium but is virtually suppressed at 0,5 μg/mL. Other factors influencing the yield of toxin in vitro are osmotic pressure, amino acid concentration, pH, and availability of suitable carbon and nitrogen sources. The factors that control toxin production in vivo are not well understood.

Pathogenesis, Pathology & Clinical Findings

Diphtheria

Diphtheria is described as «an upper respiratory tract illness characterized by sore throat, low-grade

fever, and an adherent membrane of the tonsil(s), pharynx, and/or nose».

The principal human pathogen of the group is *C. diphtheriae.* In nature, *C. diphtheriae* occurs in the respiratory tract, in wounds, or on the skin of infected persons or normal carriers. It is spread by droplets or by contact to susceptible individuals; the bacilli then grow on mucous membranes or in skin abrasions, and those that are toxigenic start producing toxin.

Diphtheria toxin is absorbed into the mucous membranes and causes destruction of epithelium and a superficial inflammatory response. The necrotic epithelium becomes embedded in exuding fibrin and red and white cells, so that a grayish «pseudomembrane» is formed - commonly over the tonsils, pharynx, or larynx. Any attempt to remove the pseudomembrane exposes and tears the capillaries and thus results in bleeding. The regional lymph nodes in the neck enlarge, and there may be marked edema of the entire neck. The diphtheria bacilli within the membrane continue to produce toxin actively. This is absorbed and results in distant toxic damage, particularly parenchymatous degeneration, fatty infiltration, and necrosis in heart muscle, liver, kidneys, and adrenals, sometimes accompanied by gross hemorrhage. The toxin also produces nerve damage, resulting often in paralysis of the soft palate, eye muscles, or extremities.

Wound or skin diphtheria occurs chiefly in the tropics. A membrane may form on an infected wound that fails to heal. However, absorption of toxin is usually slight and the systemic effects negligible. The small amount of toxin that is absorbed during skin infection promotes development of antitoxin antibodies. The «virulence» of diphtheria bacilli is due to their capacity for establishing infection, growing rapidly, and then quickly elaborating toxin that is effectively absorbed. *C. diphtheriae* does not need to be toxigenic to establish localized infection - in the nasopharynx or skin, for example - but nontoxigenic strains do not yield the localized or systemic toxic effects. *C. diphtheriae* does not actively invade deep tissues and practically never enters the bloodstream.

When diphtheritic inflammation begins in the respiratory tract, sore throat and fever usually develop. Prostration and dyspnea soon follow because of the obstruction caused by the membrane. This obstruction may even cause suffocation if not promptly relieved by intubation or tracheostomy. Irregularities of cardiac rhythm indicate damage to the heart. Later, there may be difficulties with vision, speech, swallowing, or movement of the arms or legs. All of these manifestations tend to subside spontaneously.

In general, var gravis tends to produce more severe disease than var mitis, but similar illness can be produced by all types.

Diagnostic laboratory tests

Dacron swabs from the nose, throat, or other suspected lesions must be obtained before antimicrobial drugs are administered. Swabs should be collected from beneath any visible membrane. The swab should then be placed in semisolid transport media such as Amies. Smears stained with alkaline methylene blue or Gram stain show beaded rods in typical arrangement.

Inoculate a blood agar plate (to rule out hemolytic streptococci), a Loeffler slant, and a tellurite plate (eg, cystine-tellurite agar or modified Tinsdale medium) and incubate all at 37 °C. In 12-18 hours, the Loeffler slant may yield organisms of typical «diphtheria-like» morphology. In 36-48 hours, the colonies on tellurite medium are sufficiently definite for recognition of *C. diphtheriae.*

A presumptive *C. diphtheriae* isolate should be subjected to testing for toxigenicity. Such tests are performed only in reference public health laboratories.

There are several methods, as follows:

(1) A filter paper disk containing antitoxin is placed on an agar plate. The cultures to be tested for toxigenicity are spot innoculated 7 to 9 mm away from the disk. After 48 hours of incubation, the antitoxin diffusing from the paper disk has precipitated the toxin diffusing from toxigenic cultures and has resulted in precipitate bands between the disk and the bacterial growth.

(2) Polymerase chain reaction - based methods have bee described for detection of the diphtheria toxin gene *(tox).* PCR assays for *tox* can also be used directly on patien specimens before culture results are available. A positive culture confirms a positive PCR assay. A negative culture following antibiotic therapy along with a positive PCR assay suggests that the patient probably has diphtheria.

(3) Enzyme-linked immunosorbent assays can be used to detect diphtheria toxin from clinical *C. diphtheriae* isolates.

(4) An immunochromographic strip assay allows detection of diphtheria toxin in a matter of hours. This assay is highly sensitive. Historically, toxigenicity of a *C. diphtheriae* isolate has been demonstrated

by injecting two guinea pigs with the emulsified isolate. If the guinea pig protected with diphtheria antitoxin survives while the unprotected one dies, the isolate is considered to be toxigenic. This test has largely been replaced by more modern technology.

Immunity

Assessment of immunity to diphtheria toxin for individual patients can best be made by review of documented diphtheria toxoid immunizations and primary or booster immunization if needed.

Treatment

The treatment of diphtheria rests largely on rapid suppression of toxin-producing bacteria by antimicrobial drugs and the early administration of specific antitoxin against the toxin formed by the organisms at their site of entry and multiplication. Diphtheria antitoxin is produced in various animals (horses, sheep, goats, and rabbits) by the repeated injection of purified and concentrated toxoid. Treatment with antitoxin is mandatory when there is strong clinical suspicion of diphtheria. From 20,000 to 100,000 units are injected intramuscularly or intravenously after suitable precautions have been taken (skin or conjunctival test) to rule out hypersensitivity to the animal serum. The antitoxin should be given on the day the clinical diagnosis of diphtheria is made and need not be repeated. Intramuscular injection may be used in mild cases.

Antimicrobial drugs (penicillin, erythromycin) inhibit the growth of diphtheria bacilli. Although these drugs have virtually no effect on the disease process, they arrest toxin production. They also help to eliminate coexistent streptococci and *C. diphtheriae* from the respiratory tracts of patients or carriers.

Epidemiology, Prevention & Control

Before artificial immunization, diphtheria was mainly a disease of small children. The infection occurred either clinically or subclinically at an early age and resulted in the widespread production of antitoxin in the population. An asymptomatic infection during adolescence and adult life served as a stimulus for maintenance of high antitoxin levels. Thus, most members of the population, except children, were immune.

By age 6-8 years, approximately 75% of children in developing countries where skin infections with *C. diphtheriae* are common have protective serum antitoxin levels. Absorption of small amounts of diphtheria toxin from the skin infection presumably provides the antigenic stimulus for the immune response; the amount of absorbed toxin does not produce disease.

Active immunization in childhood with diphtheria toxoid yields antitoxin levels that are generally adequate until adulthood. Young adults should be given boosters of toxoid, because toxigenic diphtheria bacilli are not sufficiently prevalent in the population of many developed countries to provide the stimulus of subclinical infection with stimulation of resistance. Levels of antitoxin decline with time, and many older persons have insufficient amounts of circulating antitoxin to protect them against diphtheria.

The principal aims of prevention are to limit the distribution of toxigenic diphtheria bacilli in the population and to maintain as high a level of active immunization as possible.

To limit contact with diphtheria bacilli to a minimum, patients with diphtheria should be isolated. Without treatment, a large percentage of infected persons continue to shed diphtheria bacilli for weeks or months after recovery (convalescent carriers). This danger may be greatly reduced by active early treatment with antibiotics.

A filtrate of broth culture of a toxigenic strain is treated with 0,3% formalin and incubated at 37 °C until toxicity has disappeared. This fluid toxoid is purified and standardized in flocculating units (Lf doses). Fluid toxoids prepared as above are adsorbed onto aluminum hydroxide or aluminum phosphate. This material remains longer in a depot after injection and is a better antigen. Such toxoids are commonly combined with tetanus toxoid (Td) and sometimes with pertussis vaccine (DPT or DaPT) as a single injection to be used in initial immunization of children. For booster injection of adults, only Td toxoids are used; these combine a full dose of tetanus toxoid with a tenfold smaller dose of diphtheria toxoid in order to diminish the likelihood of adverse reactions.

All children must receive an initial course of immunizations and boosters. Regular boosters with Td are particularly important for adults who travel to developing countries, where the incidence of clinical diphtheria may be 1000-fold higher than in developed countries, where immunization is universal.

Listeria monocytogenes

Scientific classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Listeriaceae
Genus: Listeria
Species: L. monocytogenes

The genus *Listeria* includes seven different species (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, and *L. murrayi*). Both *L. ivanovii* and *L. monocytogenes* are pathogenic in mice, but only *L. monocytogenes* is consistently associated with human illness. There are 13 serotypes of *L. monocytogenes* that can cause disease, but more than 90 percent of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b.

Morphology & Identification

A. MORPHOLOGY

L. monocytogenes is a Gram-positive, nonspore-forming, motile, facultatively anaerobic, rod-shaped bacterium. This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. Although *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20−25 °C), the organism does not synthesize flagella at body temperatures (37 °C).

B. CULTURE AND GROWTH CHARACTERISTICS

Listeria grows on media such as Mueller-Hinton agar. Identification is enhanced if the primary cultures are done on agar containing sheep blood, because the characteristic small zone of hemolysis can be observed around and under colonies. Isolation can be enhanced if the tissue is kept at 4 °C for some days before inoculation into bacteriologic media. The organism is a facultative anaerobe and is catalase-positive, oxidase-negative and expresses a beta hemolysin, which causes destruction of red blood cells. *Listeria* produces acid but not gas in a variety of carbohydrates. The motility at room temperature and hemolysin production are primary findings that help differentiate listeria from coryneform bacteria.

C. VIRULANCE FACTORS

Growth at low temperatures: a peculiar property of *L. monocytogenes* that affects its food-borne transmission is the ability to multiply at low temperatures.

Motility: *Listeria* is also acquired by ingestion and must also find a way to attach to the intestinal mucosa. Virulence is associated with another type of motility: the ability of the bacteria to move themselves into, within and between host cells by polymerization of host cell actin at one end of the bacterium («growing actin tails») that can propel the bacteria through cytoplasm.

Adherence and invasion: the bacterium is thought to attach to epithelial cells of the GI tract by means of D-galactose residues on the bacterial surface which adhere to D-galactose receptors on the host cells. The bacterium displays the protein or carbohydrate ligand on its surface and the host displays the amino acid or sugar residue to which the ligand binds. The bacteria are then taken up by induced phagocytosis, analogous to the situation in *Shigella*. An 80 kDa membrane protein called internalin probably mediates invasion. A complement receptor on macrophages has been shown to be the internalin receptor, as well.

After engulfment, the bacterium may escape from the phagosome before phagolysosome fusion occurs mediated by a toxin, which also acts as a hemolysin, listeriolysin O (LLO). Survival of the bacterium within the phagolysosome also occurs, aided by the bacterium's ability to produce catalase and superoxide dismutase which neutralize the effects of the phagocytic oxidative burst.

Other determinants of virulence: *L. monocytogenes* produces two other hemolysins besides LLO: phosphatidylinositol-specific phospholipase C (PI-PLC) and phosphatidylcholine-specific phospholipase C (PC-PLC). Unlike LLO, which lyses host cells by forming a pore in the cell membrane, these phospholipases disrupt membrane lipids such as phosphatidylinositol and phosphatidylcholine (lecithin).

The bacterium also produces a Zn^{2+} dependent protease which may act as some sort of exotoxin. Mutations in the encoding gene (mpl) reduce virulence in the mouse model.

Pathogenesis, Pathology & Clinical Findings

L. monocytogenes causes listeriosis in animals and humans. *L. monocytogenes* enters the body through the gastrointestinal tract after ingestion of contaminated foods such as cheese or vegetables. It has a cell wall surface protein called internalin that interacts with E-cadherin, a receptor on epithelial cells, promoting phagocytosis into the epithelial cells. After phagocytosis, the bacterium is enclosed in a phagolysosome, where the low pH activates the bacterium to produce listeriolysin O. This enzyme lyses the membrane of the phagolysosome and allows the *Listeria* to escape into the cytoplasm of the epithelial cell. The organisms proliferate and ActA, another listerial surface protein, induces host cell actin polymerization, which propels them to the cell membrane. Pushing against the host cell membrane, they cause formation of elongated protrusions called filopods. These filopods are ingested by adjacent epithelial cells, macrophages, and hepatocytes, the *Listeria* are released, and the cycle begins again. *L. monocytogenes* can move from cell to cell without being exposed to antibodies, complement, or polymorphonuclear cells.

There are two forms of perinatal human listeriosis. Early onset-syndrome (granulomatosis infantiseptica) is the result of infection in utero and is a disseminated form of the disease characterized by neonatal sepsis, pustular lesions and granulomas containing *L. monocytogenes* in multiple organs. Death may occur before or after delivery. The late-onset syndrome causes the development of meningitis between birth and the third week of life; it is often caused by serotype IVb and has a significant mortality rate.

Adults can develop *Listeria* meningoencephalitis, bacteremia, and (rarely) focal infections. Meningoencephalitis and bacteremia occur most commonly in immunosuppressed patients, in whom *Listeria* is one of the more common causes of meningitis. Clinical presentation of *Listeria* meningitis in these patients varies from insidious to fulminant and is nonspecific.

Spontaneous infection occurs in many domestic and wild animals. In ruminants (eg, sheep) *Listeria* may cause meningoencephalitis with or without bacteremia. In smaller animals (eg, rabbits, chickens), there is septicemia with focal abscesses in the liver and heart muscle and marked monocytosis.

Many antimicrobial drugs inhibit *Listeria in vitro*. Clinical cures have been obtained with ampicillin, with erythromycin, or with intravenous trimethoprim-sulfamethoxazole. Cephalosporins and fluoroquinolones are not active against *L. monocytogenes*. Ampicillin plus gentamicin is often recommended for therapy, but gentamicin does not enter host cells and may not help treat the *Listeria* infection.

Diagnostic laboratory tests

The organism grows well on blood or nutrient agar and in conventional blood culture broths. On blood agar, the colonies usually are surrounded by a narrow band of β-haemolysis resembling that of B-streptococci. It can be differentiated from B-streptococci by Gram-stain and by motility testing at 20-25 °C and at 37 °C. *L. monocytogenes* ferments glucose, producing principally lactic acid without gas. It elaborates catalase, hydrolyzes esculin, and produces acetoin (Vogt-Proskauer test). Instillation into the conjunctival sac of a rabbit produces a purulent conjunctivitis followed by a keratis (Anton test). On the basis of somatic (O) and flagellar (H) Ags, 17 serotypes have been described. Serotypes 1a, 1b, and 4b account for more than 90% of clinical isolates. Serological and phage typing can be helpful in the investigation of common source outbreaks. The methods for analysis of food are complex and time-consuming. The present FDA method requires 24 and 48 hours of enrichment, followed by a variety of other tests. Total time to identification is from 5 to 7 days.

Molecular-biological diagnosis: polymer chain reaction (PCR) is used for *Listeria* species identification, for the detection of virulence factors or for classification in the main *Listeria* groups which correspond to the serovars.

Pulse field gel electrophoresis (PFGE) is used for the fine typing and elucidation of epidemiological chains of infection.

Immunity

Immunity to *L. monocytogenes* is primarily cell-mediated, as demonstrated by the intracellular location of infection and by the marked association of infection and conditions of impaired cell-mediated immunity such as pregnancy, AIDS, lymphoma, and organ transplantation. Immunity can be transferred by sensitized lymphocytes but not by antibodies.

Treatment

If diagnosed early enough, antibiotic treatment of pregnant women or immunocompromised individuals can prevent serious consequences of the disease. Antibiotics effective against *Listeria* species include

ampicillin, vancomycin, ciprofloxacin, linezolid and azithromycin.

Epidemiology, Prevention & Control

Reservoirs of *L. monocytogenes* are present in the environment, human and animal populations. Transmission of the disease causing bacteria can occur through:

A. FOODBORNE

L. monocytogenes has been associated with foods such as unpasteurized milk, cheeses, ice cream, raw vegetables, raw and smoked sausages, raw and cooked poultry, all types of raw meats, and raw and smoked fish. *L. monocytogenes* introduced as a foodborne disease initially manifests itself as a gastrointestinal illness before spreading through the blood stream to affect the brain and the nervous system.

B. Person to person spread

L. monocytogenes is spread from person to person in nosocomial and nursery settings.

C. DIRECT INOCULATION

Direct contact with infectious material. This is more common in people with lesions in their hands and arms and when they come in contact with the environment that is infected with *L. monocytogenes*.

D. IN UTERO/ PARENTAL TRANSMISSION

L. monocytogenes can be transmitted to the unborn fetus through an infected mother during her pregnancy term.

Prevention

Do not drink raw (unpasteurized) milk, and do not eat foods that have unpasteurized milk in them.

Wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods.

Rinse raw produce thoroughly under running tap water before eating.

Keep uncooked meats, poultry, and seafood separate from vegetables, fruits, cooked foods, and ready-to-eat foods.

Thoroughly cook raw food from animal sources, such as meat, poultry, or seafood to a safe internal temperature.

Wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods.

Consume perishable and ready-to-eat foods as soon as possible.

Persons in higher risk groups should heat hot dogs, cold cuts, and deli meats before eating them.

Pathogenic mycobacteria

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores. Although they do not stain readily, once stained they resist decolorization by acid or alcohol and are therefore called «acid-fast» bacilli. *Mycobacterium tuberculosis* causes tuberculosis and is a very important pathogen of humans. *Mycobacterium leprae* causes leprosy. *Mycobacterium avium-intracellulare* (*M. avium* complex, or MAC) and other atypical mycobacteria frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems. There are more than 50 *Mycobacterium* species, including many that are saprophytes.

Scientific classification

Kingdom: Bacteria
Phylum: Actinobacteria
Class: Actinobacteria
Order: Actinomycetales
Family: Mycobacteriaceae
Genus: Mycobacterium
Species: M. tuberculosis, M. leprae, M. bovis

Mycobacterium tuberculosis

Morphology & Identification

A. MORPHOLOGY

M. tuberculosis is aerobic, non-spore forming, non-motile, slightly curved or straight rods (0,2 to 0,6 μm by 1,0 to 10 μm) which may branch. On artificial media, coccoid and filamentous forms are seen with variable morphology from one species to another. *Mycobacterium* cannot be classified as either gram positive or Gram-negative.

The constituents listed below are found mainly in cell walls.

LIPIDS

Mycobacterium are rich in lipids. These include mycolic acids (long-chain fatty acids C78-C90), waxes, and phosphatides. In the cell, the lipids are largely bound to proteins and polysaccharides. Muramyl dipeptide (from peptidoglycan) complexed with mycolic acids can cause granuloma formation; phospholipids induce caseous necrosis. Lipids are to some extent responsible for acidfastness. Their removal with hot acid destroys acid-fastness, which depends on both the integrity of the cell wall and the presence of certain lipids. Acid-fastness is also lost after sonication of mycobacterial cells. Analysis of lipids by gas chromatography reveals patterns that aid in classification of different species.

Virulent strains of tubercle bacilli form microscopic «serpentine cords» in which acid-fast bacilli are arranged in parallel chains. Cord formation is correlated with virulence. A «cord factor» (trehalose-6,6′-dimycolate) has been extracted from virulent bacilli with petroleum ether. It inhibits migration of leukocytes, causes chronic granulomas, and can serve as an immunologic «adjuvant»**.**

PROTEINS

Each type of *Mycobacterium* contains several proteins that elicit the tuberculin reaction. Proteins bound to a wax fraction can, upon injection, induce tuberculin sensitivity. They can also elicit the formation of a variety of antibodies.

POLYSACCHARIDES

Mycobacterium contain a variety of polysaccharides. Their role in the pathogenesis of disease is uncertain. They can induce the immediate type of hypersensitivity and can serve as antigens in reactions with sera of infected persons.

B. CULTURE AND GROWTH CHARACTERISTICS

True tubercle bacilli are characterized by «acid-fastness» - ie, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria.

A. CULTURE

The media for primary culture of *Mycobacterium* should include a nonselective medium and a selective medium. Selective media contain antibiotics to prevent the overgrowth of contaminating bacteria and fungi. There are three general formulations that can be used for both the nonselective and selective media.

1. Semisynthetic Agar Media - these media (eg, Middlebrook 7H10 and 7H11) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol; the 7H11 medium contains casein hydrolysate also. The albumin neutralizes the toxic and inhibitory effects of fatty acids in the specimen or medium. Large inocula yield growth on these media in several weeks. Because large inocula may be necessary these media may be less sensitive than other media for primary isolation of *Mycobacterium*.

The semisynthetic agar media are used for observing colony morphology, for susceptibility testing, and, with added antibiotics and malachite green, as selective media.

2. Inspissated Egg Media - these media contain defined salts, glycerol, and complex organic substances (eg, fresh eggs or egg yolks, potato flour, and other ingredients in various combinations). Malachite green is included to inhibit other bacteria. Small inocula in specimens from patients will grow on these media in 3-6 weeks. These media with added antibiotics are used as selective media.

3. Broth Media - Broth media (eg, Middlebrook 7H9 and 7H12) support the proliferation of small inocula. Ordinarily, *Mycobacterium* grow in clumps or masses because of the hydrophobic character of the cell surface. If Tweens (water-soluble esters of fatty acids) are added, they wet the surface and thus permit dispersed growth in liquid media. Growth is often more rapid than on complex media.

B. GROWTH CHARACTERISTICS

Mycobacterium are obligate aerobes and derive energy from the oxidation of many simple carbon compounds. Increased CO_2 tension enhances growth. Biochemical activities are not characteristic, and the growth rate is much slower than that of most bacteria. The doubling time of tubercle bacilli is about 18 hours. Saprophytic forms tend to grow more rapidly, to proliferate well at 22-33 °C, to produce more pigment, and to be less acid-fast than pathogenic forms.

C. REACTION TO PHYSICAL AND CHEMICAL AGENTS

Mycobacterium tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth. Dyes (eg, malachite green) or antibacterial agents (eg, penicillin) that are bacteriostatic to other bacteria can be incorporated into media without inhibiting the growth of tubercle bacilli. Acids and alkalies permit the survival of some exposed tubercle bacilli and

are used to help eliminate contaminating organisms and for «concentration» of clinical specimens. Tubercle bacilli are resistant to drying and survive for long periods in dried sputum.

D. VIRULANCE FACTORS

The virulence of *M. tuberculosis* is extraordinarily complicated and multifaceted. Although the organism apparently does not produce any toxins, it possesses a huge repertoire of structural and physiological properties that have been recognized for their contribution to mycobacterial virulence and to pathology of tuberculosis.

The tubercle bacillus can bind directly to mannose receptors on macrophages via the cell wall-associated mannosylated glycolipid, LAM, or indirectly via certain complement receptors or Fc receptors.

MTB can grow intracellularly. This is an effective means of evading the immune system.

Antigen 85 complex is composed of a group of proteins secreted by MTB that are known to bind fibronectin. These proteins may aid in walling off the bacteria from the immune system and may facilitate tubercle formation.

Because of MTB's slow generation time, the immune system may not readily recognize the bacteria or may not be triggered sufficiently to eliminate them.

High lipid concentration in cell wall accounts for impermeability and resistance to antimicrobial agents, resistance to killing by acidic and alkaline compounds in both the intracellular and extracellular environment, and resistance to osmotic lysis via complement deposition and attack by lysozyme.

It has been shown recently that *M. tuberculosis* produces pili during human infection, which could be involved in initial colonization of the host.

Pathogenesis, Pathology & Clinical Findings

Tuberculosis is a contagious airborne disease caused by the bacteria *M. tuberculosis*. Tuberculosis is widespread and deadly, and causes the highest number of deaths worldwide.

STAGES OF THE DISEASE

The following stages that will be explained are for a MTB - sensitive host. It should be realized that, as stated previously, only a small percent of MTB infections progress to disease and even a smaller percent progress all the way to stage 5. Usually the host will control the infection at some point.

Stage 1

Droplet nuclei are inhaled. One droplet nuclei contains no more than 3 bacilli. Droplet nuclei are so small that they can remain air-borne for extended periods of time. The most effective (infective) droplet nuclei tend to have a diameter of 5 micrometers. Droplet nuclei are generated by during talking coughing and sneezing. Coughing generates about 3000 droplet nuclei. Talking for 5 minutes generates 3000 droplet nuclei but singing generates 3000 droplet nuclei in one minute. Sneezing generates the most droplet nuclei by far, which can spread to individuals up to 10 feet away.

Stage 2

Begins 7-21 days after initial infection. MTB multiplies virtually unrestricted within unactivated macrophages until the macrophages burst. Other macrophages begin to extravasate from peripheral blood. These macrophages also phagocytose MTB, but they are also unactivated and hence can not destroy the bacteria.

Stage 3

At this stage lymphocytes begin to infiltrate. The lymphocytes, specifically T-cells, recognize processed and presented MTB antigen in context of MHC molecules. This results in T-cell activation and the liberation of cytokines including gamma interferon (IFN). The liberation of IFN causes in the activation of macrophages. These activated macrophages are now capable of destroying MTB.

It is at this stage that the individual becomes tuberculin-positive. This positive tuberculin reaction is the result of the host developing a vigorous cell mediated immune (CMI) response. A CMI response must be mounted to control an MTB infection. An antibody mediated immune (AMI) will not aid in the control of a MTB infection because MTB is intracellular and if extracellular, it is resistant to complement killing due to the high lipid concentration in its cell wall.

Although a CMI response is necessary to control an MTB infection, it is also responsible for much of the pathology associated with tuberculosis. Activated macrophages may release lytic enzymes and reactive intermediates that facilitate the development of immune pathology. Activated macrophages and T-cells also secrete cytokines that may also play a role in the development of immune pathology, including Interleukin 1 (IL-l), tumor necrosis factor (TNF), and gamma IFN.

It is also at this stage that tubercle formation begins. The center of the tubercle is characterized by «caseation necrosis», meaning it takes on a semi-solid or «cheesy» consistency. MTB cannot multiply within these tubercles because of the low pH and anoxic environment. MTB can, however, persist within these tubercles for extended periods.

Stage 4

Although many activated macrophages can be found surrounding the tubercles, many other macrophages present remain unactivated or poorly activated. MTB uses these macrophages to replicate, and hence, the tubercle grows.

The growing tubercle may invade a bronchus. If this happens, MTB infection can spread to other parts of the lung. Similarly the tubercle may invade an artery or other blood supply line. The hematogenous spread of MTB may result in extrapulmonary tuberculosis otherwise known as milliary tuberculosis. The name «milliary» is derived from the fact that metastasizing tubercles are about the same size as a millet seed, a grain commonly grown in Africa.

The secondary lesions caused by milliary TB can occur at almost any anatomical location, but usually involve the genitourinary system, bones, joints, lymph nodes and peritoneum. These lesions are of two types:

1. Exudative lesions result from the accumulation of PMN's around MTB. Here the bacteria replicate with virtually no resistance. This situation gives rise to the formation of a «soft tubercle».

2. Productive or granulomatous lesions occur when the host becomes hypersensitive to tuberculoproteins. This situation gives rise to the formation of a «hard tubercle».

Stage 5

For unknown reasons, the caseous centers of the tubercles liquefy. This liquid is very conducive to MTB growth, and the organism begins to rapidly multiply extracellularly. After time, the large antigen load causes the walls of nearby bronchi to become necrotic and rupture. This results in cavity formation. This also allows MTB to spill into other airways and rapidly spread to other parts of the lung.

As stated previously, only a very small percent of MTB infections result in disease, and even a smaller percentage of MTB infections progress to an advanced stage. Usually the host will begin to control the infection at some point. When the primary lesion heals, it becomes fibrous and calcifies. When this happens the lesion is referred to as the Ghon complex. Depending on the size and severity, the Ghon complex may never subside. Typically, the Ghon complex is readily visible upon chest X-ray.

Small metastatic foci containing low numbers of MTB may also calcify. However, in many cases these foci will contain viable organisms. These foci are referred to Simon foci. The Simon foci are also visible upon chest X-ray and are often the site of disease reactivation.

Diagnostic laboratory tests

A tuberculin skin test is done to find people who have tuberculosis. The test is done by putting a small amount of TB protein under the top layer of skin on inner forearm - the tuberculin test. In an individual who has not had contact with *Mycobacterium*, there's no reaction. An individual who has had a primary infection with tubercle bacilli develops induration, edema, and erythema in 24-48 hours. The skin test should be read 48 or 72 hours. A positive tuberculin test indicates that an individual has been infected in the past or continue to carry viable *Mycobacterium* in some tissues. It does not imply that active disease or immunity to disease is present. Tuberculin- positive persons are at risk of developing disease from reactivation of the primary infection

Acid fast staining of clinical material, followed by smear microscopy, remains the most frequently used microbiological test for detection of TB. Fluorescence microscopy with auramine-rhodamine stain is more sensitive than acid-fast stain.

Culture of *M. tuberculosis* can be performed using selective agar media (eg, Lowenstein-Jensen or middlebrook 7H10/7H11). Incubation is at 37 °C in 5-10% CO_2 for up to 8 weeks. It is medically important to characterize and separate *M. tuberculosis* from all other species of *Mycobacterium*.

Conventional methods for identification of *Mycobacterium* include observation of rate of growth, colony morphology, pigmentation, and biochemical profiles. Growth rate separates the rapid growers < 7 days, from other mycobacteria.

Molecular probes provide a rapid, sensitive, and specific method for identification of mycobacteria. The polymerase chain reaction holds great promise for the rapid and direct detection of *M. tuberculosis* in clinical specimens - the PCR test is approved for this use.

Immunity

The live attenuated strain of *M. bovis* known as bacillus Calmette-Guérin (BCG) uses shared antigens to stimulate the development of cross-immunity to *M. tuberculosis* and *M. leprae.*

BCG vaccine contains a live attenuated strain derived from *M. bovis*. Studies of the effectiveness of BCG vaccine range from no protection to 70-80% protection. However, the vaccine is 70-80% effective against the most severe forms of the disease, such as TB meningitis in children. It is less effective in preventing respiratory disease, which is the more common form in adults.

BCG vaccine must be administered intradermally, normally into the lateral aspect of the left upper arm at the level of the insertion of the deltoid muscle. The left arm is recommended by the World Health Organization.

No further immunisation should be given in the arm used for BCG immunisation for at least three months because of the risk of regional lymphadenitis. BCG should ideally be given at the same time as other live vaccines such as measles, mumps and rubella (MMR). If live vaccines cannot be administered simultaneously, a four-week interval is recommended. A single dose should be given of:

0,05 ml for infants under 12 months;

0,1 ml for children aged 12 months or older and adults.

BCG is ineffective at protecting against adult pulmonary tuberculosis in the parts of the world where a good vaccine is most needed. Many new vaccines are in development. The new candidates include live attenuated *M. tuberculosis*, recombinant BCG, DNA vaccines and fusion proteins with novel adjuvants, and all aim to provide a stronger and longer-lasting immune response in heterogeneous populations.

Treatment

Because administration of a single drug often leads to the development of a bacterial population resistant to that drug, effective regimens for the treatment of TB must contain multiple drugs to which the organisms are susceptible. Tuberculosis is usually treated with four different antimicrobial agents. The course of drug therapy usually lasts from 6-9 months. The most commonly used drugs are rifampin (RIF) isoniazid (INH), pyrazinamide a (PZA) and ethambutol (EMB) or streptomycin (SM).

Epidemiology, Prevention & Control

The most frequent source of infection is the human who excretes, particularly from the respiratory tract, large numbers of tubercle bacilli. Close contact (eg, in the family) and massive exposure (eg, in medical personnel) make transmission by droplet nuclei most likely.

Susceptibility to tuberculosis is a function of the risk of acquiring the infection and the risk of clinical disease after infection has occurred. For the tuberculin-negative person, the risk of acquiring tubercle bacilli depends on exposure to sources of infectious bacilli - principally sputum-positive patients. This risk is proportionate to the rate of active infection in the population, crowding, socioeconomic disadvantage, and inadequacy of medical care.

Prevention:

Promote and effective treatment of patients with active tuberculosis

Drug treatment of asymptomatic tuberculin-positive persons (eg, children)-receive immunosuppressive drugs

Nonspecific factors may reduce host resistance include starvation, gastrectomy, and suppression of cellular immunity by drugs

Immunization: various living avirulent tubercle bacilli, particularly BCG (Bacillus Calmette-Guerin-an attenuated bovine organism). Vaccination is a substitute for primary infection with virulent tubercle bacilli without the danger inherent in the latter given to children

The eradication of tuberculosis in cattle and the pasteurization of milk have greatly reduced *M. bovis* infections.

Mycobacterium leprae

Morphology & Identification

A. MORPHOLOGY

The etiological agent of leprosy is *M. leprae*. It is a strongly acid-fast rod-shaped organism with parallel sides and rounded ends. In size and shape it closely resembles the tubercle bacillus. It occurs in large numbers in the lesions of lepromatous leprosy, chiefly in masses within the lepra cells, often grouped together like

bundles of cigars or arranged in a palisade. Chains are never seen. Most striking are the intracellular and extracellular masses, known as globi, which consist of clumps of bacilli in capsular material. Under the electron microscope the bacillus appears to have a great variety of forms. The commonest is a slightly curved filament 3-10 m in length containing irregular arrangements of dense material sometimes in the shape of rods. Short rod-shaped structures can also be seen (identical with the rod-shaped inclusions within the filaments) and also dense spherical forms. Some of the groups of bacilli can be seen to have a limiting membrane.

B. VIRULANCE FACTORS

The bacteria express many virulence factors that allow for invasion of the nerve cells and nutritional access.

Iron utilization

Although some genes from the closely related *M. tuberculosis* have been deleted in *M. leprae,* some iron utilization genes have been conserved to help the pathogen acquire nutrients for growth. NRAMP proteins, which are coded by one particular conserved gene, allow transportation of iron into the macrophage for survival.

Waxy exterior

Bacteria of the *Mycobacterium* Genus are defined by their waxy exterior coat. In *M. leprae*, the exterior allows for intake into the macrophage and into some dendritic cells, in which it can survive. The terminal mannose caps on the waxy mycobacterial ligand, lipoarabinomannan, of the pathogen are recognized by the PRR of the macrophage to allow for phagocytosis.

Macrophage invasion

M. leprae survives and replicates in macrophages, dividing to approximately 100 organisms per cell. The bacteria prevent phagosome and lysosome fusion to avoid degradation. In the event that the bacteria are absorbed into the phagolysosome, *M. leprae* has the ability to survive emission of reactive oxygen species.

Schwann cell invasion

The major target of *M. leprae* is the Schwann cell. The optimal temperature of the bacteria corresponds to the temperature in the peripheral nerves. To access the cells, *M. leprae* gets into the lymphatic system and the blood vessels. Once in the area, *M. leprae* binds to the Schwann cell via laminin-binding protein. The bacteria are thought to then enter through the vascular epithelium into the cell. The infection remains localized to the peripheral nervous system by rolling and binding to exposed Schwann cells.

Drug resistance

M. leprae has many mechanisms of drug resistance to allow it to continue to survive despite antimicrobial presence.

Pathogenesis, Pathology & Clinical Findings

Leprosy infection presents in a continuum, ranging from the mildest indeterminate form to the most severe lepromatous type. Symptoms and physical findings vary depending on the stage of disease and level of infection. Symptoms of leprosy are generally so slight that the disease is not recognized until a cutaneous eruption is present. 90% of patient present with numbness first, sometimes years before the skin lesions appear. Temperature is the first sensation that is lost. Patients cannot sense extremes of hot or cold. The next sensation lost is light touch, then pain, and finally deep pressure. These losses are especially apparent in the hands and feet. A hypopigmented macule is often the first cutaneous lesion.

M. leprae replicates intracellularly inside histocytes and nerve cells and has two forms. One form is tuberculoid, which induces a cell-mediated response that limits its growth. Through this form *M. leprae* multiplies at the site of entry, usually the skin, invading and colonizing Schwann cells. The microbe then induces T-helper lymphocytes, epitheloid cells, and giant cell infiltration of the skin, causing infected individuals to exhibit large flattened patches with raised and elevated red edges on their skin. These patches have dry, pale, hairless centers, accompanied by a loss of sensation on the skin. The loss of sensation may develop as a result of invasion of the peripheral sensory nerves. The macule at the cutaneous site of entry and the loss of pain sensation are key clinical indications that an individual has a tuberculoid form of leprosy.

The second form of leprosy is the lepromatous form. This form of the microbe proliferates within the macrophages at the site of entry. It also grows within the epithelial tissues of the face and ear lobes. The suppressor T-cells that are induced are numerous, however the epithelioid and giant cells are rare or absent. With cell-mediated immunity impaired, large numbers of *M. leprae* appear in the macrophages and the infected patients develop papules at the entry site, marked by a folding of the skin. Gradual destruction of cutaneous

nerves lead to what is referred to as «classic lion face»**.** Extensive penetration of this microbe may lead to severe body damage; for example the loss of bones, fingers, and toes.

Diagnostic laboratory tests

Laboratory studies include the following:

- Skin biopsy, nasal smears, or both are used to assess for acid-fast bacilli using Fite stain. Biopsies should be full dermal thickness taken from an edge of the lesion that appears most active.

- Serologic assays can be used to detect phenolic glycolipid-1 (specific for *M. leprae*) and lipoarabinomannan (commonly seen in mycobacteria).

- Molecular probes detect 40-50% of cases missed on prior histologic evaluation. Since probes require a minimum amount of genetic material (ie, 104 DNA copies), they can fail to identify paucibacillary leprosy.

Immunity

Science has discovered that over 95 percent of people could be immune to leprosy, due to protection and exposure. However, some populations may be more susceptible to the disease based on genetic factors. The PARK2/PACRG gene could contribute to susceptibility to *M. leprae* infection. In addition, the Vitamin D receptor (VDR) gene is associated with both polar types of the disease. Innate immunity can fail in an instance of susceptibility, so the adaptive immune response works to eradicate the infection through T cells.

Treatment

The current standard treatment for *M. leprae* infection is a Multi-drug Treatment (MDT) which consists of corticosteroids and antimicrobials. For paucibacillary leprosy, rifampicin and dapsone are used, while rifampicin, clofazimine, and dapsone are used in multibacillary leprosy. Rifampicin, ofloxacin, and minocycline can be combined in single lesion paucibacillary leprosy. Oral prednisolone can be used in secondary complications such as neuropathy and eye problems. Drops can be used to dilate the eye and stimulate relaxation to help the healing process. Minocycline or ofloxacin can be used in the event of a rifampicin allergy, resistance, or presence of a disease antagonistic to rifampicin. Interestingly, the disease can sometimes be self-limiting and cure itself independently of drug treatment.

Epidemiology, Prevention & Control

Transmission of leprosy is most likely to occur when small children are exposed for prolonged periods to heavy shedders of bacilli. Nasal secretions are the most likely infectious material for family contacts. The incubation period is probably 2-10 years. Without prophylaxis, about 10% of exposed children may acquire the disease. Treatment tends to reduce and abolish the infectivity of patients.

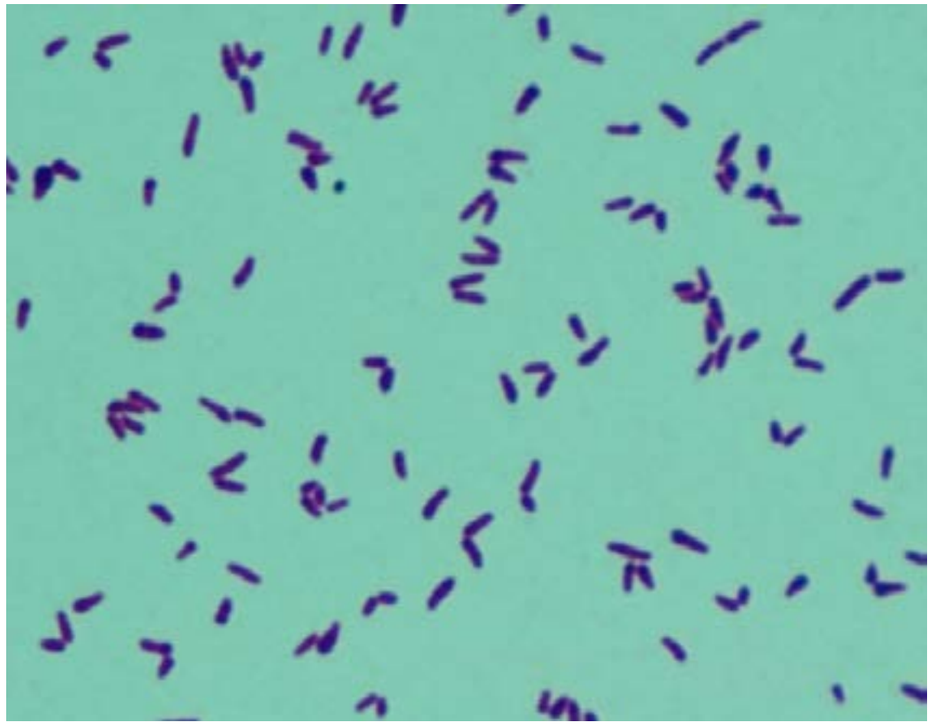
VACCINATION

A proper vaccination for *M. leprae* would provide protective immunity. One vaccine has shown positive results in protecting the inoculated person from infection. The *M. bovis* BCG vaccine proved effective in India and Brazil.

ERADICATION ATTEMPTS

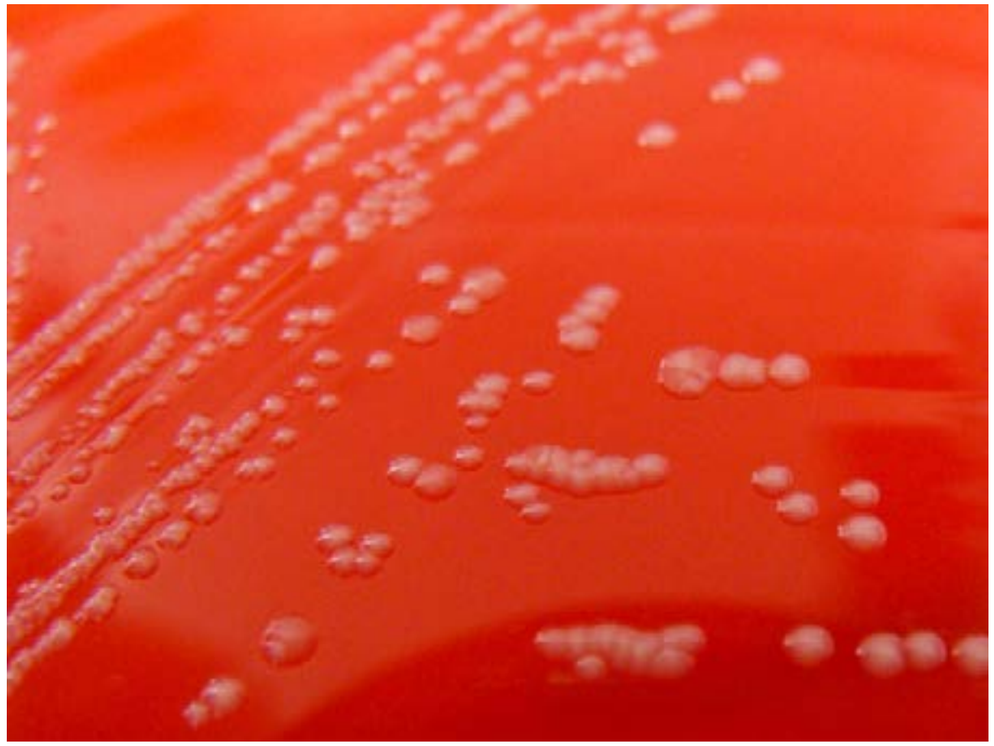
Global efforts to eradicate leprosy has resulted in elimination of the disease from 119 of 122 countries thought to be highly affected. With proper prevention measures, early detection, and treatment, numbers of new cases would decrease and those already infected could be cured.

9 Class – Illustrations



Corynebacterium diphtheriae (light microscopy)

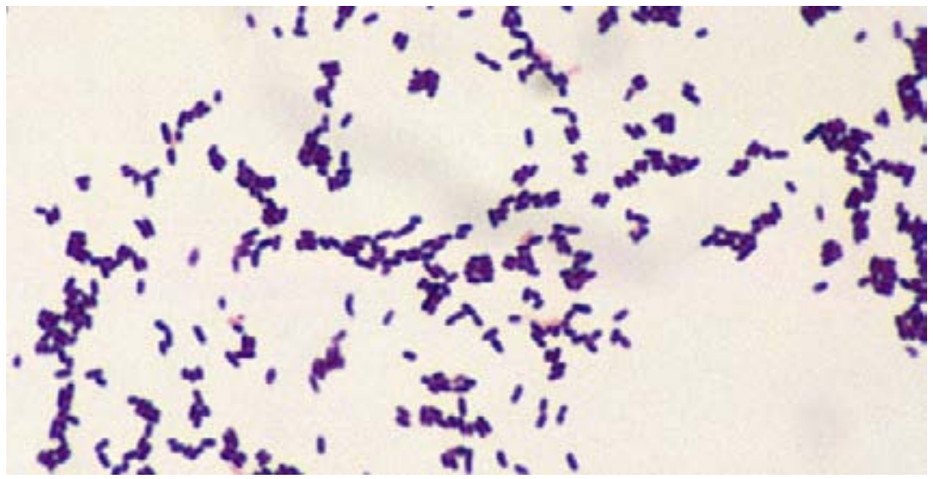
C. diphtheriae on tellurite agar



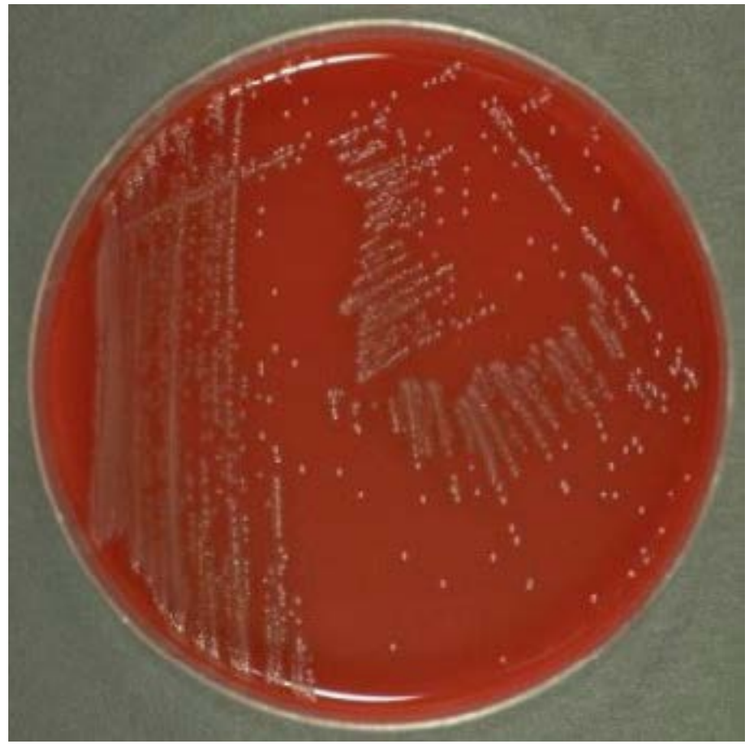
C. diphtheriae on blood agar

Diphtheria

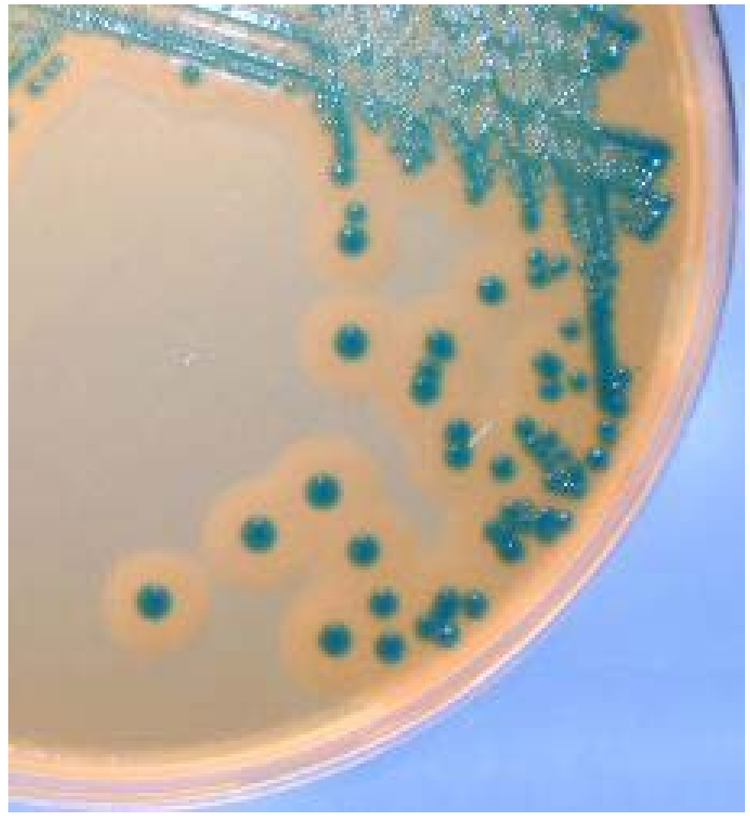
Loeffler's serum for *C. diphtheriae*



Listeria monocytogenes (Gram-stain)

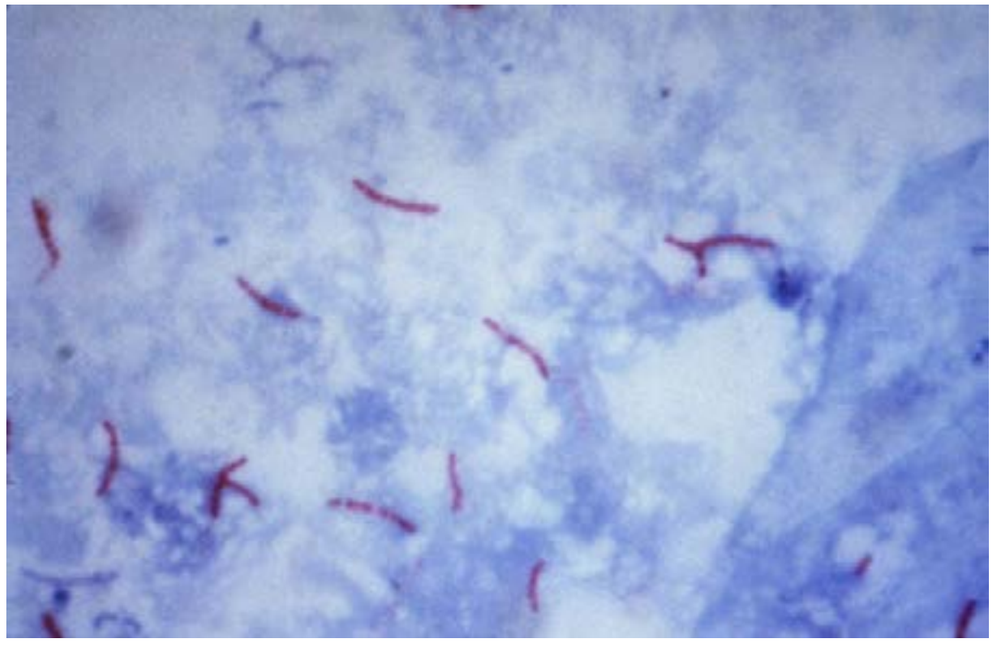


Listeria on blood agar



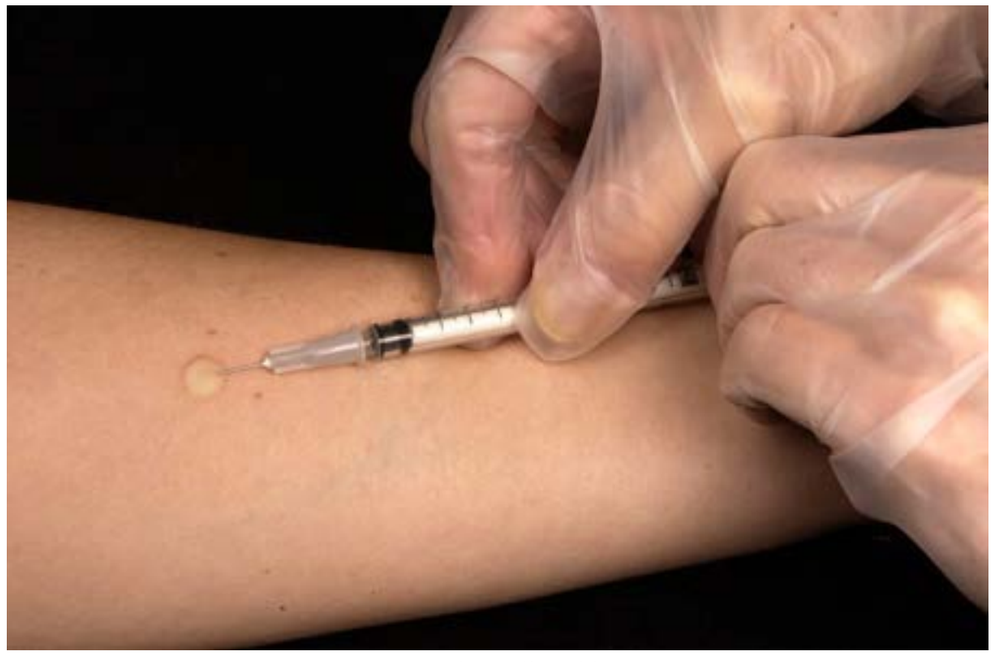
L. monocytogenes on Listeria Chromogenic Agar (ISO)

Listeria Latex Test, latex slide agglutination test for isolated colonies



Mycobacterium tuberculosis (Ziehl-Neelsen stain)

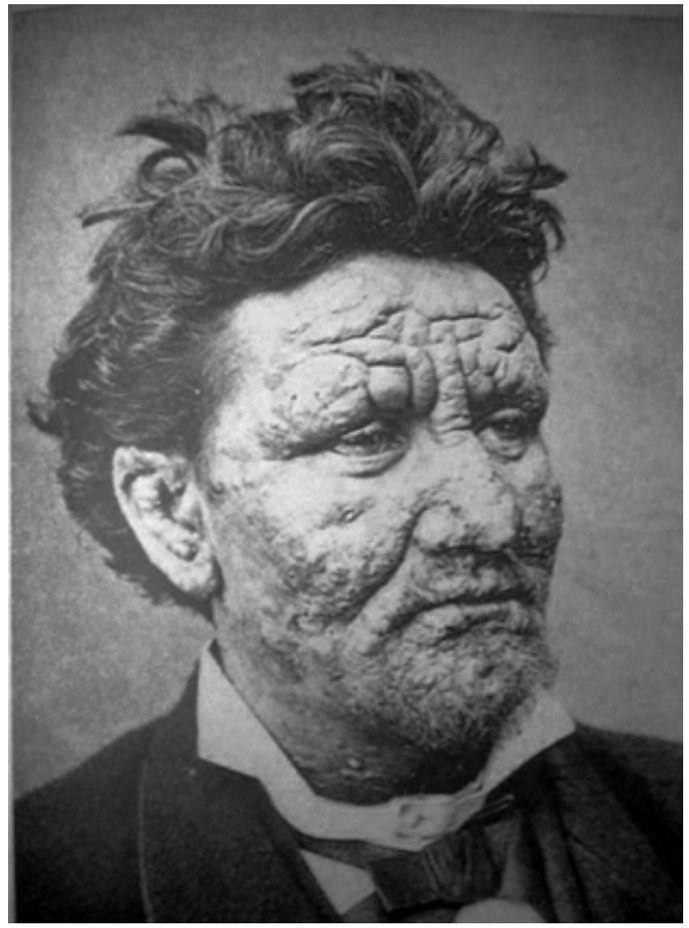
M. tuberculosis (Lowenstein Jensen Medium)



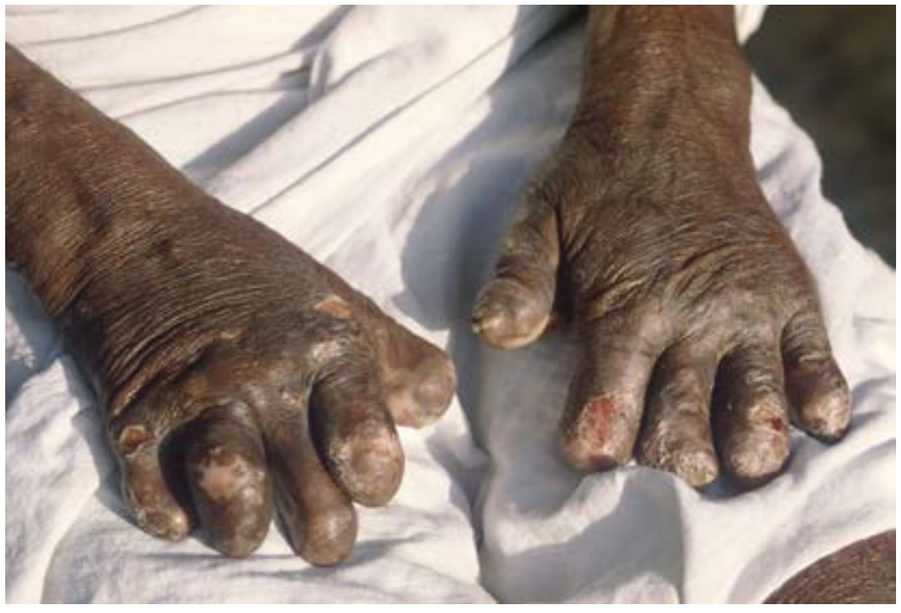
Mantoux tuberculin (skin test)

BCG vaccine

Scanning electron micrograph of *Mycobacterium leprae*



Leprosy



Hands deformed by leprosy

10 Class - Pathogenic spirochetes. *Treponema*. Non venereal forms of syphilis. Bejel, pints, frambesia. Pathogenic *Leptospira*. *Borrelia*. Lyme disease

Pathogenic spirochetes

The spirochetes are a large, heterogeneous group of spiral, motile bacteria. One family (Spirochaetaceae) of the order Spirochaetales consists of three genera of free-living, large spiral organisms. The other family (*Treponemataceae*) includes three genera whose members are human pathogens: (1) *Treponema*, (2) *Borrelia*, and (3) *Leptospira*.

The spirochetes have many structural characteristics in common, as typified by *Treponema pallidum.* They are long, slender, helically coiled, spiral or corkscrewshaped, Gram-negative bacilli. *T. pallidum* has an outer sheath or glycosaminoglycan coating. Inside the sheath is the outer membrane, which contains peptidoglycan and maintains the structural integrity of the organisms. Endoflagella (axial filaments) are the flagella-like organelles in the periplasmic space encased by the outer membrane. The endoflagella begin at each end of the organism and wind around it, extending to and overlapping at the midpoint. Inside the endoflagella is the inner membrane (cytoplasmic membrane) that provides osmotic stability and covers the protoplasmic cylinder. A series of cytoplasmic tubules (body fibrils) are inside the cell near the inner membrane. Treponemes reproduce by transverse fission.

Treponema pallidum pallidum

Scientific classification

Kingdom: Bacteria
Phylum: Spirochaetes
Order: Spirochaetales
Family: Spirochaetaceae
Genus: Treponema
Species: T. pallidum pallidum

Morphology & Identification

A. MORPHOLOGY

Slender spirals measuring about 0,2 μm in width and 5-15 μm in length. The spiral coils are regularly spaced at a distance of 1 μm from one another. The organisms are actively motile, rotating steadily around their endoflagella even after attaching to cells by their tapered ends. The long axis of the spiral is ordinarily straight but may sometimes bend, so that the organism forms a complete circle for moments at a time, returning then to its normal straight position.

The spirals are so thin that they are not readily seen unless immunofluorescent stain or darkfield illumination is employed. They do not stain well with aniline dyes, but they can be seen in tissues when stained by a silver impregnation method.

B. CULTURE AND GROWTH CHARACTERISTICS

Pathogenic *T. pallidum* has never been cultured continuously on artificial media, in fertile eggs, or in tissue culture. Nonpathogenic treponemes (eg, Reiter strain) can be cultured anaerobically in vitro. They are saprophytes antigenically related to *T. pallidum.*

T. pallidum is a microaerophilic organism; it survives best in 1-4% oxygen. The saprophytic Reiter strain grows on a defined medium of 11 amino acids, vitamins, salts, minerals, and serum albumin.

In proper suspending fluids and in the presence of reducing substances, *T. pallidum* may remain motile for 3-6 days at 25 °C. In whole blood or plasma stored at 4 °C, organisms remain viable for at least 24 hours, which is of potential importance in blood transfusions.

Drying kills the spirochete rapidly, as does elevation of the temperature to 42 °C. Treponemes are rapidly immobilized and killed by trivalent arsenical, mercury, and bismuth (contained in drugs of historical interest in the treatment of syphilis).

C. VIRULANCE FACTORS

The fact that *T. pallidum* cannot be cultured in vitro has markedly limited the characterization of its antigens. The outer membrane surrounds the periplasmic space and the peptidoglycan-cytoplasmic membrane complex. Membrane proteins are present that contain covalently bound lipids at their amino terminals. The

lipids appear to anchor the proteins to the cytoplasmic or outer membranes and keep the proteins inaccessible to antibodies.

The endoflagella are in the periplasmic space. *T. pallidum* subspecies *pallidum* has hyaluronidase that breaks down the hyaluronic acid in the ground substance of tissue and presumably enhances the invasiveness of the organism. The protein profiles of *T. pallidum* (all the subspecies) are indistinguishable; more than 100 protein antigens have been noted. The endoflagella are composed of three core proteins that are homologous to other bacterial flagellin proteins, plus an unrelated sheath protein. Cardiolipin is an important component of the treponemal antigens.

Pathogenesis, Pathology & Clinical Findings

A. ACQUIRED SYPHILIS

Natural infection with *T. pallidum* is limited to the human host. Human infection is usually transmitted by sexual contact, and the infectious lesion is on the skin or mucous membranes of genitalia. In 10-20% of cases, however, the primary lesion is intrarectal, perianal, or oral. It may be anywhere on the body. *T. pallidum* can probably penetrate intact mucous membranes, or it may enter through a break in the epidermis.

Spirochetes multiply locally at the site of entry, and some spread to nearby lymph nodes and then reach the bloodstream. In 2-10 weeks after infection, a papule develops at the site of infection and breaks down to form an ulcer with a clean, hard base («hard chancre»). The inflammation is characterized by a predominance of lymphocytes and plasma cells. This «primary lesion» always heals spontaneously, but 2-10 weeks later the «secondary» lesions appear. These consist of a red maculopapular rash anywhere on the body, including the hands and feet, and moist, pale papules (condylomas) in the anogenital region, axillas, and mouth. There may also be syphilitic meningitis, chorioretinitis, hepatitis, nephritis (immune complex type), or periostitis. The secondary lesions also subside spontaneously. Both primary and secondary lesions are rich in spirochetes and highly infectious. Contagious lesions may recur within 3-5 years after infection, but thereafter the individual is not infectious. Syphilitic infection may remain subclinical, and the patient may pass through the primary or secondary stage (or both) without symptoms or signs yet develop tertiary lesions.

In about 30% of cases, early syphilitic infection progresses spontaneously to complete cure without treatment. In another 30%, the untreated infection remains latent (principally evident by positive serologic tests). In the remainder, the disease progresses to the «tertiary stage», characterized by the development of granulomatous lesions (gummas) in skin, bones, and liver; degenerative changes in the central nervous system (meningovascular syphilis, paresis, tabes); or cardiovascular lesions (aortitis, aortic aneurysm, aortic valve insufficiency). In all tertiary lesions, treponemes are very rare, and the exaggerated tissue response must be attributed to hypersensitivity to the organisms. However, treponemes can occasionally be found in the eye or central nervous system in late syphilis.

B. CONGENITAL SYPHILIS

A pregnant syphilitic woman can transmit *T. pallidum* to the fetus through the placenta beginning in the 10th to 15th weeks of gestation. Some of the infected fetuses die, and miscarriages result; others are stillborn at term. Others are born live but develop the signs of congenital syphilis in childhood: interstitial keratitis, Hutchinson's teeth, saddlenose, periostitis, and a variety of central nervous system anomalies. Adequate treatment of the mother during pregnancy prevents congenital syphilis. The reagin titer in the blood of the child rises with active infection but falls with time if antibody was passively transmitted from the mother. In congenital infection, the child makes IgM antitreponemal antibody.

Diagnostic laboratory tests

A. SPECIMENS

Speciments include tissue fluid expressed from early surface lesions for demonstration of spirochetes; blood serum for serologic tests.

B. DARKFIELD EXAMINATION

A drop of tissue fluid or exudate is placed on a slide and a coverslip pressed over it to make a thin layer. The preparation is then examined under oil immersion with darkfield illumination for typical motile spirochetes. Treponemes disappear from lesions within a few hours after the beginning of antibiotic treatment.

C. IMMUNOFLUORESCENCE

Tissue fluid or exudate is spread on a glass slide, air dried, and sent to the laboratory. It is fixed, stained with a fluorescein-labeled antitreponeme serum, and examined by means of immunofluorescence microscopy for typical fluorescent spirochetes.

D. SEROLOGIC TESTS FOR SYPHILIS (STS)

These tests use either nontreponemal or treponemal antigens.

1. Nontreponemal Antigen Tests

The antigens employed are lipids extracted from normal mammalian tissue. The purified cardiolipin from beef heart is a diphosphatidylglycerol. Lecithin and cholesterol are added to enhance reaction with syphilitic «reagin» antibodies. Reagin is a mixture of IgM and IgG antibodies directed against the cardiolipin-cholesterol-lecithin complex. The VDRL (Venereal Disease Research Laboratory) and RPR (rapid plasma reagin) tests are nontreponemal antigen tests used most commonly. The toluidine red unheated serum test (TRUST) also is available. All of the tests are based on the fact that the particles of the lipid antigen remain dispersed with normal serum but flocculate when combining with reagin. The VDRL test requires microscopic examination to detect flocculation, whereas the RPR and TRUST have added colored particles and can be read without microscopic magnification. Results develop within a few minutes, particularly if the suspension is agitated. The tests lend themselves to automation and to use for surveys because of their low cost.

Positive VDRL or RPR tests develop after 2-3 weeks of untreated syphilitic infection and are positive in high titer in secondary syphilis. Positive VDRL or RPR tests revert to negative in 6-18 months after effective treatment of syphilis. The VDRL test can also be performed on spinal fluid and becomes positive after 4-8 weeks of infection. Reagin antibodies do not reach the cerebrospinal fluid from the bloodstream but are probably formed in the central nervous system in response to syphilitic infection.

The flocculation tests can give quantitative results. An estimate of the amount of reagin present in serum can be made by performing the tests with twofold dilutions of serum and expressing the titer as the highest dilution that gives a positive result. Quantitative results are valuable in establishing a diagnosis - especially in neonates - and in evaluating the effect of treatment.

Nontreponemal tests are subject to «biologic» falsepositive results attributable to the occurrence of «reagins» in a variety of human disorders. Prominent among them are other infections (malaria, leprosy, measles, infectious mononucleosis, etc), vaccinations, collagen-vascular diseases (systemic lupus erythematosus, polyarteritis nodosa, rheumatic disorders), and other conditions.

2. Treponemal Antibody Tests

- FLUORESCENT TREPONEMAL ANTIBODY (FTA-ABS) TEST

This is a test employing indirect immunofluorescence (killed *T. pallidum* + patient's serum + labeled antihuman gamma globulin). It shows excellent specificity and sensitivity for syphilis antibodies if the patient's serum has been absorbed with sonicated Reiter spirochetes prior to the FTA test. The FTA-ABS test is the first to become positive in early syphilis, is routinely positive in secondary syphilis, and usually remains positive many years after effective treatment. The test thus cannot be used to judge the efficacy of treatment. The presence of IgM FTA in the blood of newborns is good evidence of in utero infection (congenital syphilis).

- TREPONEMA PALLIDUM-PARTICLE AGGLUTINATION (TP-PA) TEST

These are the *T. pallidum* hemagglutination (TPHA) and microhemagglutination for *T. pallidum* (MHA-TP) tests. Particles are sensitized with *T. pallidum* subspecies *pallidum* antigens. The test is performed with diluted serum. Antibodies against *T. pallidum* react with the sensitized particles. A mat of agglutinated particles indicates a positive result. These tests are similar to the FTA-ABS test in specificity and sensitivity.

Immunity

A person with active or latent syphilis or yaws appears to be resistant to superinfection with *T. pallidum.* However, if early syphilis or yaws is treated adequately and the infection is eradicated, the individual again becomes fully susceptible. The various immune responses usually fail to eradicate the infection or arrest its progression.

Treatment

Penicillin in concentrations of 0,003 unit/mL has definite treponemicidal activity, and penicillin is the treatment of choice. Syphilis of less than 1 year's duration is treated by a single injection of benzathine penicillin G intramuscularly. In older or latent syphilis, benzathine penicillin G intramuscularly is given three times at weekly intervals. In neurosyphilis, the same therapy is acceptable, but larger amounts of intravenous penicillin are sometimes recommended. Other antibiotics, eg, tetracyclines or erythromycin, can occasionally be substituted. Treatment of gonorrhea is thought to cure incubating syphilis. Prolonged follow-up is essential. In neurosyphilis, treponemes occasionally survive such treatment. Severe neurologic relapses of treated syphilis have occurred in patients with acquired immunodeficiency syndrome (AIDS) who are infected with

both HIV and *T. pallidum*. A typical Jarisch-Herxheimer reaction may occur within hours after treatment is begun. It is due to the release of toxic products from dying or killed spirochetes.

Epidemiology, Prevention & Control

With the exceptions of congenital syphilis and the rare occupational exposure of medical personnel, syphilis is acquired through sexual exposure. Reinfection in treated persons is common. An infected person may remain contagious for 3-5 years during «early» syphilis. «Late» syphilis, of more than 5 years' duration, is usually not contagious. Consequently, prevention measures depend on (1) prompt and adequate treatment of all discovered cases; (2) follow-up on sources of infection and contacts so they can be treated; and (3) safe sex with condoms. Several sexually transmitted diseases can be transmitted simultaneously. Therefore, it is important to consider the possibility of syphilis when any one sexually transmitted disease has been found.

DISEASES RELATED TO SYPHILIS

These diseases are all caused by treponemes closely related to *T. pallidum.* All give positive treponemal and nontreponemal serologic tests for syphilis, and some cross-immunity can be demonstrated in experimental animals and perhaps in humans. None are sexually transmitted diseases; all are commonly transmitted by direct contact. None of the causative organisms have been cultured on artificial media.

BEJEL

Bejel (due to *T. pallidum* subspecies *endemicum*) occurs chiefly in Africa but also in the Middle East, in Southeast Asia, and elsewhere, particularly among children, and produces highly infectious skin lesions; late visceral complications are rare.

Bejel affects the mucous membranes of the mouth, then the skin and bones. The initial mouth sore may not be noticed. Moist patches then develop in the mouth. They resolve over a period of months to years. During this time, people have few or no symptoms. Then, sores may develop on the trunk and limbs.

Lumps develop in long bones, mainly leg bones, and in the tissues around the mouth, nose, and roof of the mouth (palate). These lumps destroy tissue, causing bones to be deformed and disfiguring the face. Penicillin is the drug of choice.

YAWS

Yaws is endemic, particularly among children, in many humid, hot tropical countries. It is caused by *T. pallidum* subspecies *pertenue.* The primary lesion, an ulcerating papule, occurs usually on the arms or legs. Transmission is by person-to-person contact in children under age 15. Transplacental, congenital infection does not occur. Scar formation of skin lesions and bone destruction are common, but visceral or nervous system complications are very rare. It has been debated whether yaws represents a variant of syphilis adapted to transmission by nonsexual means in hot climates. There appears to be cross-immunity between yaws and syphilis. Diagnostic procedures and therapy are similar to those for syphilis. The response to penicillin treatment is dramatic.

PINTA

Pinta is caused by *T. pallidum* subspecies *carateum* and occurs endemically in all age groups in Mexico, Central and South America, the Philippines, and some areas of the Pacific. The disease appears to be restricted to darkskinned races. The primary lesion, a nonulcerating papule, occurs on exposed areas. Some months later, flat, hyperpigmented lesions appear on the skin; depigmen tation and hyperkeratosis take place years afterward. Late cardiovascular and nervous system involvement occurs very rarely. Pinta is transmitted by nonsexual means, either by direct contact or through the agency of flies or gnats. Diagnosis and treatment are the same as for syphilis.

Pathogenic *Leptospira*

Scientific classification

Kingdom: Bacteria
Phylum: Spirochaetes
Order: Spirochaetales
Family: Leptospiraceae
Genus: Leptospira
Species: L. interrogans, L. biflexa

The traditional classification system is based on biochemical and serologic specificity to differentiate between the pathogenic species, *Leptospira interrogans,* and the free-living nonpathogenic species, *Leptospira*

biflexa. The species are further broken down to more than 200 serovars of *L. interrogans* and more than 60 serovars of *L. biflexa.* The serovars are further organized into serogroups of *L. interrogans* and serogroups of *L. biflexa.* The serogroups are based on shared antigenicity and are primarily for laboratory use. A second classification system is based on DNA-DNA hybridization studies, which have demonstrated a high degree of heterogeneity within the two species of the traditional classification. There are three genera in the DNA relatedness classification: *Leptospira* and two nonpathogenic genera. The genus *Leptospira* contains several species of pathogens and nonpathogens that do not correspond to the species in the traditional classification.

Morphology & Identification

A. MORPHOLOGY

Leptospira are tightly coiled, thin, flexible spirochetes 5-15 μm long, with very fine spirals 0,1-0,2 μm wide; one end is often bent, forming a hook. They are motile, with hooked ends and paired axial flagella (one on each end), enabling them to burrow into tissue. Motion is marked by continual spinning on the long axis.

B. CULTURE AND GROWTH CHARACTERISTICS

Leptospira grow best under aerobic conditions at 28-30 °C in serum-containing semisolid media (Fletcher's, Stuart's, others). After 1-2 weeks the *Leptospira* produce a diffuse zone of growth near the top of the tube and later a ring of growth at a level in the tube corresponding to the level of the optimal oxygen tension for the organisms. The culture media can be made selective for *Leptospira* by addition of neomycin or 5-fluorouracil.

Leptospira derive energy from oxidation of long-chain fatty acids and cannot use amino acids or carbohydrates as major energy sources. Ammonium salts are a main source of nitrogen. *Leptospira* can survive for weeks in water, particularly at alkaline pH.

Like most bacteria, *Leptospira* require iron for growth. *L. interrogans* and *L. biflexa* have the ability to acquire iron in different forms. A TonB-dependent receptor required for utilization of the ferrous form of the iron has been identified in *L. biflexa*, and an ortholog of the receptor is encoded in the genome of *L. interrogans*. *L. interrogans* can also obtain iron from heme, which is bound to most of the iron in the human body. The HbpA hemin-binding protein, which may be involved in the uptake of hemin, has been identified on the surface of *L. interrogans*. Although other pathogenic species of *Leptospira* and *L. biflexa* lack HbpA, yet another hemin-binding protein, LipL41, may account for their ability to use hemin as a source of iron. Although they do not secrete siderophores, *L. biflexa* and *L. interrogans* may be capable of obtaining iron from siderophores secreted by other microorganisms.

C. VIRULANCE FACTORS

The main strains («serovars») of *L. interrogans* isolated from humans or animals in different parts of the world are all serologically related and exhibit cross-reactivity in serologic tests. This indicates considerable overlapping in antigenic structure, and quantitative tests and antibody absorption studies are necessary for a specific serologic diagnosis. The outer envelope contains large amounts of lipopolysaccharide (LPS) of antigenic structure that is variable from one strain to another. This variation forms the basis for the serologic classification of the *Leptospira* species. It also determines the specificity of the human immune response to *Leptospira*.

Pathogenesis, Pathology & Clinical Findings

Human infection usually results from leptospires, often in bodies of water, entering the body through breaks in the skin (cuts and abrasions) and mucus membranes (mouth, nose, conjunctivae). Ingestion is considered to be less important. After an incubation period of 1-2 weeks, there is a variable febrile onset during which spirochetes are present in the bloodstream. They then establish themselves in the parenchymatous organs (particularly liver and kidneys), producing hemorrhage and necrosis of tissue and resulting in dysfunction of those organs (jaundice, hemorrhage, nitrogen retention). The illness is often biphasic. After initial improvement, the second phase develops when the IgM antibody titer rises. It manifests itself often as «aseptic meningitis» with intense headache, stiff neck, and pleocytosis of the cerebrospinal fluid. Nephritis and hepatitis may also recur, and there may be skin, muscle, and eye lesions. The degree and distribution of organ involvement vary in the different diseases produced by different *Leptospira* in various parts of the world. Many infections are mild or subclinical. Hepatitis is frequent in patients with leptospirosis. It is often associated with elevation of serum creatine phosphokinase, whereas that enzyme is present in normal concentrations in viral hepatitis.

Kidney involvement in many animal species is chronic and results in the shedding of large numbers of *Leptospira* in the urine; this is probably the main source of environmental contamination resulting in infection

of humans. Human urine also may contain spirochetes in the second and third weeks of disease.

Agglutinating, complement-fixing, and lytic antibodies develop during the infection. Serum from convalescent patients protects experimental animals against an otherwise fatal infection. The immunity resulting from infection in humans and animals appears to be serovar-specific.

Diagnostic laboratory tests

A. SPECIMENS

Specimens consist of aseptically collected blood in a heparin tube, cerebrospinal fluid, or tissues for microscopic examination and culture. Urine should be collected using great care to avoid contamination. Serum is collected for agglutination tests.

B. MICROSCOPIC EXAMINATION

Darkfield examination or thick smears stained by the Giemsa technique occasionally show *Leptospira* in fresh blood from early infections. Darkfield examination of centrifuged urine may also be positive. Fluorescein-conjugated antibodies or other immunohistochemical techniques can be used also.

C. CULTURE

Whole fresh blood or urine can be cultured in Fletcher's semisolid or other medium. Selective and nonselective media should be used. Because of inhibitory substances in blood, only 1 or 2 drops should be placed in each of five tubes containing 5 mL of medium. Up to 0,5 mL of cerebrospinal fluid can be used. One drop of undiluted urine can be used followed by 1 drop each of tenfold serially diluted urine - for a total of four tubes. Tissue approximately 5 mm in diameter should be crushed and used as the inoculum. Growth is slow, and cultures should be kept for at least 8 weeks.

D. ANIMAL INOCULATION

A sensitive technique for the isolation of *Leptospira* consists of the intraperitoneal inoculation of young hamsters or guinea pigs with fresh plasma or urine. Within a few days, spirochetes become demonstrable in the peritoneal cavity; on the death of the animal (8-14 days), hemorrhagic lesions with spirochetes are found in many organs.

E. SEROLOGY

Agglutinating antibodies attaining very high titers (1:10,000 or higher) develop slowly in leptospiral infection, reaching a peak 5-8 weeks after infection. The reference laboratory standard for detection of leptospiral antibody uses microscopic agglutination of live organisms. It is highly sensitive but can be hazardous. Agglutination of the live suspensions is most specific for the serovar of the infecting *Leptospira* Indirect hemagglutination of red blood cells with adsorbed *Leptospira* is sometimes used. A variety of enzyme immunoassays have also been described. Because of geographic variation in the distribution of serovars, the tests may have different sensitivities and specificities in different geographic areas.

Immunity

Serovar-specific immunity follows infection, but reinfection with different serovars may occur.

Treatment

Treatment of mild leptospirosis should be with oral doxycycline, ampicillin, or amoxicillin. Treatment of moderate or severe disease should be with intravenous penicillin or ampicillin.

Epidemiology, Prevention & Control

The leptospiroses are essentially animal infections; human infection is only accidental, following contact with water or other materials contaminated with the excreta of animal hosts. Rats, mice, wild rodents, dogs, swine, and cattle are the principal sources of human infection. They excrete *Leptospira* in urine both during the active illness and during the asymptomatic carrier state. *Leptospira* remain viable in stagnant water for several weeks; drinking, swimming, bathing, or food contamination may lead to human infection. Persons most likely to come in contact with water contaminated by rats (eg, miners, sewer workers, farmers, and fishermen) run the greatest risk of infection. Children acquire the infection from dogs more frequently than do adults. Control consists of preventing exposure to potentially contaminated water and reducing contamination by rodent control. Doxycycline, 200 mg orally once weekly during heavy exposure, is effective prophylaxis. Dogs can receive distemper-hepatitis-leptospirosis vaccinations.

Pathogenic *Borrelia*

Scientific classification

Kingdom: Bacteria
Phylum: Spirochaetes
Order: Spirochaetales
Family: Spirochaetaceae
Genus: Borrelia
Species: B. recurrentis, B. burgdorferi

Borrelia recurrentis

Morphology & Identification

A. MORPHOLOGY

The *Borrelia* form irregular spirals 10-30 μm long and 0.3 μm wide. The distance between turns varies from 2 μm 4 μm. The organisms are highly flexible and move both by rotation and by twisting. *Borrelia* stain readily with bacteriologic dyes as well as with blood stains such as Giemsa's stain or Wright's stain.

B. CULTURE AND GROWTH CHARACTERISTICS

The organism can be cultured in fluid media containing blood, serum, or tissue; but it rapidly loses its pathogenicity for animals when transferred repeatedly in vitro. Multiplication is rapid in chick embryos when blood from patients is inoculated onto the chorioallantoic membrane.

Little is known of the metabolic requirements or activity of *Borrelia*. At 4 °C, the organisms survive for several months in infected blood or in culture. In some ticks (but not in lice), spirochetes are passed from generation to generation.

C. VIRULENCE FACTORS

The body has its way of recognizing and enhancing the recognition of foreign invaders called opsonization, which targets them for destruction through a cascade of reactions known as the complement system. Recent studies show that *B. recurrentis* expresses a multifunctional surface lipoprotein, termed HcpA, that exploits the host's proteins and offers resistance to complement attack and opsonization while increasing the potential to invade the host's tissues. Since HcpA outlines the high virulence potential of *B. recurrentis*, it makes a good target for therapeutic treatment of LBRF, however, none have been created yet. It was also found that this spirochete binds to the PLG (human plasminogen/Plasmin) receptor on endothelium cells and exploits their increased proteolytic capacity to breach tight junctions of endothelium, cross basement membranes, and to initiate patho-physiological processes in the affected organs. Another novel approach is its ability to undergo antigenic variation, meaning that once the innate immune system is able to identify and start fighting off the first antigenic type, another antigenic type appears. This impairs the host immunes system from being able to clear the infection and explains why there are multiple recurrences of fever.

Pathogenesis, Pathology & Clinical Findings

Relapsing fever in epidemic form is caused by *Borrelia recurrentis,* transmitted by the human body louse.

The incubation period is 3-10 days. The onset is sudden, with chills and an abrupt rise of temperature. During this time, spirochetes abound in the blood. The fever persists for 3-5 days and then declines, leaving the patient weak but not ill. The afebrile period lasts 4-10 days and is followed by a second attack of chills, fever, intense headache, and malaise. There are from three to ten such recurrences, generally of diminishing severity. During the febrile stages (especially when the temperature is rising), organisms are present in the blood; during the afebrile periods, they are absent.

Antibodies against the spirochetes appear during the febrile stage, and the attack is probably terminated by their agglutinating and lytic effects. These antibodies may select out antigenically distinct variants that multiply and cause a relapse. Several distinct antigenic varieties of *Borrelia* may be isolated from a single patient's sequential relapses, even following experimental inoculation with a single organism.

Diagnostic laboratory tests

A. SPECIMENS

Blood specimens are obtained during the rise in fever, for smears and animal inoculation.

B. SMEARS

Thin or thick blood smears stained with Wright's or Giemsa's stain reveal large, loosely coiled spirochetes

among the red cells.

C. ANIMAL INOCULATION

White mice or young rats are inoculated intraperitoneally with blood. Stained films of tail blood are examined for spirochetes 2-4 days later.

D. SEROLOGY

Spirochetes grown in culture can serve as antigens for CF tests, but the preparation of satisfactory antigens is difficult. Patients suffering from epidemic (louse-borne) relapsing fever may develop a positive VDRL.

Immunity

Immunity following infection is usually of short duration.

Treatment

The great variability of the spontaneous remissions of relapsing fever makes evaluation of chemotherapeutic effectiveness difficult. Tetracyclines, erythromycin, and penicillin are all believed to be effective. Treatment for a single day may be sufficient to terminate an individual attack.

Epidemiology, Prevention & Control

Relapsing fever is endemic in many parts of the world. Its main reservoir is the rodent population, which serves as a source of infection for ticks of the genus *Ornithodorus*. The distribution of endemic foci and the seasonal seasonal incidence of the disease are largely determined by the ecology of the ticks in different areas.

Spirochetes are present in all tissues of the tick and may be transmitted by the bite or by crushing the tick. The tick-borne disease is not epidemic. However, when an infected individual harbors lice, the lice become infected by sucking blood; 4-5 days later, they may serve as a source of infection for other individuals. The infection of the lice is not transmitted to the next generation, and the disease is the result of rubbing crushed lice into bite wounds. Severe epidemics may occur in louse-infected populations, and transmission is favored by crowding, malnutrition, and cold climate.

In endemic areas, human infection may occasionally result from contact with the blood and tissues of infected rodents. The mortality rate of the endemic disease is low, but in epidemics it may reach 30%.

Prevention is based on avoidance of exposure to ticks and lice and on delousing (cleanliness, insecticides).

Borrelia burgdorferi

Morphology & Identification

A. MORPHOLOGY

B. burgdorferi is a spiral organism 20-30 μm long and 0,2-0,3 μm wide. The distance between turns varies from 2 μm to 4 μm. The organisms have variable numbers (7-11) of endoflagella and are highly motile. *B. burgdorferi* stains readily with acid and aniline dyes and by silver impregnation techniques.

B. CULTURE AND GROWTH CHARACTERISTICS

B. burgdorferi grows most readily in a complex liquid medium, Barbour-Stoenner-Kelly medium (BSK II). Rifampin, fosfomycin (phosphonomycin), and amphotericin B can be added to BSK II to reduce the rate of culture contamination by other bacteria and fungi. *B. burgdorferi* has been most easily isolated from erythema migrans skin lesions; isolation of the organism from other sites has been difficult. The organism can also be cultured from ticks. Because culture of the organism is a complex and specialized procedure with a low diagnostic yield, it is seldom used.

C. VIRULENCE FACTORS

Once *B. burgdorferi* enters the host, the main goal is immune system avoidance through various virulence factors. The bacteria do not secret any toxins, and their virulence comes from the exploitation of the immune system. The inflammation the immune system produces in response to this bacteria results in the disease. The tactics used by this spirochaete involve survival more than outright destruction of host cells.

Immune System Avoidance

One of the main virulence factors of this bacteria is continuous variation of its surface antigens. The lipoprotein VlsE does this through recombination of silent cassettes. This virulence factor significantly reduces the effectiveness of the adaptive immune system in responding to the invader.

B. burgdorferi also use the tactic of binding to complement H factor through BbCRASPs. These H factors are used by the host immune system to avoid activating complement for self cells. By exploiting this factor, the bacteria can avoid lysis by the alternative complement pathway. This reduces the effectiveness of

the innate immune system in destruction of the pathogen.

The impediment of the generation of reactive oxygen species and chemotaxis of neutrophils can contribute to the bacteria's avoidance of phagocytosis. By avoiding this process of destruction, the bacteria can then spread to other parts of the body.

Motility, Adhesins, and Chemotaxis

About 6% of this bacteria's genome is devoted to the important virulence factor motility. This spirochaete uses a rotor mechanism to propel itself through blood, but the unique portion of its motility is the set of ribbons in its periplasmic space. Another important portion of its motility are the twin motors on either end of the flagella. The extremely fast motility of this organism allows it to escape phagocytosis of large cells such as the macrophage. Another virulence factor is the use of adhesins such as integrins, proteoglycans, laminin, and fibronectin.

The lipoprotein OspC is important to the bacteria in using plasminogen, which can digest fibrin and large glycoproteins. Once the bacteria begins moving from the blood stream, the utilization of this enzyme becomes important in making a pathway to the extracellular matrix. While in the extracellular matrix, the bacteria can more easily hide from the immune system because there are fewer leukocytes in this tissue.

The bacteria have a two-component chemotaxis system called CheA-CheY. This system can signal to the flagellar motors when environmental signals are detected. These signals can either be favorable, such as nutrient detection, or non-favorable such as repellant molecules. Methylcellulose and hyaluronic acid which are found in the extraceullular matrix are known chemoattractants of this spirochaete.

Quorum Sensing and Biofilms

The bacteria can also engage in quorum sensing and biofilm development. The biofilm can help protect the bacteria from the immune system as well as antibiotics. Quorum sensing involves microbes sensing cell density thresholds and releasing signals to other cells to alter gene expression. Biofilms allow bacteria at the bottom to survive antibiotics because lower concentrations of the drug will reach them.

Pathogenesis, Pathology & Clinical Findings

Lyme disease is caused by the spirochete *B. burgdorferi* and is transmitted to humans by the bite of a small ixodes tick.

The transmission of *B. burgdorferi* to humans is by injection of the organism in tick saliva or by regurgitation of the tick's midgut contents. The organism adheres to proteoglycans on host cells; this is mediated by a borrelial glycosaminoglycan receptor. After injection by the tick, the organism migrates out from the site, producing the characteristic skin lesion. Dissemination occurs by lymphatics or blood to other skin and musculoskeletal sites and to many other organs.

Lyme disease, like other spirochetal diseases, occurs in stages with early and late manifestations. A unique skin lesion that begins 3 days to 4 weeks after a tick bite often marks the initial stage. The lesion, erythema migrans, begins as a flat reddened area near the tick bite and slowly expands, with central clearing. With the skin lesion there is often a flu-like illness with fever, chills, myalgia, and headache. The second stage occurs weeks to months later and includes arthralgia and arthritis; neurologic manifestations with meningitis, facial nerve palsy, and painful radiculopathy; and cardiac disease with conduction defects and myopericarditis. The third stage begins months to years later with chronic skin, nervous system, or joint involvement. Spirochetes have been isolated from all of these sites, and it is likely that some of the late manifestations are caused by deposition of antigen-antibody complexes.

Diagnostic laboratory tests

In some symptomatic patients, the diagnosis of early Lyme disease can be established clinically by observing the unique skin lesion. When this skin lesion is not present and at later stages of the disease, which must be differentiated from many other diseases, it is necessary to perform diagnostic laboratory tests. There is, however, no one test that is both sensitive and specific.

A. SPECIMENS

Blood is obtained for serologic tests. Cerebrospinal fluid or joint fluid can be obtained, but culture usually is not recommended. These specimens and others can be used to detect *B. burgdorferi* DNA by the polymerase chain reaction.

B. SMEARS

B. burgdorferi has been found in sections of biopsy specimens, but examination of stained smears is

an insensitive method for diagnosis of Lyme disease. *B. burgdorferi* in tissue sections can sometimes be identified using antibodies and immunohistochemical methods.

C. CULTURE

Culture is generally not performed because it takes 6-8 weeks to complete and lacks sensitivity.

D. MOLECULAR PROBES

The polymerase chain reaction assay has been applied to detection of *B. burgdorferi* DNA in many body fluids. It is rapid, sensitive, and specific, but it does not differentiate between DNA from live *B. burgdorferi* in active disease and between DNA from dead *B. burgdorferi* in treated or inactive disease. It has about 85% sensitivity when applied to synovial fluid samples, but the sensitivity is much lower when it is applied to cerebrospinal fluid samples from patients with neuroborreliosis.

E. SEROLOGY

Serologic study has been the mainstay for the diagnosis of Lyme disease, but 3-5% of normal people and persons with other diseases (eg, rheumatoid arthritis, many infectious diseases) may be seropositive by some assays. Because the prevalence of Lyme disease is low, there is a much greater likelihood that a positive test is from a person who does not have Lyme disease than from a person who does have the disease (a positive predictive value on the order of 1-2%). Thus, serology for Lyme disease should only be done when there are highly suggestive clinical findings, and a diagnosis of Lyme disease should not be based on a positive test in the absence of such clinical findings.

The most widely used tests are the indirect fluorescent antibody (IFA) and enzyme immunoassays (EIA or ELISA). Many variations of these assays using different antigen preparations, techniques, and end points have been marketed. The immunoblot (Western blot) assay is sometimes performed to confirm results obtained by other tests. *B. burgdorferi* antigens are electrophoretically separated, transferred to a nitrocellulose membrane, and reacted with a patient's serum. Interpretation of the immunoblot is based on the number and molecular size of antibody reactions with the *B. burgdorferi* proteins.

Immunity

The immunologic response to *B. burgdorferi* develops slowly. Acute phase sera are positive in 30-40% of patients, and sera obtained 2-4 weeks later are positive in 60-70%. At 4-6 weeks after infection, about 90% of patients have IgG reactive with *B. burgdorferi.* The antibody response continues to expand for months to years and appears to be directed sequentially against a series of *B. burgdorferi* proteins, culminating in the development of IgG antibodies to OspA and OspB. Early antimicrobial treatment decreases the antibody response. Antibody titers fall slowly after treatment, but most patients with later manifestation of Lyme disease remain seropositive for years.

Treatment

Early infection, either local or disseminated, should be treated with doxycycline or amoxicillin - or an alternative - for 20-30 days. Treatment relieves early symptoms and promotes resolution of skin lesions. Doxycycline may be more effective than amoxicillin in preventing late manifestations. Established arthritis may respond to large doses of penicillin. In refractory cases, ceftriaxone has been effective. Nearly 50% of patients treated with doxycycline or amoxicillin early in the course of Lyme disease develop minor late complications (headache, joint pains, etc). Long-standing Lyme arthritis can be treated with doxycycline or amoxicillinplus probenecid for 30 days or longer.

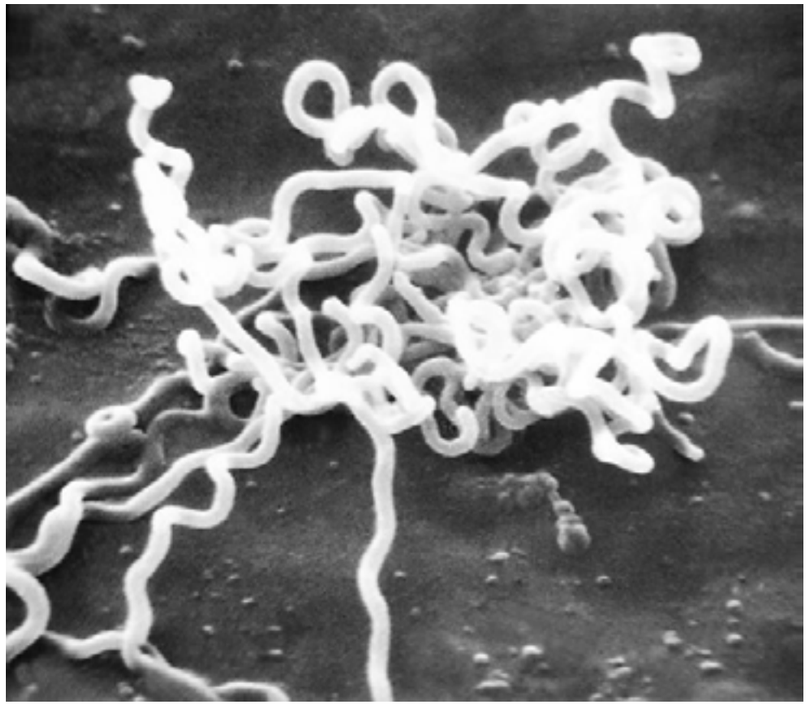
Epidemiology, Prevention & Control

B. burgdorferi is transmitted by a small tick of the genus *Ixodes.* In Europe, the vector is *Ixodes ricinus,* and other tick vectors appear to be important in other areas of the world. Mice and deer constitute the main animal reservoirs of *B. burgdorferi*, but other rodents and birds may also be infected. Most exposures are in May through July, when the nymphal stage of the ticks is most active; however, the larval stage (August and September) and adult stage (spring and fall) also feed on humans and can transmit *B. burgdorferi*.

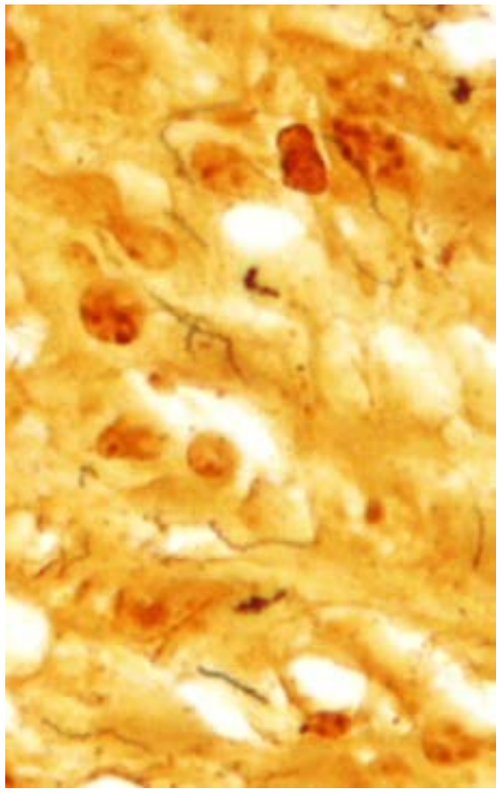
Prevention is based on avoidance of exposure to ticks. Long sleeves and long pants tucked into socks are recommended. Careful examination of the skin for ticks after being outdoors can locate ticks for removal before they transmit *B. burgdorferi*.

Environmental control of ticks using application of insecticides has provided modest success in reducing the number of nymphal ticks for a season.

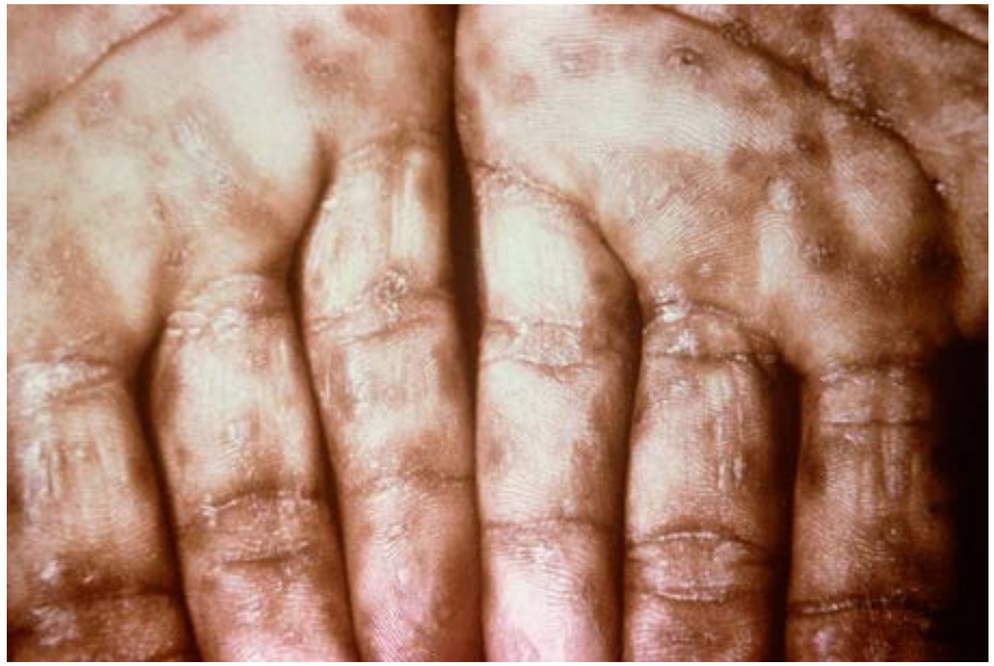
10 Class – Illustrations



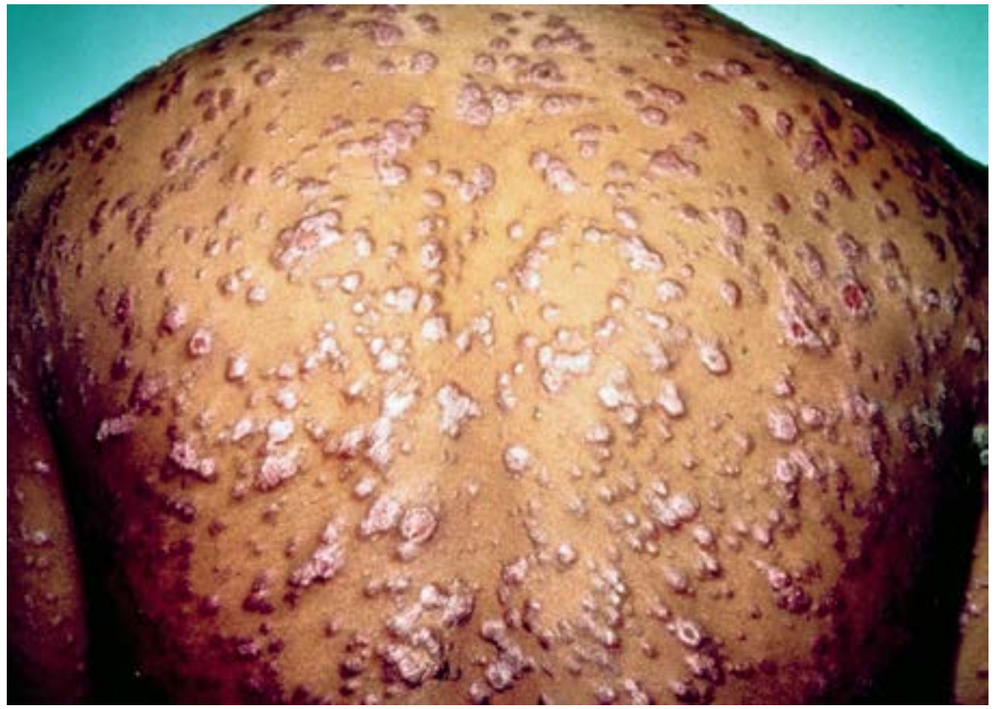
An electron micrograph of *T. pallidum*



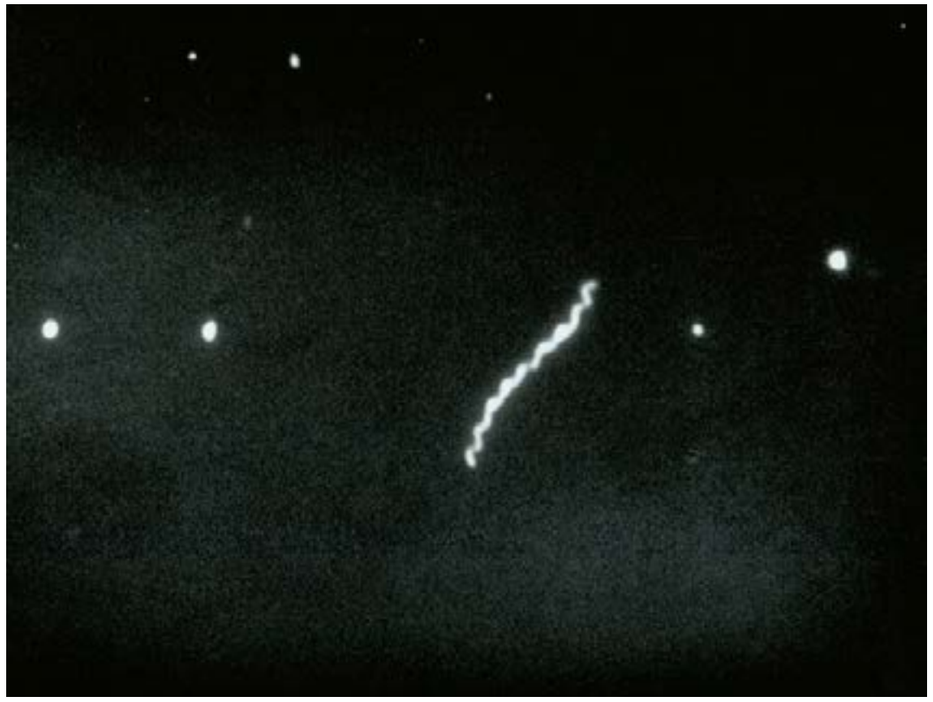
Micrograph showing *T. pallidum(black and thin)*



Typical presentation of secondary syphilis with a rash on the palms of the hands

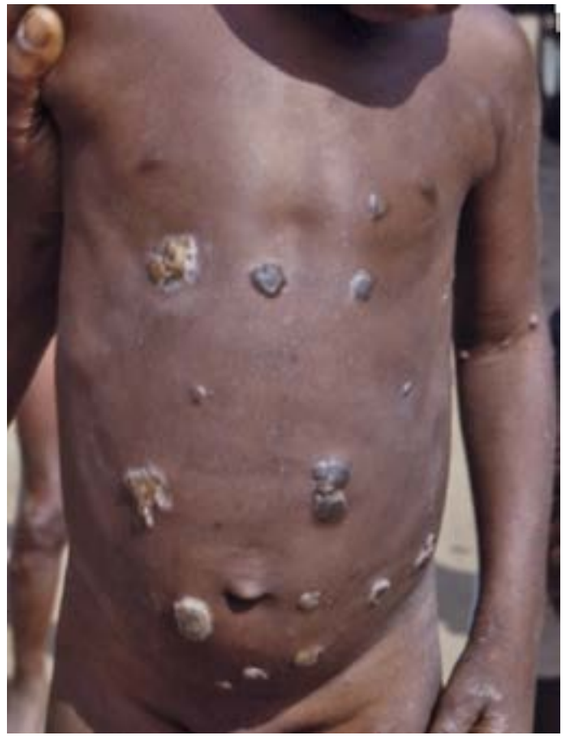


Reddish papules and nodules over much of the body due to secondary syphilis

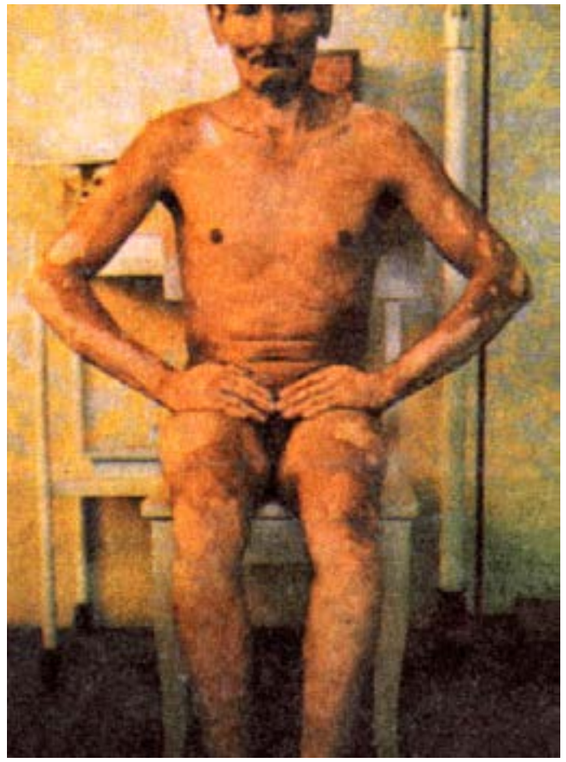


Darkfield examination of *T. pallidum*

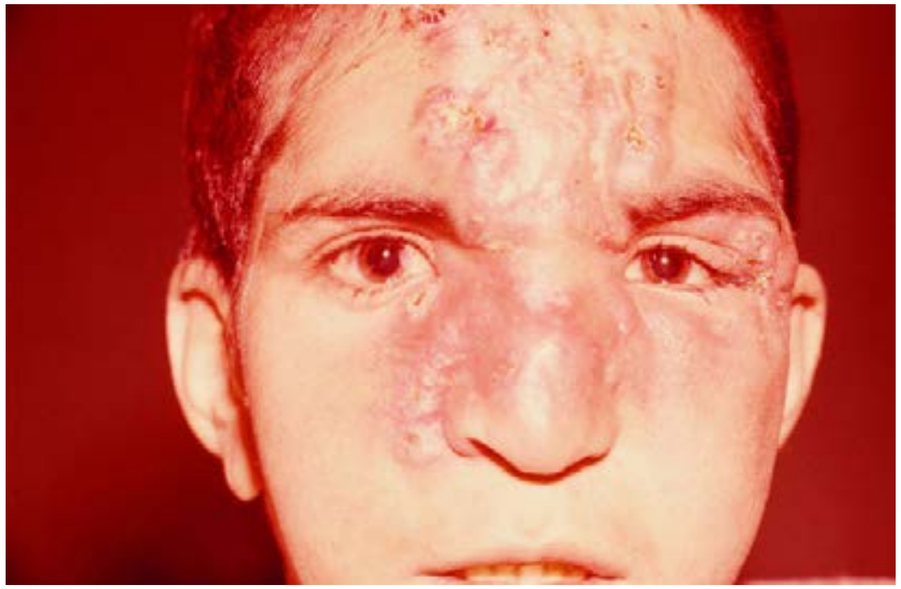
T. pallidum-Particle Agglutination (TPA) Test



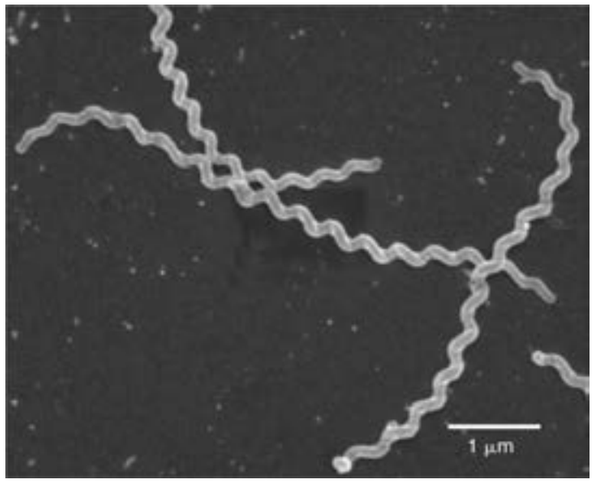
A Nigerian Boy with Yaws



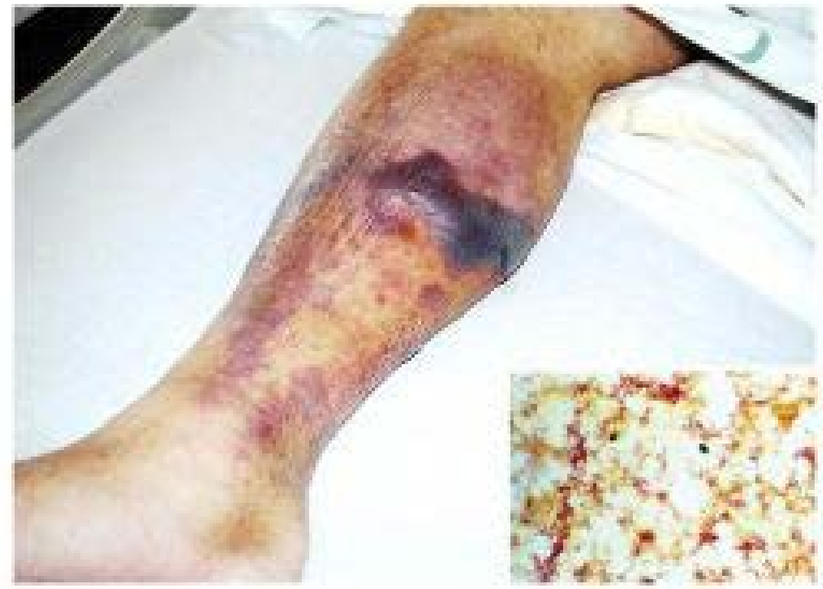
Hypopigmented Skin Lesions of Pinta



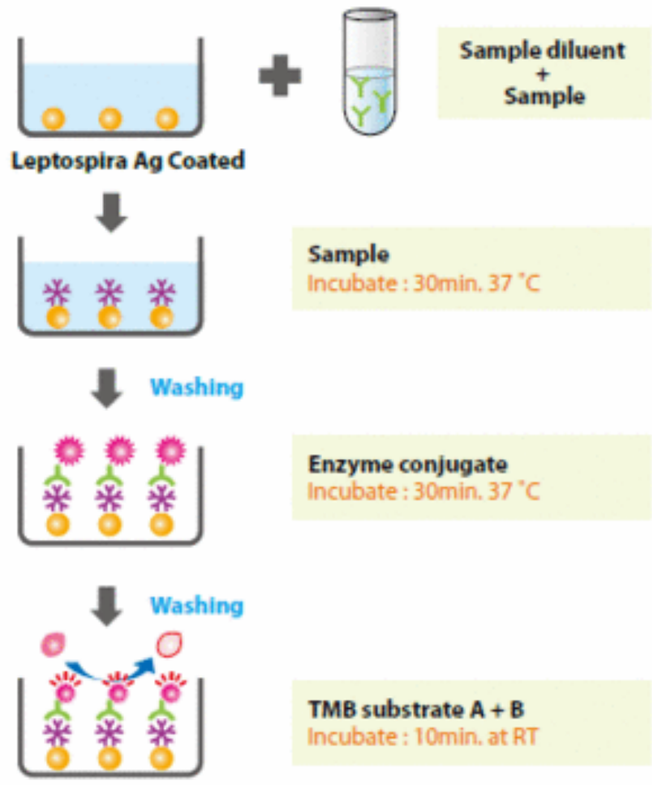
Disfiguring infiltration of the nose, glabella, and forehead
with clustered nodules in left interciliary region
of boy with endemic syphilis.



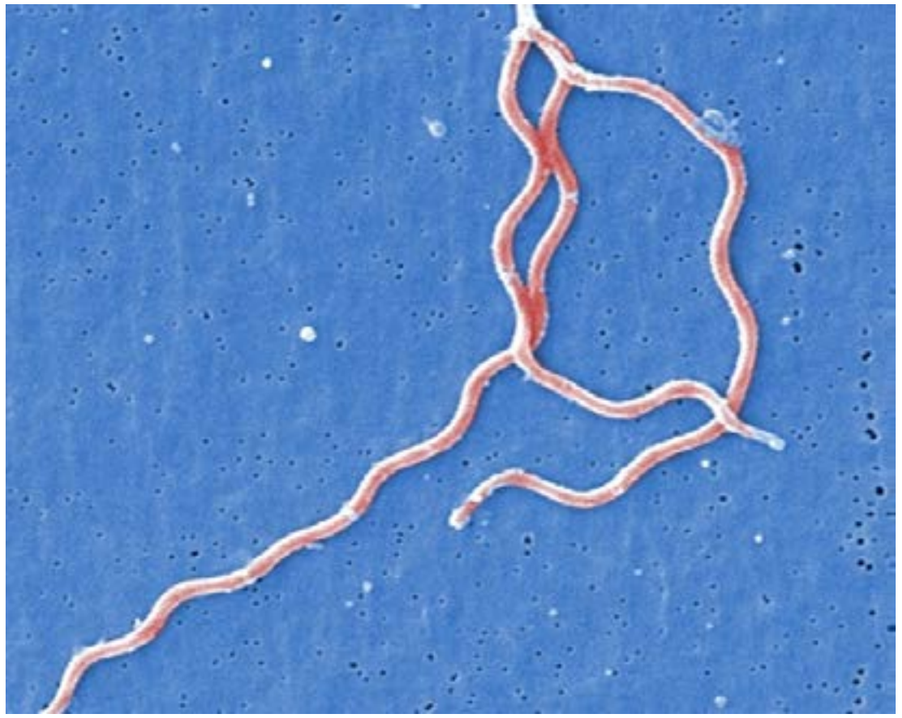
An electron micrograph of *Leptospira*



Leptospirosis rash in human



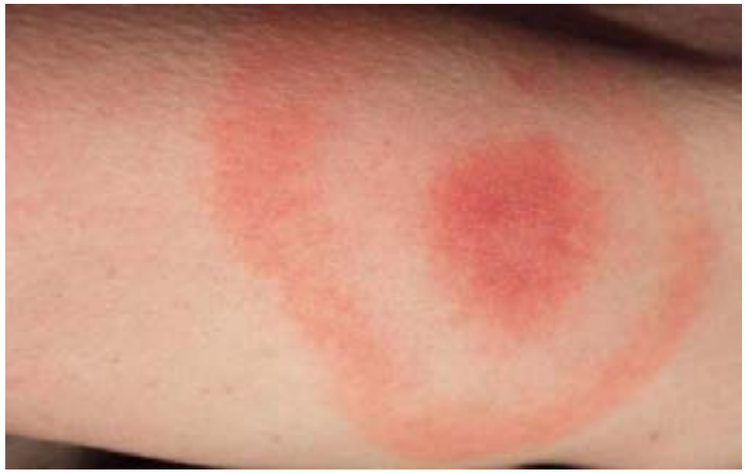
Cartoon rendering of the ELISA method for
detection of leptospiral antigens



Borrelia burgdorferi scanning electron

B. burgdorferi: darkfield microscopy micrograph

Ticks as vectors for *B. burgdorferi*



Lyme disease

ELISA lyme disease test accuracy

11 Class - The repetition of examined material. Submodule-2

1. The causative agent of anthrax. General description of the properties: cultural, biochemical, toxin, antigenic structure. Pathogenesis of the disease. Clinical forms. Laboratory diagnosis. The Ascoli reaction. Treatment and prevention. Anthropoids.

2. Pathogenic anaerobes. General comparative characteristic of anaerobic bacteria, their importance in human pathology. Anaerobes in surgery.

3. Causative agents of the tetanus. General characteristic of the properties. Pathogenesis of the disease in humans and animals. Laboratory diagnostic. Prevention and treatment. The value of microbial associations in the occurrence of the pathological process.

4. *Clostridium botulinum*. Ecology, pathogen properties, characteristic of the botulinum toxin. Pathogenesis of the disease. Laboratory diagnosis. Treatment and prevention.

5. Methods of specific prevention and treatment of wounds anaerobic infections.

6. *Corynebacterium*. The causative agent of diphtheria. Biovar characteristics. Factors of pathogenicity and toxicity. Pathogenesis of the disease. Laboratory diagnostic. Differentiation of diphtheria pathogen and saprophytic *Corynebacterium*. Treatment and prevention.

7. Pathogenic Mycobacteria. Types and properties of *Mycobacterium tuberculosis*. Toxicity. Cord factor. Pathogenesis of the disease. Immunity and its features. Laboratory diagnostic. Treatment and prevention.

8. *Mycobacterium leprae*. General characteristic of the properties of causative leprosy agent. Clinical forms. Pathogenesis. Laboratory diagnosis. Prevention, treatment.

9. Mild fungal infections. Pathogenic Actinomycetes. General characteristics, types, properties, environment. The diseases they cause. Laboratory diagnosis.

10. *Nocardia*, nocardiosis characteristic. Laboratory diagnosis. Treatment.

11. Pathogenic spirochetes. Syphilis Treponems. Ecology, characteristic properties. The pathogenesis of syphilis. Stages of the disease. Immunity, chancre immunity. Laboratory diagnosis. Treatment and prevention.

12. Notvenereal forms of syphilis. Characteristics of the Bejel, Framboesia, Pinta. Transmission, laboratory diagnosis. Prevention.

13. Pathogen *Leptospira*. Causative agents of the leptospirosis. General characteristics of cultural and morphological properties. Pathogenicity to humans and animals. The pathogenesis of leptospirosis. Laboratory diagnosis. Prevention. Treatment.

14. *Borrelia* as causative agents of relapsing fever. Characteristics of environmental pathogens endemic and epidemic reverse typhus. Laboratory diagnosis. Treatment. Pathogenesis of the disease. Prevention.

15. Lyme borreliosis. Characteristics of pathogens. Laboratory diagnostic. Treatment. Pathogenesis of the disease. Prevention.

12 Class - *Rickettsia*. Brill-Zinnsser disease. Epidemic and endemic typhus. Spotted fever - south Asian, vesicular. Paroxysmal fever. Q-fever. *Bartonella. Ehrlichia*

RICKETTSIA

Properties of *Rickettsia*

Rickettsia are pleomorphic coccobacilli, appearing either as short rods (0,3 × 1-2 μm) or as cocci (0,3 μm in diameter). They do not stain well with Gram stain but are readily visible under the light microscope when stained with Giemsa, Gimenez, acridine orange, or other stains. *Rickettsia* grow readily in yolk sacs of embryonated eggs. Pure preparations of *Rickettsia* for use in laboratory testing can be obtained by differential centrifugation of yolk sac suspensions. Many strains of *Rickettsia* also grow in cell culture. In cell culture, the generation time is 8-10 hours at 34 °C. For reasons of biosafety, isolation of *Rickettsia* should be done only in reference laboratories.

Rickettsia have Gram-negative cell wall structures that include peptidoglycan-containing muramic acid and diaminopimelic acid. The typhus and spotted fever groups contain lipopolysaccharide. The cell wall proteins include the surface proteins OmpA and OmpB, which are important in the humoral immune response and provide the basis for serotyping. *Rickettsia* grow in different parts of the cell. Those of the typhus group are usually found in the cytoplasm; those of the spotted fever group, in the nucleus. *Coxiella* grow only in cytoplasmic vacuoles. Rickettsial growth is enhanced in the presence of sulfonamides, and rickettsial diseases are made more severe by these drugs. Tetracyclines and chloramphenicol inhibit the growth of *Rickettsia* and can be therapeutically effective.

Most *Rickettsia* survive only for short times outside of the vector or host. *Rickettsia* are quickly destroyed by heat, drying, and bactericidal chemicals. Dried feces of infected lice may contain infectious *Rickettsia prowazekii* for months at room temperature. *Coxiella burnetii*, which causes Q fever, is the rickettsial agent most resistant to drying. This organism may survive pasteurization at 60 °C for 30 minutes and can survive for months in dried feces or milk. This may be due to the formation of endospore-like structures by *Coxiella burnetii.*

Rickettsial Antigens & Serology

The direct immunofluorescent antibody test can be used to detect *Rickettsia* in ticks and sections of tissues. The test has been most useful to detect *R. rickettsii* in skin biopsy specimens to aid in the diagnosis of Rocky Mountain spotted fever; however, the test is performed in only a few reference laboratories. Serologic evidence of infection occurs no earlier than the second week of illness for any of the rickettsial diseases. Thus, serologic tests are useful only to confirm the diagnosis, which is based on clinical findings (eg, fever, headache, rash) and epidemiologic information (eg, tick bite). Therapy for potentially severe diseases, such as Rocky Mountain spotted fever and typhus, should be instituted before seroconversion occurs.

A variety of serologic tests have been used to diagnose rickettsial diseases. Most of these tests are performed only in reference laboratories. Antigens for the complement fixation test to diagnose Q fever and for the indirect immunofluorescence, latex agglutination, and enzyme immunoassay for Rocky Mountain spotted fever are commercially available. Reagents for other tests are prepared only in public health or other reference laboratories. The indirect fluorescent antibody technique may be the most widely used method, because of the availability of reagents and the ease with which it can be performed. The test is relatively sensitive, requires little antigen, and can be used to detect IgM and IgG. *Rickettsia* partially purified from infected yolk sac material are tested with dilutions of a patient's serum. Reactive antibody is detected with a fluorescein-labeled antihuman globulin. The results indicate the presence of partly species-specific antibodies, but cross-reactions are observed.

Pathology

Rickettsia, except for *C. burnetii*, multiply in endothelial cells of small blood vessels and produce vasculitis. The cells become swollen and necrotic; there is thrombosis of the vessel, leading to rupture and necrosis. Vascular lesions are prominent in the skin, but vasculitis occurs in many organs and appears to be the basis of hemostatic disturbances. Disseminated intravascular coagulation and vascular occlusion may develop. In the brain, aggregations of lymphocytes, polymorphonuclear leukocytes, and macrophages are associated with the blood vessels of the gray matter; these are called typhus nodules. The heart shows similar

lesions of the small blood vessels. Other organs may also be involved.

Immunity

In cell cultures of macrophages, *Rickettsia* are phagocytosed and replicate intracellularly even in the presence of antibody. The addition of lymphocytes from immune animals stops this multiplication in vitro. Infection in humans is followed by partial immunity to reinfection from external sources, but relapses occur.

Clinical Findings

Except for Q fever, in which there is no skin lesion, rickettsial infections are characterized by fever, headache, malaise, prostration, skin rash, and enlargement of the spleen and liver.

A. TYPHUS GROUP

1. Epidemic Typhus (*Rickettsia prowazekii*);
2. Endemic Typhus (*Rickettsia typhi*);
3. Scrub Typhus (*Orientia tsutsugamushi*).

B. SPOTTED FEVER GROUP

1. Rocky Mountain spotted fever;
2. Rickettsialpox.

C. Q FEVER

1. Q fever (*C. burnetii*).

Laboratory Findings

Isolation of *Rickettsia* is technically difficult and is of only limited usefulness in diagnosis. It is also hazardous. Whole blood (or emulsified blood clot) is inoculated into guinea pigs, mice, or eggs. *Rickettsia* are recovered most frequently from blood drawn soon after onset of illness. If the guinea pigs fail to show disease (fever, scrotal swellings, hemorrhagic necrosis, and death), serum is collected for antibody tests to determine if the animal has had an inapparent infection. Some *Rickettsia* can infect mice, and *Rickettsia* are seen in smears of peritoneal exudate. In Rocky Mountain spotted fever, skin biopsies taken from patients between the fourth and eighth days of illness may reveal *Rickettsia* by immunofluorescence stain.

The most widely used serologic tests are indirect immunofluorescence and complement fixation. An antibody rise should be demonstrated during the course of the illness. In Rocky Mountain spotted fever, the antibody response may not occur until after the second week of illness.

The polymerase chain reaction has been used to help diagnose Rocky Mountain spotted fever, other diseases of the spotted fever group, murine typhus, scrub typhus, and Q fever. The sensitivity of the method for Rocky Mountain spotted fever is about 70%, comparable to that of skin biopsy with immunocytology.

Treatment

Tetracyclines are effective provided treatment is started early. Tetracycline is given daily orally and continued for 3-4 days after defervescence. In severely ill patients, the initial doses can be given intravenously. Sulfonamides enhance the disease and are contraindicated.

The antibiotics do not free the body of *Rickettsia*, but they do suppress their growth. Recovery depends in part upon the immune mechanisms of the patient.

Control

Control must rely on breaking the infection chain, treating patients with antibiotics, and immunizing when possible. Patients with rickettsial disease who are free from ectoparasites are not contagious and do not transmit the infection.

A. PREVENTION OF TRANSMISSION BY BREAKING THE CHAIN OF INFECTION

1. Epidemic Typhus - delousing with insecticide;
2. Murine Typhus - rat-proofing buildings and using rat poisons;
3. Scrub Typhus - clearing from campsites the secondary jungle vegetation in which rats and mites live;
4. Spotted Fever - similar measures for the spotted fevers may be used; clearing of infested land; personal prophylaxis in the form of protective clothing such as high boots, socks worn over trousers; tick repellents; and frequent removal of attached ticks;
5. Rickettsialpox - elimination of rodents and their parasites from human domiciles.

B. PREVENTION OF TRANSMISSION OF Q FEVER BY ADEQUATE PASTEURIZATION OF MILK

The presently recommended conditions of «high-temperature, short-time» pasteurization at 71,5 °C for

15 seconds are adequate to destroy viable *Coxiella*.

C. PREVENTION BY VACCINATION

There is no vaccine for Rocky Mountain spotted fever, for the other diseases of the spotted fever group, or for the diseases in the typhus group. For *Coxiella burnetti* there is an investigational vaccine made from infected egg yolk sacs. This vaccine has been used for laboratory workers who handle live *C. burnetti*.

EHRLICHIA

Properties of *Ehrlichia*

Ehrlichia are small (0,5 μm) Gram-negative bacteria. They infect circulating leukocytes where they multiply within phagocytic vacuoles, forming clusters with inclusion-like appearance. These clusters of *Ehrlichia* are called morulae, which is derived from the Latin word for mulberry. The *Ehrlichia* and *Chlamydia* resemble each other in that both are found in intracellular vacuoles. The *Ehrlichia*, however, are like the *Rickettsia* in that they are able to synthesize ATP; the *Chlamydia* are not able to synthesize ATP.

Clinical Findings

The clinical manifestations of ehrlichiosis in humans are nonspecific: fever, chills, headache, myalgia, nausea or vomiting, anorexia, and weight loss. These manifestations are very similar to those of Rocky Mountain spotted fever without the rash. *E. chaffeensis* frequently and *A. phagocytophilum* less often cause severe or fatal illness. Seroprevalence studies suggest that subclinical ehrlichiosis occurs frequently.

Laboratory Findings

The diagnosis is confirmed by observing typical morulae in white blood cells. The indirect fluorescent antibody test can also be used to confirm the diagnosis. Antibodies are measured against *E. chaffeensis* and *A. phagocytophilum*. *E. chaffeensis* is also used as the substrate for *E. ewingii*, because the two species share antigens. Seroconversion from < 1:64 to ≥ 1:128 or a fourfold or greater rise in titer makes a confirmed serologic diagnosis of human monocytotropic ehrlichiosis in a patient with a clinically compatible illness.

Multiple methods have been described for PCR detection of *Ehrlichia* in EDTA-anticoagulated blood. Culture using a variety of tissue culture cell lines also can be used. PCR and culture are performed in reference laboratories and in a small number of commercial laboratories.

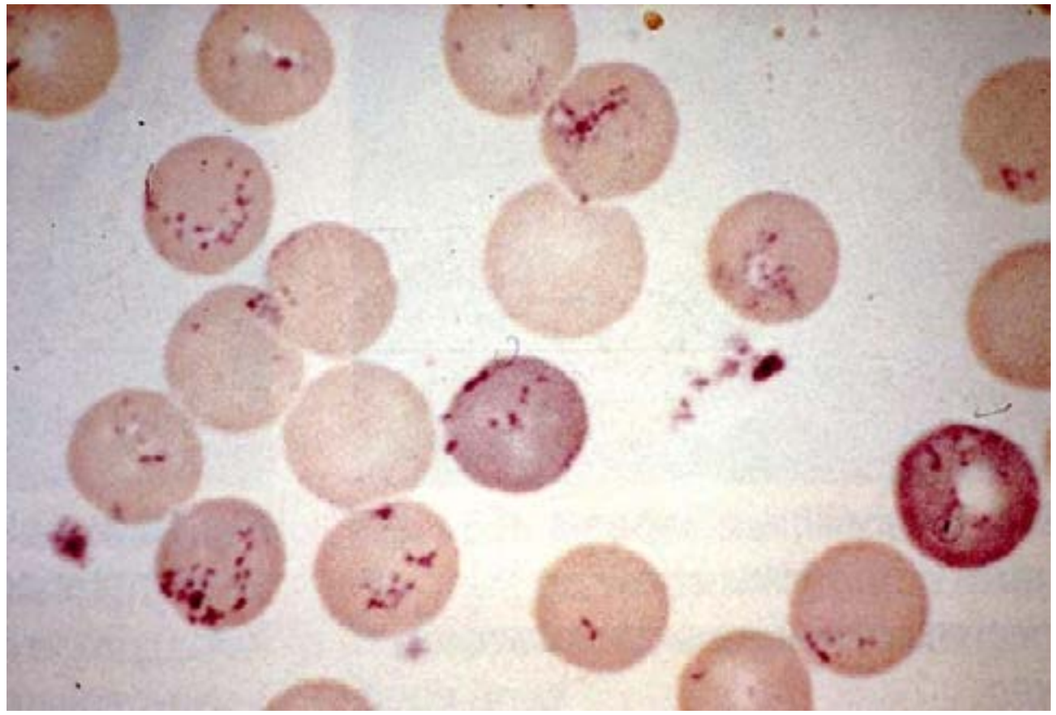
Treatment

Tetracycline, commonly in the form of doxycycline, is cidal for *Ehrlichia* and is the treatment of choice. Rifamycins also are ehrlichiacidal.

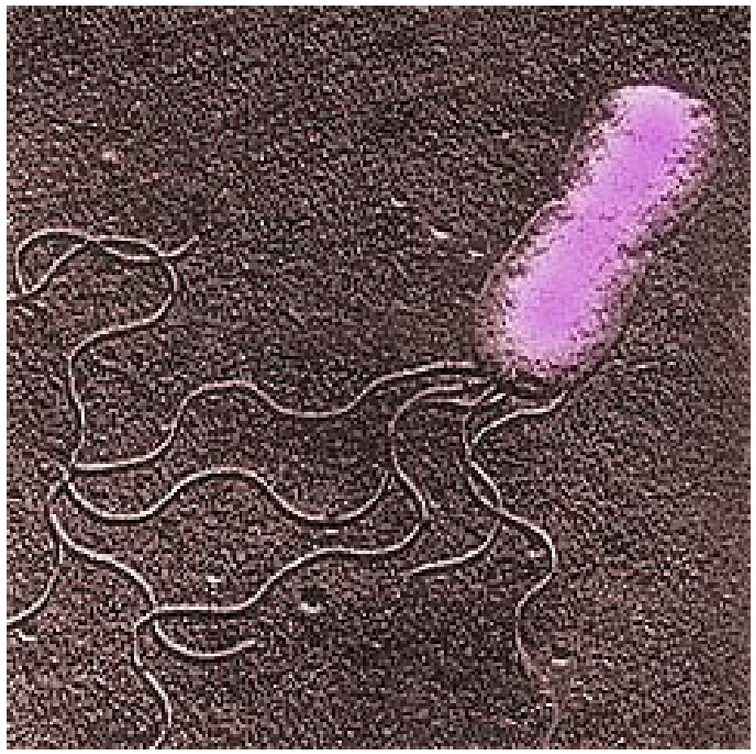
Epidemiology & Prevention

The incidence of human ehrlichioses is not well defined. In Oklahoma, which has the highest incidence of Rocky Mountain spotted fever, human monocytotropic ehrlichiosis is at least as common. Human granulocytotropic ehrlichiosis is thought to occur at a rate of about 15 cases per 100,000 population in upper Midwestern Oklahoma and at higher rates in selected counties. More than 90% of cases occur between mid April and October, and more than 80% of cases are in men. Most patients give histories of tick exposure in the month before onset of illness. Cases of human monocytotropic ehrlichiosis have occurred in over 30 states, primarily in the south-central and southeastern United States. This area corresponds to the area of distribution of the Lone Star tick, *Amblyomma americanum*. Cases of human monocytotropic ehrlichiosis in the western United States and in Europe and Africa suggest other tick vectors such as Dermacentor variabilis. Cases of human granulocytotropic ehrlichiosis occur in the upper Midwest and East Coast states and in West Coast states. These areas correspond to the distribution of the tick vectors *Ixodes scapularis* and *Ixodes pacificus,* respectively.

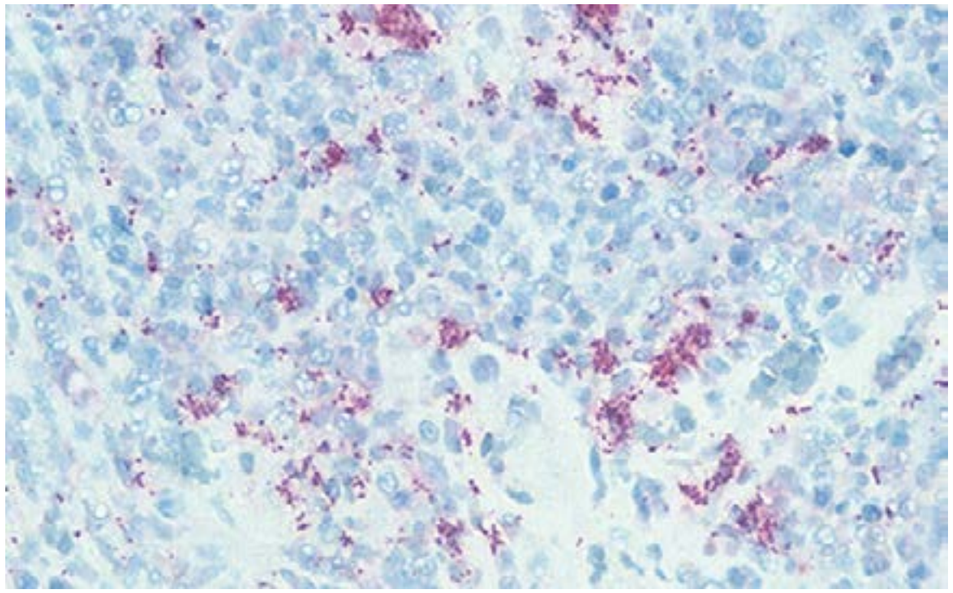
12 Class – Illustrations



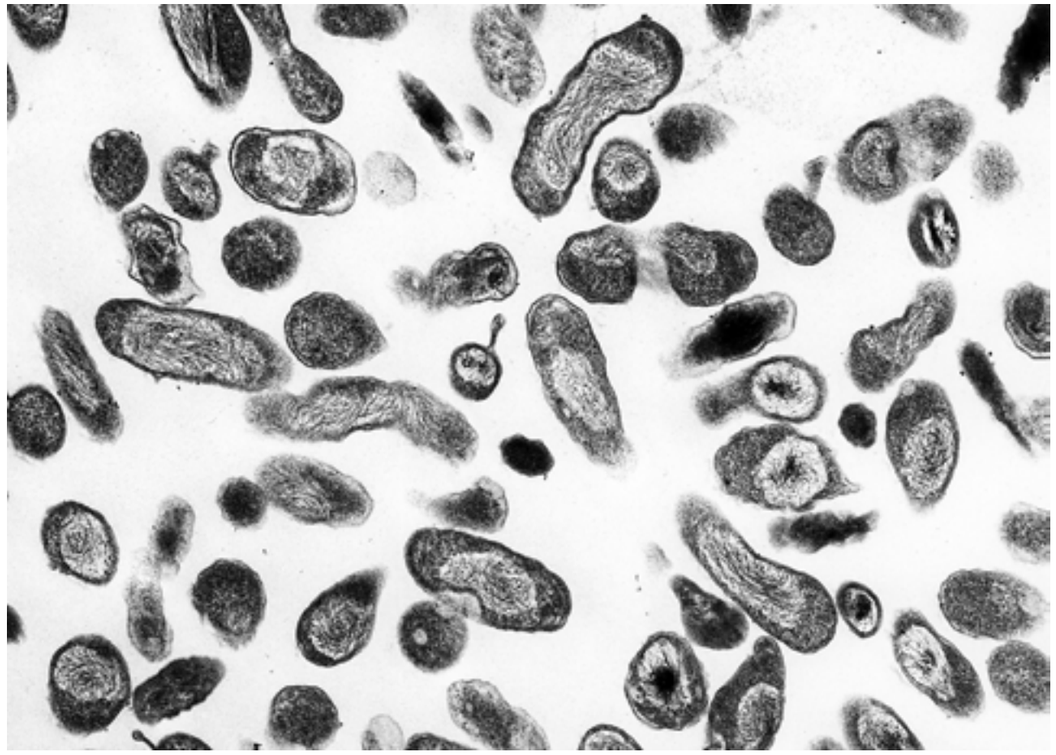
Stained *Bartonella bacilliformis* in blood of an Oroyo fever infected human



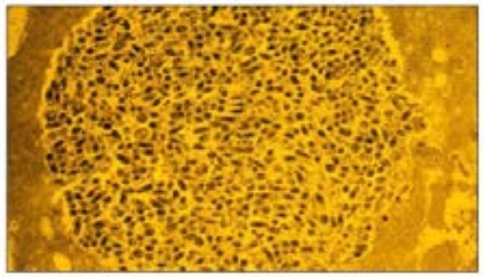
Bartonella bacilliformis from a culture showing flagella



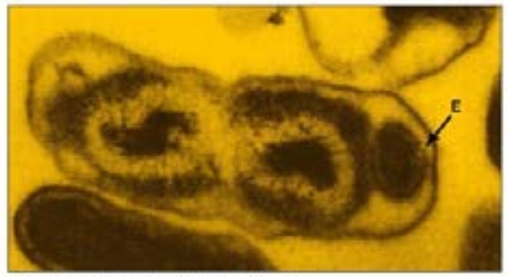
Bartonella henselae antibodies (immunohistochemistry-paraffin)



Coxiella burnetii, the Q-fever causing agent

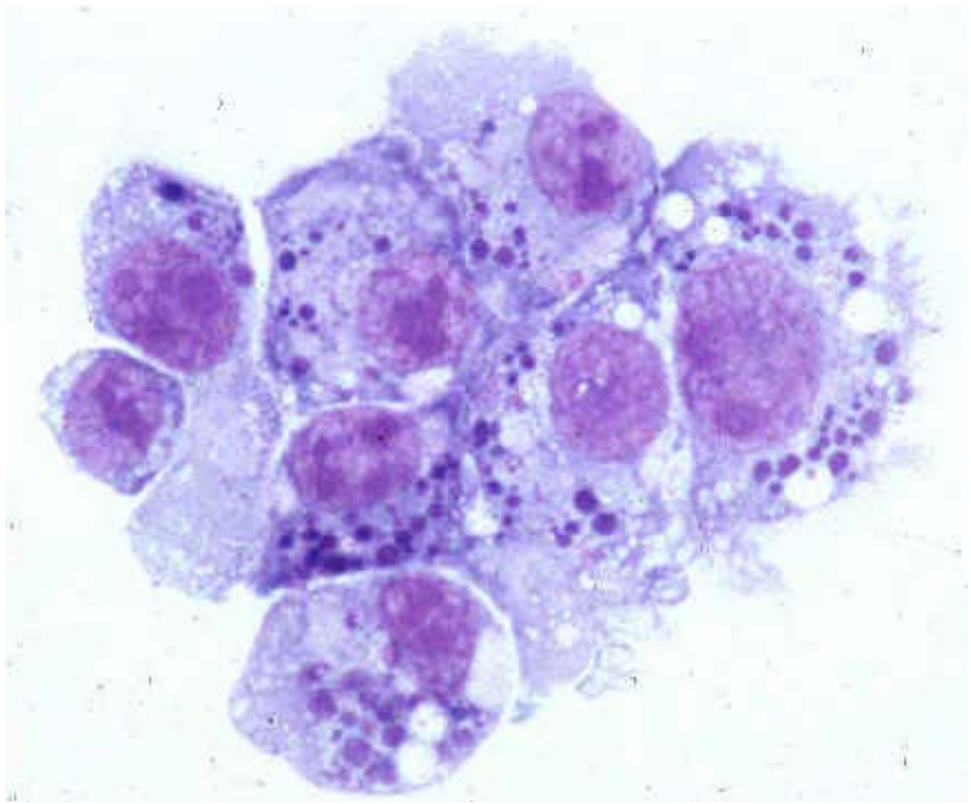


(a) *Coxiella burnetii* gowing in placental cell.

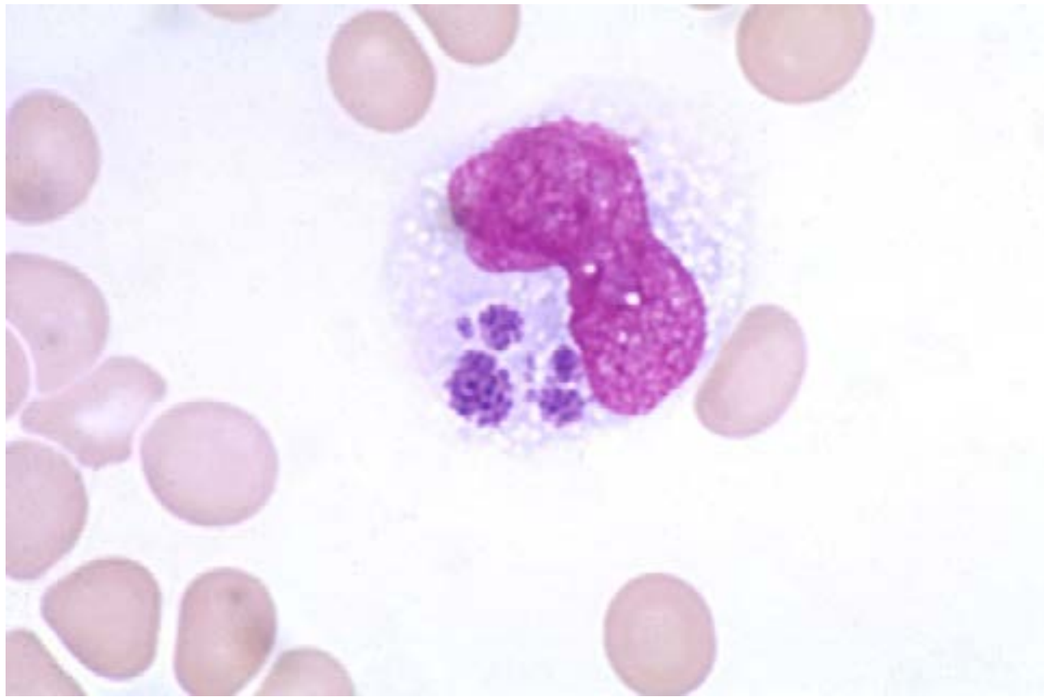


(b) This cell has just divided; notice the endospore-like body (E), which is probably responsible for the relative resistance of the organism.

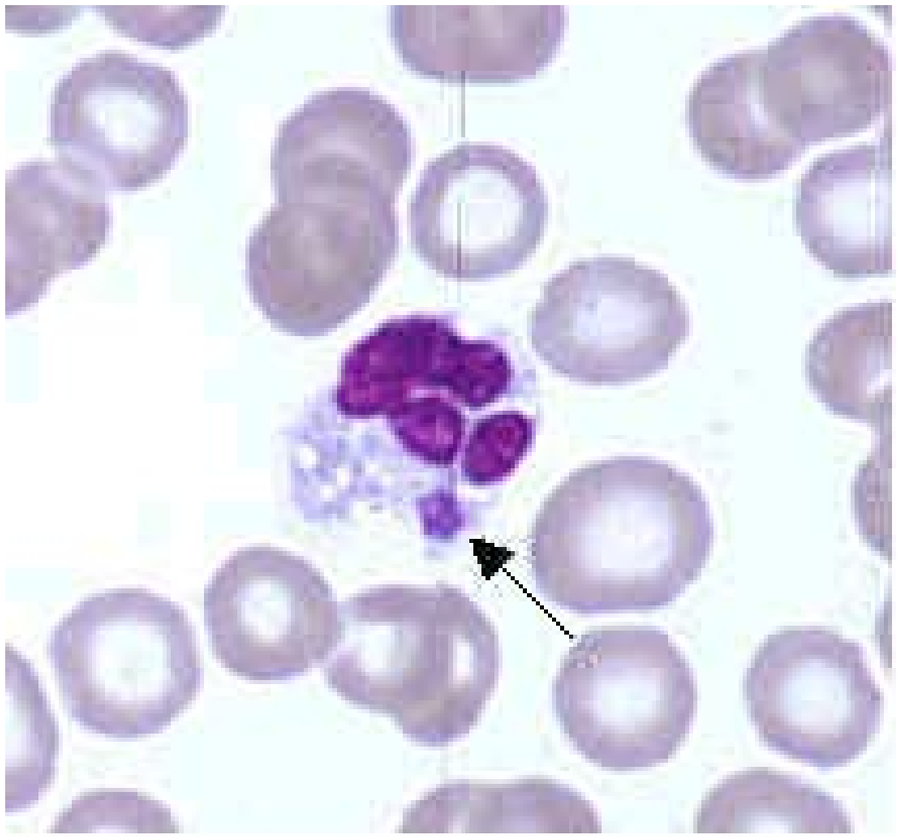
Coxiella burnetii



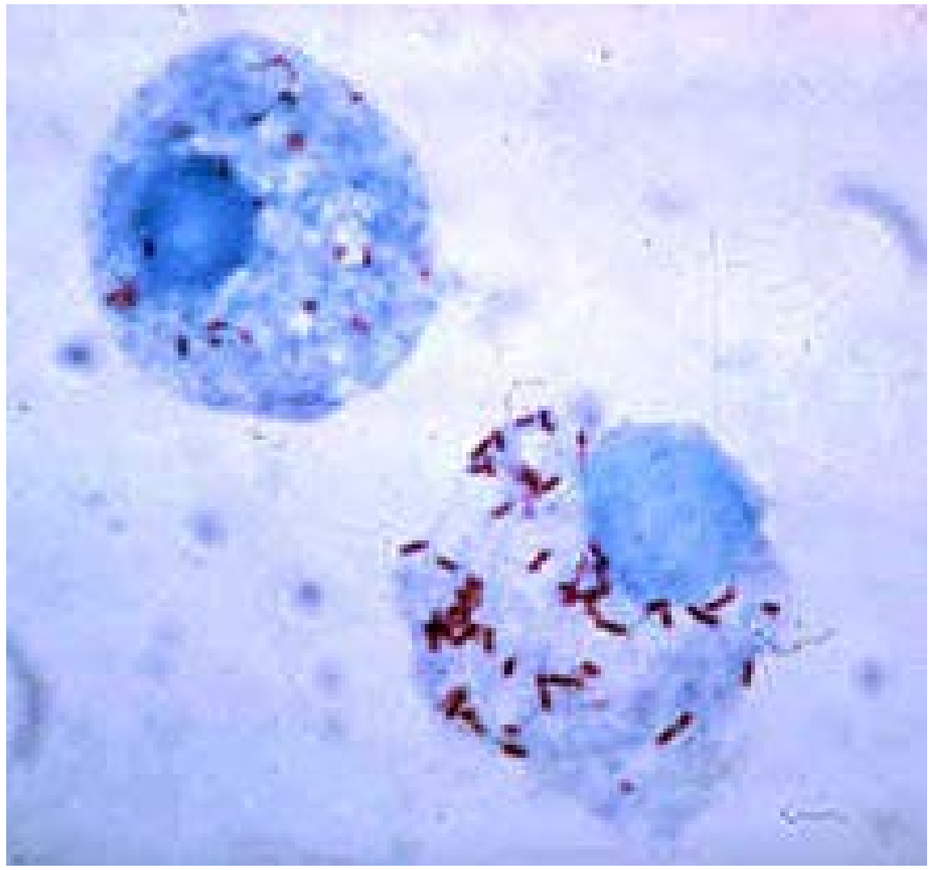
Diff-Quik Stain of *Ehrlichia chaffeensis* in DH82 cells, 1000x CDC



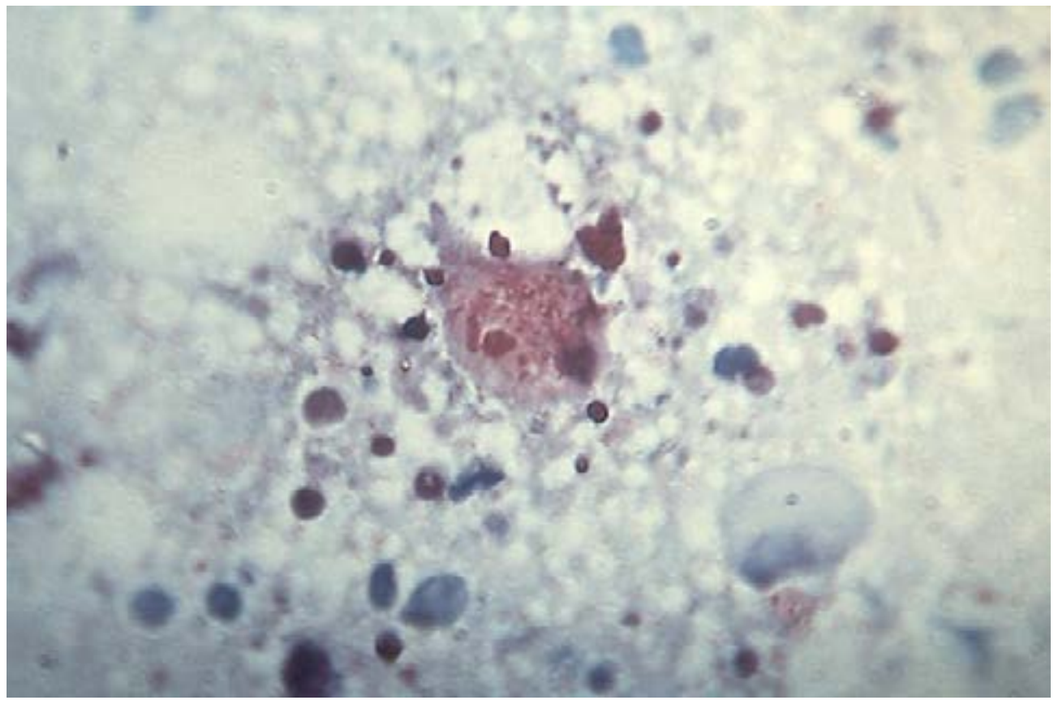
Clumps of *Ehrlichia chaffeensis* in neutrophil



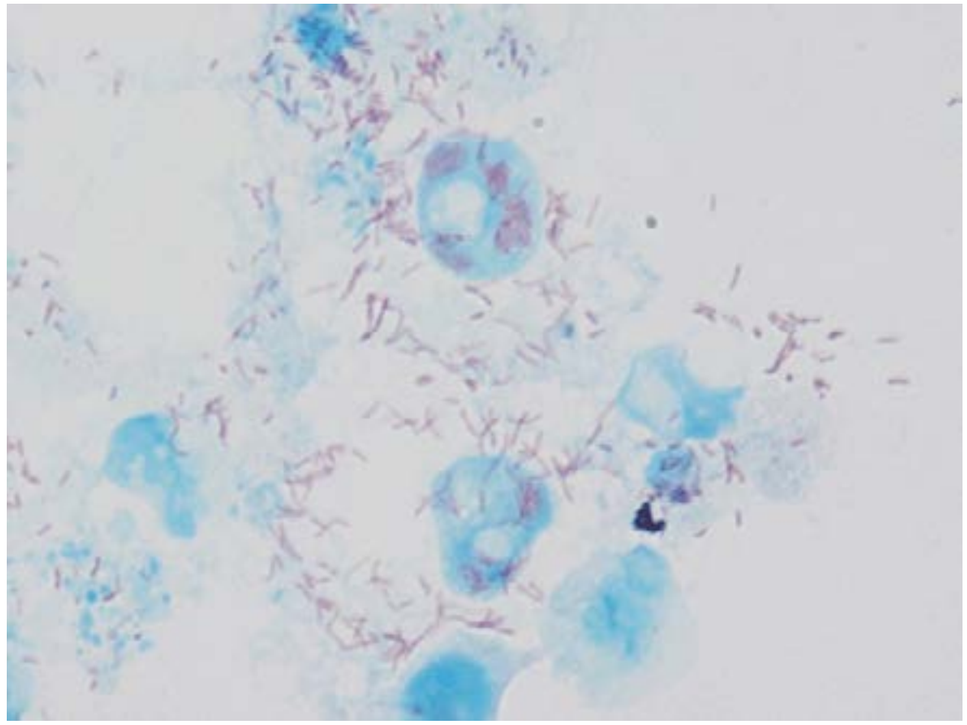
Morula of *Ehrlichia ewingii* in neutrophil



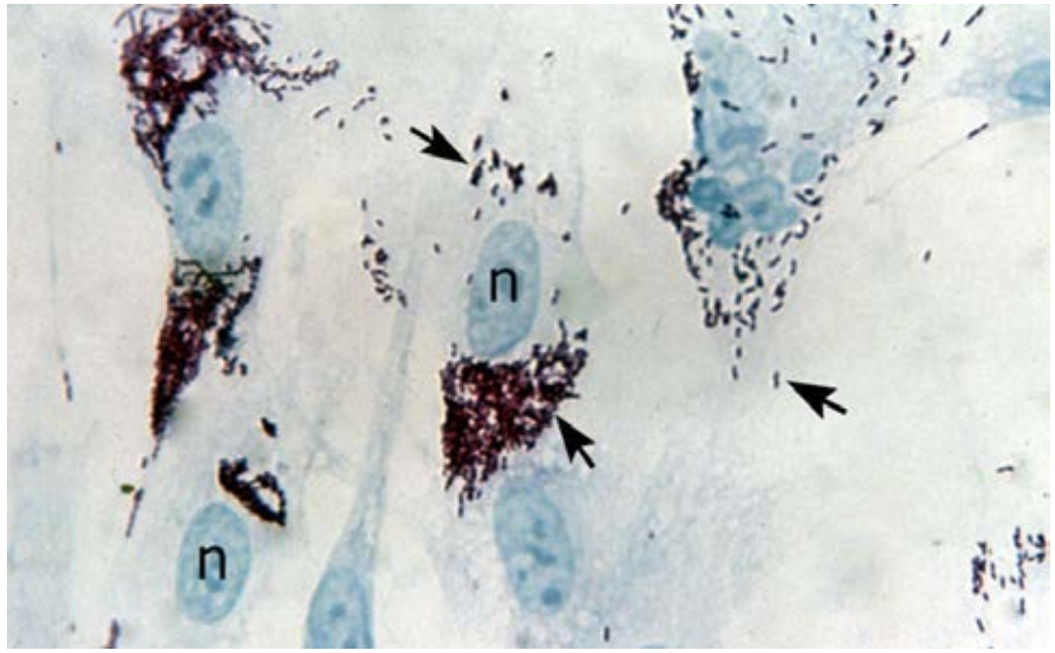
Cells infected by *Rickettsia rickettsii*



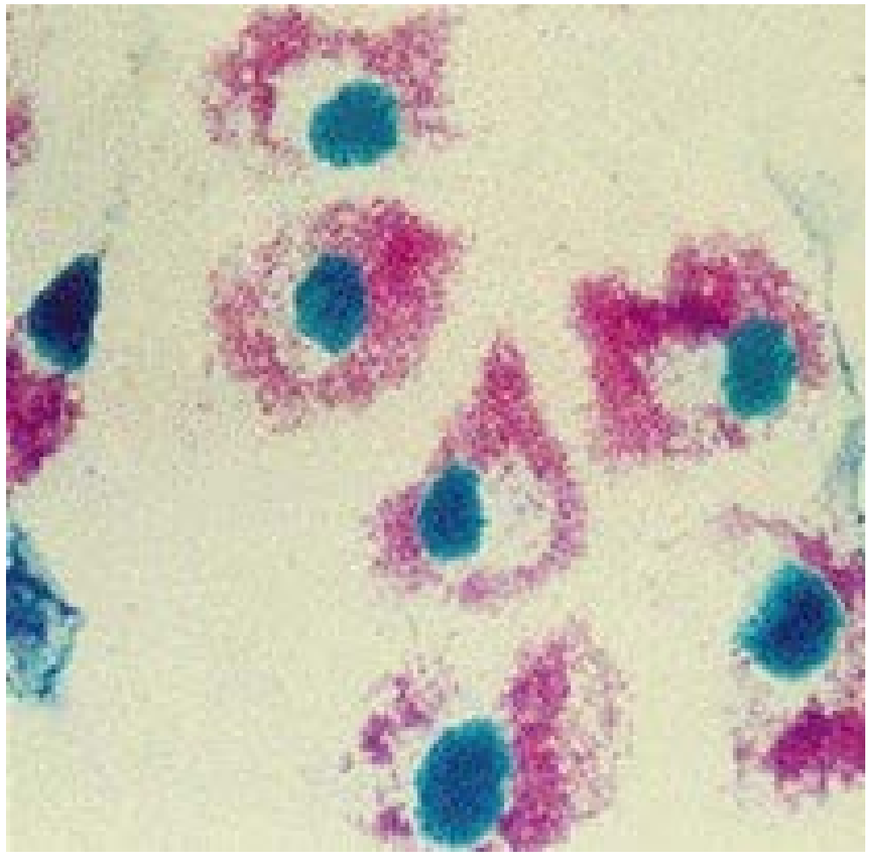
Presence of intracellular Rocky Mountain spotted fever bacteria, *Rickettsia rickettsii*



Rickettsia conorii detected in hemolymph from infected *Rhipicephalus sanguineus* adult ticks using Gimenes staining



Rickettsia prowazekii bacteria growing incide human fibroblasts. The black arrows point to the bacteria and the ‘n’ indicates host cell nuclei



Rickettsia prowazekii

13 Class - Pathogenic *Chlamydia*. Pathogenic *Mycoplasma*, *Ureaplasma*

CHLAMYDIA

Structure & Chemical Composition

In *Chlamydia*, the outer cell wall resembles the cell wall of Gram-negative bacteria. It has a relatively high lipid content. It is rigid but does not contain a typical bacterial peptidoglycan; however, the chlamydial genome contains the genes needed for peptidoglycan synthesis.

Penicillin-binding proteins occur in *Chlamydia*, and chlamydial cell wall formation is inhibited by penicillins and other drugs that inhibit transpeptidation of bacterial peptidoglycan. Lysozyme has no effect on chlamydial cell walls. N-acetylmuramic acid appears to be absent from chlamydial cell walls. Both DNA and RNA are present in elementary and reticulate bodies. The reticulate bodies contain about four times as much RNA as DNA, whereas the elementary bodies contain about equal amounts of RNA and DNA. In elementary bodies, most DNA is concentrated in the electrondense central nucleoid. Most RNA exists in ribosomes.

The circular genome of *Chlamydia* (MW 7 X 108) is similar to that of bacterial chromosomes. Multiple chlamydial genomes have been sequenced providing insight into the basic biology of the organisms. For example, *Chlamydia* have a type III secretion system, which may allow them to inject effector proteins into host cells as part of the infectious process.

Staining Properties

Chlamydia have distinctive staining properties (similar to those of *Rickettsia*). Elementary bodies stain purple with Giemsa stain - in contrast to the blue of host cell cytoplasm. The larger, noninfective reticulate bodies stain blue with Giemsa stain. The Gram reaction of *Chlamydia* is negative or variable and is not useful in identification of the agents. Chlamydial particles and inclusions stain brightly by immunofluorescence, with group-specific, species-specific, or serovar-specific antibodies.

Fully formed, mature intracellular inclusions of *C. trachomatis* are compact masses near the nucleus which are dark purple when stained with Giemsa stain because of the densely packed mature particles. If stained with dilute Lugol's iodine solution, some of the inclusions of *C. trachomatis* (but not *C. pneumoniae* or *C. psittaci*) appear brown because of the glycogen matrix that surrounds the particles. Inclusions of *C. psittaci* are diffuse intracytoplasmic aggregates.

Antigens

Chlamydia possess shared group (genus)-specific antigens. These are heat-stable lipopolysaccharides with 2-keto-3-deoxyoctanoic acid as an immunodominant component. Antibody to these genus-specific antigens can be detected by CF and immunofluorescence. Species-specific or serovar-specific antigens are mainly outer membrane proteins. Specific antigens can best be detected by immunofluorescence, particularly using monoclonal antibodies. Specific antigens are shared by only a limited number of *Chlamydia*, but a given organism may contain several specific antigens. There are at least 15 serovars of *C. trachomatis*; these include A, B, Ba, C-K, and L1-L3. Several serovars of *C. psittaci* can be demonstrated by complement fixation (CF) and microimmunofluorescence tests. Only one serovar of *C. pneumoniae* has been described.

TRACHOMA

Clinical Findings

In experimental human infections, the incubation period for chlamydial conjunctival infection is 3-10 days. In endemic areas, initial infection occurs in early childhood, and the onset of the long-term consequence, trachoma, is insidious. Chlamydial infection is often mixed with bacterial conjunctivitis in endemic areas, and the two together produce the clinical picture. The earliest symptoms of trachoma are lacrimation, mucopurulent discharge, conjunctival hyperemia, and follicular hypertrophy. Microscopic examination of the cornea reveals epithelial keratitis, subepithelial infiltrates, and extension of limbal vessels into the cornea (pannus). As the pannus extends downward across the cornea, there is scarring of the conjunctiva, eyelid deformities (entropion, trichiasis), and added insult caused by eyelashes sweeping across the cornea. With secondary bacterial infection, loss of vision progresses over a period of years. There are, however, no systemic symptoms or signs of infection.

Laboratory Diagnosis

A. CULTURE

Typical cytoplasmic inclusions are found in epithelial cells of conjunctival scrapings stained with fluorescent antibody or by the Giemsa method. These occur most frequently in the early stages of the disease and on the upper tarsal conjunctiva. Inoculation of conjunctival scrapings into cycloheximide-treated McCoy cell cultures permits growth of *C. trachomatis* if the number of viable infectious particles is sufficiently large. Centrifugation of the inoculum into the cells increases the sensitivity of the method. The diagnosis can sometimes be made in the first passage after 2-3 days of incubation by looking for inclusions by immunofluorescence or staining with iodine or Giemsa stain.

B. SEROLOGY

Infected individuals often develop both group antibodies and serovar-specific antibodies in serum and in eye secretions. Immunofluorescence is the most sensitive method for their detection. Neither ocular nor serum antibodies confer significant resistance to reinfection.

C. MOLECULAR METHODS

Developing countries, where trachoma is endemic, generally do not have the resources to apply PCR or other molecular methods to the diagnosis of *C. trachomatis* infections of the eye. Developed countries have relatively little trachoma and little need for such tests. Thus, the molecular methods have been developed for the diagnosis of genital infections. Only research projects have used PCR in studies of trachoma.

Treatment

Clinical trials, in villages with endemic trachoma, using mass azithromycin treatment show that infection and clinical disease are greatly decreased at 6 and 12 months post therapy; this is true even with single dose therapy. Thus, azithromycin has replaced erythromycin and doxycycline in the mass treatment of endemic trachoma. Topical therapy is of little value.

Epidemiology & Control

It is believed that more than 400 million people throughout the world have trachoma and that 20 million are blinded by it. The disease is most prevalent in Africa, Asia, and the Mediterranean basin, where hygienic conditions are poor and water is scarce. In such hyperendemic areas, childhood infection may be universal, and severe blinding disease (resulting from frequent bacterial superinfection) is common. In the United States, trachoma occurs sporadically in some areas, and endemic foci persist.

The WHO has initiated the S-A-F-E program to eliminate blinding trachoma and at least markedly reduce clinically active disease. The S-A-F-E program is as follows: surgery for deformed eyelids; periodic Azithromycin therapy; face washing and hygiene; and, environmental improvement such as building latrines and decreasing the number of flies that feed on conjunctival exudates. It is clear that improved socioeconomic conditions enhance the disappearance of endemic trachoma.

GENITAL INFECTION

Laboratory Diagnosis

A. CULTURE

Collect endocervical specimens following removal of discharge and secretions from the cervix. A swab or cytology brush is used to scrape epithelial cells from 1-2 cm deep into the endocervix. A similar method is used to collect specimens from the vagina, urethra, or conjunctiva. Biopsy specimens of the uterine tube or epididymis can also be cultured. Dacron, cotton, rayon, or calcium alginate on a plastic shaft should be used to collect the specimen; some other swab materials and wooden shafts are toxic to *Chlamydia*. The swab specimens should be placed in a *Chlamydia* transport medium and kept at refrigerator temperature before transport to the laboratory. McCoy cells are grown in monolayers on coverslips in dram or shell vials. Some laboratories use flat-bottomed microdilution trays, but cultures by this method are not as sensitive as those achieved with the shell vial method. The McCoy cells are treated with cycloheximide to inhibit metabolism and increase the sensitivity of isolation of the *Chlamydia*. The inoculum from the swab specimen is centrifuged onto the monolayer and incubated at 35-37 °C for 48-72 hours. A second monolayer can be inoculated, and after incubation it can be sonicated and passaged to another monolayer to enhance sensitivity. The monolayers are examined by direct immunofluorescence to visualize the cytoplasmic inclusions. Chlamydial cultures by this method are about 80% sensitive but 100% specific.

B. DIRECT CYTOLOGIC EXAMINATION (DIRECT FLUORESCENT ANTIBODY OR DFA) AND ENZYME-LINKED IMMUNOASSAY (EIA)

Commercially available DFA and EIA assays to detect *C. trachomatis* can be used in laboratories that lack the expertise or facilities to perform culture. Specimens are collected with techniques similar to those used to collect specimens for culture. Urine specimens may be used with some of the tests. The DFA uses monoclonal antibodies directed against a species-specific antigen on the chlamydial major outer membrane protein (MOMP). The EIA detects the presence of genus-specific lipopolysaccharide antigens extracted from elementary bodies in the specimen. The sensitivity of the DFA is 80-90%, and the specificity is 98-99%; the sensitivity of EIA is 80-95%, and the specificity is 98-99% when compared with culture.

C. NUCLEIC ACID DETECTION

The specimens used for the molecular methods to diagnose *C. trachomatis* are the same as those used for culture; urine may be tested as well. One commercial method uses a chemiluminescent DNA probe that hybridizes to a species-specific sequence of *Chlamydia* 16S rRNA; chlamydiae have up to 104 copies of the 16S rRNA. Once the hybrids are formed they are absorbed onto beads, and the amount of chemiluminescence is then detected in a luminometer. The overall sensitivity and specificity of this method are about 85% and 98-99%, respectively.

Nucleic acid amplification tests have also been developed and marketed. One test is based on the polymerase chain reaction (PCR) and another on the ligase chain reaction (LCR). These tests are much more sensitive than culture and other nonamplification tests and have required redefinition of sensitivity in the laboratory documentation of chlamydial infection. The specificity of the tests appears to be close to 100%. The nucleic acid amplification tests are the tests of choice to diagnose genital *C. trachomatis* infections.

D. SEROLOGY

Because of the relatively great antigenic mass of *Chlamydia* in genital tract infections, serum antibodies occur much more commonly than in trachoma and are of higher titer. A titer rise occurs during and after acute chlamydial infection. Because of the high prevalence of chlamydial genital tract infections in some societies, there is a high background of antichlamydial antibodies in the population; serologic tests to diagnose genital tract chlamydial infections generally are not useful. In genital secretions (eg, cervical), antibody can be detected during active infection and is directed against the infecting immunotype (serovar).

Treatment

It is essential that chlamydial infections be treated simultaneously in both sex partners and in offspring to prevent reinfection. Tetracyclines (eg, doxycycline) are commonly used in nongonococcal urethritis and in nonpregnant infected females. Azithromycin is effective and can be given to pregnant women. Topical tetracycline or erythromycin is used for inclusion conjunctivitis, sometimes in combination with a systemic drug.

Epidemiology & Control

Genital chlamydial infection and inclusion conjunctivitis are sexually transmitted diseases that are spread by contact with infected sex partners. Neonatal inclusion conjunctivitis originates in the mother's infected genital tract. Prevention of neonatal eye disease depends upon diagnosis and treatment of the pregnant woman and her sex partner. As in all sexually transmitted diseases, the presence of multiple etiologic agents (gonococci, treponemes, trichomonads, herpes, etc) must be considered. Instillation of erythromycin or tetracycline into the newborn's eyes does not prevent development of chlamydial conjunctivitis. The ultimate control of this- and all- sexually transmitted disease depends on safe sex practices and on early diagnosis and treatment of infected persons.

LYMPHOGLANULOMA VENEREUM

Properties of the Agent

The particles contain CF heat-stable chlamydial group antigens that are shared with all other *Chlamydia*. They also contain one of three serovar antigens (L1-L3), which can be defined by immunofluorescence.

Clinical Findings

Several days to several weeks after exposure, a small, evanescent papule or vesicle develops on any part of the external genitalia, anus, rectum, or elsewhere. The lesion may ulcerate, but usually it remains unnoticed and heals in a few days. Soon thereafter, the regional lymph nodes enlarge and tend to become matted and painful. In males, inguinal nodes are most commonly involved both above and below Poupart's ligament, and

the overlying skin often turns purplish as the nodes suppurate and eventually discharge pus through multiple sinus tracts. In females and in homosexual males, the perirectal nodes are prominently involved, with proctitis and a bloody mucopurulent anal discharge. Lymphadenitis may be most marked in the cervical chains.

During the stage of active lymphadenitis, there are often marked systemic symptoms including fever, headaches, meningismus, conjunctivitis, skin rashes, nausea and vomiting, and arthralgias. Meningitis, arthritis, and pericarditis occur rarely. Unless effective antimicrobial drug treatment is given at that stage, the chronic inflammatory process progresses to fibrosis, lymphatic obstruction, and rectal strictures.

The lymphatic obstruction may lead to elephantiasis of the penis, scrotum, or vulva. The chronic proctitis of women or homosexual males may lead to progressive rectal strictures, rectosigmoid obstruction, and fistula formation.

Laboratory Diagnosis

A. SMEARS

Pus, buboes, or biopsy material may be stained, but particles are rarely recognized.

B. CULTURE

Suspected material is inoculated into McCoy cell cultures. The inoculum can be treated with an aminoglycoside (but not with penicillin) to lessen bacterial contamination. The agent is identified by morphology and serologic tests.

C. SEROLOGY

Antibodies are commonly demonstrated by the CF reaction. The test becomes positive 2-4 weeks after onset of illness, at which time skin hypersensitivity can sometimes also be demonstrated. In a clinically compatible case, a rising antibody level or a single titer of more than 1:64 is good evidence of active infection. If treatment has eradicated the lymphogranuloma venereum infection, the CF titer falls. Serologic diagnosis of lymphogranuloma venereum can employ immunofluorescence, but the antibody is broadly reactive with many chlamydial antigens.

Immunity

Untreated infections tend to be chronic, with persistence of the agent for many years. Little is known about active immunity. The coexistence of latent infection, antibodies, and cell-mediated reactions is typical of many chlamydial infections.

Treatment

The sulfonamides and tetracyclines have been used with good results, especially in the early stages. In some drug-treated persons there is a marked decline in complement-fixing antibodies, which may indicate that the infective agent has been eliminated from the body. Late stages require surgery.

Epidemiology & Control

Although the highest incidence of lymphogranuloma venereum has been reported from subtropical and tropical areas, the infection occurs all over the world. The disease is most often spread by sexual contact, but not exclusively so. The portal of entry may sometimes be the eye (conjunctivitis with an oculoglandular syndrome). The genital tracts and rectums of chronically infected (but at times asymptomatic) persons serve as reservoirs of infection. Laboratory personnel exposed to aerosols of *C. trachomatis* serovars L1-L3 can develop a chlamydial pneumonitis with mediastinal and hilar adenopathy. If the infection is recognized, treatment with tetracycline or erythromycin is effective.

The measures used for the control of other sexually transmitted diseases apply also to the control of lymphogranuloma venereum. Case-finding and early treatment and control of infected persons are essential.

RESPIRATORY INFECTIONS

Properties of the Agent

C. pneumoniae produces round, dense, glycogen-negative inclusions that are sulfonamide-resistant, much like *C. psittaci*.The elementary bodies sometimes have a pear-shaped appearance. The genetic relatedness of *C. pneumoniae* isolates is > 95%. Only on serovar has been demonstrated.

Clinical Findings

Most infections with *C. pneumoniae* are asymptomatic or associated with mild illness, but severe disease has been reported. There are no signs or symptoms that specifically differentiate *C. pneumoniae* infections from those caused by many other agents. Both upper and lower airway disease occur. Pharyngitis is common.

Sinusitis and otitis media may occur and be accompanied by lower airway disease. An atypical pneumonia similar to that caused by *Mycoplasma pneumoniae* is the primary recognized illness. Five to 20 percent of community-acquired pneumonia in young persons is thought to be caused by *C. pneumoniae*.

Laboratory Diagnosis

A. SMEARS

Direct detection of elementary bodies in clinical specimens using fluorescent antibody techniques is insensitive. Other stains do not effectively demonstrate the organism.

B. CULTURE

Swab specimens of the pharynx should be put into a *Chlamydia* transport medium and placed at 4 °C; *C. pneumoniae* is rapidly inactivated at room temperature. It grows poorly in cell culture, forming inclusions smaller than those formed by the other *Chlamydia*. *C. pneumoniae* grows better in HL and HEp-2 cells than in HeLa 229 or McCoy cells; the McCoy cells are widely used to culture *C. trachomatis*. The sensitivity of the culture is increased by incorporation of cycloheximide into the cell culture medium to inhibit the eukaryotic cell metabolism and by centrifugation of the inoculum onto the cell layer. Growth is better at 35 °C than 37 °C. After 3 days' incubation, the cells are fixed and inclusions detected by fluorescent antibody staining with genus- or species-specific antibody or, preferably, with a *C. pneumoniae*-specific monoclonal antibody conjugated with fluorescein. Giemsa staining is insensitive, and the glycogen-negative inclusions do not stain with iodine. It is moderately difficult to grow *C. pneumonia* - as evidenced by the number of isolates described compared with the incidence of infection.

C. SEROLOGY

Serology using the microimmunofluorescence test is the most sensitive method for diagnosis of *C. pneumoniae* infection. The test is species-specific and can detect IgG or IgM antibodies by using the appropriate reagents. Primary infection yields IgM antibody after about 3 weeks followed by IgG antibody at 6-8 weeks. In reinfection, the IgM response may be absent or minimal and the IgG response occurs in 1-2 weeks. The following criteria have been suggested for the serologic diagnosis of *C. pneumoniae* infection: a single IgM titer of ≥ 1:16; a single IgG titer of ≥ 1:512; and a fourfold rise in either the IgM or IgG titers.

The complement fixation test can be used, but it is group-reacting, does not differentiate *C. pneumoniae* infection from psittacosis or lymphogranuloma venereum, and is less sensitive than the microimmunofluorescence test.

Immunity

Little is known about active or potentially protective immunity. Prolonged infections can occur with *C. pneumoniae*, and asymptomatic carriage may be common.

Treatment

C. pneumoniae is susceptible to the macrolides and tetracyclines and to some fluoroquinolones. Treatment with doxycycline, azithromycin, or clarithromycin appears to significantly benefit patients with *C. pneumoniae* infection, but there are only limited data on the efficacy of antibiotic treatment. Reports indicate that the symptoms may continue or recur after routine courses of therapy with erythromycin, doxycycline, or tetracycline, and these drugs should be given for 10- to 14-day courses.

Epidemiology

Infection with *C. pneumoniae* is common. Worldwide, 30-50% of people have antibody to *C. pneumoniae.* Few young children have antibody, but after the age of 6-8 years, the prevalence of antibody increases through young adulthood. Infection is both endemic and epidemic, with multiple outbreaks attributed to *C. pneumoniae*. There is no known animal reservoir, and transmission is presumed to be from person to person, predominantly by the airborne route.

Lines of evidence suggesting that *C. pneumoniae* is associated with atherosclerotic coronary artery and cerebrovascular disease consist of seroepidemiologic studies, detection of *C. pneumoniae* in atherosclerotic tissues, cell culture studies, animal models, and trials of prevention using antibiotic agents. The association appears valid, but additional work is needed before causation can be considered established or disproved.

PSITTACOSIS

Properties of the Agent

C. psittaci can be propagated in embryonated eggs, in mice and other animals, and in some cell cultures. The heat-stable group-reactive CF antigen resists proteolytic enzymes and appears to be a lipopolysaccharide.

Treatment of *C. psittaci* infection with deoxycholate and trypsin yields extracts that contain group-reactive CF antigens, whereas the cell walls retain the species-specific antigen. Antibodies to the species-specific antigen are able to neutralize toxicity and infectivity.

Specific serovars characteristic for certain mammalian and avian species may be demonstrated by immunofluorescence typing. Neutralization of infectivity of the agent by specific antibody or cross-protection of immunized animals can also be used for serotyping, and the results parallel those of immunofluorescence typing.

Pathogenesis & Pathology

The agent enters through the respiratory tract, is found in the blood during the first 2 weeks of the disease, and may be found in the sputum at the time the lung is involved. Psittacosis causes a patchy inflammation of the lungs in which consolidated areas are sharply demarcated. The exudate is predominantly mononuclear. Only minor changes occur in the large bronchioles and bronchi. The lesions are similar to those found in pneumonitis caused by some viruses and mycoplasmas. Liver, spleen, heart, and kidney are often enlarged and congested.

Clinical Findings

A sudden onset of illness taking the form of influenza or nonbacterial pneumonia in a person exposed to birds is suggestive of psittacosis. The incubation period averages 10 days. The onset is usually sudden, with malaise, fever, anorexia, sore throat, photophobia, and severe headache.

The disease may progress no further, and the patient may improve in a few days. In severe cases, the signs and symptoms of bronchial pneumonia appear at the end of the first week of the disease. The clinical picture often resembles that of influenza, nonbacterial pneumonia, or typhoid fever. The mortality rate may be as high as 20% in untreated cases, especially in the elderly.

Laboratory Diagnosis

A. CULTURE

Culture of *C. psittaci* can be dangerous, and detection of the organism using immunoassays or polymerase chain reaction is preferred. If necessary, *C. psittaci* can be cultured from blood or sputum or from lung tissue by culture in tissue culture cells, embryonated eggs, or mice.

Isolation of *C. psittaci* is confirmed by the serial transmission, its microscopic demonstration, and serologic identification.

B. DETECTION OF C PSITTACI

Antigen detection by direct fluorescent antibody staining or by immunoassay or molecular diagnosis by polymerase chain reaction is done in reference or research laboratories.

C. SEROLOGY

A diagnosis of psittacosis is usually confirmed by demonstrating complement-fixing or microimmunofluorescent antibodies in serum specimens. A confirmed case is one with a positive culture or associated with a compatible clinical illness plus a fourfold or greater change in antibody titer to at least 1:32 or a microimmunofluorescence IgM titer of at least 1:16. A probable case is one associated with a compatible illness linked epidemiologically with a confirmed case or a titer of at least 1:32 in a single specimen.

The complement fixation test is cross-reactive with *C. trachomatis* and *C. pneumoniae*. The microimmunofluorescence test (MIF) is more sensitive and specific than the CF test, but cross-reactions do occur. MIF allows detection of IgM and IgG. Although antibodies usually develop within 10 days, the use of antibiotics may delay their development for 20-40 days or suppress it altogether.

In live birds, infection is suggested by a positive CF test and an enlarged spleen or liver. This can be confirmed by demonstration of particles in smears or sections of organs and by passage of the agent in mice and eggs.

D. MOLECULAR METHODS

Multiple PCR assays have been developed to detect *C. psittaci* in respiratory tract specimens, vascular tissues, serum, and mononuclear cells from peripheral blood.

These tests hold great promise of being more sensitive than culture or serology in detecting *C. psittaci*. None of the tests is approved by the FDA for clinical use, and until they are approved they will remain for research use only.

Immunity

Immunity in animals and humans is incomplete. A carrier state in humans can persist for 10 years after recovery. During this period, the agent may continue to be excreted in the sputum. Live or inactivated vaccines induce only partial resistance in animals. They have not been used in humans.

Treatment

Because of the difficulty in obtaining laboratory confirmation of *C. psittaci* infection, most infections are treated based only on the clinical diagnosis. Information on therapeutic efficacy comes from several clinical trials. Azithromycin, clarithromycin, and erythromycin (and doxycycline in adults) clear most, but not all, respiratory *C. psittaci* infections. All the patients improve clinically, even those with persistent infection.

Epidemiology & Control

Outbreaks of human disease can occur whenever there is close and continued contact between humans and infected birds that excrete or shed large amounts of infectious agent. Birds often acquire infection as fledglings in the nest, may develop diarrheal illness or no illness, and often carry the infectious agent for their normal life span. When subjected to stress (eg, malnutrition, shipping), birds may become sick and die. The agent is present in tissues (eg, spleen) and is often excreted in feces by healthy birds. The inhalation of infected dried bird feces is a common method of human infection.

Another source of infection is the handling of infected tissues (eg, in poultry rendering plants) and inhalation of an infected aerosol. Birds kept as pets have been an important source of human infection. Foremost among these were the many imported psittacine birds. Latent infections often flared up in these birds during transport and crowding, and sick birds excreted exceedingly large quantities of infectious agent. Control of bird shipment, quarantine, testing of imported birds for psittacosis infection, and prophylactic tetracyclines in bird feed have helped to control this source. Pigeons kept for racing or as pets or raised for squab meat have been important sources of infection. Pigeons populating buildings and thoroughfares in many cities, if infected, shed relatively small quantities of agent.

MYCOPLASMA

Morphology & Identification

A. TYPICAL ORGANISMS

Mycoplasma cannot be studied by the usual bacteriologic methods because of the small size of their colonies and the plasticity and delicacy of their individual cells (owing to the lack of a rigid cell wall). Growth in fluid media gives rise to many different forms. Growth on solid media consists principally of plastic protoplasmic masses of indefinite shape that are easily distorted. These structures vary greatly in size, ranging from 50 to 300 nm in diameter. The morphology appears different according to the method of examination (eg, darkfield, immunofluorescence, Giemsa-stained films from solid or liquid media, agar fixation).

B. CULTURE

Many strains of *Mycoplasma* grow in heart infusion peptone broth with 2% agar (pH 7,8) to which about 30% human ascitic fluid or animal serum (horse, rabbit) has been added. Following incubation at 37 °C for 48-96 hours, there may be no turbidity; however, Giemsa stains of the centrifuged sediment show the characteristic pleomorphic structures, and subculture on solid media yields minute colonies.

After 2-6 days on diphasic (broth over agar) and agar medium incubated in a Petri dish that has been sealed to prevent evaporation, isolated colonies measuring 20-500 μm can be detected with a hand lens. These colonies are round, with a granular surface and a dark center typically buried in the agar. They can be subcultured by cutting out a small square of agar containing one or more colonies and streaking this material on a fresh plate or dropping it into liquid medium. The organisms can be stained for microscopic study by placing a similar square on a slide and covering the colony with a cover-glass onto which an alcoholic solution of methylene blue and azure has been poured and then evaporated (agar fixation). Such slides can also be stained with specific fluorescent antibody.

C. GROWTH CHARACTERISTICS

Mycoplasma are unique in microbiology because of (1) their extremely small size and (2) their growth on complex but cell-free media. *Mycoplasma* pass through filters with 450-nm pore size and thus are comparable to *Chlamydia* or large viruses. However, parasitic *Mycoplasma* grow on cell-free media that contain lipoprotein and sterol. The sterol requirement for growth and membrane synthesis is unique.

Many *Mycoplasma* use glucose as a source of energy; *Ureaplasm*a require urea. Some human

Mycoplasma produce peroxides and hemolyze red blood cells. In cell cultures and in vivo, *Mycoplasma* develop predominantly at cell surfaces. Many established animal and human cell culture lines carry *Mycoplasma* as contaminants.

D. VARIATION

The extreme pleomorphism of *Mycoplasma* is one of their principal characteristics.

Antigenic Structure

Many antigenically distinct species of *Mycoplasma* have been isolated from animals (eg, mice, chickens, turkeys). In humans, at least 14 species can be identified, including *M. hominis*, *Mycoplasma salivarium*, *Mycoplasma orale*, *Mycoplasma fermentans*, *M. pneumoniae*, *M. genitalium*, *U. urealyticum*, and others.

The species are classified by biochemical and serologic features. The CF antigens of *Mycoplasma* are glycolipids. Antigens for ELISA tests are proteins. Some species have more than one serotype.

Pathogenesis

Many pathogenic *Mycoplasma* have flask-like or filamentous shapes and have specialized polar tip structures that mediate adherence to host cells. These structures are a complex group of interactive proteins, adhesins, and adherence-accessory proteins. The proteins are proline-rich, which influences the protein folding and binding and is important in the adherence to cells. The *Mycoplasma* attach to the surfaces of ciliated and nonciliated cells, probably through the mucosal cell sialoglycoconjugates and sulfated glycolipids. Some *Mycoplasma* lack the distinct tip structures but use adhesion proteins or have alternative mechanisms to adhere to host cells. The subsequent events in infection are less well understood but may include several factors as follows: direct cytotoxicity through generation of hydrogen peroxide and superoxide radicals; cytolysis mediated by antigen-antibody reactions or by chemotaxis and action of mononuclear cells; and competition for and depletion of nutrients.

MYCOPLASMAL INFECTION

The *Mycoplasma* appear to be host-specific, being communicable and potentially pathogenic only within a single host species. In animals, *Mycoplasma* appear to be intracellular parasites with a predilection for mesothelial cells (pleura, peritoneum, synovia of joints). Several extracellular products can be elaborated (eg, hemolysins).

A. INFECTION OF HUMANS

Mycoplasma have been cultivated from human mucous membranes and tissues, particularly from the genital, urinary, and respiratory tracts. *Mycoplasma* are part of the normal flora of the mouth and can be grown from normal saliva, oral mucous membranes, sputum, or tonsillar tissue. *M. salivarium*, *M. orale*, and other *Mycoplasma* can be recovered from the oral cavities of many healthy adults, but an association with clinical disease is uncertain. *M. hominis* is found in the oropharynx of less than 5% of adults. *M. pneumoniae* in the oropharynx is generally associated with disease.

Some *Mycoplasma* are inhabitants of the genitourinary tract, particularly in females. In both men and women, genital carriage of *Mycoplasma* is directly related to the number of lifetime sex partners. *M. hominis* can be cultured from 1-5% of asymptomatic men and 30-70% of asymptomatic women; the rates increase to 20% and over 90% positive for men and women, respectively, in sexually transmitted disease clinics. *U. urealyticum* is found in the genital tracts of 5-20% of sexually active men and 40-80% of sexually active women. Approximately 20% of women attending sexually transmitted disease clinics have *M. genitalium* in their lower genital tracts. Other *Mycoplasma* also occur in the lower genital tract.

B. INFECTION OF ANIMALS

Bovine pleuropneumonia is a contagious, occasionally lethal disease of cattle associated with pneumonia and pleural effusion. The disease probably has an airborne spread. *Mycoplasma* are found in inflammatory exudates.

Agalactia of sheep and goats in the Mediterranean area is a generalized infection with local lesions in the skin, eyes, joints, udder, and scrotum; it leads to atrophy of lactating glands in females. *Mycoplasma* are present in blood early and in milk and exudates later. In poultry, several economically important respiratory diseases are caused by *Mycoplasma*. The organisms can be transmitted from hen to egg to chick.

Swine, dogs, rats, mice, and other species harbor *Mycoplasma* that can produce infection involving particularly the pleura, peritoneum, joints, respiratory tract, and eye. In mice, a *Mycoplasma* of spiral shape (spiroplasma) can induce cataracts.

C. INFECTION OF PLANTS

Aster yellows, corn stunt, and other plant diseases appear to be caused by *Mycoplasma*. They are transmitted

by insects and can be suppressed by tetracyclines.

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens consist of throat swabs, sputum, inflammatory exudates, and respiratory, urethral, or genital secretions.

B. MICROSCOPIC EXAMINATION

Direct examination of a specimen for *Mycoplasma* is useless. Cultures are examined as described above.

C. CULTURES

The material is inoculated onto special solid media and incubated for 3-10 days at 37 °C with 5% CO_2 (under microaerophilic conditions), or into special broth and incubated aerobically. One or two transfers of media may be necessary before growth appears that is suitable for microscopic examination by staining or immunofluorescence. Colonies may have a «fried egg» appearance on agar.

D. SEROLOGY

Antibodies develop in humans infected with *Mycoplasma* and can be demonstrated by several methods. CF tests can be performed with glycolipid antigens extracted with chloroform-methanol from cultured *Mycoplasma*. HI tests can be applied to tanned red cells with adsorbed *Mycoplasma* antigens. Indirect immunofluorescence may be used. The test that measures growth inhibition by antibody is quite specific. With all these serologic techniques, there is adequate specificity for different human *Mycoplasma* species, but a rising antibody titer is required for diagnostic significance because of the high incidence of positive serologic tests in normal individuals. *M. pneumoniae* and *M. genitalium* are serologically cross-reactive.

Treatment

Many strains of *Mycoplasma* are inhibited by a variety of antimicrobial drugs, but most strains are resistant to penicillins, cephalosporins, and vancomycin. Tetracyclines and erythromycins are effective both in vitro and in vivo and are, at present, the drugs of choice in mycoplasmal pneumonia. Some *Ureaplasma* are resistant to tetracycline.

Epidemiology, Prevention, & Control

Isolation of infected livestock will control the highly contagious pleuropneumonia and agalactia. No vaccines are available. Mycoplasmal pneumonia behaves like a communicable viral respiratory disease.

MYCOPLASMA PNEUMONIAE & ATYPICAL PNEUMONIAS

Pathogenesis

M. pneumoniae is transmitted from person to person by means of infected respiratory secretions. Infection is initiated by attachment of the organism's tip to a receptor on the surface of respiratory epithelial cells.

Attachment is mediated by a specific adhesin protein on the differentiated terminal structure of the organism. During infection, the organisms remain extracellular.

Clinical Findings

Mycoplasmal pneumonia is generally a mild disease. The clinical spectrum of *M. pneumoniae* infection ranges from asymptomatic infection to serious pneumonitis, with occasional neurologic and hematologic (ie, hemolytic anemia) involvement and a variety of possible skin lesions.

Bullous myringitis occurs in spontaneous cases and in experimentally inoculated volunteers. The incubation period varies from 1 to 3 weeks. The onset is usually insidious, with lassitude, fever, headache, sore throat, and cough. Initially, the cough is nonproductive, but it is occasionally paroxysmal. Later there may be blood-streaked sputum and chest pain. Early in the course, the patient appears only moderately ill, and physical signs of pulmonary consolidation are often negligible compared to the striking consolidation seen on x-rays. Later, when the infiltration is at a peak, the illness may be severe. Resolution of pulmonary infiltration and clinical improvement occur slowly over 1-4 weeks. Although the course of the illness is exceedingly variable, death is very rare and is usually attributable to cardiac failure. Complications are uncommon, but hemolytic anemia may occur. The most common pathologic findings are interstitial and peribronchial pneumonitis and necrotizing bronchiolitis. Other diseases possibly related to *M. pneumoniae* include erythema multiforme; central nervous system involvement, including meningitis, meningoencephalitis, and mono- and polyneuritis; myocarditis; pericarditis; arthritis; and pancreatitis.

Common causes of community-acquired bacterial pneumonia, in addition to *Mycoplasma pneumoniae*,

include *Streptococcus pneumoniae*, *Legionella pneumophila*, *Chlamydia pneumoniae*, and *Haemophilus influenzae*. The clinical presentations of these infections can be very similar, and recognition of the subtleties of signs and symptoms is important. The causative organisms must be determined by sputum examination and culture, blood culture, and other tests.

Laboratory Tests

The diagnosis of *M. pneumoniae* pneumonia is largely made by the clinical recognition of the syndrome. Laboratory tests are of secondary value. The white cell count may be slightly elevated. A sputum Gram stain is of value in not suggesting some other bacterial pathogen (eg, *Streptococcus pneumoniae*). The causative *Mycoplasma* can be recovered by culture from the pharynx and from sputum, but culture is a highly specialized test and is almost never done to diagnose *M. pneumoniae* infection. Cold hemagglutinins for group O human erythrocytes appear in about 50% of untreated patients, in rising titer, with the maximum reached in the third or fourth week after onset. A titer of 1:64 or more supports the diagnosis of *M. pneumoniae* infection. There is a rise in specific antibodies to *M. pneumoniae* that is demonstrable by complement fixation (CF) tests; acute and convalescent phase sera are necessary to demonstrate a fourfold rise in the CF antibodies. EIA to detect IgM and IgG antibodies can be highly sensitive and specific, but may not be readily available. PCR assay of specimens from throat swabs or other clinical material can be diagnostic, but is generally performed only in reference laboratories.

Treatment

Tetracyclines or erythromycins can produce clinical improvement but do not eradicate the *Mycoplasma*.

Epidemiology, Prevention, & Control

M. pneumoniae infections are endemic all over the world. In populations of children and young adults, where close contact prevails, and in families, the infection rate may be high (50-90%), but the incidence of pneumonitis is variable (3-30%). For every case of frank pneumonitis, there exist several cases of milder respiratory illness. *M. pneumoniae* is apparently transmitted mainly by direct contact involving respiratory secretions. Second attacks are infrequent. The presence of antibodies to *M. pneumoniae* has been associated with resistance to infection but may not be responsible for it. Cell-mediated immune reactions occur. The pneumonic process may be attributed in part to an immunologic response rather than only to infection by *Mycoplasma*.

MYCOPLASMA HOMINIS

Mycoplasma hominis has been associated with a variety of diseases but is a demonstrated cause in only a few of them. The evidence for a causal relationship in disease is from culture and serologic studies. *M. hominis* can be cultured from the upper urinary tract in about 10% of patients with pyelonephritis. *M. hominis* is strongly associated with infection of the uterine tubes (salpingitis) and tubo-ovarian abscesses; the organism can be isolated from the uterine tubes of about 10% of patients with salpingitis but not from women with no signs of disease. Women with salpingitis more commonly have antibodies against *M. hominis* than women with no disease. *M. hominis* has been isolated from the blood of about 10% of women who have postabortal or postpartum fever and occasionally from joint fluid cultures of patients with arthritis.

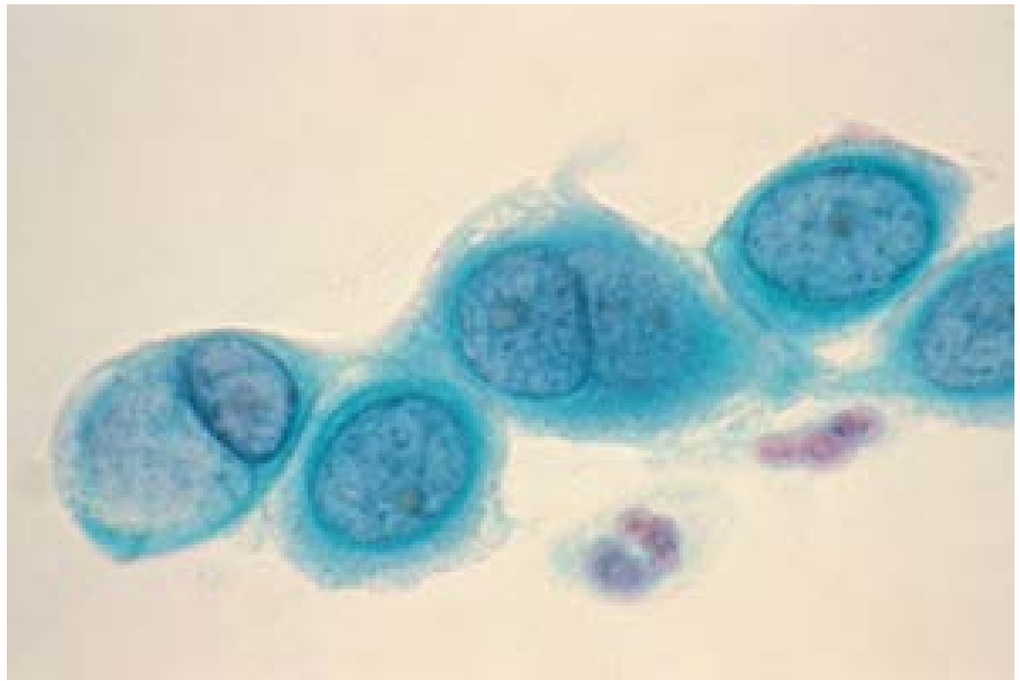
UREAPLASMA UREALYTICUM

Ureaplasma urealyticum, like *M. hominis*, has been associated with a variety of diseases but is a demonstrated cause in only a few of them. *U. urealyticum*, which requires 10% urea for growth, probably causes nongonococcal urethritis in some men, but a majority of cases of nongonococcal urethritis are caused by *Chlamydia trachomatis*. *U. urealyticum* is common in the female genital tract, where the association with disease is weak. *U. urealyticum* has been associated with lung disease in premature low-birth-weight infants who acquired the organism during birth, but a causal effect has not been clearly demonstrated. The evidence that *U. urealyticum* is associated with involuntary infertility is at best marginal.

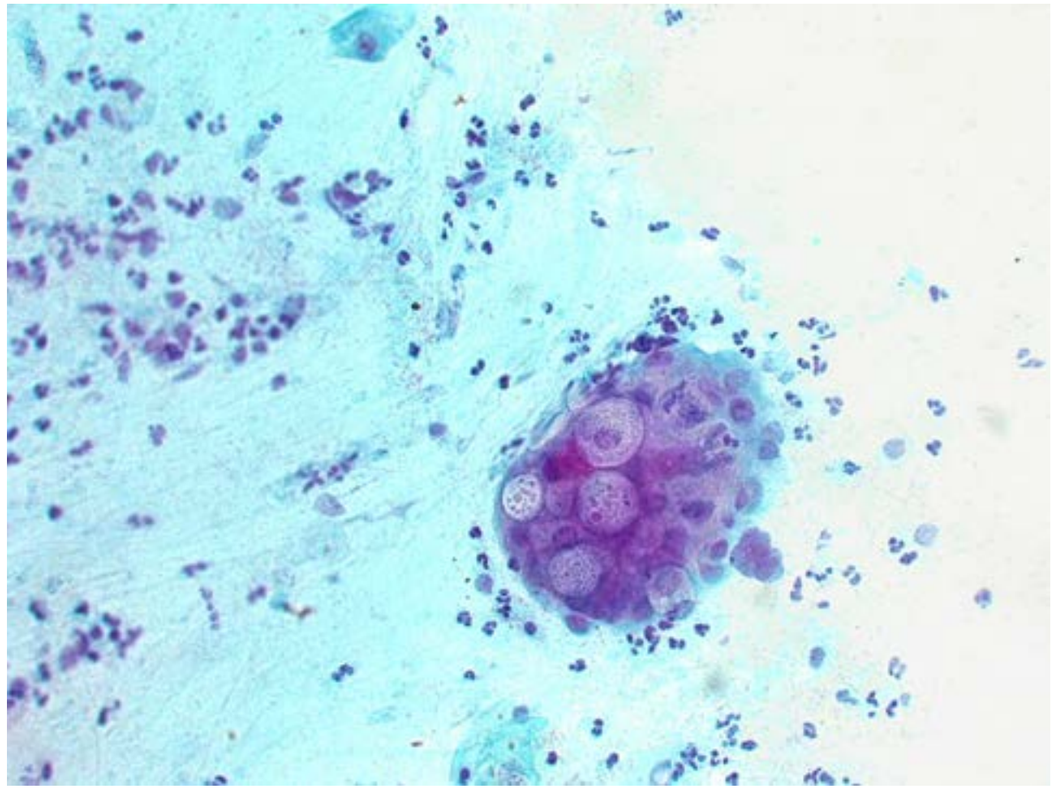
MYCOPLASMA GENITALIUM

Mycoplasma genitalium was originally isolated from urethral cultures of two men with nongonococcal urethritis, but culture of *M. genitalium* is difficult, and subsequent observations have been based on data obtained by using the PCR, molecular probes, and serologic tests. The data suggest that *M. genitalium* in men is associated with some cases of acute as well as chronic nongonococcal urethritis. In women, *M. genitalium* has been associated with a variety of infections such as cervicitis, endometritis, salpingitis, and infertility.

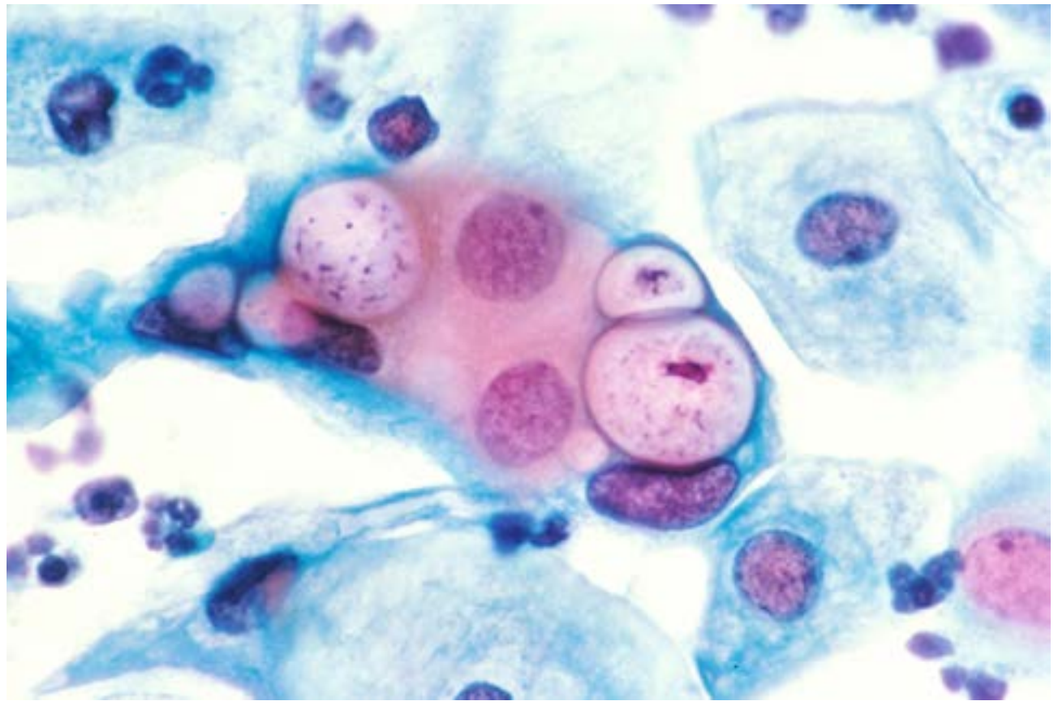
13 Class – Illustrations



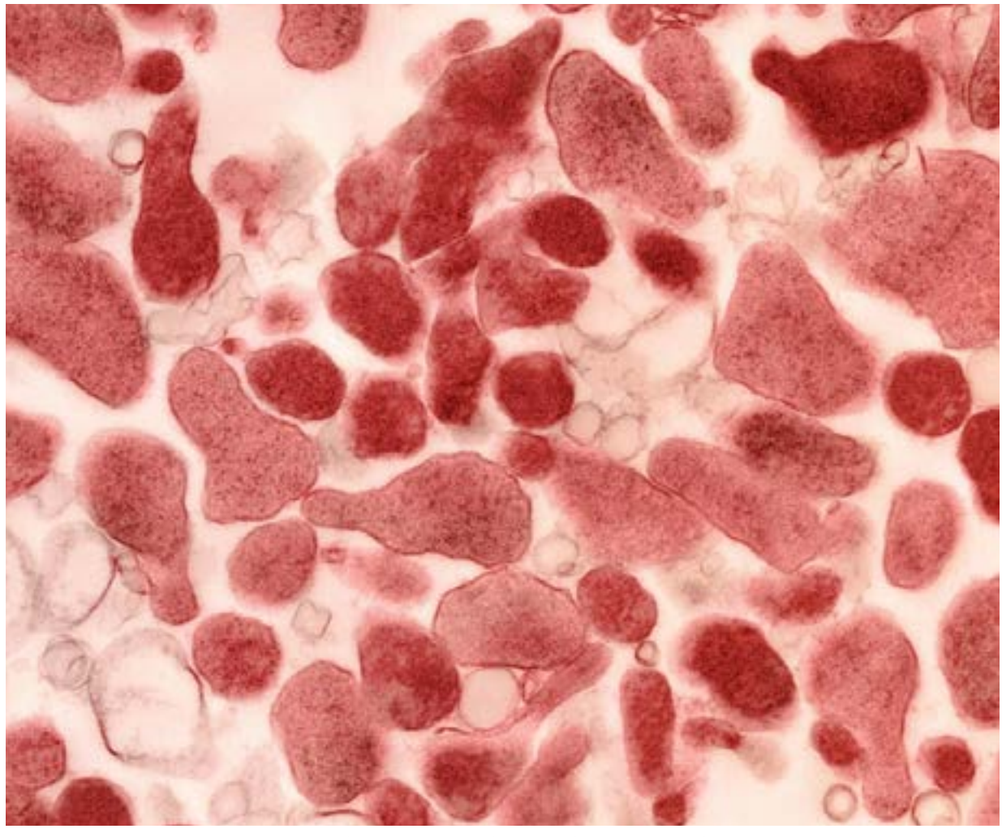
Chlamydia trachomatis (thin preparate, Papanicolaou stain)



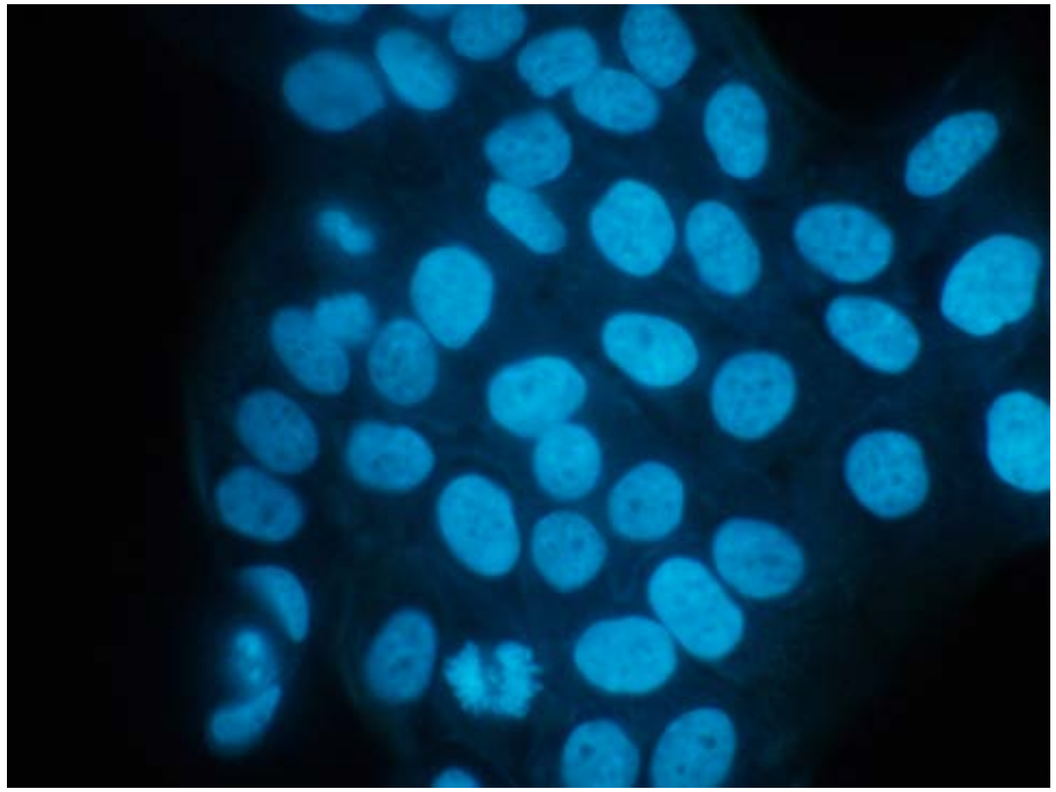
Chlamydia trachomatis inclusion bodies



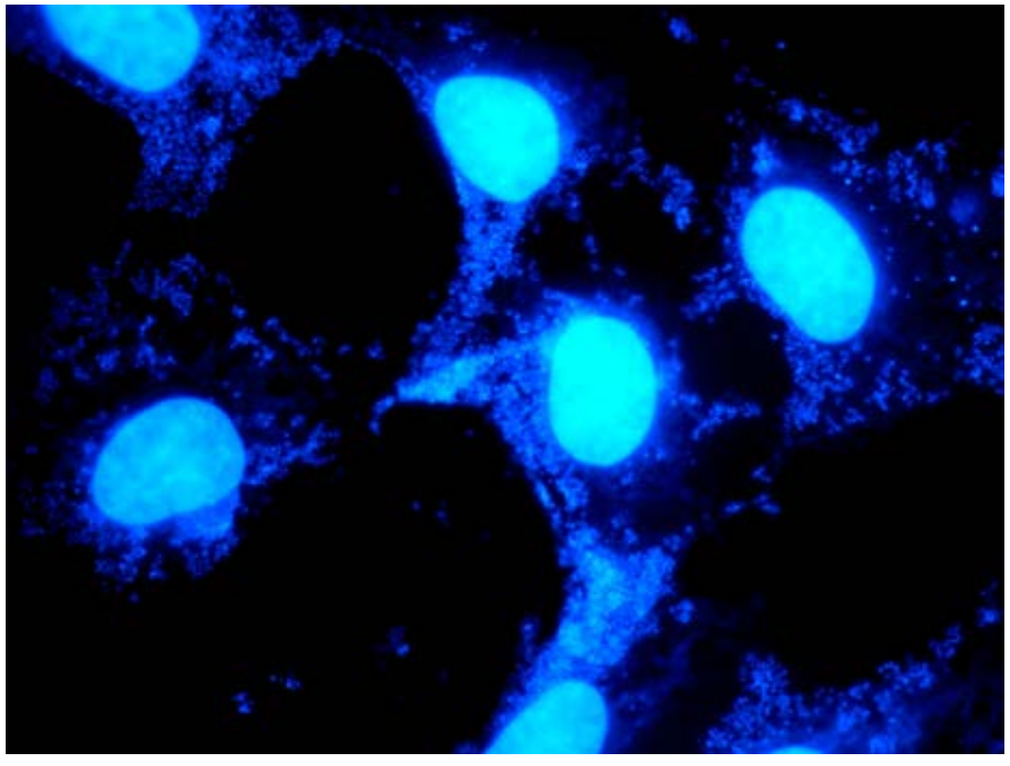
Chlamydia trachomatis (Papanicolaou stain)



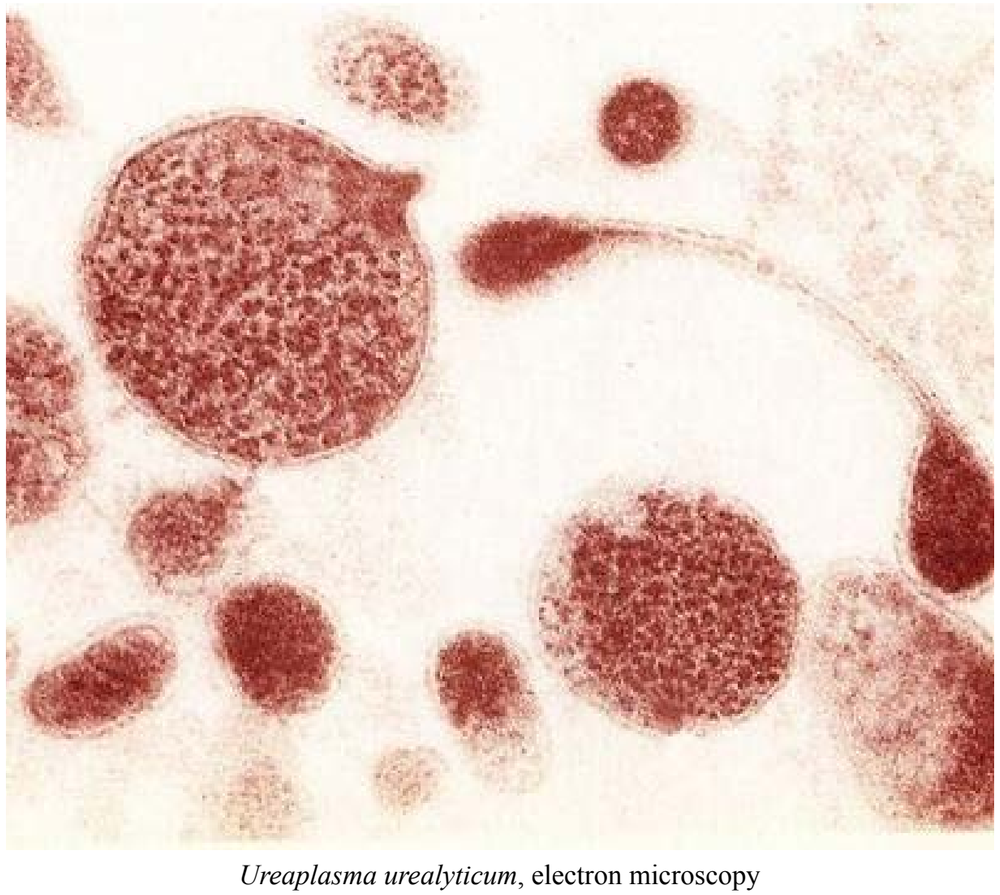
Mycoplasma genitalium, microscope view



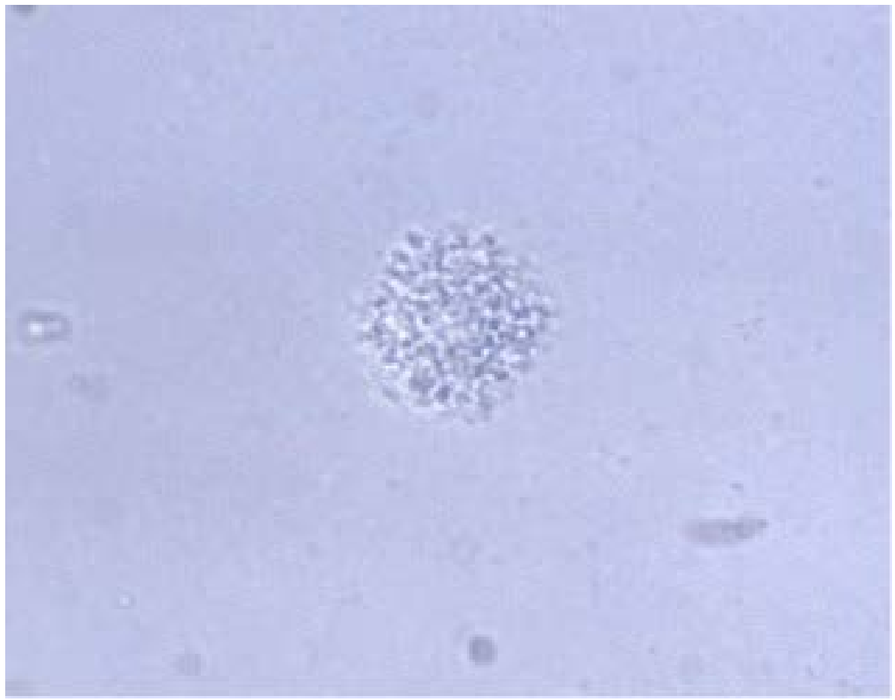
Mycoplasma genitalium, fluorescent staining



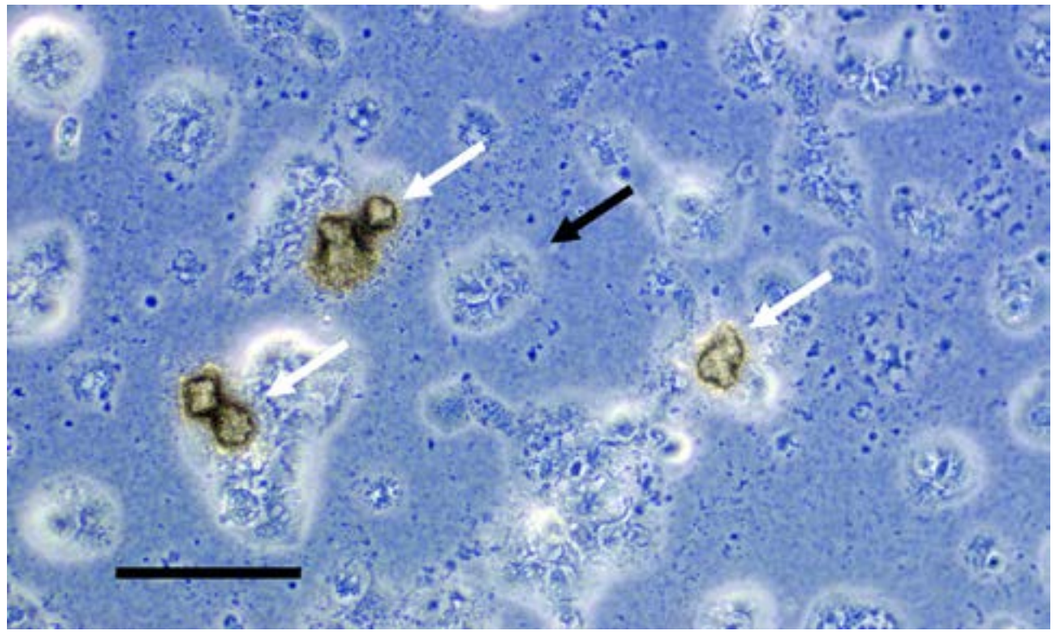
Mycoplasma genitalium



Ureaplasma urealyticum, electron microscopy



Ureaplasma urealyticum, electron microscopy (without staining)



Ureaplasma urealyticum meningitis in an adult patient

14 Class - Fungi. Mold fungi. Actinomycetes. *Nocardia*. Mycotoxicoses

ACTINOMYCETES

The aerobic Actinomycetes are a large, diverse group of Gram-positive bacilli with a tendency to form chains or filaments. They are related to the *Corynebacterium* and include multiple genera of clinical significance such as *Mycobacterium* and saprophytic organisms such as *Streptomyces*. As the bacilli grow, the cells remain together after division to form elongated chains of bacteria (1 μm in width) with occasional branches. The extent of this process varies in different taxa. It is rudimentary in some actinomycetes - the chains are short, break apart after formation, and resemble diphtheroids; others develop extensive substrate or aerial filaments (or both); and either may produce spores or fragment into coccobacillary forms. Members of the aerobic *Actinomycetales* can be categorized on the basis of the acid fast stain. *Mycobacterium* are truly positive acid fast organisms; weakly positive genera include *Nocardia*, *Rhodococcus*, and a few others of clinical significance. *Streptomyces* and *Actinomadura*, two agents that cause actinomycotic mycetomas, are acid fast stain negative.

NOCARDIOSIS

The genus *Nocardia* continues to undergo extensive taxonomic reclassification. New species continue to be recognized and at least 16 species have been implicated as causes of human infections. Nocardiosis is caused by infection with *Nocardia asteroides* complex or, less frequently, *Nocardia brasiliensis* or *Nocardia otitidiscaviarum*, and only rarely by other species of *Nocardia*. The *Nocardia asteroides* complex includes *Nocardia abscessus*, *Nocardia farcinica*, *Nocardia nova*, and others. The importance of the complex is that its members tend to have variable antimicrobial susceptibility, which can influence treatment. The pathogenic *Nocardia*, like many nonpathogenic species of *Nocardia*, are found worldwide in soil and water. Nocardiosis is initiated by inhalation of these bacteria. The usual presentation is as a subacute to chronic pulmonary infection that may disseminate to other organs, usually the brain or skin. *Nocardia* are not transmitted from person to person.

Morphology & Identification

Nocardia species are aerobic and grow on a variety of media. Over the course of several days to a week or more, they develop heaped, irregular, waxy colonies. Strains vary in their pigmentation from white to orange to red.

These bacteria are Gram-positive, catalase-positive, and partially acid-fast bacilli. They produce urease and can digest paraffin. *Nocardia* form extensive branching substrates and aerial filaments that fragment after formation, breaking into coccobacillary cells. The cell walls contain mycolic acids that are shorter-chained than those of *Mycobacterium*. They are considered to be weakly acid-fast, but if they are stained with the routine acid-fast reagent (carbol-fuchsin) but decolorized with 1-4% sulfuric acid instead of the stronger acid-alcohol decolorant, most isolates will stain acid-fast. The species of nocardia are identified by most clinical laboratories by routine phenotypic tests. However, laboratories with molecular capability are using sequencing methodologies and RFLP of amplified gene fragments such as hsp.

Pathogenesis & Clinical Findings

In most cases, nocardiosis is an opportunistic infection associated with several risk factors, most of which impair the cell-mediated immune responses: corticosteroid treatment, immunosuppression, organ transplantation, AIDS, tuberculosis, and alcoholism. Nocardiosis begins as chronic lobar pneumonia, and a variety of symptoms may occur, including fever, weight loss, and chest pain. The clinical manifestations are not distinctive and mimic tuberculosis and other infections. Pulmonary consolidations may develop, but granuloma formation and caseation are rare.

The usual pathologic process is abscess formation. Spread from the lung often involves the central nervous system, where abscesses develop in the brain, leading to a variety of clinical presentations. Some patients have subclinical lung involvement and present with brain lesions. Dissemination may also occur to the skin, kidney, or elsewhere.

Diagnostic Laboratory Tests

Specimens consist of sputum, pus, spinal fluid, and biopsy material. Gram-stained smears reveal Gram-positive bacilli, coccobacillary cells, and branching filaments. With the modified acid-fast stain, most isolates will be acid-fast. *Nocardia* species grow on most laboratory media. Serologic tests are unreliable at present.

Treatment

The treatment of choice is trimethoprim-sulfamethoxazole. If patients fail to respond, a number of other antibiotics have been used with success, such as amikacin, imipenem, minocycline, linezolide, and cefotaxime. Surgical drainage or resection may be required.

ACTINOMYCETOMA

Mycetoma (Madura foot) is a localized, slowly progressive, chronic infection that begins in subcutaneous tissue and spreads to adjacent tissues. It is destructive and often painless. In many cases the cause is a soil fungus that has been implanted into the subcutaneous tissue by minor trauma.

An actinomycetoma is a mycetoma caused by filamentous branching bacteria. The actinomycetoma granule is composed of tissue elements and Gram-positive bacilli and bacillary chains or filaments (1 μm in diameter). The most common causes of actinomycetoma are *Nocardia asteroides*, *Nocardia brasiliensis*, *Streptomyces somaliensis*, and *Actinomadura madurae*. *N. brasiliensis* may be acid-fast. These and other pathogenic actinomycetes are differentiated by biochemical tests and chromatographic analysis of cell wall components. Actinomycetomas respond well to various combinations of streptomycin, trimethoprim-sulfamethoxazole, and dapsone if therapy is begun early before extensive damage has occurred.

Oftentimes students are confused by the terms, Actinomycetes and Actinomycosis. The former have been described above; the latter is an infection caused by members of the anaerobic gram positive genus, *Actinomyces*. The disease Actinomycosis is discussed below.

ACTINOMYCOSIS

Actinomycosis is a chronic suppurative and granulomatous infection that produces pyogenic lesions with interconnecting sinus tracts that contain granules composed of microcolonies of the bacteria embedded in tissue elements. The etiologic agents are several closely related members of the normal flora of the mouth and gastrointestinal tract. Most cases are due to *Actinomyces israelii*, *Actinomyces naeslundii*, and related anaerobic or facultative bacteria. Based on the site of involvement, the three common forms are cervicofacial, thoracic, and abdominal actinomycosis. Regardless of site, infection is initiated by trauma that introduces these endogenous bacteria into the mucosa.

Often, in addition to the primary agent of actinomycosis, there are concomitant bacteria present. Some of these are relatively fastidious Gram-negative bacilli such as *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Eikenella corrodens*, and *Capnocytophaga* species. Occasionally, staphylococci, streptococci, or enteric Gram-negative bacilli are found.

Morphology & Identification

Most strains of *A. israelii* and the other agents of actinomycosis are facultative anaerobes that grow best in an atmosphere with increased carbon dioxide. On enriched medium, such as brain-heart infusion agar, young colonies (24-48 hours) produce Gram-positive substrate filaments that fragment into short chains, diphtheroids, and coccobacilli. After a week, these «spider» colonies develop into white, heaped-up «molar tooth» colonies. In thioglycolate broth, *A. israelii* grows below the surface in compact colonies. Species are identified based on cell wall chemotype and biochemical reactions.

The sulfur granules found in tissue are yellowish in appearance, up to 1 mm in size, and are composed of macrophages, other tissue cells, fibrin, and the bacteria. Eosinophilic club-shaped enlargements of the bacterial cells often project from the periphery of the granule.

Pathogenesis & Pathology

Regardless of the body site, the natural history is similar. The bacteria bridge the mucosal or epithelial surface of the mouth, respiratory tract, or lower gastrointestinal tract-associated with dental caries, gingivitis, surgical complication, or trauma. Aspiration may lead to pulmonary infection. The organisms grow in an anaerobic niche, induce a mixed inflammatory response, and spread with the formation of sinuses, which contain the granules and may drain to the surface. The infection causes swelling and may spread to neighboring organs, including the bones. There is often superinfection with other endogenous bacteria.

Clinical Findings

Cervicofacial disease presents as a swollen, erythematous process in the jaw area. With progression, the mass becomes fluctuant, producing draining fistulas. The disease will extend to contiguous tissue, bone, and lymph nodes of the head and neck. The symptoms of thoracic actinomycosis resemble those of a subacute

pulmonary infection: mild fever, cough, and purulent sputum. Eventually, lung tissue is destroyed, sinus tracts may erupt to the chest wall, and invasion of the ribs may occur. Abdominal actinomycosis often follows a ruptured appendix or an ulcer. In the peritoneal cavity, the pathology is the same, but any of several organs may be involved, including the kidneys, vertebrae, and liver. Genital actinomycosis is a rare occurrence in women that results from colonization of an intrauterine device with subsequent invasion.

Diagnostic Laboratory Tests

Pus from draining sinuses, sputum, or specimens of tissue are examined for the presence of sulfur granules. The granules are hard, lobulated, and composed of tissue and bacterial filaments, which are club-shaped at the periphery. Specimens are cultured in thioglycolate broth and on brain-heart infusion blood agar plates, which are incubated anaerobically or under elevated carbon dioxide conditions. Growth is examined for typical morphology and biochemical reactions. The main agents of actinomycosis are catalase-negative, whereas most other actinomycetes are catalase-positive. Surface lesions may also contain other bacterial species.

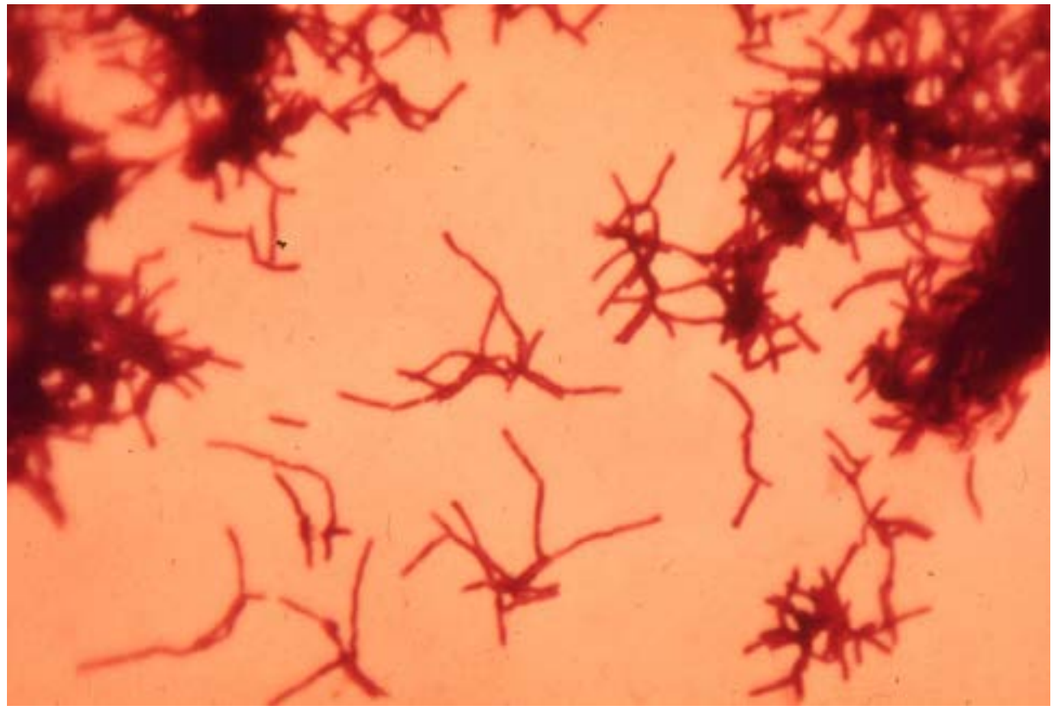
Treatment

Prolonged administration (6-12 months) of a penicillin is effective in many cases. Clindamycin or erythromycin is effective in penicillin-allergic patients. However, drugs may penetrate the abscesses poorly, and some of the tissue destruction may be irreversible. Surgical excision and drainage may also be required.

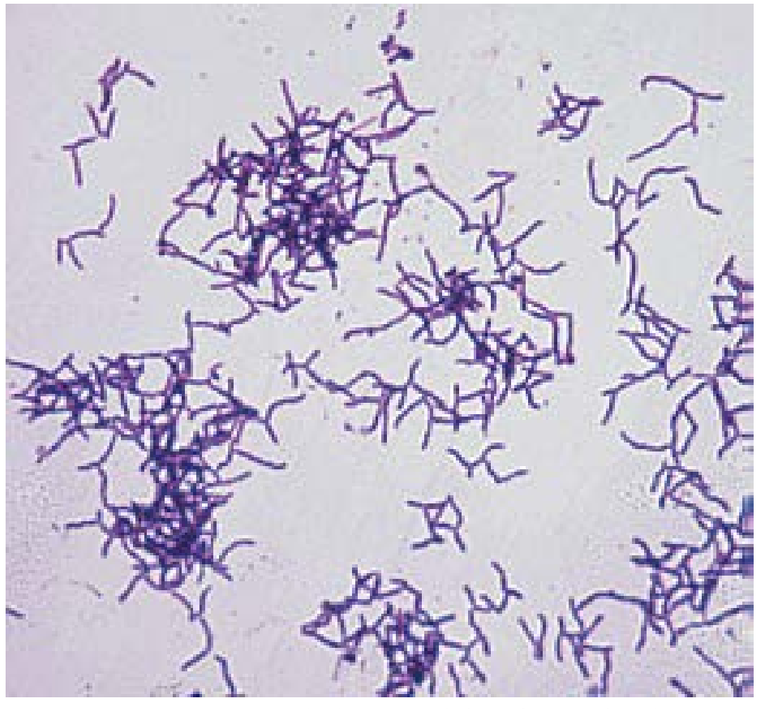
Epidemiology

Because *A. israelii* and the related agents of actinomycosis are endogenous members of the bacterial flora, they cannot be eliminated. Some individuals with recurrent infections are given prophylactic penicillin, especially prior to dental procedures.

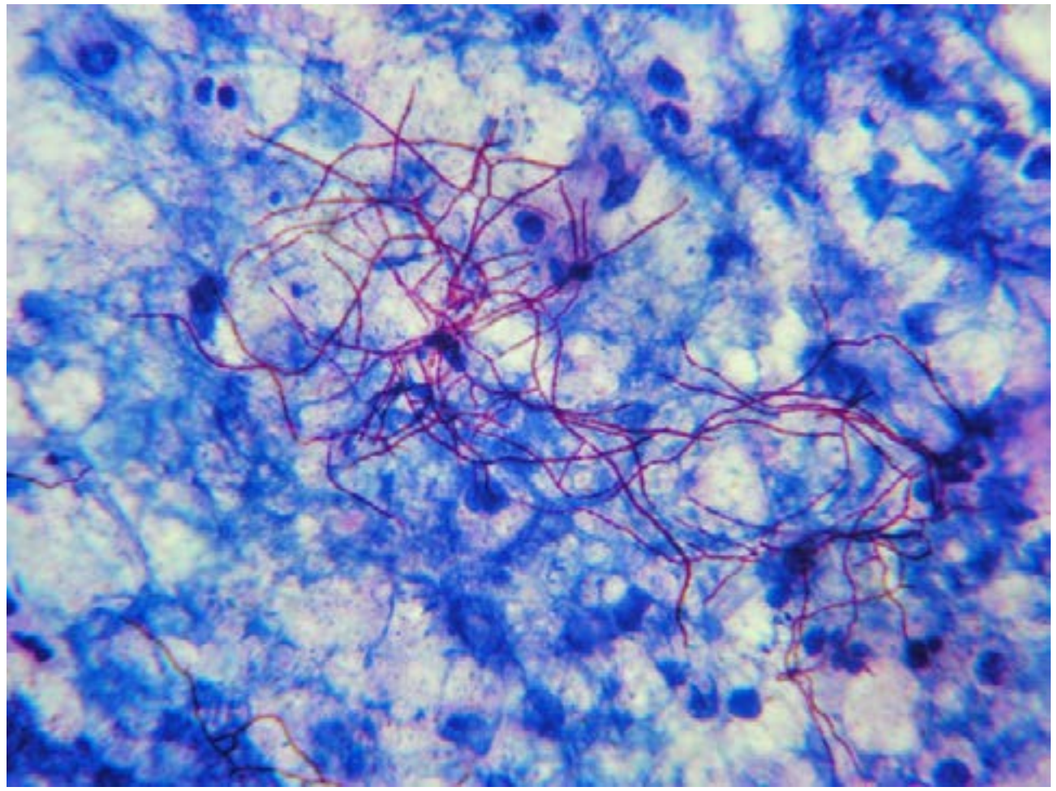
14 Class – Illustrations



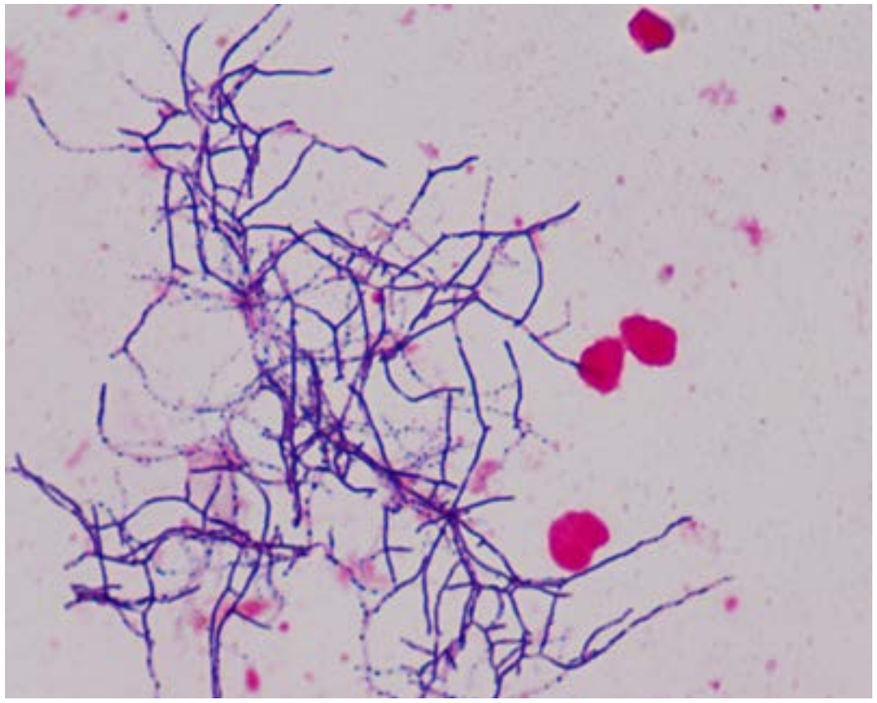
Actinomyces israelii (Gram stained broth culture)



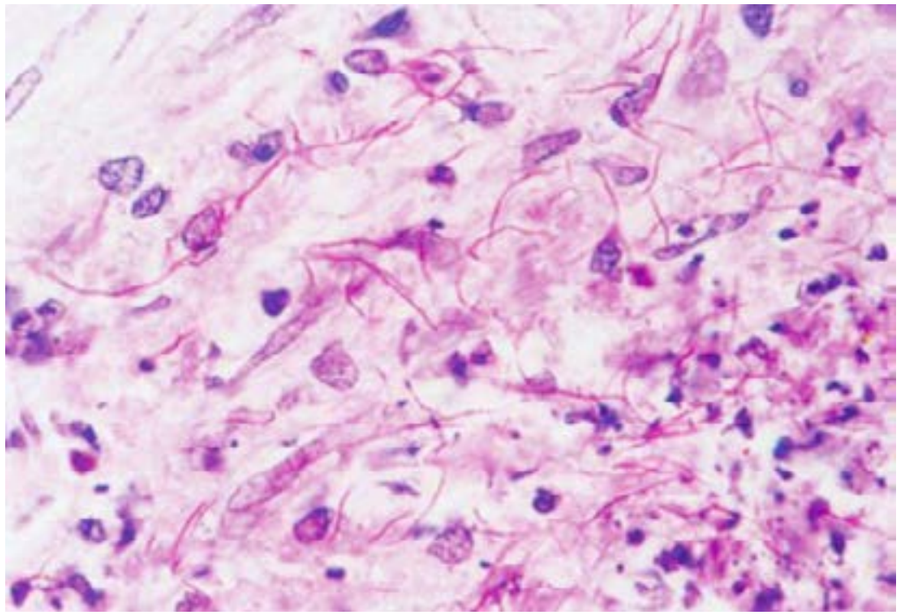
Actinomyces israelii (Gram staining, from culture)



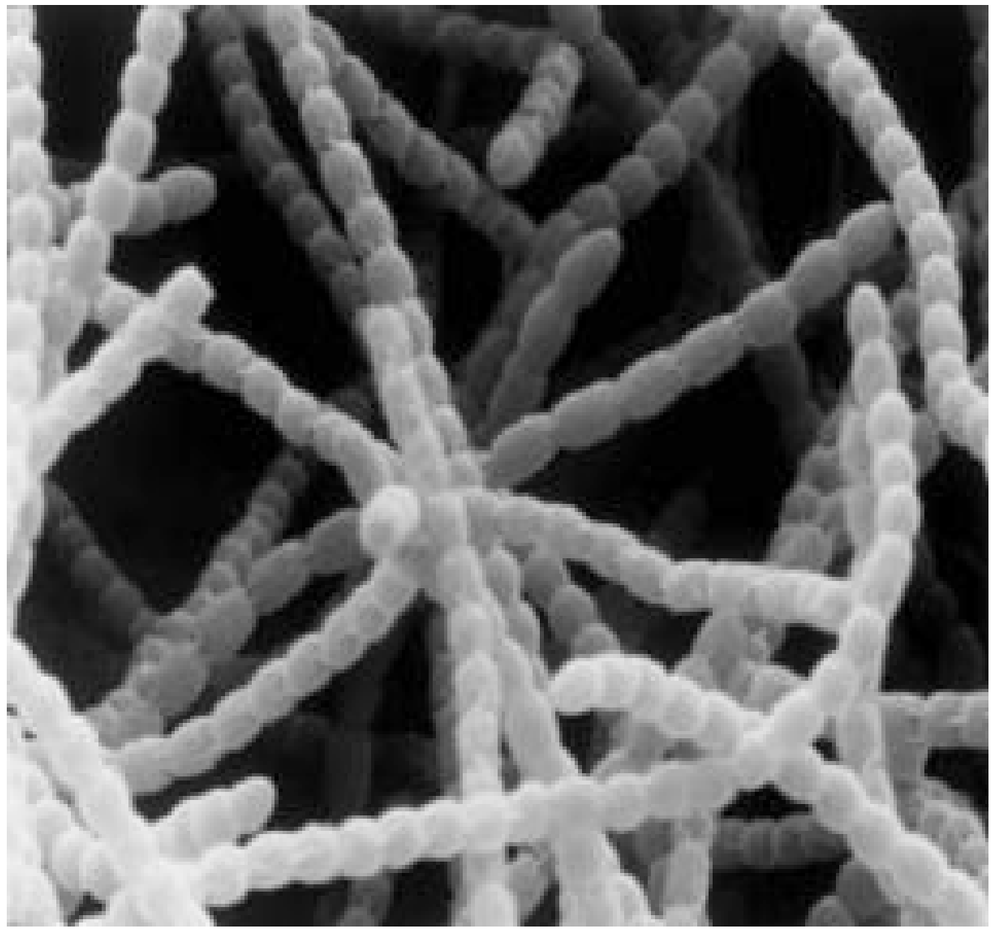
Nocardia asteroides in abdominal abscess (Partial acid fast stain)



Nocardia asteroides (Gram staining)

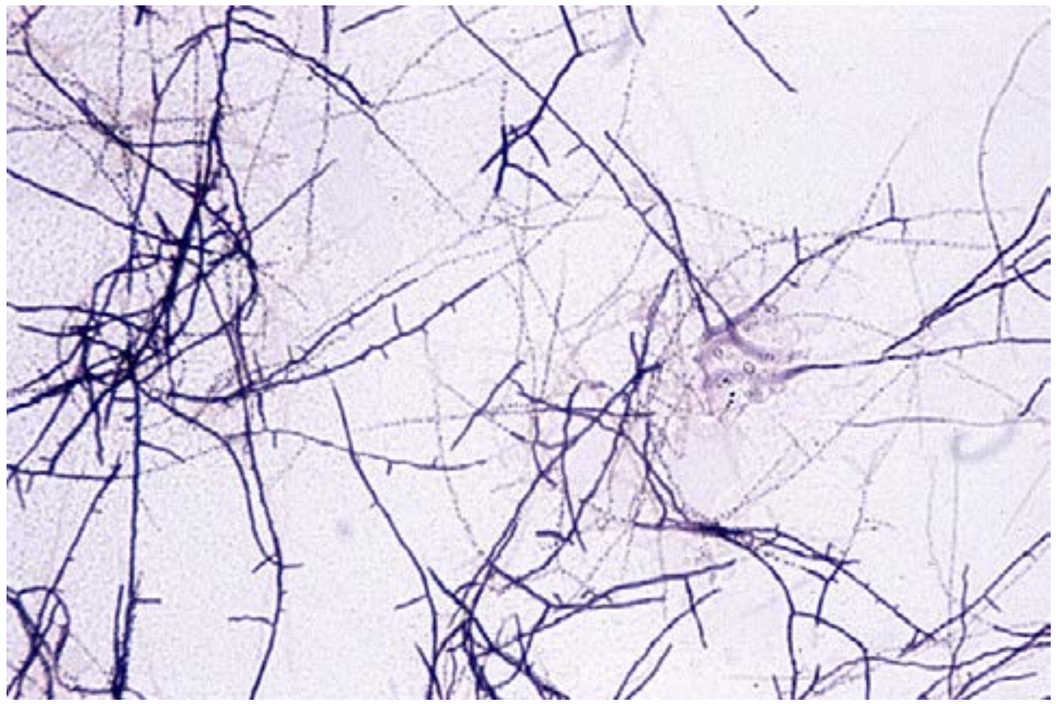


Nocardia asteroides



Streptomyces griseus (scanning electron microscope)

Streptomyces spp. mixed colonies on Petri plates



Streptomyces spp. (Gram staining)

15 Class - Superficial mycoses. Superficial candidiasis. Histoplasmosis. Blastomycosis. Chromomycoses. Sporotrichosis

SUPERFICIAL MYCOSES

PITYRIASIS VERSICOLOR

Pityriasis versicolor is a chronic mild superficial infection of the stratum corneum caused by *Malassezia globosa*, *M. restricta*, and other members of the *M. furfur* complex. Invasion of the cornified skin and the host responses are both minimal. Discrete, serpentine, hyper- or hypopigmented maculae occur on the skin, usually on the chest, upper back, arms, or abdomen.

The lesions are chronic and occur as macular patches of discolored skin that may enlarge and coalesce, but scaling, inflammation, and irritation are minimal. Indeed, this common affliction is largely a cosmetic problem. *Malassezia* species are lipophilic yeasts, and most require lipid in the medium for growth. The diagnosis is confirmed by direct microscopic examination of scrapings of infected skin, treated with 10-20% KOH or stained with calcofluor white. Short unbranched hyphae and spherical cells are observed. The lesions also fluoresce under Wood's lamp. Pityriasis versicolor is treated with daily applications of selenium sulfide. Topical or oral azoles are also effective. Rarely, *Malassezia* may cause an opportunistic fungemia in patients - usually infants - receiving total parenteral nutrition, as a result of contamination of the lipid emulsion. In most cases, the fungemia is transient and corrected by replacing the fluid and intravenous catheter. Some individuals develop folliculitis due to

Malassezia. Species of *Malassezia.* are considered part of the microbial flora and can be isolated from normal skin and scalp. They have been implicated as a cause of or contributor to seborrheic dermatitis, or dandruff. This hypothesis is supported by the observation that many cases are alleviated by treatment with ketoconazole.

TINEA NIGRA

Tinea nigra (or tinea nigra palmaris) is a superficial chronic and asymptomatic infection of the stratum corneum caused by the dematiaceous fungus *Hortaea* (*Exophiala*) *werneckii*.

This condition is more prevalent in warm coastal regions and among young women. The lesions appear as a dark (brown to black) discoloration, often on the palm. Microscopic examination of skin scrapings from the periphery of the lesion will reveal branched, septate hyphae and budding yeast cells with melaninized cell walls. Tinea nigra will respond to treatment with keratolytic solutions, salicylic acid, or azole antifungal drugs.

PIEDRA

Black piedra is a nodular infection of the hair shaft caused by *Piedraia hortai*. White piedra, due to infection with *Trichosporon* species, presents as larger, softer, yellowish nodules on the hairs. Axillary, pubic, beard, and scalp hair may be infected. Treatment for both types consists of removal of hair and application of a topical antifungal agent. Piedra is endemic in tropical underdeveloped countries.

CUTANEOUS MYCOSES

Cutaneous mycoses are caused by fungi that infect only the superficial keratinized tissue (skin, hair, and nails). The most important of these are the dermatophytes, a group of about 40 related fungi that belong to three genera:

Microsporum, *Trichophyton*, and *Epidermophyton*. *Dermatophytes* are probably restricted to the nonviable skin because most are unable to grow at 37 °C or in the presence of serum. Dermatophytoses are among the most prevalent infections in the world. Although they can be persistent and troublesome, they are not debilitating or life-threatening - yet millions of dollars are expended annually in their treatment. Being superficial, dermatophyte (ringworm) infections have been recognized since antiquity. In skin they are diagnosed by the presence of hyaline, septate, branching hyphae or chains of arthroconidia. In culture, the many species are closely related and often difficult to identify.

They are speciated on the basis of subtle differences in the appearance of the colonies and microscopic morphology as well as a few vitamin requirements. Despite their similarities in morphology, nutritional requirements, surface antigens, and other features, many species have developed keratinases, elastases, and

other enzymes that enable them to be quite host-specific. For some species of dermatophytes, a sexual reproductive state has been discovered, and all dermatophytes with a sexual form produce ascospores and belong to the teleomorphic genus *Arthroderma*.

Dermatophytes are classified as geophilic, zoophilic, or anthropophilic depending on whether their usual habitat is soil, animals, or humans. Several dermatophytes that normally reside in soil or are associated with particular animal species are still able to cause human infections. In general, as a species evolves from habitation in soil to a specific animal or human host, it loses the ability to produce asexual conidia and to reproduce sexually. Anthropophilic species, which cause the greatest number of human infections, cause relatively mild and chronic infections in humans, produce few conidia in culture, and may be difficult to eradicate. Conversely, geophilic and zoophilic dermatophytes, being less adapted to human hosts, produce more acute inflammatory infections that tend to resolve more quickly. Dermatophytes are acquired by contact with contaminated soil or with infected animals or humans.

Some anthropophilic species are geographically restricted, but others, such as *Epidermophyton floccosum*, *Trichophyton mentagrophytes var interdigitale*, *T. rubrum*, and *T. tonsurans*, are globally distributed. The most common geophilic species causing human infections is *Microsporum gypseum*. Cosmopolitan zoophilic species (and their natural hosts) include *Microsporum canis* (dogs and cats), *Microsporum gallinae* (fowl), *Microsporum nanum* (pigs), *Trichophyton equinum* (horses), and *Trichophyton verrucosum* (cattle).

Morphology & Identification

Dermatophytes are identified by their colonial appearance and microscopic morphology after growth for 2 weeks at 25 °C on Sabouraud's dextrose agar. *Trichophyton* species, which may infect hair, skin, or nails, develop cylindric, smooth-walled macroconidia and characteristic microconidia. Depending on the variety, colonies of *T. mentagrophytes* may be cottony to granular; both types display abundant grape-like clusters of spherical microconidia on terminal branches. Coiled or spiral hyphae are commonly found in primary isolates. The typical colony of *T. rubrum* has a white, cottony surface and a deep red, non-diffusible pigment when viewed from the reverse side of the colony. The microconidia are small and piriform (pear-shaped). *T. tonsurans* produces a flat, powdery to velvety colony on the obverse surface that becomes reddish-brown on reverse; the microconidia are mostly elongate.

Microsporum species tend to produce distinctive multicellular macroconidia with echinulate walls. Both types of conidia are borne singly in these genera. *M. canis* forms a colony with a white cottony surface and a deep yellow color on reverse; the thick-walled, 8- to 15-celled macroconidia frequently have curved or hooked tips. *M. gypseum* produces a tan, powdery colony and abundant thin-walled, four- to six-celled macroconidia.

Microsporum species infect only hair and skin. *Epidermophyton floccosum*, which is the only pathogen in this genus, produces only macroconidia, which are smooth-walled, clavate, two- to four-celled, and formed in groups of two or three. The colonies are usually flat and velvety with a tan to olive-green tinge. *E. floccosum* infects the skin and nails but not the hair.

In addition to gross and microscopic morphology, a few nutritional or other tests, such as growth at 37 °C or a test for in vitro hair perforation, are useful in differentiating certain species.

Epidemiology & Immunity

Dermatophyte infections begin in the skin after trauma and contact. There is evidence that host susceptibility may be enhanced by moisture, warmth, specific skin chemistry, composition of sebum and perspiration, youth, heavy exposure, and genetic predisposition. The incidence is higher in hot, humid climates and under crowded living conditions. Wearing shoes provides warmth and moisture, a setting for infections of the feet. The source of infection is soil or an infected animal in the case of geophilic and zoophilic dermatophytes, respectively. The conidia can remain viable for long periods. Anthropophilic species may be transmitted by direct contact or through fomites, such as contaminated towels, clothing, shared shower stalls, and similar examples.

Trichophytin is a crude antigen preparation that can be used to detect immediate- or delayed-type hypersensitivity to dermatophytic antigens. Many patients who develop chronic, noninflammatory dermatophyte infections have poor cell-mediated immune responses to dermatophyte antigen. These patients often are atopic and have immediate-type hypersensitivity and elevated IgE concentrations. In the normal host, immunity to dermatophytosis varies in duration and degree depending on the host, the site, and the species of fungus causing the infection.

Clinical Findings

Dermatophyte infections were mistakenly termed ringworm or tinea because of the raised circular lesions. The clinical forms are based on the site of involvement. A single species is able to cause more than one type of clinical infection. Conversely, a single clinical form, such as tinea corporis, may be caused by more than one dermatophyte species. Very rarely, immunocompromised patients may develop systemic infection by a dermatophyte.

A. TINEA PEDIS (ATHLETE'S FOOT)

Tinea pedis is the most prevalent of all dermatophytoses. It usually occurs as a chronic infection of the toe webs.

Other varieties are the vesicular, ulcerative, and moccasin types, with hyperkeratosis of the sole. Initially, there is itching between the toes and the development of small vesicles that rupture and discharge a thin fluid. The skin of the toe webs becomes macerated and peels, whereupon cracks appear that are prone to develop secondary bacterial infection. When the fungal infection becomes chronic, peeling and cracking of the skin are the principal manifestations, accompanied by pain and pruritus.

B. TINEA UNGUIUM (ONYCHOMYCOSIS)

Nail infection may follow prolonged tinea pedis. With hyphal invasion, the nails become yellow, brittle, thickened, and crumbly. One or more nails of the feet or hands may be involved.

C. TINEA CORPORIS, TINEA CRURIS, AND TINEA MANUS

Dermatophytosis of the glabrous skin commonly gives rise to the annular lesions of ringworm, with a clearing, scaly center surrounded by a red advancing border that may be dry or vesicular. The dermatophyte grows only within dead, keratinized tissue, but fungal metabolites, enzymes, and antigens diffuse through the viable layers of the epidermis to cause erythema, vesicle formation, and pruritus. Infections with geophilic and zoophilic dermatophytes produce more irritants and are more inflammatory than anthropophilic species. As hyphae age, they often form chains of arthroconidia. The lesions expand centrifugally and active hyphal growth is at the periphery, which is the most likely region from which to obtain material for diagnosis. Penetration into the newly forming stratum corneum of the thicker plantar and palmar surfaces accounts for the persistent infections at those sites.

When the infection occurs in the groin area, it is called tinea cruris, or jock itch. Most such infections involve males and present as dry, itchy lesions that often start on the scrotum and spread to the groin.

Tinea manus refers to ringworm of the hands or fingers. Dry scaly lesions may involve one or both hands, single fingers, or two or more fingers.

D. TINEA CAPITIS AND TINEA BARBAE

Tinea capitis is dermatophytosis or ringworm of the scalp and hair. The infection begins with hyphal invasion of the skin of the scalp, with subsequent spread down the keratinized wall of the hair follicle. Infection of the hair takes place just above the hair root. The hyphae grow downward on the nonliving portion of the hair and at the same rate as the hair grows upward. The agar or Sabouraud's agar slants containing cycloheximide and chloramphenicol to suppress mold and bacterial growth, incubated for 1-3 weeks at room temperature, and further examined in slide cultures if necessary. Species are identified on the basis of colonial morphology (growth rate, surface texture, and any pigmentation), microscopic morphology (macroconidia, microconidia), and, in some cases, nutritional requirements.

Treatment

Therapy consists of thorough removal of infected and dead epithelial structures and application of a topical antifungal chemical or antibiotic. To prevent reinfection, the area should be kept dry, and sources of infection, such as an infected pet or shared bathing facilities, should be avoided.

A. TINEA CAPITIS

Scalp infections are treated with griseofulvin for 4-6 weeks. Frequent shampoos and miconazole cream or other topical antifungal agents may be effective if used for weeks. Alternatively, ketoconazole, itraconazole, and terbinafine are all quite effective.

B. TINEA CORPORIS, TINEA PEDIS, AND RELATED INFECTIONS

The most effective drugs are itraconazole and terbinafine. However, a number of topical preparations may be used, such as miconazole nitrate, tolnaftate, and clotrimazole. If applied for at least 2-4 weeks, the cure rates are usually 70-100%. Treatment should be continued for 1-2 weeks after clearing of the lesions. For troublesome cases, a short course of oral griseofulvin can be administered.

C. TINEA UNGUIUM

Nail infections are the most difficult to treat, often requiring months of oral itraconazole or terbinafine as well as surgical removal of the nail. Relapses are common.

SUBCUTANEOUS MYCOSES

The fungi that cause subcutaneous mycoses normally reside in soil or on vegetation. They enter the skin or subcutaneous tissue by traumatic inoculation with contaminated material. In general, the lesions become granulomatous and expand slowly from the area of implantation.

Extension via the lymphatics draining the lesion is slow except in sporotrichosis. These mycoses are usually confined to the subcutaneous tissues, but in rare cases they become systemic and produce life-threatening disease.

SPOROTHRIX SCHENCKII

Sporothrix schenckii is a thermally dimorphic fungusthat lives on vegetation. It is associated with a variety of plants - grasses, trees, sphagnum moss, rose bushes, and other horticultural plants. At ambient temperatures, it grows as a mold, producing branching, septate hyphae and conidia, and in tissue or in vitro at 35-37 °C as a small budding yeast. Following traumatic introduction into the skin, *S. schenckii* causes sporotrichosis, a chronic granulomatous infection. The initial episode is typically followed by secondary spread with involvement of the draining lymphatics and lymph nodes.

Morphology & Identification

S. schenckii grows well on routine agar media, and at room temperature the young colonies are blackish and shiny, becoming wrinkled and fuzzy with age. Strains vary in pigmentation from shades of black and gray to whitish. The organism produces branching, septate hyphae and distinctive small (3-5 μm) conidia, delicately clustered at the ends of tapering conidiophores. Isolates may also form larger conidia directly from the hyphae. *S. schenckii* is thermally dimorphic, and at 35 °C on a rich medium it converts to growth as small, often multiply budding yeast cells that are variable in shape but often fusiform (about 1-3 to 3-10 μm).

Antigenic Structure

Heat-killed saline suspensions of cultures or carbohydrate fractions (sporotrichin) will elicit positive delayed skin tests in infected humans or animals. A variety of serologic tests have been developed, and most patients, as well as some normal individuals, have specific or cross-reactive antibodies.

Pathogenesis & Clinical Findings

The conidia or hyphal fragments of *S. schenckii* are introduced into the skin by trauma. Patients frequently recall a history of trauma associated with outdoor activities and plants. The initial lesion is usually located on the extremities but can be found anywhere (children often present with facial lesions). About 75% of cases are lymphocutaneous; ie, the initial lesion develops as a granulomatous nodule that may progress to form a necrotic or ulcerativelesion. Meanwhile, the draining lymphatics become thickened and cord-like. Multiple subcutaneous nodules and abscesses occur along the lymphatics.

Fixed sporotrichosis is a single nonlymphangitic nodule that is limited and less progressive. The fixed lesion is more common in endemic areas such as Mexico, where there is a high level of exposure and immunity in the population. Immunity limits the local spread of the infection.

There is usually little systemic illness associated with these lesions, but dissemination may occur, especially in debilitated patients. Rarely, primary pulmonary sporotrichosis results from inhalation of the conidia. This manifestation mimics chronic cavitary tuberculosis and tends to occur in patients with impaired cell-mediated immunity.

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens include biopsy material or exudate from granulous or ulcerative lesions.

B. MICROSCOPIC EXAMINATION

Although specimens can be examined directly with KOH or calcofluor white stain, the yeasts are rarely found. Even though they are sparse in tissue, the sensitivity of histopathologic sections is enhanced with routine fungal cell wall stains, such as Gomori's methenamine silver, which stains the cell walls black, or the periodic acid-Schiff stain, which imparts a red color to the cell walls. Alternatively, they can be identified ab fluorescent antibody staining. The yeasts are 3-5 μm in diameter and spherical to elongated.

Another structure termed an asteroid body is often seen in tissue, particularly in endemic areas such as Mexico, South Africa, and Japan. In hematoxylin and eosin-stained tissue, the asteroid body consists of a central basophilic yeast cell surrounded by radiating extensions of eosinophilic material, which are depositions of antigen-antibody complexes and complement.

C. CULTURE

The most reliable method of diagnosis is culture. Specimens are streaked on inhibitory mold agar or Sabouraud's agar containing antibacterial antibiotics and incubated at 25-30 °C. The identification is confirmed by growth at 35 °C and conversion to the yeast form.

D. SEROLOGY

Agglutination of yeast cell suspensions or of latex particles coated with antigen occurs in high titer with sera of infected patients but is not always diagnostic.

Treatment

In some cases, the infection is self-limited. Although the oral administration of saturated solution of potassium iodide in milk is quite effective, it is difficult for many patients to tolerate. Oral itraconazole or another of the azoles is the treatment of choice. For systemic disease, amphotericin B is given.

Epidemiology & Control

S. schenckii occurs worldwide in close association with plants. For example, cases have been linked to contact with sphagnum moss, rose thorns, decaying wood, pine straw, prairie grass, and other vegetation. About 75% of cases occur in males, either because of increased exposure or because of an X-linked difference in susceptibility. The incidence is higher among agricultural workers, and sporotrichosis is considered an occupational risk for forest rangers, horticulturists, and workers in similar occupations. Prevention includes measures to minimize accidental inoculation and the use of fungicides, where appropriate, to treat wood. Animals are also susceptible to sporotrichosis.

CHROMOBLASTOMYCOSIS

Chromoblastomycosis (chromomycosis) is a subcutaneous mycotic infection caused by traumatic inoculation by any of five recognized fungal agents that reside in soil and vegetation. All are dematiaceous fungi, having melaninized cell walls: *Phialophora verrucosa*, *Fonsecaea pedrosoi*, *Rhinocladiella aquaspersa*, *Fonsecaea compacta*, and *Cladophialophora carrionii*. The infection is chronic and characterized by the slow development of progressive granulomatous lesions that in time induce hyperplasia of the epidermal tissue.

Morphology & Identification

The dematiaceous fungi are similar in their pigmentation, antigenic structure, morphology, and physiologic properties. The colonies are compact, deep brown to black, and develop a velvety, often wrinkled surface. The agents of chromoblastomycosis are identified by their modes of conidiation. In tissue they appear the same, producing spherical brown cells (4-12 μm in diameter) termed muriform or sclerotic bodies that divide by transverse septation. Septation in different planes with delayed separation may give rise to a cluster of four to eight cells. Cells within superficial crusts or exudates may germinate into septate, branching hyphae.

A. *PHIALOPHORA VERRUCOSA*

The conidia are produced from flask-shaped phialides with cup-shaped collarettes. Mature, spherical to oval conidia are extruded from the phialide and usually accumulate around it.

B. *CLADOPHIALOPHORA (CLADOSPORIUM) CARRIONII*

Species of *Cladophialophora* and *Cladosporium* produce branching chains of conidia by distal (acropetalous) budding. The terminal conidium of a chain gives rise to the next conidium by a budding process. Species are identified based on differences in the length of the chains and the shape and size of the conidia. *C. carrionii* produces elongated conidiophores with long, branching chains of oval conidia.

C. *RHINOCLADIELLA AQUASPERSA*

This species produces lateral or terminal conidia from a lengthening conidiogenous cell - a sympodial process. The conidia are elliptical to clavate.

D. *FONSECAEA PEDROSOI*

Fonsecaea is a polymorphic genus. Isolates may exhibit (1) phialides; (2) chains of blastoconidia, similar to *Cladosporium* species; or (3) sympodial, rhinocladiella-type conidiation. Most strains of *F. pedrosoi* form short branching chains of blastoconidia as well as sympodial conidia.

E. *FONSECAEA COMPACTA*

The blastoconidia produced by *F. compacta* are almost spherical, with a broad base connecting the conidia. These structures are smaller and more compact than those of *F. pedrosoi*.

Pathogenesis & Clinical Findings

The fungi are introduced into the skin by trauma, often of the exposed legs or feet. Over months to years, the primary lesion becomes verrucous and wart-like with extension along the draining lymphatics. Cauliflower like nodules with crusting abscesses eventually cover the area. Small ulcerations or «black dots» of hemopurulent material are present on the warty surface. Rarely, elephantiasis may result from secondary infection, obstruction, and fibrosis of lymph channels.

Dissemination to other parts of the body is very rare, though satellite lesions can occur due either to local lymphatic spread or to autoinoculation. Histologically, the lesions are granulomatous and the dark sclerotic bodies may be seen within leukocytes or giant cells.

Diagnostic Laboratory Tests

Specimens of scrapings or biopsies from lesions are placed in 10% KOH and examined microscopically for dark, spherical cells. Detection of the sclerotic bodies is diagnostic of chromoblastomycosis regardless of the etiologic agent. Tissue sections reveal granulomas and extensive hyperplasia of the dermal tissue.

Specimens should be cultured on inhibitory mold agar or Sabouraud's agar with antibiotics. The dematiaceous species is identified by its characteristic conidial structures, as described above. There are many similar saprophytic dematiaceous molds, but they differ from the pathogenic species in being unable to grow at 37 °C and being able to digest gelatin.

Treatment

Surgical excision with wide margins is the therapy of choice for small lesions. Chemotherapy with flucytosine or itraconazole may be efficacious for larger lesions. Local applied heat is also beneficial. Relapse is common.

Epidemiology

Chromoblastomycosis occurs mainly in the tropics. The fungi are saprophytic in nature, probably occurring on vegetation and in soil. The disease occurs chiefly on the legs of barefoot agrarian workers following traumatic introduction of the fungus. Chromoblastomycosis is not communicable. Wearing shoes and protecting the legs probably would prevent infection.

PHAEOHYPHOMYCOSIS

Phaeohyphomycosis is a term applied to infections characterized by the presence of darkly pigmented septate hyphae in tissue. Both cutaneous and systemic infections have been described. The clinical forms vary from solitary encapsulated cysts in the subcutaneous tissue to sinusitis to brain abscesses. Over 100 species of dematiaceous molds have been associated with various types of phaeohyphomycotic infections. They are all exogenous molds that normally exist in nature. Some of the more common causes of subcutaneous phaeohyphomycosis are *Exophiala jeanselmei*, *Phialophora richardsiae*, *Bipolaris spicifera*, and *Wangiella dermatitidis*. These species and others (eg, *Exserohilum rostratum*, *Alternaria* species, and *Curvularia* species) may be implicated also in systemic phaeohyphomycosis. The incidence of phaeohyphomycosis and the range of pathogens have been increasing in recent years in both immunocompetent and compromised patients.

In tissue, the hyphae are large (5-10 μm in diameter), often distorted and may be accompanied by yeast cells, but these structures can be differentiated from other fungi by the melanin in their cell walls. Specimens are cultured on routine fungal media to identify the etiologic agent. In general, itraconazole or flucytosine is the drug of choice for subcutaneous phaeohyphomycosis. Brain abscesses are usually fatal, but when recognized they are managed with amphotericin B and surgery. The leading cause of cerebral phaeohyphomycosis is *Cladophialophora bantiana*.

MYCETOMA

Mycetoma is a chronic subcutaneous infection induced by traumatic inoculation with any of several saprophytic species of fungi or actinomycetous bacteria that are normally found in soil. The clinical features defining mycetoma are local swelling and interconnecting - often draining - sinuses that contain granules, which are - microcolonies of the agent embedded in tissue material.

An actinomycetoma is a mycetoma caused by an actinomycete; a eumycetoma (maduromycosis, Madura foot) is a mycetoma caused by a fungus. The natural history and clinical features of both types of

mycetoma are similar, but actinomycetomas may be more invasive, spreading from the subcutaneous tissue to the underlying muscle. Of course, the therapy is different. Mycetoma occurs worldwide but more often among impoverished people who do not wear shoes.

Mycetomas occur only sporadically outside the tropics and are particularly prevalent in India, Africa, and Latin America.

Morphology & Identification

The fungal agents of mycetoma include, among others, *Pseudallescheria boydii*, *Madurella mycetomatis*, *Madurella grisea*, *Exophiala jeanselmei*, and *Acremonium falciforme*.

In the United States, the prevalent species is *P. boydii*, which is homothallic and has the ability to produce ascospores in culture. *E. jeanselmei* and the *Madurella* species are dematiaceous molds. These molds are identified primarily by their mode of conidiation. *P. boydii* may also cause pseudallescheriasis, which is a systemic infection in compromised patients.

In tissue, the mycetoma granules may range up to 2 mm in size. The color of the granule may provide information about the agent. For example, the granules of mycetoma caused by *P. boydii* and *A. falciforme* are white; those of *M. grisea* and *E. jeanselmei* are black; and *M. mycetomatis* produces a dark red to black granule.

These granules are hard and contain intertwined, septate hyphae (3-5 μm in width). The hyphae are typically distorted and enlarged at the periphery of the granule.

Pathogenesis & Clinical Findings

Mycetoma develops after traumatic inoculation with soil contaminated with one of the agents. Subcutaneous tissues of the feet, lower extremities, hands, and exposed areas are most often involved. Regardless of the agent, the pathology is characterized by suppuration and abscess formation, granulomas, and the formation of draining sinuses containing the granules. This process may spread to contiguous muscle and bone. Untreated lesions persist for years and extend deeper and peripherally, causing deformation and loss of function.

Very rarely, *P. boydii* may disseminate in an immunocompromised host or produces infection of a foreign body (eg, a cardiac pacemaker).

Diagnostic Laboratory Tests

Granules can be dissected out from the pus or biopsy material for examination and culture on appropriate media. The granule color, texture, and size and the presence of hyaline or pigmented hyphae (or bacteria) are helpful in determining the causative agent. Draining mycetomas are often superinfected with staphylococci and streptococci.

Treatment

The management of eumycetoma is difficult, involving surgical debridement or excision and chemotherapy. *P. boydii* is treated with topical nystatin or miconazole.

Itraconazole, ketoconazole, and even amphotericin B can be recommended for *Madurella* infections and flucytosine for *E. jeanselmei*. Chemotherapeutic agents must be given for long periods to adequately penetrate these lesions.

Epidemiology & Control

The organisms producing mycetoma occur in soil and on vegetation. Barefoot farm laborers are therefore commonly exposed. Properly cleaning wounds and wearing shoes are reasonable control measures.

ENDEMIC MYCOSES

Each of the four primary systemic (dimorphic) mycoses - coccidioidomycosis, histoplasmosis, blastomycosis, and paracoccidioidomycosis - is geographically restricted to specific areas of endemicity. The fungi that cause coccidioidomycosis and histoplasmosis exist in nature in dry soil or in soil mixed with guano, respectively. The agents of blastomycosis and paracoccidioidomycosis are presumed to reside in nature, but their habitats have not been clearly defined. Each of these four mycoses is caused by a thermally dimorphic fungus, and most infections are initiated in the lungs following inhalation of the respective conidia.

Only a few infections lead to disease, which may involve dissemination from the lungs to other organs. With rare exceptions, these mycoses are not transmissible among humans or other animals.

For all of these infections, the initial host defenses are provided by the alveolar macrophages, which are usually capable of inactivating the conidia and inducing a robust immune response. This process typically

leads to granulomatous inflammation and the production of both antibodies and cell-mediated immunity. The induction of Th1 cytokines (eg, interleukin-12, interferon-γ, tumor necrosis factor α) will amplify the cellular defenses, activating macrophages and enhancing their fungicidal capacity. In an immunocompetent host, these responses lead to resolution of the inflammatory lesions. However, residual granulomata may retain dormant organisms with the potential for subsequent reactivation, constituting a latent form of the disease. Within the endemic areas for these fungi, most infections occur in immunocompetent individuals, but persons with impaired cellular immunity, such as patients with HIV/AIDS, have an increased risk of serious infection.

COCCIDIOIDES IMMITIS & COCCIDIOIDES POSADASII

Coccidioides immitis and *C. posadasii* are phenotypically indistinguishable soil molds that cause coccidioidomycosis. The infection is endemic in well-circumscribed semiarid regions of the southwestern United States, Central America, and South America. Infection is usually self-limited; dissemination is rare but always serious, and it may be fatal.

Morphology & Identification

C. posadasii was recently recognized by DNA-based analyses as a distinct species and a frequent cause of coccidioidomycosis. However, since it cannot be readily identified in the laboratory and since the clinical manifestations are the same with either *C. immitis* or *C. posadasii*, only the former, more familiar species name will be used in this chapter.

On most laboratory media, *C. immitis* produces a white to tan cottony colony. The hyphae form chains of arthroconidia (arthrospores), which often develop in alternate cells of a hypha. These chains fragment into individual arthroconidia, which are readily airborne and highly resistant to adverse environmental conditions. These small arthroconidia (3 x 6 µm) remain viable for years and are highly infectious. Following their inhalation, the arthroconidia become spherical and enlarge, forming spherules that contain endospores. Spherules can also be produced in the laboratory by cultivation on a complex medium.

In histologic sections of tissue, sputum, or other specimens, the spherules are diagnostic of *C. immitis*. At maturity, the spherules have a thick, doubly refractile wall and may attain a size of 80 µm in diameter. The spherule becomes packed with endospores (2-5 µm in size). Eventually, the wall ruptures to release the endospores, which may develop into new spherules.

Antigenic Structure

Coccidioidin is a crude antigen preparation extracted from the filtrate of a liquid mycelial culture of *C. immitis*. Spherulin is produced from a filtrate of a broth culture of spherules. In standardized doses, both antigens elicit positive delayed skin reactions in infected persons.

They have also been used in a variety of serologic tests to measure serum antibodies to *C. immitis*.

Pathogenesis & Clinical Findings

Inhalation of arthroconidia leads to a primary infection that is asymptomatic in 60% of individuals. The only evidence of infection is the development of serum precipitins and conversion to a positive skin test within 2-4 weeks. The precipitins will decline, but the skin test often remains positive for a lifetime. The other 40% of individuals develop a self-limited influenza-like illness with fever, malaise, cough, arthralgia, and headache.

This condition is called valley fever, San Joaquin Valley fever, or desert rheumatism. After 1-2 weeks, about 15% of these patients develop hypersensitivity reactions, which present as a rash, erythema nodosum, or erythema multiforme. On radiographic examination, patients typically show hilar adenopathy along with pulmonary infiltrates, pneumonia, pleural effusions, or nodules. Pulmonary residua occur in about 5%, usually in the form of a solitary nodule or thin-walled cavity.

Less than 1% of persons infected with *C. immitis* develop secondary or disseminated coccidioidomycosis, which is often debilitating and life-threatening. The risk factors for systemic coccidioidomycosis include heredity, sex, age, and compromised cell-mediated immunity. The disease occurs more frequently in certain racial groups. In decreasing order of risk, these are Filipinos, African-Americans, Native Americans, Hispanics, and Asians. There is clearly a genetic component to the immune response to *C. immitis*. Males are more susceptible than females, with the exception of women who are pregnant, which may relate to differences in the immune response or a direct effect of sex hormones on the fungus. For example, *C. immitis* has estrogen-binding proteins, and elevated levels of estradiol and progesterone stimulate its growth. The young and the aged are also at greater risk. Because cell-mediated immune responses are required for adequate

resistance, patients with AIDS and other conditions of cellular immunosuppression are at risk for disseminated coccidioidomycosis.

Some individuals develop a chronic but progressive pulmonary disease with multiplying or enlarging nodules or cavities. Dissemination will usually occur within a year after the primary infection. The spherules and endospores are spread by direct extension or hematogenously. A number of extrapulmonary sites may be involved, but the most frequent organs are the skin, the bones and joints, and the meninges. There are distinctive clinical manifestations associated with *C. immitis* infections in each of these and other areas of the body.

Dissemination occurs when the immune response is inadequate to contain the pulmonary foci. In most persons, a positive skin test signifies a strong cell-mediated immune response and protection against reinfection.

However, if such individuals become immunocompromised by taking cytotoxic drugs or by disease (eg, AIDS), dissemination can occur many years after primary infection (reactivation disease). Coccidioidomycosis in AIDS patients often presents with a rapidly fatal diffuse reticulonodular pneumonitis. Because of the radiologic overlap between this disease and pneumocystis pneumonia and the different therapies for these two entities, it is important to be aware of the possibility of coccidioidal pneumonia in AIDS patients.

Blood cultures are often positive for *C. immitis*.

On histologic examination, the coccidioidal lesions contain typical granulomas with giant cells and interspersed suppuration. A diagnosis can be made by finding spherules and endospores. The clinical course is often characterized by remissions and relapses.

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens for culture include sputum, exudate from cutaneous lesions, spinal fluid, blood, urine, and tissue biopsies.

B. MICROSCOPIC EXAMINATION

Materials should be examined fresh (after centrifuging, if necessary) for typical spherules. KOH or calcofluor white stain will facilitate finding the spherules and endospores. These structures are often found in histologic preparations.

C. CULTURES

Cultures on inhibitory mold agar, Sabouraud's agar, or blood agar slants can be incubated at room temperature or at 37 °C. The media can be prepared with or without antibacterial antibiotics and cycloheximide to inhibit contaminating bacteria or saprophytic molds, respectively. Because the arthroconidia are highly infectious, suspicious cultures are examined only in a biosafety cabinet. Identification must be confirmed by detection of a *C. immitis*-specific antigen, animal inoculation, or use of a specific DNA probe.

D. SEROLOGY

Within 2-4 weeks after infection, IgM antibodies to coccidioidin can be detected with a latex agglutination test. Specific IgG antibodies are detected by the immunodiffusion (ID) or complement fixation (CF) test.

With resolution of the primary episode, these antibodies decline within a few months. In contrast, in disseminated coccidioidomycosis, the CF antibody titer continues to rise. Titers above 1:32 are indicative of dissemination, and their fall during treatment suggests improvement. However, CF titers < 1:32 do not exclude coccidioidomycosis. Indeed, only half of the patients with coccidioidal meningitis have elevated serum antibodies, but antibody levels in the cerebrospinal fluid are usually high. In AIDS patients with coccidioidomycosis, these serologic tests are often negative.

E. SKIN TEST

The coccidioidin skin test reaches maximum induration (≥ 5 mm in diameter) between 24 and 48 hours after cutaneous injection of 0,1 mL of a standardized dilution.

If patients with disseminated disease become anergic, the skin test will be negative, which implies a very poor prognosis. Cross-reactions with antigens of other fungi may occur. Spherulin is more sensitive than coccidioidin in detecting reactors. Reactions to skin tests tend to diminish in size and intensity years after primary infection in persons residing in endemic areas, but skin testing exerts a booster effect. Following recovery from primary infection, there is usually immunity to reinfection.

Treatment

In most persons, symptomatic primary infection is selflimited and requires only supportive treatment,

although itraconazole may reduce the symptoms. However, patients who have severe disease require treatment with amphotericin B, which is administered intravenously. This regimen may be followed by several months of oral therapy with itraconazole. Cases of coccidioidal meningitis have been treated with oral fluconazole, which has good penetration of the central nervous system; however, long-term therapy is required, and relapses have occurred. The azoles are not more efficacious than amphotericin B, but they are easier to administer and associated with fewer and less severe side effects. The newer lipid emulsions of amphotericin B promise to deliver higher doses with less toxicity. Surgical resection of pulmonary cavities is sometimes necessary and often curative.

Epidemiology & Control

The areas of endemicity for *C. immitis* are semiarid regions, resembling the Lower Sonoran Life Zone. They include the southwestern states - particularly the San Joaquin and Sacramento Valleys of California, areas around Tucson and Phoenix in Arizona, the Rio Grand valley - and similar areas in Central and South America. Within these regions, *C. immitis* can be isolated from the soil and indigenous rodents, and the level of skin test reactivity in the population indicates that many humans have been infected. The infection rate is highest during the dry months of summer and autumn, when dust is most prevalent. A high incidence of infection and disease may follow dust storms. During an epidemic of coccidioidomycosis in the San Joaquin.

Valley of California in 1991-1993, the rate of coccidioidomycosis increased more than tenfold. Increased precipitation in the spring months of these years has been suggested as an environmental stimulus.

The disease is not communicable from person to person, and there is no evidence that infected rodents contribute to its spread. Some measure of control can be achieved by reducing dust, paving roads and air-fields, planting grass or crops, and using oil sprays.

HISTOPLASMA CAPSULATUM

Histoplasma capsulatum is a dimorphic soil saprophyte that causes histoplasmosis, the most prevalent pulmonary mycotic infection in humans and animals. In nature, *H. capsulatum* grows as a mold in association with soil and avian habitats, being enriched by alkaline nitrogenous substrates in guano. *H. capsulatum* and histoplasmosis, which is initiated by inhalation of the conidia, occur worldwide. However, the incidence varies considerably, and most cases occur in the United States. *H. capsulatum* received its name from the appearance of the yeast cells in histopathologic sections; however, it is neither a protozoan nor does it have a capsule.

Morphology & Identification

At temperatures below 37 °C, primary isolates of *H. capsulatum* often develop brown mold colonies, but the appearance varies. Many isolates grow slowly, and specimens require incubation for 4-12 weeks before colonies develop. The hyaline, septate hyphae produce microconidia (2-5 μm) and large, spherical thick-walled macroconidia with peripheral projections of cell wall material (8-16 μm). In tissue or in vitro on rich medium at 37 °C, the hyphae and conidia convert to small, oval yeast cells (2 x 4 μm). In tissue, the yeasts are typically seen within macrophages, as *H. capsulatum* is a facultative intracellular parasite. In the laboratory, with appropriate mating strains, a sexual cycle can be demonstrated, yielding *Ajellomyces capsulatus*, a teleomorph that produces ascospores.

Antigenic Structure

Histoplasmin is a crude mycelial broth culture filtrate antigen. After initial infection, which is asymptomatic in over 95% of individuals, a positive delayed type skin test to histoplasmin is acquired. Antibodies to both yeast and mycelial antigens can be measured serologically.

Pathogenesis & Clinical Findings

After inhalation, the conidia develop into yeast cells and are engulfed by alveolar macrophages, where they are able to replicate. Within macrophages, the yeasts may disseminate to reticuloendothelial tissues such as the liver, spleen, bone marrow, and lymph nodes. The initial inflammatory reaction becomes granulomatous.

In over 95% of cases, the resulting cell-mediated immune response leads to the secretion of cytokines that activate macrophages to inhibit the intracellular growth of the yeasts. Some individuals, such as immunocompetent persons who inhale a heavy inoculum, develop acute pulmonary histoplasmosis, which is a self-limited flu-like syndrome with fever, chills, myalgias, headaches, and nonproductive cough. On radiographic examination, most patients will have hilar lymphadenopathy and pulmonary infiltrates or nodules.

These symptoms resolve spontaneously without therapy, and the granulomatous nodules in the lungs or

other sites heal with calcification.

Chronic pulmonary histoplasmosis occurs most often in men and is usually a reactivation process, the breaking down of a dormant lesion that may have been acquired years before. This reactivation is usually precipitated by pulmonary damage such as emphysema.

Severe disseminated histoplasmosis develops in a small minority of infected individuals - particularly infants, the elderly, and the immunosuppressed, including AIDS patients. The reticuloendothelial system is especially apt to be involved, with lymphadenopathy, enlarged spleen and liver, high fever, anemia, and a high mortality rate without antifungal therapy. Mucocutaneous ulcers of the nose, mouth, tongue, and intestine can occur. In such individuals, histologic study reveals focal areas of necrosis within granulomas in many organs. The yeasts may be present in macrophages in the blood, liver, spleen, and bone marrow.

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens for culture include sputum, urine, scrapings from superficial lesions, bone marrow aspirates, and buffy coat blood cells. Blood films, bone marrow slides, and biopsy specimens may be examined microscopically. In disseminated histoplasmosis, bone marrow cultures are often positive.

B. MICROSCOPIC EXAMINATION

The small ovoid cells may be observed within macrophages in histologic sections stained with fungal stains (eg, Gomori's methenamine silver, periodic acid-Schiff, or calcofluor white) or in Giemsa-stained smears of bone marrow or blood.

C. CULTURE

Specimens are cultured in rich media, such as glucose-cysteine blood agar at 37 °C and on Sabouraud's agar or inhibitory mold agar at 25-30 °C. Cultures must be incubated for a minimum of 4 weeks. The laboratory should be alerted if histoplasmosis is suspected because special blood culture methods, such as lysis-centrifugation or fungal broth medium, can be used to enhance the recovery of *H. capsulatum*.

D. SEROLOGY

CF tests for antibodies to histoplasmin or the yeast cells become positive within 2-5 weeks after infection. CF titers rise during progressive disease and then decline to very low levels when the disease is inactive. With progressive disease, the CF titers are ≥ 1:32. Because cross-reactions may occur, antibodies to other fungal antigens are routinely tested. In the ID test, precipitins to two *H. capsulatum*-specific antigens are detected. The presence of antibodies to the H antigen often signifies active histoplasmosis, while antibodies to the M antigen may arise from repeated skin testing or past exposure. One of the most sensitive tests is a radioassay or enzyme immunoassay for circulating antigen of *H. capsulatum*. Nearly all patients with disseminated histoplasmosis have a positive test for antigen in the serum or urine; the antigen level drops following successful treatment and recurs during relapse. Despite cross-reactions with other mycoses, this test for antigen is more sensitive than conventional antibody tests in AIDS patients with histoplasmosis.

E. SKIN TEST

The histoplasmin skin test becomes positive soon after infection and remains positive for years. It may become negative in progressive disseminated histoplasmosis.

Repeated skin testing stimulates serum antibodies in sensitive individuals, interfering with the diagnostic interpretation of the serologic tests.

Immunity

Following initial infection, most persons appear to develop some degree of immunity. Immunosuppression may lead to reactivation and disseminated disease.

AIDS patients may develop disseminated histoplasmosis through reactivation or new infection.

Treatment

Acute pulmonary histoplasmosis is managed with supportive therapy and rest. Itraconazole is the treatment for mild to moderate infection. In disseminated disease, systemic treatment with amphotericin B is often curative, though patients may need prolonged treatment and monitoring for relapses. Patients with AIDS typically relapse despite therapy that would be curative in other patients. Therefore, AIDS patients require maintenance therapy with itraconazole.

Epidemiology & Control

The incidence of histoplasmosis is highest in the United States, where the endemic areas include the

central and eastern states and in particular the Ohio River Valley and portions of the Mississippi River Valley. Numerous outbreaks of acute histoplasmosis have resulted from exposure of many persons to large inocula of conidia.

These occur when *H. capsulatum* is disturbed in its natural habitat, ie, soil mixed with bird feces (eg, starling roosts, chicken houses) or bat guano (caves). Birds are not infected, but their excrement provides superb culture conditions for growth of the fungus. Conidia are also spread by wind and dust. The largest urban outbreak of histoplasmosis occurred in Indianapolis.

In some highly endemic areas, 80-90% of residents have a positive skin test by early adulthood. Many will have miliary calcifications in the lungs. Histoplasmosis is not communicable from person to person. Spraying formaldehyde on infected soil may destroy *H. capsulatum*. In Africa, in addition to the usual pathogen, there is a stable variant, *H. capsulatum* var *duboisii*, which causes African histoplasmosis. This form differs from the usual disease by causing less pulmonary involvement and more skin and bone lesions with abundantgiant cells that contain the yeasts, which are larger and more spherical.

BLASTOMYCES DERMATITIDIS

Blastomyces dermatitidis is a thermally dimorphic fungus that grows as a mold in culture, producing hyaline, branching septate hyphae and conidia. At 37 °C or in the host, it converts to a large, singly budding yeast cell.

B. dermatitidis causes blastomycosis, a chronic infection with granulomatous and suppurative lesions that is initiated in the lungs, whence dissemination may occur to any organ but preferentially to the skin and bones. The disease has been called North American blastomycosis because it is endemic and most cases occur in the United States and Canada. Despite this high prevalence in North America, blastomycosis has been documented in Africa, South America, and Asia. It is endemic for humans and dogs in the eastern United States.

Morphology & Identification

When *B. dermatitidisis* is grown on Sabouraud's agar at room temperature, a white or brownish colony develops, with branching hyphae bearing spherical, ovoid, or piriform conidia (3-5 µm in diameter) on slender terminal or lateral conidiophores. Larger chlamydospores (7-18 µm) may also be produced. In tissue or culture at 37 °C, *B. dermatitidis* grows as a thick-walled, multinucleated, spherical yeast (8-15 µm) that usually produces single buds. The bud and the parent yeast are attached with a broad base, and the bud often enlarges to the same size as the parent yeast before they become detached. The yeast colonies are wrinkled, waxy, and soft.

Antigenic Structure

Extracts of culture filtrates of *B. dermatitidis* contain blastomycin, probably a mixture of antigens. As a skin test reagent, blastomycin lacks specificity and sensitivity. Patients are often negative or lose their reactivity, and false-positive cross-reactions occur in people exposed to other fungi. Consequently, skin test surveys of the population to determine the level of exposure have not been conducted. The diagnostic value of blastomycin as an antigen in the CF test is also questionable because cross-reactions are common; however, many patients with widespread blastomycosis have high CF titers. In the ID test, using adsorbed reference antisera, antibodies can be detected to a specific *B. dermatitidis* antigen, designated antigen A. More reliable is an enzyme immunoassay for antigen A. The immunodominant motif probably responsible for generating a protective cell-mediated immune response is part of a cell-surface and secreted protein (WI-1).

Pathogenesis & Clinical Findings

Human infection is initiated in the lungs. Mild and self-limited cases have been documented, but their frequency is unknown because there is no adequate skin or serologic test with which to assess subclinical or resolved primary infections. The most common clinical presentation is a pulmonary infiltrate in association with a variety of symptoms indistinguishable from other acute lower respiratory infections (fever, malaise, night sweats, cough, and myalgias). Patients can also present with chronic pneumonia. Histologic examination reveals a distinct pyogranulomatous reaction with neutrophils and noncaseating granulomas. When dissemination occurs, skin lesions on exposed surfaces are most common. They may evolve into ulcerated verrucous granulomas with an advancing border and central scarring. The border is filled with microabscesses and has a sharp, sloping edge. Lesions of bone, the genitalia (prostate, epididymis, and testis), and the central nervous system also occur; other sites are less frequently involved. Although immunosuppressed patients,

including those with AIDS, may develop blastomycosis, it is not as common in these patients as are other systemic mycoses.

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens consist of sputum, pus, exudates, urine, and biopsies from lesions.

B. MICROSCOPIC EXAMINATION

Wet mounts of specimens may show broadly attached buds on thick-walled yeast cells. These may also be apparent in histologic sections.

C. CULTURE

Colonies usually develop within 2 weeks on Sabouraud's or enriched blood agar at 30 °C. The identification is confirmed by conversion to the yeast form after cultivation on a rich medium at 37 °C, by extraction and detection of the *B. dermatitidis*-specific antigen A, or by a specific DNA probe.

D. SEROLOGY

Antibodies can be measured by the CF and ID tests. In the EIA, high antibody titers to antigen A are associated with progressive pulmonary or disseminated infection. Overall, serologic tests are not as useful for the diagnosis of blastomycosis as they are in the case of the other endemic mycoses.

Treatment

Severe cases of blastomycosis are treated with amphotericin B. In patients with confined lesions, a 6-month course of itraconazole is very effective.

Epidemiology

Blastomycosis is a relatively common infection of dogs (and rarely other animals) in endemic areas. Blastomycosis cannot be transmitted by animals or humans.

Unlike *C. immitis* and *H. capsulatum*, *B. dermatitidis* has only rarely (and not reproducibly) been isolated from the environment, so its natural habitat is unknown. However, the occurrence of several small outbreaks has linked *B. dermatitidis* to rural river banks.

PARACOCCIDIOIDES BRASILIENSIS

Paracoccidioides brasiliensis is the thermally dimorphic fungal agent of paracoccidioidomycosis (South American blastomycosis), which is confined to endemic regions of Central and South America.

Morphology & Identification

Cultures of the mold form of *P. brasiliensis* grow very slowly and produce chlamydospores and conidia. The features are not distinctive. At 36 °C, on rich medium, it forms large, multiply budding yeast cells (up to 30 µm). The yeasts are larger and have thinner walls than those of *B. dermatitidis*. The buds are attached by a narrow connection.

Pathogenesis & Clinical Findings

P. brasiliensis is inhaled, and initial lesions occur in the lung. After a period of dormancy that may last for decades, the pulmonary granulomas may become active, leading to chronic, progressive pulmonary disease or dissemination. Most patients are 30-60 years of age, and over 90% are men. A few patients (≤ 10%), typically less than 30 years of age, develop an acute or subacute progressive infection with a shorter incubation time. In the usual case of chronic paracoccidioidomycosis, the yeasts spread from the lung to other organs, particularly the skin and mucocutaneous tissue, lymph nodes, spleen, liver, adrenals, and other sites. Many patients present with painful sores involving the oral mucosa. Histology usually reveals either granulomas with central caseation or microabscesses. The yeasts are frequently observed in giant cells or directly in exudates from mucocutaneous lesions.

Skin test surveys have been conducted using an antigen extract, paracoccidioidin, which may cross-react with coccidioidin or histoplasmin.

Diagnostic Laboratory Tests

In sputum, exudates, biopsies, or other material from lesions, the yeasts are often apparent on direct microscopic examination with KOH or calcofluor white. Cultures on Sabouraud's or yeast extract agar are incubated at room temperature and confirmed by conversion to the yeast form by in vitro growth at 36 °C. Serologic testing is most useful for diagnosis. Antibodies to paracoccidioidin can be measured by the CF or ID test. Healthy persons in endemic areas do not have antibodies to *P. brasiliensis*. In patients, titers tend to

correlate with the severity of disease.

Treatment

Itraconazole appears to be most effective against paracoccidioidomycosis, but ketoconazole and trimethoprim-sulfamethoxazole are also efficacious. Severe disease can be treated with amphotericin B.

Epidemiology

Paracoccidioidomycosis occurs mainly in rural areas of Latin America, particularly among farmers. The disease manifestations are much more frequent in males than in females, but infection and skin test reactivity occur equally in both sexes. Since *P. brasiliensis* has only rarely been isolated from nature, its natural habitat has not been defined. As with the other endemic mycoses, paracoccidioidomycosis is not communicable.

OPPORTUNISTIC MYCOSES

Patients with compromised host defenses are susceptible to ubiquitous fungi to which healthy people are exposed but usually resistant. In many cases, the type of fungus and the natural history of the mycotic infection are determined by the underlying predisposing condition of the host. As members of the normal microbial flora, *Candida* and related yeasts are endogenous opportunists. Other opportunistic mycoses are caused by exogenous fungi that are globally present in soil, water, and air. The more common pathogens will be discussed, but the incidence and the roster of fungal species causing serious mycotic infections in compromised individuals continue to increase.

In patients with AIDS, the susceptibility and incidence of opportunistic mycoses are inversely correlated with the CD4 lymphocyte count.

CANDIDA & RELATED YEASTS

Several species of the yeast genus *Candida* are capable of causing candidiasis. They are members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. *Candida* species colonize the mucosal surfaces of all humans during or soon after birth, and the risk of endogenous infection is ever-present. Candidiasis is the most common systemic mycosis, and the most common agents are *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. guilliermondii*, and *C. dubliniensis*. The widespread use of fluconazole has precipitated the emergence of more azole-resistant species, such as *C. krusei* and *C. lusitaniae*.

Morphology & Identification

In culture or tissue, *Candida* species grow as oval, budding yeast cells (3-6 µm in size). They also form pseudohyphae when the buds continue to grow but fail to detach, producing chains of elongated cells that are pinched or constricted at the septations between cells. Unlike other species of *Candida*, *C. albicans* is dimorphic; in addition to yeasts and pseudohyphae, it can also produce true hyphae. On agar media or within 24 hours at 37 °C or room temperature, *Candida* species produce soft, cream-colored colonies with a yeasty odor. Pseudohyphae are apparent as submerged growth below the agar surface. Two simple morphologic tests distinguish *C. albicans*, the most common pathogen, from other species of *Candida*. After incubation in serum for about 90 minutes at 37 °C, yeast cells of *C. albicans* will begin to form true hyphae or germ tubes, and on nutritionally deficient media *C. albicans* produces large, spherical chlamydospores. Sugar fermentation and assimilation tests can be used to confirm the identification and speciate the more common *Candida* isolates, such as *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. kefyr*, *C. krusei*, and *C. lusitaniae*; *C. glabrata* is unique among these pathogens because it produces only yeast cells and no pseudohyphal forms.

Antigenic Structure

The use of adsorbed antisera have defined two serotypes of *C. albicans*: A (which includes *C. tropicalis*) and B.

Many other antigens have been characterized, including secreted proteases, an immunodominant enolase, and heat shock proteins.

Pathogenesis & Pathology

Superficial (cutaneous or mucosal) candidiasis is established by an increase in the local census of *Candida* and damage to the skin or epithelium that permits local invasion by the yeasts and pseudohyphae. Systemic candidiasis occurs when *Candida* enters the bloodstream and the phagocytic host defenses are inadequate to contain the growth and dissemination of the yeasts. From the circulation, *Candida* can infect the kidneys, attach to prosthetic heart valves, or produce candidal infections almost anywhere (eg, arthritis, meningitis, endophthalmitis). The local histology of cutaneous or mucocutaneous lesions is characterized

by inflammatory reactions varying from pyogenic abscesses to chronic granulomas. The lesions contain abundant budding yeast cells and pseudohyphae. Large increases of *Candida* in the intestinal tract often follow the administration of oral antibacterial antibiotics, and the yeasts can enter the circulation by crossing the intestinal mucosa.

Clinical Findings

A. CUTANEOUS AND MUCOSAL CANDIDIASIS

The risk factors associated with superficial candidiasis include AIDS, pregnancy, diabetes, young or old age, birth control pills, and trauma (burns, maceration of the skin).

Thrush can occur on the tongue, lips, gums, or palate. It is a patchy to confluent, whitish pseudomembranous lesion composed of epithelial cells, yeasts, and pseudohyphae.

Thrush develops in most patients with AIDS. Other risk factors include treatment with corticosteroids or antibiotics, high levels of glucose, and cellular immunodeficiency. Yeast invasion of the vaginal mucosa leads to vulvovaginitis, characterized by irritation, pruritus, and vaginal discharge. This condition is often preceded by factors such as diabetes, pregnancy, or antibacterial drugs that alter the microbial flora, local acidity, or secretions. Other forms of cutaneous candidiasis include invasion of the skin. This occurs when the skin is weakened by trauma, burns, or maceration. Intertriginous infection occurs in moist, warm parts of the body such as the axillae, groin, and intergluteal or inframammary folds; it is most common in obese and diabetic individuals. The infected areas become red and moist and may develop vesicles. Interdigital involvement between the fingers follows repeated prolonged immersion in water; it is most common in homemakers, bartenders, cooks, and vegetable and fish handlers.

Candidal invasion of the nails and around the nail plate causes onychomycosis, a painful, erythematous swelling of the nail fold resembling a pyogenic paronychia, which may eventually destroy the nail.

B. SYSTEMIC CANDIDIASIS

Candidemia can be caused by indwelling catheters, surgery, intravenous drug abuse, aspiration, or damage to the skin or gastrointestinal tract. In most patients with normal host defenses, the yeasts are eliminated and candidemia is transient. However, patients with compromised innate phagocytic defenses may develop occult lesions anywhere, especially the kidney, skin (maculonodular lesions), eye, heart, and meninges. Systemic candidiasis is most often associated with chronic administration of corticosteroids or other immunosuppressive agents; with hematologic diseases such as leukemia, lymphoma, and aplastic anemia; or with chronic granulomatous disease.

Candidal endocarditis is frequently associated with deposition and growth of the yeasts and pseudohyphae on prosthetic heart valves or vegetations. Kidney infections are usually a systemic manifestation, whereas urinary tract infections are often associated with Foley catheters, diabetes, pregnancy, and antibacterial antibiotics.

C. CHRONIC MUCOCUTANEOUS CANDIDIASIS

Most forms of this rare disease have onset in early childhood, are associated with cellular immunodeficiencies and endocrinopathies, and result in chronic superficial disfiguring infections of any or all areas of skin or mucosa.

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens include swabs and scrapings from superficial lesions, blood, spinal fluid, tissue biopsies, urine, exudates, and material from removed intravenous catheters.

B. MICROSCOPIC EXAMINATION

Tissue biopsies, centrifuged spinal fluid, and other specimens may be examined in Gram-stained smears for pseudohyphae and budding cells. Skin or nail scrapings are first placed in a drop of 10% potassium hydroxide (KOH) and calcofluor white.

C. CULTURE

All specimens are cultured on fungal or bacteriologic media at room temperature or at 37 °C. Yeast colonies are examined for the presence of pseudohyphae. *C. albicans* is identified by the production of germ tubes or chlamydospores. Other *Candida* isolates are speciated with a battery of biochemical reactions. The interpretation of positive cultures varies with the specimen.

Positive cultures from normally sterile body sites are significant. The diagnostic value of a quantitative urine culture depends on the integrity of the specimen and the yeast census. Contaminated Foley catheters

may lead to «false-positive» urine cultures. Positive blood cultures may reflect systemic candidiasis or transient candidemia due to a contaminated intravenous line.

Sputum cultures have no value because *Candida* species are part of the oral flora. Cultures of skin lesions are confirmatory.

D. SEROLOGY

In general, the currently available serologic tests have limited specificity or sensitivity. Serum antibodies and cell-mediated immunity are demonstrable in most people as a result of lifelong exposure to *Candida*. In systemic candidiasis, antibody titers to various candidal antigens may be elevated, but there are no clear criteria for establishing a diagnosis serologically. The detection of circulating cell wall mannan, using a latex agglutination test or an enzyme immunoassay, is much more specific, but the test lacks sensitivity because many patients are only transiently positive or because they do not develop significant and detectable antigen titers until late in the disease. A promising new serological test for circulating β-glucan, which is found in the cell walls of many fungal species, is currently under evaluation.

Immunity

The basis of resistance to candidiasis is complex and incompletely understood. Cell-mediated immune responses, especially CD4 cells, are important in controlling mucocutaneous candidiasis, and the neutrophil is probably crucial for resistance to systemic candidiasis.

Treatment

Thrush and other mucocutaneous forms of candidiasis are usually treated with topical nystatin or oral ketoconazole or fluconazole. Systemic candidiasis is treated with amphotericin B, sometimes in conjunction with oral flucytosine, fluconazole, or caspofungin. The clearing of cutaneous lesions is accelerated by eliminating contributing factors such as excessive moisture or antibacterial drugs. Chronic mucocutaneous candidiasis responds well to oral ketoconazole and other azoles, but patients have a genetic cellular immune defect and often require lifelong treatment.

It is often difficult to establish an early diagnosis of systemic candidiasis - the clinical signs are not definitive, and cultures are often negative. Furthermore, there is no established prophylactic regimen for patients at risk, though treatment with an azole or with a short course of low-dose amphotericin B is often indicated for febrile or debilitated patients who are immunocompromised and do not respond to antibacterial therapy.

Epidemiology & Control

The most important preventive measure is to avoid disturbing the normal balance of microbial flora and intact host defenses. Candidiasis is not communicable, since virtually all persons normally harbor the organism.

CRYPTOCOCCUS NEOFORMANS AND *C. GATTII*

C. neoformans and *C. gattii* are basidiomycetous yeasts with large polysaccharide capsules. *C. neoformans* occurs worldwide in nature and is isolated readily from dry pigeon feces. *C. gattii* is less common and typically associated with trees in tropical areas. Both species cause cryptococcosis, which follows inhalation of desiccated yeast cells or possibly the smaller basidiospores. From the lungs, these neurotropic yeasts typically migrate to the central nervous system where they cause meningoencephalitis. However, they also have the capacity to infect many other organs (e.g., skin, eyes, prostate). *C. neoformans* occurs in immunocompetent persons but more often in patients with HIV/AIDS, hematogenous malignancies, and other immunosuppressive conditions. Cryptococcosis due to *C. gattii* is rarer and usually associated with apparently normal hosts.

Morphology & Identification

In culture, *Cryptococcus* species produce whitish mucoid colonies within 2-3 days. Microscopically, in culture or clinical material, the spherical budding yeast cells (5-10 µm in diameter) are surrounded by a thick non-staining capsule. All species of *Cryptococcus*, including several nonpathogenic species, are encapsulated and possess urease. However, *C. neoformans* and *C. gattii* differ from nonpathogenic species by the abilities to grow at 37 °C and the production of laccase, a phenol oxidase, which catalyzes the formation of melanin from appropriate phenolic substrates (eg, catecholamines). Both the capsule and laccase are well-characterized virulence factors. Clinical isolates are identified by demonstrating the production of laccase or a specific pattern of carbohydrate assimilations. Adsorbed antisera have defined five serotypes (A-D and AD); strains

of *C. neoformans* may possess serotype A, D, or AD, and isolates of *C. gattii* may have serotype B or C. In addition to their capsular serotypes, the two species differ in their genotypes, ecology, some biochemical reactions, and clinical manifestations. Sexual reproduction can be demonstrated in the laboratory, and successful mating results in the production of mycelia and basidiospores; the corresponding teleomorphs of the two varieties are *Filobasidiella neoformans* var *neoformans* (serotypes A and D) and *Filobasidiella neoformans* var *bacillispora* (serotypes B and C).

Antigenic Structure

The capsular polysaccharides, regardless of serotype, have a similar structure: they are long, unbranched polymers consisting of an α-1,3-linked polymannose backbone with β-linked monomeric branches of xylose and glucuronic acid. During infection, the capsular polysaccharide is solubilized in spinal fluid, serum, or urine and can be detected by an enzyme immunoassay or by the agglutination of latex particles coated with antibody to the polysaccharide. With proper controls, this test is diagnostic of cryptococcosis. Patient antibodies to the capsule can also be measured, but they are not used in diagnosis.

Pathogenesis

Infection is initiated by inhalation of the yeast cells, which in nature are dry, minimally encapsulated, and easily aerosolized. The primary pulmonary infection may be asymptomatic or may mimic an influenza-like respiratory infection, often resolving spontaneously. In patients who are compromised, the yeasts may multiply and disseminate to other parts of the body but preferentially to the central nervous system, causing cryptococcal meningoencephalitis. Other common sites of dissemination include the skin, adrenals, bone, eye, and prostate gland. The inflammatory reaction is usually minimal or granulomatous.

Clinical Findings

The major clinical manifestation is chronic meningitis, which can resemble a brain tumor, brain abscess, degenerative central nervous system disease, or any mycobacterial or fungal meningitis. Cerebrospinal fluid pressure and protein may be increased and the cell count elevated, whereas the glucose is normal or low.

Patients may complain of headache, neck stiffness, and disorientation. In addition, there may be lesions in skin, lungs, or other organs.

The course of cryptococcal meningitis may fluctuate over long periods, but all untreated cases are ultimately fatal. About 5-8% of patients with AIDS develop cryptococcal meningitis. The infection is not transmitted from person to person.

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens include spinal fluid, tissue, exudates, sputum, blood, and urine. Spinal fluid is centrifuged before microscopic examination and culture.

B. MICROSCOPIC EXAMINATION

Specimens are examined in wet mounts, both directly and after mixing with India ink, which delineates the capsule.

C. CULTURE

Colonies develop within a few days on most media at room temperature or 37 °C. Media with cycloheximide inhibit *Cryptococcus* and should be avoided. Cultures can be identified by growth at 37 °C and detection of urease. Alternatively, on an appropriate diphenolic substrate, the phenol oxidase (or laccase) of *C. neoformans* and *C. gattii* produces melanin in the cell walls and colonies develop a brown pigment.

D. SEROLOGY

Tests for capsular antigen can be performed on cerebrospinal fluid and serum. The latex slide agglutination test for cryptococcal antigen is positive in 90% of patients with cryptococcal meningitis. With effective treatment, the antigen titer drops - except in AIDS patients, who often maintain high antigen titers for long periods.

Treatment

Combination therapy of amphotericin B and flucytosine has been considered the standard treatment for cryptococcal meningitis, though the benefit from adding flucytosine remains controversial. Amphotericin B (with or without flucytosine) is curative in most patients. Since AIDS patients with cryptococcosis will almost always relapse when amphotericin B is with-drawn, they require suppressive therapy with fluconazole. Fluconazole offers excellent penetration of the central nervous system. Recent evidence has shown that

HIV/AIDS patients treated with highly active antiretroviral therapy (HAART) have a lower incidence of cryptococcosis, and cases have a much better prognosis.

Epidemiology & Control

Bird droppings (particularly pigeon droppings) enrich for the growth of *C. neoformans* and serve as a reservoir of infection. The organism grows luxuriantly in pigeon excreta, but the birds are not infected. In addition to patients with AIDS or hematologic malignancies, patients being maintained on corticosteroids are highly susceptible to cryptococcosis.

ASPERGILLOSIS

Aspergillosis is a spectrum of diseases that may be caused by a number of *Aspergillus* species. *Aspergillus* species are ubiquitous saprobes in nature, and aspergillosis occurs worldwide. *A. fumigatus* is the most common human pathogen, but many others, including *A. flavus*, *A. niger*, and *A. terreus*, may cause disease. This mold produces abundant small conidia that are easily aerosolized. Following inhalation of these conidia, atopic individuals often develop severe allergic reactions to the conidial antigens. In immunocompromised patients - especially those with leukemia, stem cell transplant patients, and individuals taking corticosteroids - the conidia may germinate to produce hyphae that invade the lungs and other tissues.

Morphology & Identification

Aspergillus species grow rapidly, producing aerial hyphae that bear characteristic conidial structures: long conidiophores with terminal vesicles on which phialides produce basipetal chains of conidia. The species are identified according to morphologic differences in these structures, including the size, shape, texture, and color of the conidia.

Pathogenesis

In the lungs, alveolar macrophages are able to engulf and destroy the conidia. However, macrophages from corticosteroid-treated animals or immunocompromised patients have a diminished ability to contain the inoculum. In the lung, conidia swell and germinate to produce hyphae that have a tendency to invade preexisting cavities (aspergilloma or fungus ball) or blood vessels.

Clinical Findings

A. ALLERGIC FORMS

In some atopic individuals, development of IgE antibodies to the surface antigens of *Aspergillus* conidia elicits an immediate asthmatic reaction upon subsequent exposure. In others, the conidia germinate and hyphae colonize the bronchial tree without invading the lung parenchyma. This phenomenon is characteristic of allergic bronchopulmonary aspergillosis, which is clinically defined as asthma, recurrent chest infiltrates, eosinophilia, and both type I (immediate) and type III (Arthus) skin test hypersensitivity to *Aspergillus* antigen.

Many patients produce sputum with *Aspergillus* and serum precipitins. They have difficulty breathing and may develop permanent lung scarring. Normal hosts exposed to massive doses of conidia can develop extrinsic allergic alveolitis.

B. ASPERGILLOMA AND EXTRAPULMONARY COLONIZATION

Aspergilloma occurs when inhaled conidia enter an existing cavity, germinate, and produce abundant hyphae in the abnormal pulmonary space. Patients with previous cavitary disease (eg, tuberculosis, sarcoidosis, emphysema) are at risk. Some patients are asymptomatic; others develop cough, dyspnea, weight loss, fatigue, and hemoptysis. Cases of aspergilloma rarely become invasive. Localized, noninvasive infections (colonization) by *Aspergillus* species may involve the nasal sinuses, the ear canal, the cornea, or the nails.

C. INVASIVE ASPERGILLOSIS

Following inhalation and germination of the conidia, invasive disease develops as an acute pneumonic process with or without dissemination. Patients at risk are those with lymphocytic or myelogenous leukemia and lymphoma, stem cell transplant recipients, and especially individuals taking corticosteroids. The risk is much greater for patients receiving allogeneic (rather than autologous) hematopoietic stem cell transplants. In addition, AIDS patients with CD4 cell counts < 50 CD4 cells/mm^3 are predisposed to invasive aspergillosis.

Symptoms include fever, cough, dyspnea, and hemoptysis. Hyphae invade the lumens and walls of blood vessels, causing thrombosis, infarction, and necrosis. From the lungs, the disease may spread to the gastrointestinal tract, kidney, liver, brain, or other organs, producing abscesses and necrotic lesions. Without rapid treatment, the prognosis for patients with invasive aspergillosis is grave. Persons with less compromising underlying disease may develop chronic necrotizing pulmonary aspergillosis, which is a milder disease.

Diagnostic Laboratory Tests

A. SPECIMENS

Sputum, other respiratory tract specimens, and lung biopsy tissue provide good specimens. Blood samples are rarely positive.

B. MICROSCOPIC EXAMINATION

On direct examination of sputum with KOH or calcofluor white or in histologic sections, the hyphae of *Aspergillus* species are hyaline, septate, and uniform in width (about 4 μm) and branch dichotomously.

C. CULTURE

Aspergillus species grow within a few days on most media at room temperature. Species are identified according to the morphology of their conidial structures.

D. SEROLOGY

The ID test for precipitins to *A. fumigatus* is positive in over 80% of patients with aspergilloma or allergicforms of aspergillosis, but antibody tests are not helpful in the diagnosis of invasive aspergillosis. However, a serologic test for circulating cell wall galactomannan is diagnostic.

Treatment

Aspergilloma is treated with itraconazole or amphotericin B and surgery. Invasive aspergillosis requires rapid administration of either the native or lipid formulation of amphotericin B or voriconazole, often supplemented with cytokine immunotherapy. Amphotericin B-resistant strains of *A. terreus* have emerged at several leukemia treatment centers, and the new triazole, posaconazole, may be more effective for these infections. The less severe chronic necrotizing pulmonary disease may be treatable with voriconazole or itraconazole. Allergic forms of aspergillosis are treated with corticosteroids or disodium chromoglycate.

Epidemiology & Control

For persons at risk for allergic disease or invasive aspergillosis, efforts are made to avoid exposure to the conidia of *Aspergillus* species. Most bone marrow transplant units employ filtered air-conditioning systems, monitor airborne contaminants in patients' rooms, reduce visiting, and institute other measures to isolate patients and minimize their risk of exposure to the conidia of *Aspergillus* and other molds. Some patients at risk for invasive aspergillosis are given prophylactic lowdose amphotericin B or itraconazole.

MUCORMYCOSIS

Mucormycosis (zygomycosis) is an opportunistic mycosis caused by a number of molds classified in the order Mucorales of the phylum Zygomycota. These fungi are ubiquitous thermotolerant saprobes. The leading pathogens among this group of fungi are species of the genera *Rhizopus*, *Rhizomucor*, *Absidia*, *Cunninghamella* and *Mucor*. The conditions that place patients at risk include acidosis - especially that associated with diabetes mellitus - leukemias, lymphoma, corticosteroid treatment, severe burns, immunodeficiencies, and other debilitating diseases as well as dialysis with the iron chelator deferoxamine.

The major clinical form is rhinocerebral mucormycosis, which results from germination of the sporangiospores in the nasal passages and invasion of the hyphae into the blood vessels, causing thrombosis, infarction, and necrosis. The disease can progress rapidly with invasion of the sinuses, eyes, cranial bones, and brain. Blood vessels and nerves are damaged, and patients develop edema of the involved facial area, a bloody nasal exudate, and orbital cellulitis. Thoracic mucormycosis follows inhalation of the sporangiospores with invasion of the lung parenchyma and vasculature.

In both locations, ischemic necrosis causes massive tissue destruction. Less frequently, this process has been associated with contaminated wound dressings and other situations.

Direct examination or culture of nasal discharge, tissue, or sputum will reveal broad hyphae (10-15 μm) with uneven thickness, irregular branching, and sparse septations. These fungi grow rapidly on laboratory media, producing abundant cottony colonies. Identification is based on the sporangial structures. Treatment consists of aggressive surgical debridement, rapid administration of amphotericin B, and control of the underlying disease. Many patients survive, but there may be residual effects such as partial facial paralysis or loss of an eye.

PNEUMOCYSTIS JIROVECI

Pneumocystis jiroveci causes pneumonia in immunocompromised patients; dissemination is rare. Until recently, *P. jiroveci* was thought to be a protozoan, but molecular biologic studies have proved that it is a

fungus with a close relationship to ascomycetes. Pneumocystis species are present in the lungs of many animals (rats, mice, dogs, cats, ferrets, rabbits) but rarely cause disease unless the host is immunosuppressed. *P. jiroveci* is the human species, and the more familiar *P. carinii* is found only in rats.

Until the AIDS epidemic, human disease was confined to interstitial plasma cell pneumonitis in malnourished infants and immunosuppressed patients (corticosteroid therapy, antineoplastic therapy, and transplant recipients). Prior to the introduction of effective chemoprophylactic regimens, it was a major cause of death among AIDS patients. Chemoprophylaxis has resulted in a dramatic decrease in the incidence of pneumonia, but infections are increasing in other organs, primarily the spleen, lymph nodes, and bone marrow.

P. jiroveci has morphologically distinct forms: thin-walled trophozoites and cysts, which are thick-walled, spherical to elliptical (4-6 μm), and contain four to eight nuclei. Cysts can be stained with silver stain, toluidine blue, and calcofluor white. In most clinical specimens, the trophozoites and cysts are present in a tight mass that probably reflects their mode of growth in the host. *P. jiroveci* contains a surface glycoprotein that can be detected in sera from acutely ill or normal individuals.

P. jiroveci is an extracellular pathogen. Growth in the lung is limited to the surfactant layer above alveolar epithelium. In non-AIDS patients, infiltration of the alveolar spaces with plasma cells leads to interstitial plasma cell pneumonitis. Plasma cells are absent in AIDS-related pneumocystis pneumonia. Blockage of the oxygen exchange interface results in cyanosis.

To establish the diagnosis of pneumocystis pneumonia, specimens of bronchoalveolar lavage, lung biopsy tissue, or induced sputum are stained and examined for the presence of cysts or trophozoites. Appropriate stains include Giemsa, toluidine blue, methenamine silver, and calcofluor white. A specific monoclonal antibody is available for direct fluorescent examination of specimens. *Pneumocystis* cannot be cultured. While not clinically useful, serologic testing has been used to establish the prevalence of infection.

In the absence of immunosuppression, *P. jiroveci* does not cause disease. Serologic evidence suggests that most individuals are infected in early childhood, and the organism has worldwide distribution. Cell-mediated immunity presumably plays a dominant role in resistance to disease, as AIDS patients often have significant antibody titers, and pneumocystis pneumonia is not usually seen until the CD4 lymphocyte count drops below 400/μL.

Acute cases of pneumocystis pneumonia are treated with trimethoprim-sulfamethoxazole or pentamidine isethionate. Prophylaxis can be achieved with daily TMP-SMZ or aerosolized pentamidine. Other drugs are also available.

No natural reservoir has been demonstrated, and the agent may be an obligate member of the normal flora. Persons at risk are provided with chemoprophylaxis. The mode of infection is unclear, and transmission by aerosols may be possible.

OTHER OPPORTUNISTIC MYCOSES

Individuals with compromised host defenses are susceptible to infections by many of the thousands of saprobic molds that exist in nature and produce airborne spores.

Such opportunistic mycoses occur less frequently than candidiasis, aspergillosis, and mucormycosis because the fungi are less pathogenic. Advances in medicine have resulted in growing numbers of severely compromised patients in whom normally nonpathogenic fungi may become opportunistic pathogens. Devastating systemic infections have been caused by species of *Fusarium*, *Paecilomyces*, *Bipolaris*, *Curvularia*, *Alternaria*, and many others. Some opportunists are geographically restricted. For example, AIDS patients in Asia acquire systemic infections with *Penicillium marneffei*, which is a dimorphic pathogen endemic to the area. Another contributing factor is the increasing useof antifungal antibiotics, which has led to the selection of resistant fungal species and strains.

ANTIFUNGAL PROPHYLAXIS

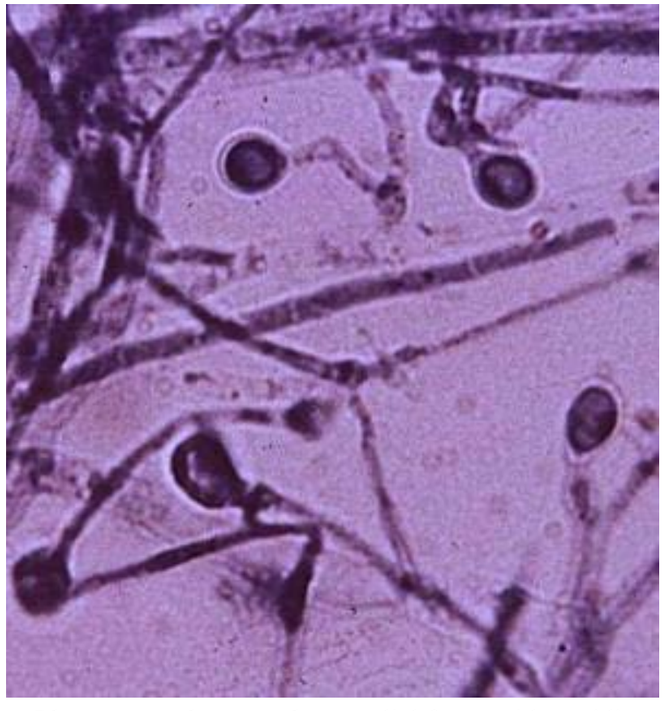
Opportunistic mycoses are increasing among immunocompromised patients, especially in patients with hematological dyscrasias (eg, leukemia), hematopoietic stem cell recipients, and solid organ transplant patients, and others receiving cytotoxic and immunosuppressive drugs (eg, corticosteroids). For example, the incidence of systemic mycoses among patients with acute lymphocytic or myelogenous leukemia is 5-20%, and among allogeneic stem cell transplant patients, 5-10%. Many of these high risk patients have depressed innate host defenses, such as a reduction in the number and/or functionality of circulating neutrophils and monocytes. In addition, AIDS patients are highly susceptible to a variety of systemic mycoses when their CD4

cell counts drop below 200 cells per cubic milliliter.

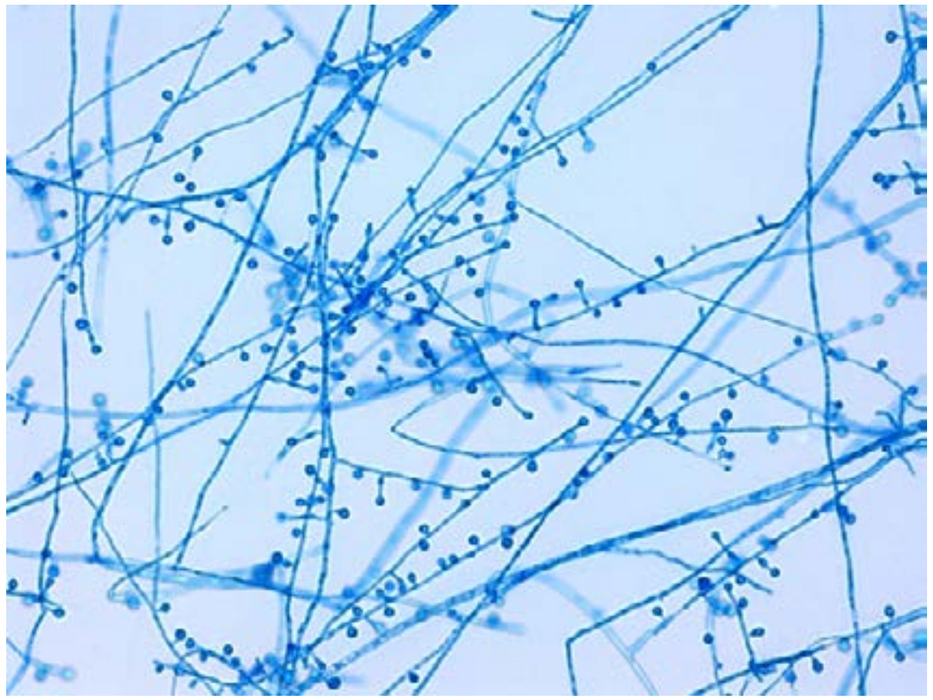
The list of invasive opportunistic pathogens include species of *Candida*, *Cryptococcus*, *Saccharomyces*, and other yeasts; *Aspergillus* and other ascomycetous molds, such as *Fusarium*, *Paecilomyces*, and *Scopulariopsis*; dematiaceous molds (eg, species of *Bipolaris*, *Phialophora*, *Cladosporium*), and zygomycetes (*Rhizopus*). Because it is usually difficult to establish a definitive diagnosis early in the course of infection, many high risk patients are treated empirically or prophylactically with antifungal drugs. However, there is no universal consensus on the criteria for administering antifungal prophylaxis or the specific chemotherapy and regimen. Rather, most tertiary care hospitals have developed their own protocols for the administration of prophylactic antifungal chemotherapy to patients at high risk for invasive mycoses.

Most hospitals will give oral fluconazole; others prescribe a short course of low-dose amphotericin B. Some of the criteria for administering antifungal prophylaxis to a patient with an underlying high risk disease or condition are persistent fever that is unresponsive to antibacterial antibiotics, neutropenia lasting more than 7 days, the observation of new and unexplained pulmonary infiltrates on radiographic examinations, or progressive, unexplained organ failure.

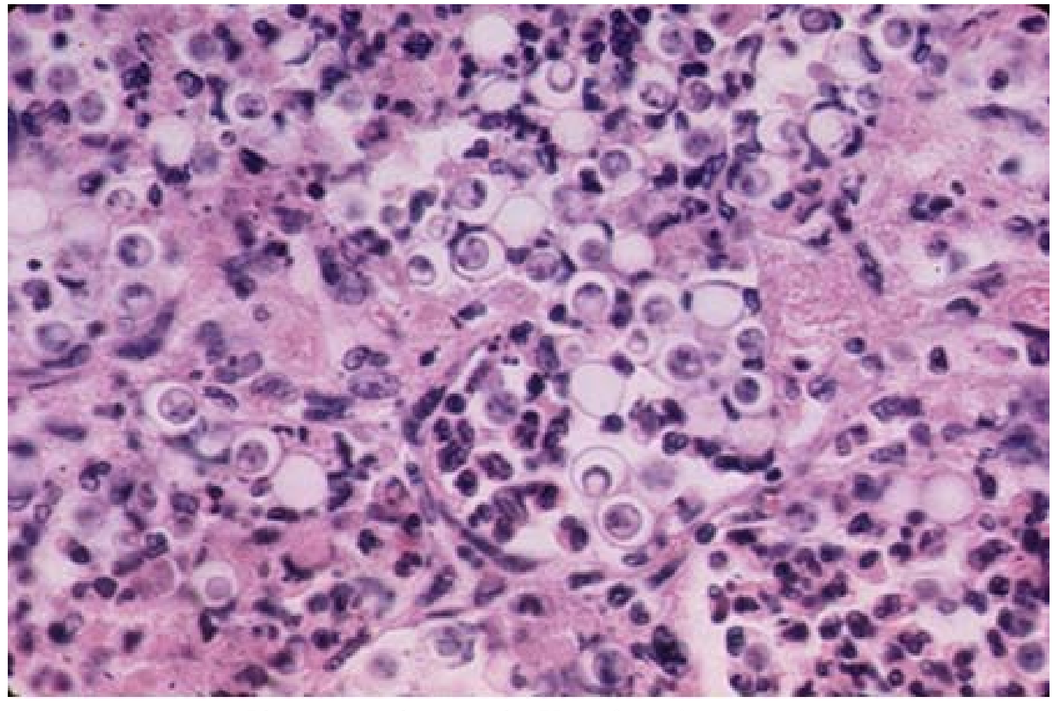
15 Class – Illustrations



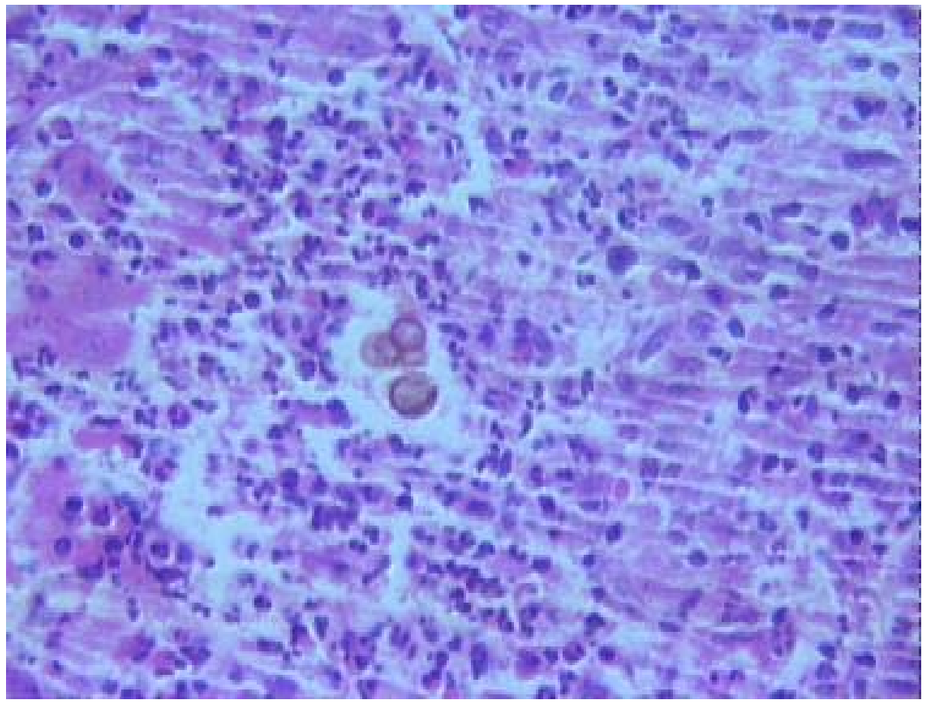
Blastomyces dermatitidis mycelial form, with conidia



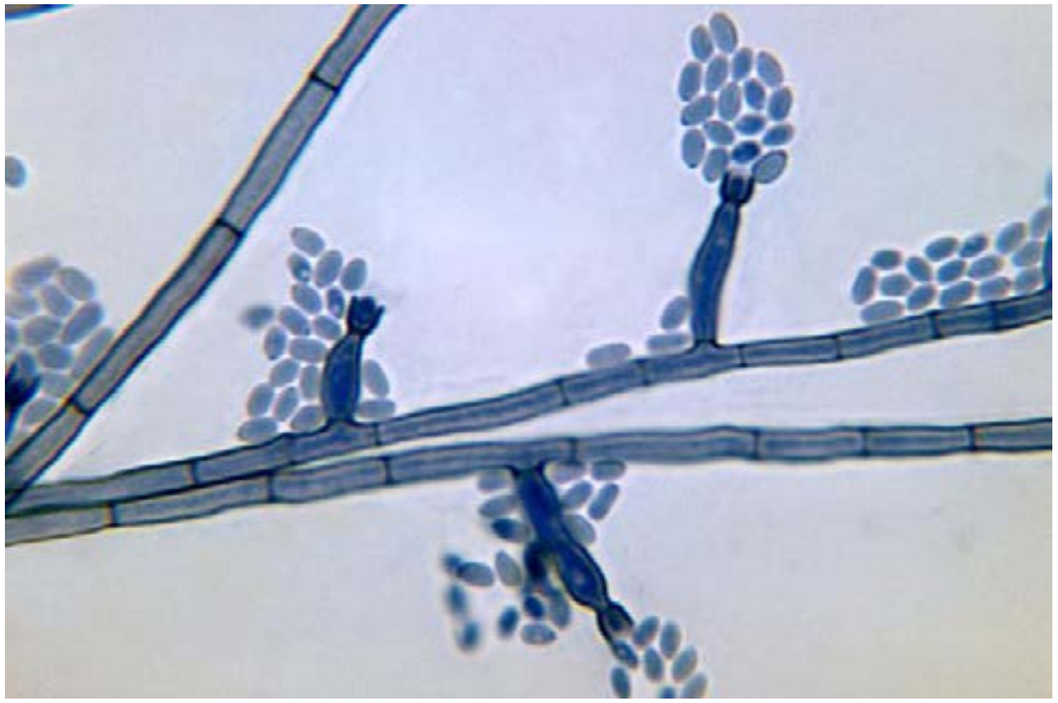
Blastomyces dermatitidis. View of the “lollipop-like” conidiophore-conidia structures extending along the length of hyphae. (400X, DMD-108)



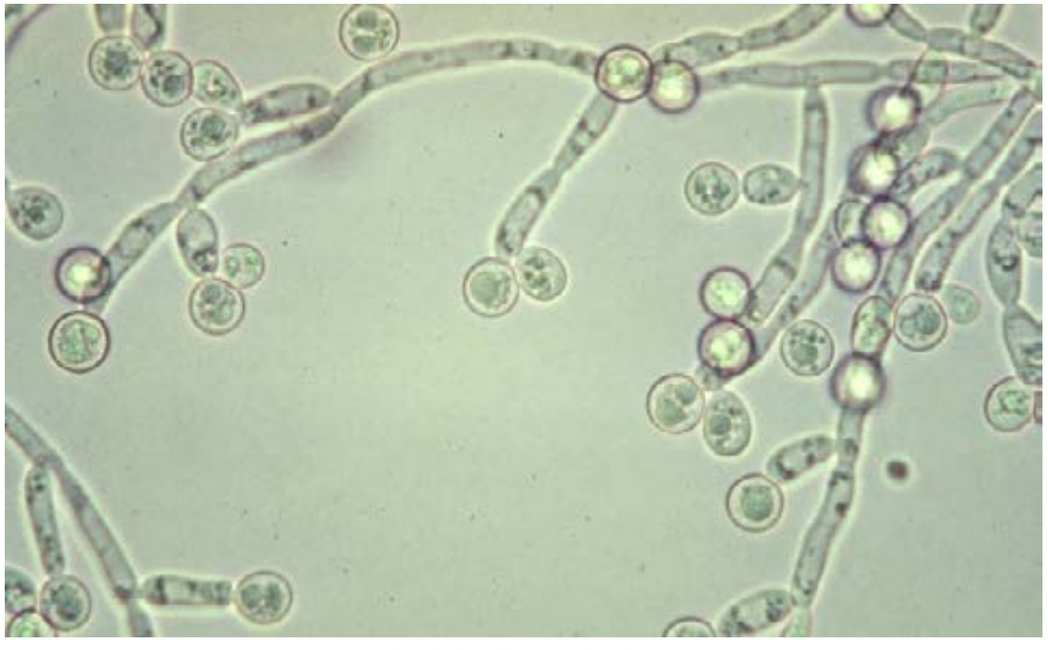
Blastomyces dermatitidis. Yeast from tissue smear.



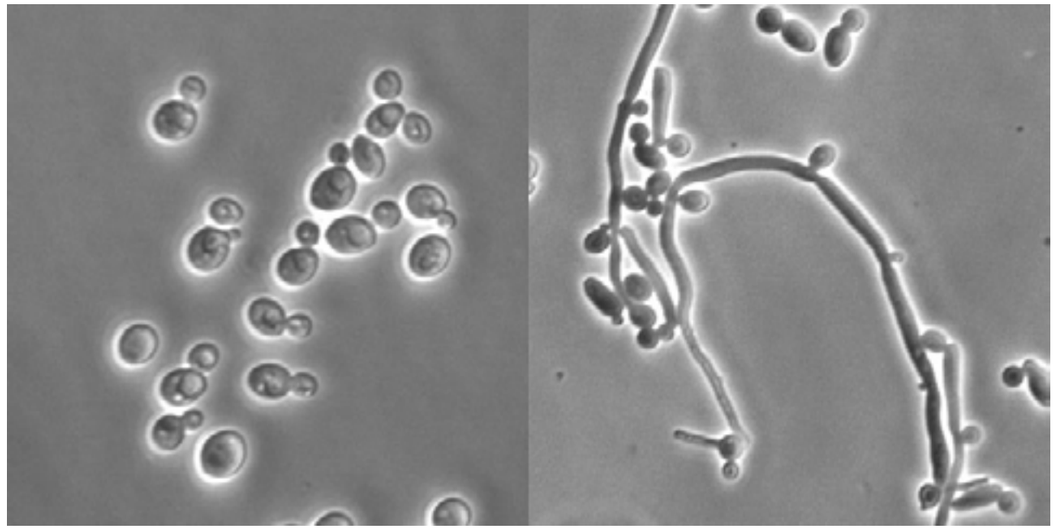
Phialophora verrucosa (thin tissue preparate)



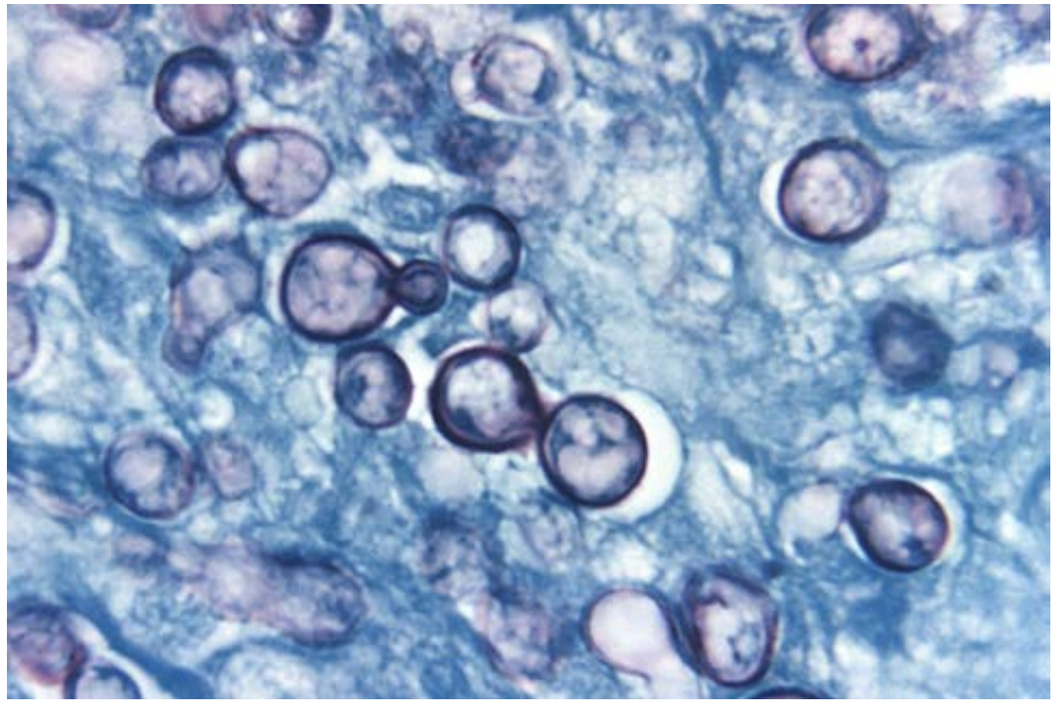
Conidia-laden conidiophores of *Phialophora verrucosa*



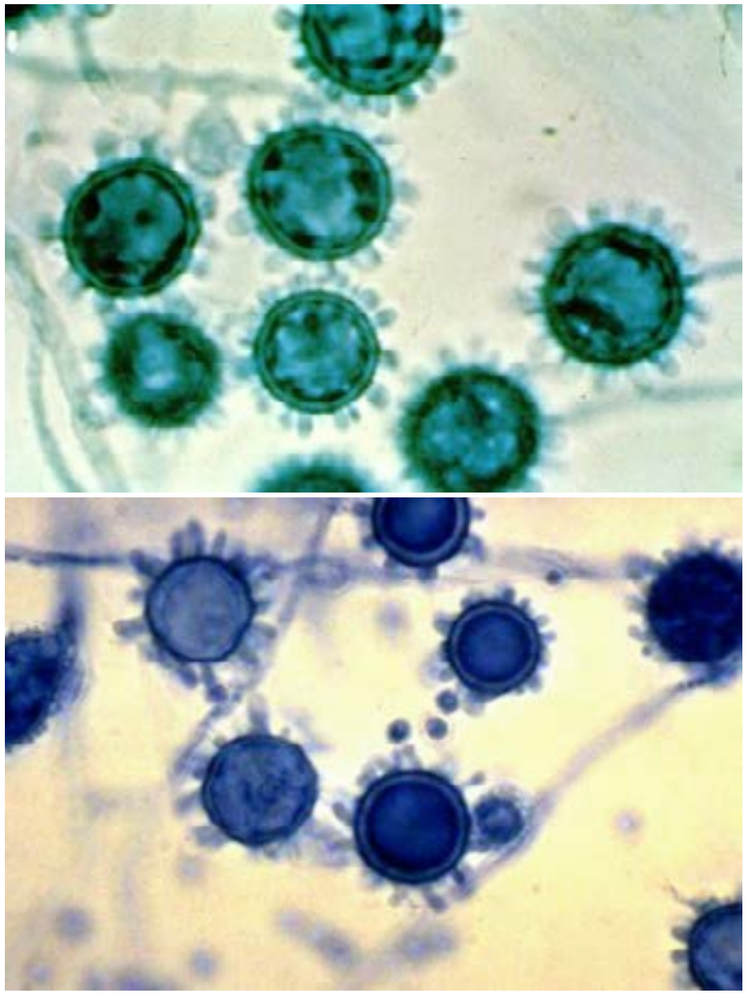
Candida albicans hyphae



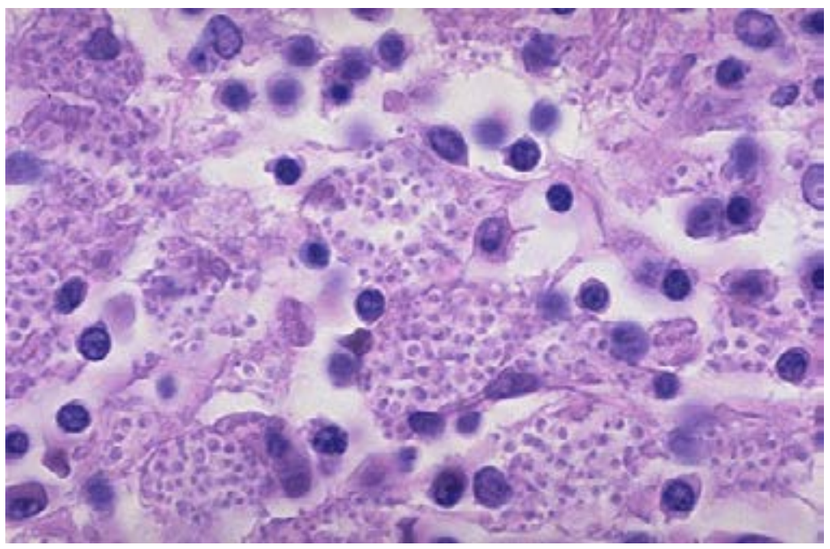
Candida albicans individual cells and hyphae



Histoplasma capsulatum

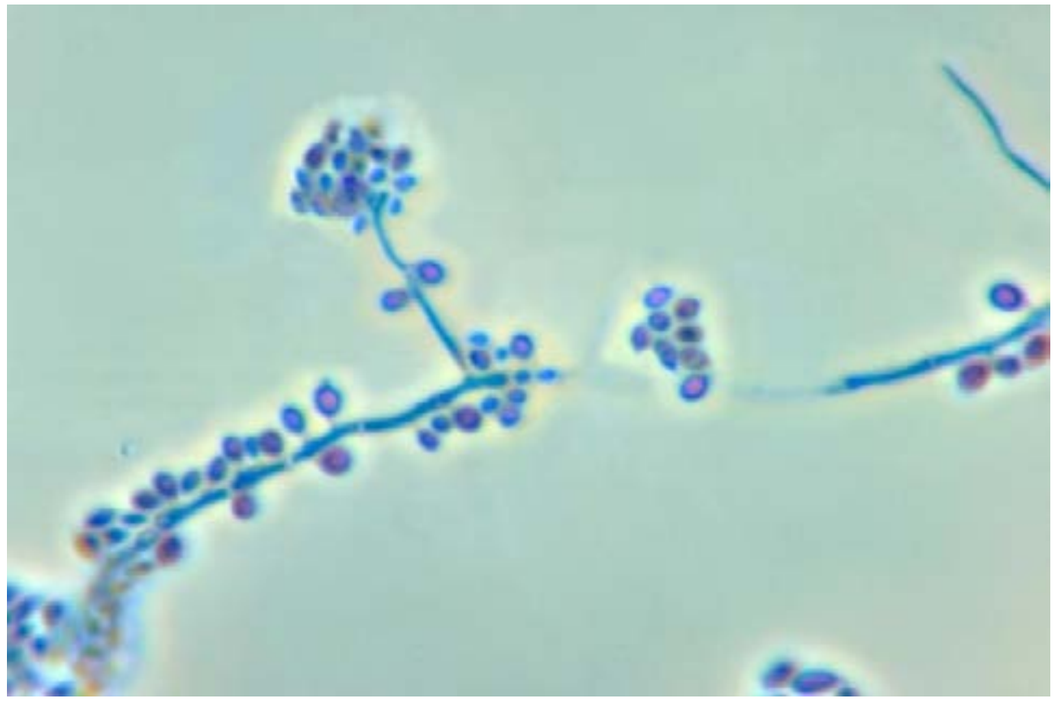


Culture of *Histoplasma capsulatum*, rounded tuberculate macroconidia

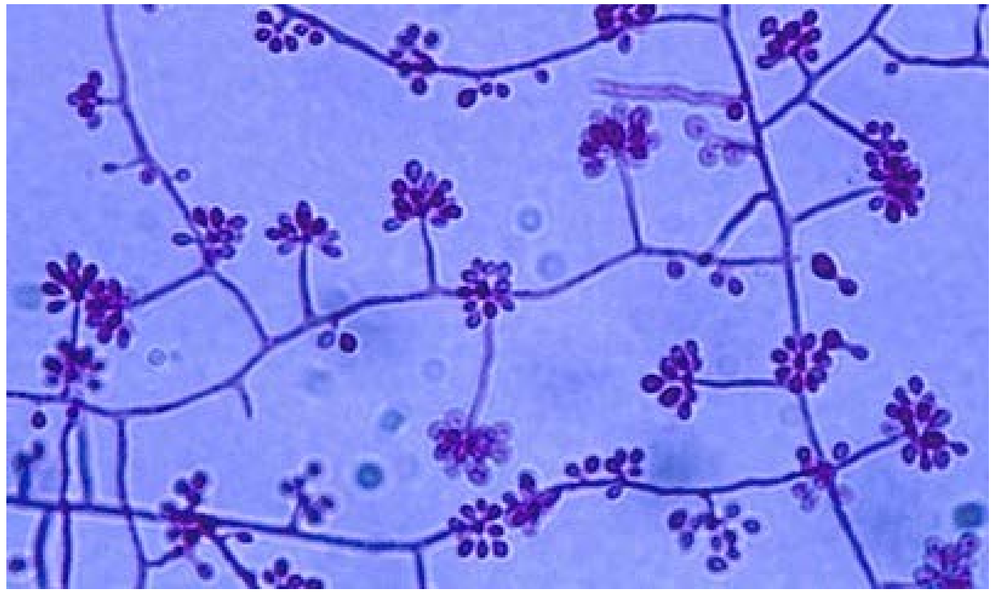


Histoplasma capsulatum within macrophages

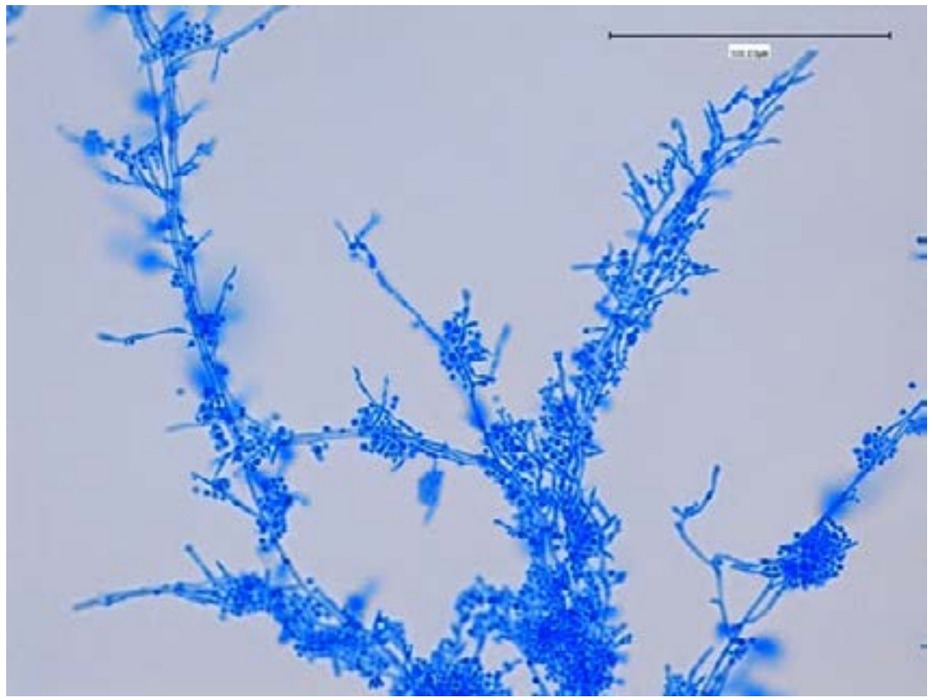
Sporothrix schenckii growing on Sabouraud agar



Micrograph of *Sporothrix schenckii* conidia stained with lactophenol cotton blue



Microscopic morphology of the saprophytic or mycelial form of *Sporothrix schenckii*



Sporothrix schenckii hyphae

16 Class - Protozoa. *Leischmania. Trypanosoma.* Chagas disease. «Sleeping» sickness. *Trichomonas. Lamblia*

The parasites of humans in the kingdom Protozoa are now classified under three phyla: Sarcomastigophora (containing the flagellates and amebas), Apicomplexa (containing the sporozoans), and Ciliophora (containing the ciliates). Within these great assemblages are found the important human parasites, conveniently listed as subphyla.

(1) Mastigophora, the flagellates, have one or more whip-like flagella and, in some cases, an undulating membrane (eg, trypanosomes). These include intestinal and genitourinary flagellates (*Giardia*, *Trichomonas*, *Dientamoeba*, *Chilomastix*) and blood and tissue flagellates (*Trypanosoma*, *Leishmania*).

(2) Sarcodina are typically ameboid and are represented in humans by species of *Entamoeba*, *Endolimax*, *Iodamoeba*, *Naegleria*, and *Acanthamoeba*.

(3) Sporozoa undergo a complex life cycle with alternating sexual and asexual reproductive phases, usually involving two different hosts (eg, arthropod and vertebrate, as in the blood forms). The class Coccidia contains the human parasites *Isospora*, *Toxoplasma*, and others. One of these, *Cryptosporidium*, has been implicated as a cause of intractable diarrhea among the immunosuppressed. Within the class Haematozoa (blood sporozoans) are the malarial parasites (*Plasmodium* species) and members of the order Piroplasmida, which includes *Babesia* species. *Pneumocystis* has recently been shown to be a member of the Fungi rather than the Protozoa. It is another opportunistic parasite of immunosuppressed individuals.

(4) Ciliophora are complex protozoa bearing cilia distributed in rows or patches, with two kinds of nuclei in each individual. *Balantidium coli*, a giant intestinal ciliate of humans and pigs, is the only human parasite representative of this group. A distinctive group, formerly listed with the Protozoa, often within the Sporozoa, is now considered a separate phylum, the Microspora. It includes the microsporidians, frequently seen as opportunistic parasites of immunosuppressed hosts.

THE HEMOFLAGELLATES

The hemoflagellates of humans include the genera *Trypanosoma* and *Leishmania*. There are two distinct types of human trypanosomes: (1) African, which causes sleeping sickness and is transmitted by tsetse flies (Glossina) - *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*; and (2) American, which causes Chagas' disease and is transmitted by conenose bugs (*Triatoma*, etc) - *Trypanosoma* (*Schizotrypanum*) *cruzi*. The genus *Leishmania*, divided into a number of species infecting humans, causes cutaneous (Oriental sore), mucocutaneous (espundia), and visceral (kala-azar) leishmaniasis. All of these infections are transmitted by sandflies (*Phlebotomus* in the Old World and *Lutzomyia* in the New World).

The genus *Trypanosoma* appears in the blood as trypomastigotes, with elongated bodies supporting a longitudinal lateral undulating membrane and a flagellum that borders the free edge of the membrane and emerges at the anterior end as a whip-like extension. The kinetoplast is a darkly staining body lying immediately adjacent to the tiny node (blepharoplast) from which the flagellum arises.

Other developmental forms among the hemoflagellates include (1) a leishmanial rounded intracellular stage, the amastigote; (2) a flagellated extracellular stage, the promastigote, a lanceolate form without an undulating membrane, with a kinetoplast at the anterior end; and (3) an epimastigote, a more elongated extracellular stage with a short undulating membrane and a kinetoplast placed more posteriorly, near the nucleus.

In *Leishmania* life cycles, only the amastigote and promastigote are found, the latter being restricted to the insect vector. In *T. cruzi,* all three developmental stages may occur in humans, and trypomastigote and epimastigote in the vector. In African trypanosomes, the latter two flagellated stages also occur in the tsetse fly vector, but only the trypomastigote in humans.

LEISHMANIA

The genus *Leishmania*, widely distributed in nature, has a number of species that are nearly identical morphologically. Differentiation therefore is based on a number of biochemical and epidemiologic criteria: electrophoretic mobility profile of a battery of isoenzymes (zymodeme pattern); excretory factor serotyping; kinetoplast DNA restriction analysis (schizodemes); lectin conjugation patterns on the parasite surface; use of monoclonal probes to detect specific antigens; promastigote growth patterns *in vitro* in the presence of antisera; developmental characteristics of promastigotes in the specific sandfly vector; and vectors, reservoir hosts, and

other epidemiologic factors. Clinical characteristics of the disease produced are traditional differentiating characteristics, but many exceptions are now recognized. Visceral leishmaniasis results from infection with members of the *Leishmania donovani* complex, which includes many different species and subspecies.

The New World forms are all carried by sandflies of the genus *Lutzomyia*. Old World leishmanias are transmitted by sandflies of the genus *Phlebotomus*. The different leishmanias present a range of clinical and epidemiologic characteristics that, for convenience only, are combined under three clinical groupings:

(1) visceral leishmaniasis (kala-azar);

(2) cutaneous leishmaniasis (Oriental sore, Baghdad boil, wet cutaneous sore, dry cutaneous sore, chiclero ulcer, uta, and other names), and

(3) mucocutaneous or nasooral leishmaniasis (espundia).

However, some species can induce several disease syndromes (eg, visceral leishmaniasis from one of the agents of cutaneous leishmaniasis or cutaneous leishmaniasis from the agent of visceral leishmaniasis). Similarly, the same clinical condition can be caused by different agents.

Morphology & Identification

A. TYPICAL ORGANISM

Only the intracellular nonflagellated amastigote (Leishman-Donovan [LD] body) occurs in mammals.

The sandfly transmits the infective promastigotes by bite. The promastigotes rapidly change to amastigotes after phagocytosis by macrophages, and then multiply, filling the cytoplasm of the macrophages. The infected cells burst, the released parasites are again phagocytosed, and the process is repeated, producing a cutaneous lesion or visceral infection depending upon the species of parasite and the host response. The amastigotes are oval, 2-6 x 1-3 µm, with a laterally placed oval vesicular nucleus and a darkstaining, rod-like kinetoplast.

B. CULTURE AND GROWTH CHARACTERISTICS

In NNN or Tobie's medium, only the promastigotes are found. *L. donovani* usually grows slowly, the promastigotes forming tangled clumps in the fluid. *L. tropica* grows more quickly, promastigotes forming small rosettes attached by their flagella in the fluid, while *L. braziliensis* may produce a wax-like surface with fewer, smaller promastigotes. In contrast, *L. mexicana* produces rapid growth of large organisms in simple blood agar medium. In tissue cultures, intracellular amastigotes may occur in addition to the extracellular promastigotes.

C. VARIATIONS

There are strain differences in virulence, tissue tropism, and biologic and epidemiologic characteristics, as well as the serologic and biochemical criteria previously noted. The New World species (or subspecies) of cutaneous and mucocutaneous leishmaniasis have been placed within the *L. mexicana* and *L. braziliensis* complexes, respectively, and the agents of visceral leishmaniasis have been placed within the *L. donovani* complex as geographically distinct species (or subspecies).

Pathogenesis, Pathology, & Clinical Findings

L. donovani, which causes kala-azar, spreads from the site of inoculation to multiply in reticuloendothelial cells, especially macrophages in spleen, liver, lymph nodes, and bone marrow. This is accompanied by marked hyperplasia of the spleen. Progressive emaciation is accompanied by growing weakness. There is irregular fever, sometimes hectic. Untreated cases with symptoms of kala-azar usually are fatal. Some forms, especially in India, develop a postcure florid cutaneous resurgence, with abundant parasites in cutaneous vesicles, 1-2 years later (post-kala-azar dermal leishmanoid).

L. tropica, *L. major*, *L. mexicana*, *L. braziliensis*, and other dermotropic forms induce a dermal lesion at the site of inoculation by the sandfly: cutaneous leishmaniasis, Oriental sore, Delhi boil, etc. Mucous membranes are rarely involved. The dermal layers are first affected, with cellular infiltration and proliferation of amastigotes intracellularly and spreading extracellularly, until the infection penetrates the epidermis and causes ulceration.

Satellite lesions may be found (hypersensitivity or recidivans type of cutaneous leishmaniasis) that contain few or no parasites, do not readily respond to treatment, and induce a strongly granulomatous scarring reaction. In Venezuela, a cutaneous disseminating form, caused by *L. mexicana* pifanoi, is known. In Ethiopia, a form known as *L. aethiopica* causes a similar nonulcerating, blistering, spreading cutaneous leishmaniasis. Both forms are typically anergic and nonreactive to skin test antigen and contain large numbers of parasites in the dermal blisters.

L. braziliensis braziliensis causes mucocutaneous or nasopharyngeal leishmaniasis in Amazonian South America. It is known by many local names. The lesions are slow-growing but extensive (sometimes 5-10 cm).

From these sites, migration appears to occur rapidly to the nasopharyngeal or palatine mucosal surfaces, where no further growth may take place for years. After months to over 20 years, relentless erosion may develop, destroying the nasal septum and surrounding regions in an often intractable, fungating, polypoid course. In such instances, death occurs from asphyxiation due to blockage of the trachea, starvation, or respiratory infection. This is the classic clinical picture of espundia, most commonly found in the Amazon basin.

At high altitudes in Peru, the clinical features (uta) resemble those of Oriental sore. *L. braziliensis* guyanensis infection frequently spreads along lymphatic routes, where it appears as a linear chain of nonulcerating lesions. *L. mexicana* infection is more typically confined to a single, indolent, ulcerative lesion that heals in about 1 year, leaving a characteristic depressed circular scar. In Mexico and Guatemala, the ears are frequently involved (chiclero ulcer), usually with a cartilage-attacking infection without ulceration and with few parasites.

Diagnostic Laboratory Tests

A. SPECIMENS

Lymph node aspirates, scrapings, and biopsies from the margin of the lesion, not the center, are important in the cutaneous forms; lymph node aspirates, blood, and spleen, liver, or bone marrow puncture are important in kala-azar. Purulent discharges are of no value for diagnosis, although nasal scrapings may be useful. An enzyme-linked immunosorbent assay (ELISA) technique using a 70-kDa antigen has been studied as a rapid and accurate field-applied tool to detect visceral leishmaniasis (in place of splenic aspiration or the direct agglutination test [DAT], which remains positive for some years after cure, a disadvantage for current diagnosis).

B. MICROSCOPIC EXAMINATION

Giemsa-stained smears and sections may show amastigotes, especially in material from kala-azar and under the rolled edges of cutaneous sores.

C. CULTURE

NNN medium is the medium most generally used. A diphasic rabbit blood agar culture, Tobie's medium, at about 26-28 °C, is especially suitable. Blood culture is satisfactory for *L. donovani* and *L. braziliensis*. Lymph node aspirates are suitable for all forms, and tissue aspirates, biopsy material, scrapings, or small biopsies from the edges of ulcers are useful for the cutaneous forms and often for kala-azar also. However, only promastigotes can be cultivated in the absence of living cells.

D. SEROLOGY

The formol-gel (aldehyde) test of Napier is a nonspecific test that detects an elevated serum globulin level in kala-azar. The IHA (indirect hemagglutination antibody) test or the IFA (indirect fluorescent antibody) test may be useful, but they lack sufficient sensitivity and may cross-react with *T. cruzi*. The ELISA test is promising, as noted, and the polymerase chain reaction (PCR), especially when combined with Southern immunoblotting, demonstrates both high sensitivity and high specificity. Both tests avoid the need for invasive diagnostic methods, such as spleen or bone marrow punctures, both painful and potentially hazardous procedures. A skin test (Montenegro test) is epidemiologically important in indicating past exposure to any of the *Leishmania*.

Immunity

Recovery from cutaneous leishmaniasis confers a solid and permanent immunity, although it usually is species-specific and may be strain-specific as well. Natural resistance varies greatly among individuals and with age and sex. Vaccination with a living inoculum from a recently isolated culture significantly reduces the incidence of Oriental sore.

Immunity to kala-azar may develop but varies with the time of treatment and condition of the patient.

Treatment

Single lesions may be cleaned, curetted, treated with antibiotics if secondarily infected, and then covered and left to heal. For larger or nonhealing forms, pentavalent antimony sodium gluconate (Pentostam, Solustibosan) is still widely used, but is now being replaced by miltefosine, an orally administered alkylphosphocholine, also used as an anti-neoplastic agent. The only FDA-approved drug for treatment of visceral leishmaniasis in the United States is liposomal amphotericin B. It is expensive but effective and well

tolerated. Miltefosine is widely used in India, where it was first tested and developed for kala-azar resistant to antimony compounds. Cycloguanil pamoate in oil (Camolar) and amphotericin B (Fungizone) can be used for espundia, which is frequently quite unresponsive to treatment. Local heat with hot water compresses (39-42 °C) applied directly 20-30 min/d for 12-30 days or by exposure to ultraviolet or infrared radiation for 20 min/d may be effective against the nonresponsive recidivans form of *L. tropica* infection. Ketoconazole (Nizoral), given daily for 4-8 weeks, has also been used successfully against cutaneous leishmaniasis.

Epidemiology, Prevention, & Control

Kala-azar, caused by *L. donovani*, is found focally in most tropical and subtropical countries. Its local distribution is related to the prevalence of specific sandfly vectors. In the Mediterranean littoral and in middle Asia and South America, domestic and wild canids are reservoirs, and in the Sudan, various wild carnivores and rodents are reservoirs of endemic kala-azar. No animal reservoirs have been found for the forms from India and Kenya. Control is aimed at destroying breeding places and dogs, where appropriate, and protecting people from sandfly bites. Oriental sore occurs mostly in the Mediterranean region, North Africa, and the Middle and Near East. The «wet» type, caused by *L. major*, is rural, and burrowing rodents are the main reservoir; the «dry» type, caused by *L. tropica*, is urban, and humans are presumably the only reservoir. For *L. braziliensis*, there are a number of wild but apparently no domestic animal reservoirs. Sandfly vectors are involved in all forms.

TRYPANOSOMA

Hemoflagellates of the genus *Trypanosoma* occur in the blood of mammals as mature elongated trypomastigotes. A multiplying epimastigote stage precedes the formation of infective trypomastigotes in the intermediate host (an insect vector) in all species of trypanosomes that infect humans. Trypanosomiasis is expressed as African sleeping sickness; Chagas' disease of the southern United States, Mexico, and Central and South America; and asymptomatic trypanosomiasis in Central and South America.

The parent form in Africa is *T. b. brucei*, which causes nagana in livestock and game animals; the two human forms are *T. b. rhodesiense* and *T. b. gambiense*. The three forms are indistinguishable morphologically but differ biochemically, ecologically, and epidemiologically.

Morphology & Identification

A. TYPICAL ORGANISMS

African *T. b. gambiense* and *T. b. rhodesiense* vary in size and shape of the body and length of the flagellum (usually 15-30 µm) but are essentially indistinguishable. A «stumpy» short form is infective to the insect host and possesses a full battery of enzymes for energy metabolism. The elongated form requires host metabolic assistance and is specialized for rapid multiplication in the richly nutritious vertebrate bloodstream. The same forms are seen in blood as in lymph node aspirates.

The blood forms of American *T. cruzi* are present during the early acute stage and at intervals thereafter in smaller numbers. They are typical trypomastigotes, varying about a mean of 20 µm, frequently curved in a C shape when fixed and stained. A large, rounded terminal kinetosome in stained preparations is characteristic. The tissue forms, which are most common in heart muscle, liver, and brain, develop from amastigotes that multiply to form an intracellular colony after invasion of the host cell or phagocytosis of the parasite. *Trypanosoma rangeli* of South and Central America infects humans without causing disease and must therefore be carefully distinguished from the pathogenic species.

B. CULTURE

T. cruzi and *T. rangeli* are readily cultivated (3-6 weeks) in the epimastigote form in fluid or diphasic media.

Diagnosis of patients in the early, blood-borne phase of infection can be aided by using the multiplying powers of parasites in laboratory-reared, clean vector insects (kissing, conenose, or triatomine bugs) that have been allowed to feed on patients.

C. VARIATION

There are variations in morphology, virulence, and antigenic constitution. The African trypanosomes of the *T. brucei* complex are remarkable in that they undergo development of a series of genetically controlled glycoprotein antigenic coats (variant surface glycoproteins, or VSGs). Successive waves of parasites in the host bloodstream are each covered with a distinct coat, one of an apparently unlimited number. This process is due to genetically induced changes in the development of the surface glycoprotein coat; it is viewed as a

means of continuously escaping the host's antibody response by producing different antigenic membranes. Each population is reduced but is promptly replaced with another antigenic type before the preceding one is eliminated.

Each *Trypanosoma* is thought to possess about 1000 VSG genes, an example of mosaic gene formation.

Pathogenesis, Pathology, & Clinical Findings

Infective trypanosomes of *T. b. gambiense* and *T. b. rhodesiense* are introduced through the bite of the tsetse fly and multiply at the site of inoculation to cause variable induration and swelling (the primary lesion), which may progress to form a trypanosomal chancre. They spread to lymph nodes, to the bloodstream, and, in terminal stages, to the central nervous system, where they produce the typical sleeping sickness syndrome: lassitude, inability to eat, tissue wasting, unconsciousness, and death.

Infective forms of *T. cruzi* do not pass to humans by triatomine bug bites (which is the mode of entry of the nonpathogenic *T. rangeli*); rather, they are introduced when infected bug feces are rubbed into the conjunctiva, the bite site, or a break in the skin. At the site of *T. cruzi* entry, there may be a subcutaneous inflammatory nodule or chagoma. Chagas' disease is common in infants. Unilateral swelling of the eyelids (Romaca's sign) is characteristic at onset, especially in children. The primary lesion is accompanied by fever, acute regional lymphadenitis, and dissemination to blood and tissues. The parasites can usually be detected within 1-2 weeks as trypomastigotes in the blood. Subsequent development depends upon the organs and tissues affected and on the nature of multiplication and release of toxins.

The African forms multiply extracellularly as trypomastigotes in the blood as well as in lymphoid tissues. *T. cruzi* multiplies mostly within reticuloendothelial cells, going through a cycle starting with large agglomerations of amastigotes. In both African and American forms, multiplication in the tissues is punctuated by phases of parasitemia with later destruction by the host of the blood forms, accompanied by bouts of intermittent fever gradually decreasing in intensity. Parasitemia is more common in *T. b. rhodesiense* and is intermittent and scant with *T. cruzi*.

The release of toxins explains much of the systemic and local reactions. The organs most seriously affected are the central nervous system and heart muscle. Interstitial myocarditis is the most common serious element in Chagas' disease. Other organs affected are the liver, spleen, and bone marrow, especially with chronic *T. cruzi* infection. Invasion or toxic destruction of nerve plexuses in the alimentary tract walls leads to megaesophagus and megacolon, especially in Brazilian Chagas' disease. Megaesophagus and megacolon are absent in Colombian, Venezuelan, and Central American Chagas' disease. Central nervous system involvement is most characteristic of African trypanosomiasis. *T. b. rhodesiense* appears in the cerebrospinal fluid in about 1 month and *T. b. gambiense* in several months, but both are present in small numbers. *T. b. gambiense* infection is chronic and leads to progressive diffuse meningoencephalitis, with death from the sleeping syndrome usually following in 1-2 years. The more rapidly fatal *T. b. rhodesiense* produces somnolence and coma only during the final weeks of a terminal infection. All three trypanosomes are transmissible through the placenta, and congenital infections occur in hyperendemic areas.

Diagnostic Laboratory Tests

A. SPECIMENS

Blood, preferably collected when the patient's temperature rises; cerebrospinal fluid; lymph node or primary lesion aspirates; or specimens obtained by iliac crest, sternal bone marrow, or spleen puncture are used.

B. MICROSCOPIC EXAMINATION

Fresh blood (or aspirated tissue in saline) is kept warm and examined immediately for the actively motile trypanosomes. Thick films may be stained with Giemsa's stain. Thin films stained with Giemsa's stain are necessary for confirmation. Centrifugation may be necessary.

Tissue smears must be stained for identification of the pretrypanosomal stages. Centrifuged cerebrospinal fluid should be similarly examined; there is seldom more than one trypanosome per milliliter. The most reliable tests are smears of blood for *T. b. rhodesiense*, of lymph gland puncture specimens for *T. b. gambiense*, and of cerebrospinal fluid for *T. b. rhodesiense* and advanced *T. b. gambiense*.

C. CULTURE

Any specimens may be inoculated into Tobie's, Wenyon's semisolid, NNN, or other media for culture of *T. cruzi* or *T. rangeli*. The organisms are grown at 22-24 °C and subcultured every 1-2 weeks. Centrifuged material is examined microscopically for trypanosomes. Culture of the African forms is unsatisfactory.

D. ANIMAL INOCULATION

T. cruzi and *T. rangeli* may be detected by inoculating blood intraperitoneally into mice (when available, pups and kittens are animals of first choice). *T. b. rhodesiense* is often detectable and *T. b. gambiense* sometimes detectable by this procedure. Trypanosomes appear in the blood in a few days after successful inoculation.

E. SEROLOGY

A positive IHA, IFA, or CF (Machado's) test provides confirmatory support in *T. cruzi* infection. Recently developed ELISAs using recombinant antigens now provide a highly specific and sensitive serodiagnostic tool for detection of *T. cruzi*. These tests are especially useful for blood bank screening. African forms cause IFA reactions after about 12 days of infection. This is especially useful for *T. b. gambiense* diagnosis. A card test for direct agglutination is valuable for field use or for rural medical stations, using lyophilized trypanosome antigen.

F. XENODIAGNOSIS

This is the method of choice in suspected Chagas' disease if other examinations are negative, especially during the early phase of disease onset. Because laboratory infection with *T. cruzi* is a distinct hazard, the test should be performed only by workers trained in the procedure. About six clean laboratory-reared triatomine bugs are fed on the patient, and their droppings are examined in 7-10 days for the various developmental forms. Defecation follows shortly after a fresh meal or may be forced by gently probing the bug's anus and then squeezing its abdomen.

Xenodiagnosis is impracticable for the African forms.

Differential Diagnosis

T. b. rhodesiense and *T. b. gambiense* are morphologically identical but may be distinguished by their geographic distribution, vector species, and clinical disease in humans. The presence of specific IgM in the cerebrospinal fluid is considered pathognomonic for the encephalitic stage of African trypanosomiasis. The differentiation of *T. cruzi* from *T. rangeli* is important. A laboratory-based procedure has been described that differentiates the two species based on total parasite DNA digestant electrophoresed on agarose gels which are then stained with ethidium bromide.

Immunity

Humans show some individual variation in natural resistance to trypanosomes. Strain-specific CF and protecting antibodies can be detected in the plasma, and these presumably lead to the disappearance of blood forms. Each relapse of African trypanosomiasis is due to a strain serologically distinct from the preceding one.

Apart from such relapses, Africans free from symptoms may still have trypanosomes in the blood.

Treatment

There is no effective drug treatment for American trypanosomiasis, although nifurtimox (Bayer 2502) plus gamma interferon may shorten the acute phase and may temporarily relieve some patients with trypomastigotes still present in the blood. Benznidazole (Rochagan) is a recently tested alternative drug. African trypanosomiasis is treated principally with suramin sodium (Germanin) or pentamidine isethionate (Lomidine). Late disease with central nervous system involvement requires melarsoprol (Mel B), as well as suramin or tryparsamide. A promising drug is eflornithine (difluoromethylornithine; DFbIO; [Ornidyl]), which works against both the blood and central nervous system phases of *T. b. gambiense* infection and the hemolymphatic stage of *T. b. rhodesiense.*

Epidemiology, Prevention, & Control

African trypanosomiasis is restricted to recognized tsetse fly belts. *T. b. gambiense*, transmitted by the streamside tsetse ***Glossina palpalis*** and several other humid forest tsetse vectors, extends from West to central Africa and produces a relatively chronic infection with progressive central nervous system involvement. *T .b. rhodesiense*, transmitted by the woodland-savanna *Glossina morsitans*, *Glossina pallidipes*, and *Glossina fuscipes*, occurs in the eastern and southeastern savannas of Africa, with foci west of Lake Victoria. It causes a smaller number of cases but is more virulent. Bushbuck and other antelopes may serve as reservoirs of *T. b. rhodesiense*, whereas humans are the principal reservoir of *T. b. gambiense*.

Control depends upon searching for and then isolating and treating patients with the disease; controlling movement of people in and out of fly belts; using insecticides in vehicles; and instituting fly control, principally with aerial insecticides and by altering habitats.

Contact with reservoir animals is difficult to control, and insect repellent is of little value against tsetse

bites. Chemoprophylaxis, eg, with suramin sodium, is difficult and short-lived.

American trypanosomiasis (Chagas' disease) is especially important in Central and South America, although infection of animals extends much more widely - eg, to Maryland and southern California. A few autochthonous human cases have been reported in Texas and southern California. Certain triatomine bugs become as domiciliated as bedbugs, and infection may be brought in by rats, opossums, or armadillos - which may spread the infection to domestic animals such as dogs and cats. Since no effective treatment is known, it is particularly important to control the vectors with residual insecticides and habitat modification, such as replacement of mud-brick (adobe) houses with thatched roofs, and to avoid contact with animal reservoirs. Chagas' disease occurs largely among people in poor economic circumstances. An estimated 20-25 million persons harbor the parasite, and many of these sustain heart damage, with the result that their ability to work and their life expectancy are sharply reduced.

INTESTINAL FLAGELLATES

GIARDIA LAMBLIA

Giardia lamblia, a flagellate, is the only common pathogenic protozoan found in the duodenum and jejunum of humans. It is the cause of giardiasis.

Giardia duodenalis is another name commonly ascribed to the parasite that causes human giardiasis; the term *Giardia intestinalis* is frequently used in Europe and *Lamblia intestinalis* in the former USSR. Much of the confusion is due to merging of species names now that human giardiasis is recognized as a zoonosis and species based on supposed single-host parasitism have been synonymized.

Pending further taxonomic clarification, the name of the species first described, *G. lamblia*, will be retained.

Morphology & Identification

A. TYPICAL ORGANISMS

The trophozoite of *G. lamblia* is a heart-shaped, symmetric organism 10-20 µm in length.

There are four pairs of flagella, two nuclei with prominent central karyosomes, and two axostyles (rod-like supporting organelles). A large concave sucking disk in the anterior portion occupies much of the ventral surface. The swaying or dancing motion of *Giardia* trophozoites in fresh preparations is unmistakable. As the parasites pass into the colon, they typically encyst. Cysts are found in the stool - often in enormous numbers.

They are thick-walled, highly resistant, 8-14 µm in length, and ellipsoid and contain two nuclei as immature, four as mature cysts.

B. CULTURE

Cultivation, though possible, is not diagnostically useful.

Pathogenesis & Clinical Findings

G. lamblia is usually only weakly pathogenic for humans. Cysts may be found in large numbers in the stools of entirely asymptomatic persons. In some persons, however, large numbers of parasites attached to the bowel wall may cause irritation and low-grade inflammation of the duodenal or jejunal mucosa, with consequent acute or chronic diarrhea associated with crypt hypertrophy, villous atrophy or flattening, and epithelial cell damage. The stools may be watery, semisolid, greasy, bulky, and foul-smelling at various times during the course of the infection. Malaise, weakness, weight loss, abdominal cramps, distention, and flatulence can occur. Children are more liable to clinical giardiasis than adults. Immunosuppressed individuals are especially liable to massive infection with severe clinical manifestations. Symptoms may continue for long periods.

Diagnostic Laboratory Tests

Diagnosis depends upon finding the distinctive cysts in formed stools, or cysts and trophozoites in liquid stools.

Development of a stool enzyme-linked immunosorbent assay (ELISA) has been shown to be both a specific and sensitive rapid diagnostic tool (Seradyn Color Vue - Giardia; LMD Laboratories). Examination of the duodenal contents may be necessary to establish the diagnosis, as cyst production may be sporadic and not found in the stool by an ovum and parasite fecal smear examination. A series of three or more stool examinations on alternate days is therefore recommended. Duodenal aspiration or use of the duodenal capsule technique (Entero-Test) may be needed in addition to fecal examination for diagnosis.

Treatment

Metronidazole (Flagyl) will clear over 90% of *G. lamblia* infections. Oral quinacrine hydrochloride (Atabrine) and furazolidone (Furoxone) are alternatives. Tinidazole (Fasigyn), used for 1-day treatment, is widely andeffectively used but is not available in the United States. Paromomycin (Humatin) may be useful in pregnancy. Treatment may be repeated if necessary. Only symptomatic patients require treatment.

Epidemiology

G. lamblia occurs worldwide. Humans are infected by ingestion of fecally contaminated water or food containing giardia cysts or by direct fecal contamination, as may occur in day care centers for children, refugee camps, institutions, or among male homosexuals. Epidemic outbreaks have been reported at ski resorts in the United States where overloading of sewage facilities or contamination of the water supply has resulted in sudden outbreaks of giardiasis. Cysts can survive in water for up to 3 months. Outbreaks among campers in wilderness areas suggest that humans may be infected with various animal giardia harbored by rodents, deer, cattle, sheep, horses, or household pets. This suggests that human infection can also be a zoonosis and that *G. lamblia* has a broad spectrum of hosts, contrary to earlier views. Extensive variation occurs in the *Giardia* complex, and though species definitions are still unresolved, it is clear that a great number of distinct and probably variable clones exist.

TRICHOMONAS

The trichomonads are flagellate protozoa with three to five anterior flagella, other organelles, and an undulating membrane. *Trichomonas vaginalis* causes the most common form of trichomoniasis in humans.

Morphology & Identification

A. TYPICAL ORGANISMS

T. vaginalis is pear-shaped, with a short undulating memrane lined with a flagellum and four anterior flagella. It measures about 10 x 7 µm, though its length may vary from 5 to 30 µm and its width from 2 to 14 µm.

The organism moves with a characteristic wobbling and rotating motion. The nonpathogenic trichomonads, *Trichomonas hominis* and *Trichomonas tenax*, cannot readily be distinguished from *T. vaginalis* when alive. For all practical purposes, trichomonads found in the mouth are *T. tenax*; in the intestine, *T. hominis*; and in the genitourinary tract (both sexes), *T. vaginalis*.

B. CULTURE

T. vaginalis may be cultivated in many solid and fluid cell-free media, in tissue cultures, and in chick embryo. Simplified trypticase serum is usually used for semen cultures.

C. GROWTH REQUIREMENTS

T. vaginalis grows best at 35-37 °C under anaerobic conditions, less well aerobically. The optimal pH for growth *in vitro* (5,5-6,0) suggests why vaginal trichomoniasis is more severe in women with higher than normal vaginal pH.

Pathogenesis, Pathology, & Clinical Findings

T. hominis and *T. tenax* are generally considered to be harmless commensals. *T. vaginalis* is capable of causing low-grade inflammation. The intensity of infection, the pH and physiologic status of the vaginal and other genitourinary tract surfaces, and the accompanying bacterial flora are among the factors affecting pathogenicity. The organisms do not survive at normal vaginal acidity of pH 3,8-4,4.

In females, the infection is normally limited to vulva, vagina, and cervix; it does not usually extend to the uterus. The mucosal surfaces may be tender, inflamed, eroded, and covered with a frothy yellow or cream-colored discharge. In males, the prostate, seminal vesicles, and urethra may be infected. Signs and symptoms in females, in addition to profuse vaginal discharge, include local tenderness, vulval pruritus, and burning. About 10% of infected males have a thin, white urethral discharge.

Diagnostic Laboratory Tests

A. SPECIMENS AND MICROSCOPIC EXAMINATION

Vaginal or urethral secretions or discharge should be examined microscopically in a drop of saline for characteristic motile trichomonads. Dried smears may be stained with hematoxylin or other stains for later study.

B. CULTURE

Culture of vaginal or urethral discharge, of prostatic secretion, or of a semen specimen may reveal

organisms when direct examination is negative.

Immunity

Infection confers no apparent immunity, although over time reinfections appear to cause less severe symptoms in women, suggesting that some resistance may develop.

Treatment

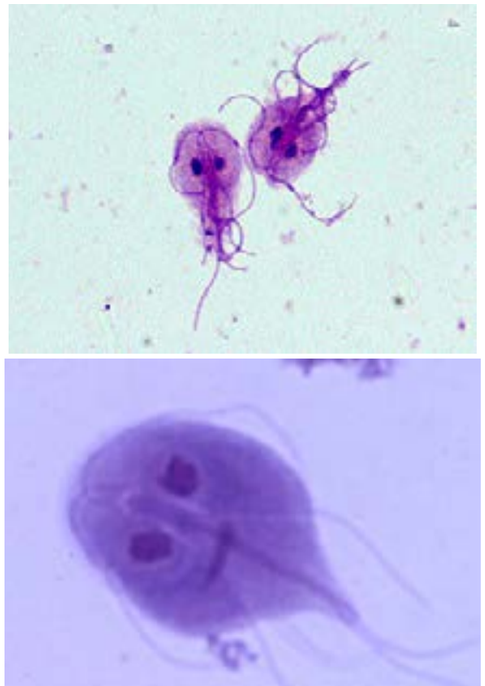
Successful treatment of vaginal infection requires destruction of the trichomonads, for which topical and systemic metronidazole (Flagyl) is best. Tinidazole (Fasigyn) and ornidazole (Tiberal) are equally effective, with fewer side effects, but are not available in the United States. The patient's sexual partner should be examined and treated simultaneously. Postmenopausal patients may require treatment with estrogens to improve the condition of the vaginal epithelium. Prostatic infection can be cured with certainty only by systemic treatment with metronidazole or one of the above-mentioned nitroimidazoles.

Epidemiology & Control

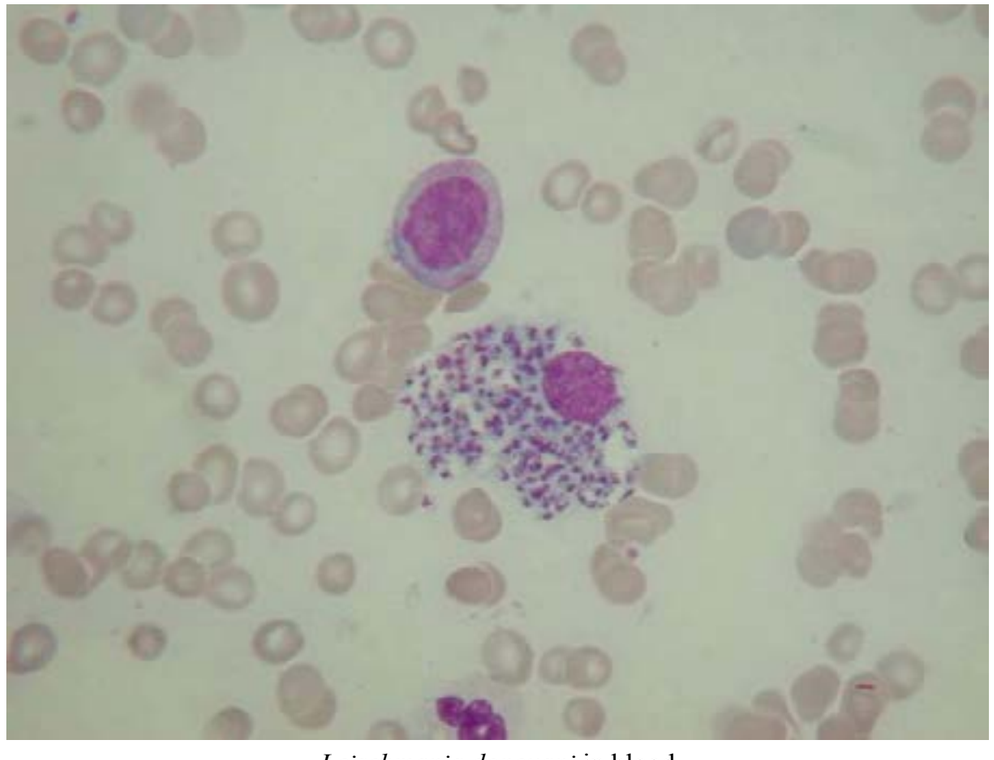
T . vaginalis is a common parasite of both males and females. Infection rates vary greatly but may be quite high (40% or higher). Transmission is by sexual intercourse, but contaminated towels, douche equipment, examination instruments, and other objects may be responsible for some new infections. Infants may be infected during birth.

Most infections, in both sexes, are asymptomatic or mild. Control of *T. vaginalis* infections always requires simultaneous treatment of both sexual partners. Mechanical protection (condom) should be used during intercourse until the infection is eradicated in both partners.

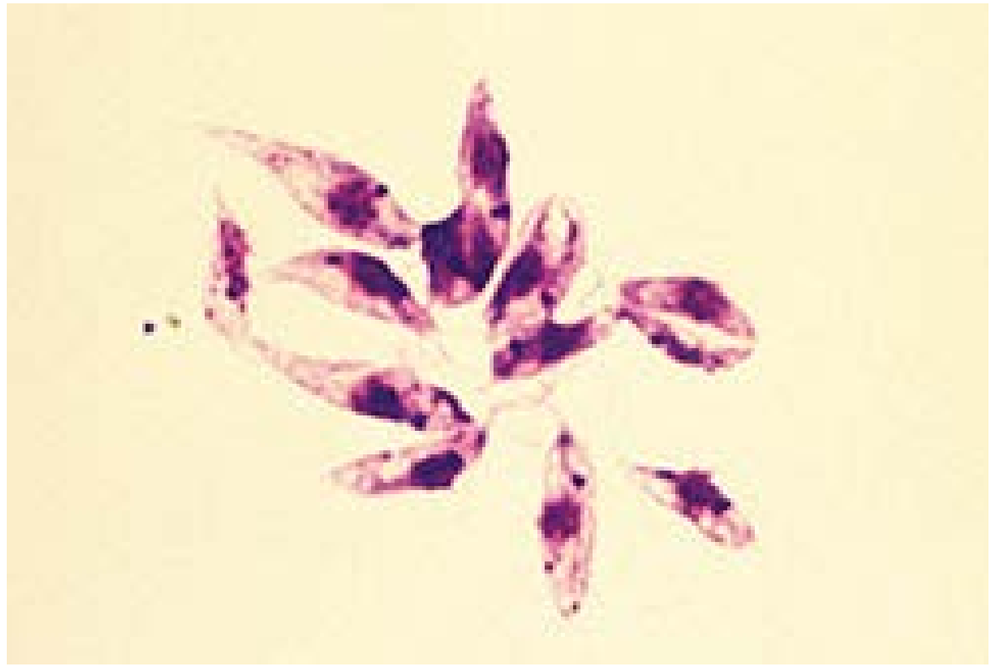
16 Class – Illustrations



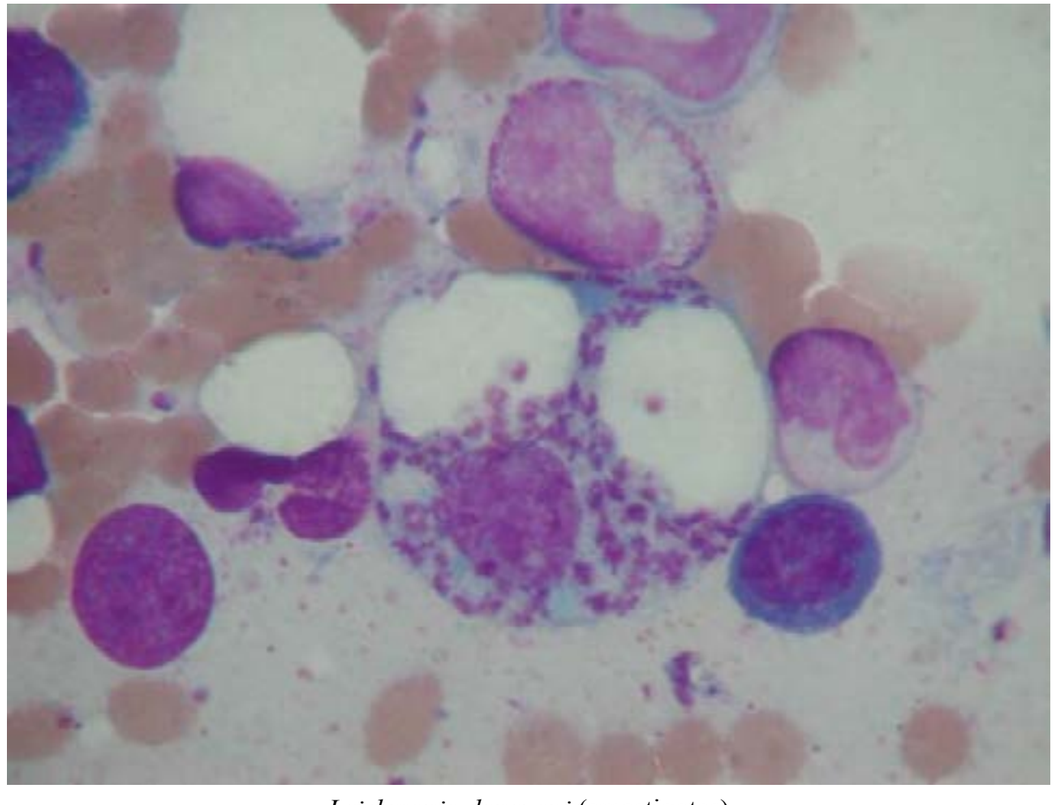
Giardia lamblia (cell morphology)



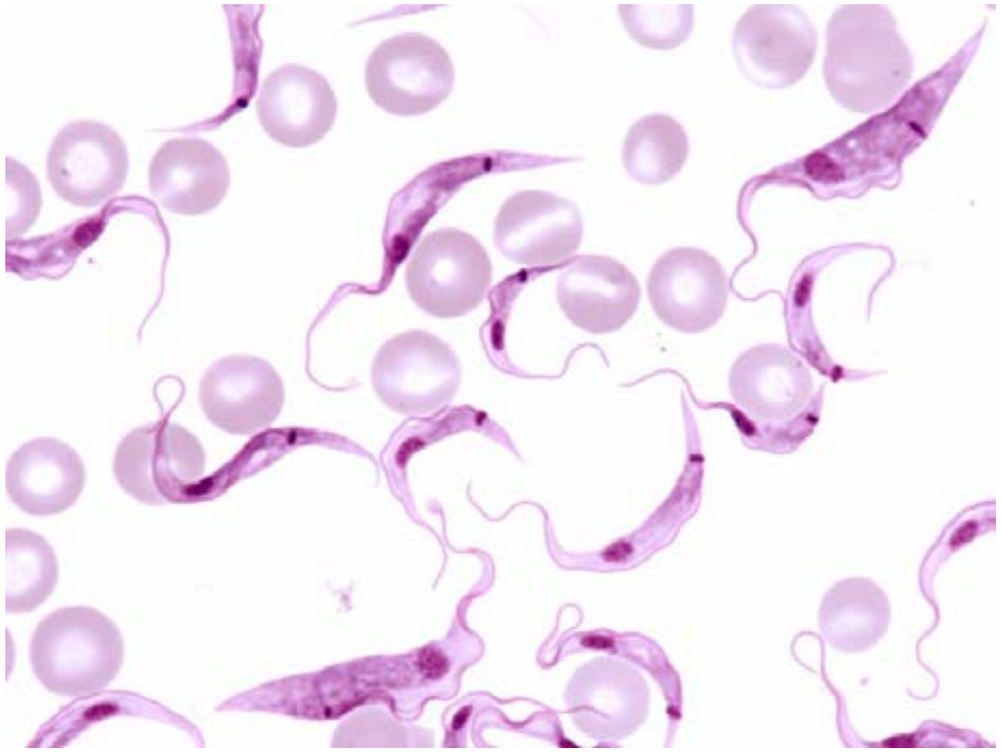
Leischmania donovani in blood



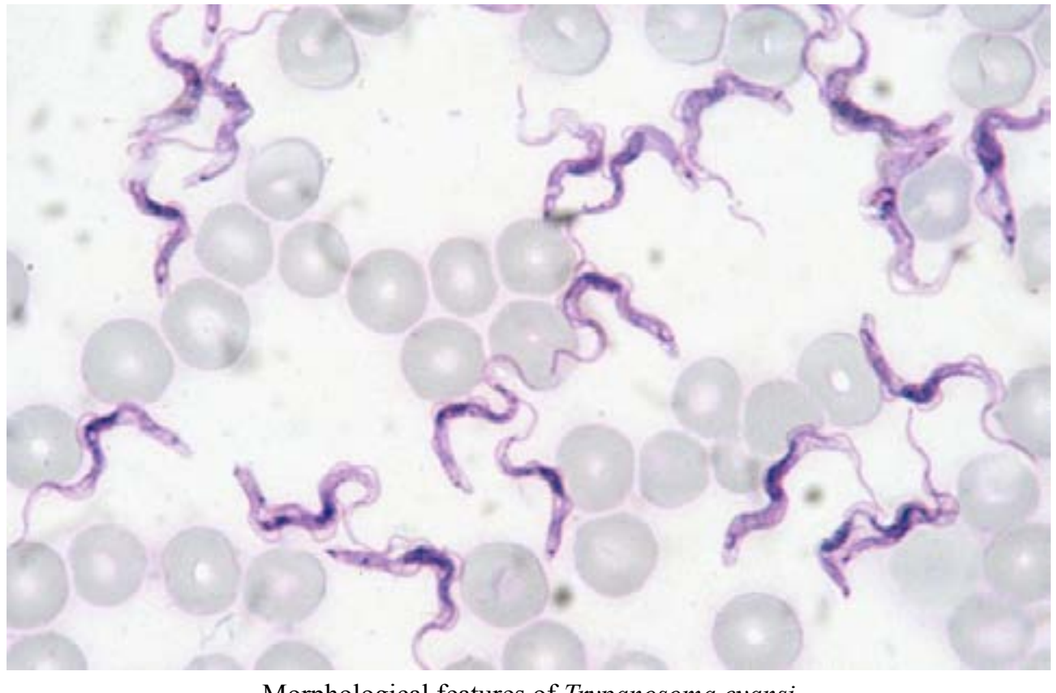
Leishmania donovani (promastigotes)



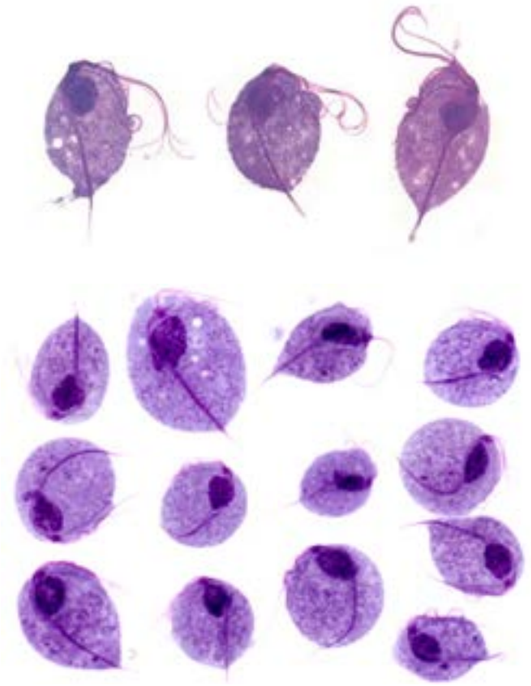
Leishmania donovani (amastigotes)



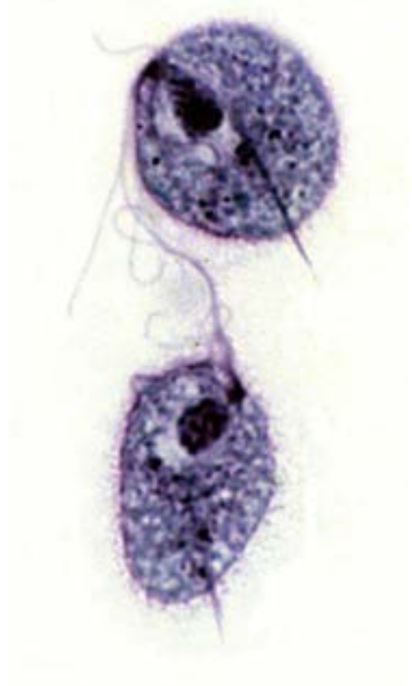
Blood smear with *Trypanosoma brucei*



Morphological features of *Trypanosoma evansi*



Trichomonas vaginalis parasites seen under a microscope



*Trichomonas vaginalis (*cell morphology)

17 Class - Sarcodina. Amebiasis. *Plasmodium malariae.* Apicomplexa. *Toxoplasma. Balantidium*

INTESTINAL AMEBAS

ENTAMOEBA HISTOLYTICA

Entamoeba histolytica is a common parasite in the large intestine of humans, certain other primates, and some other animals. Many cases are asymptomatic except in humans or among animals living under stress (eg, zoo-held primates).

Morphology & Identification

A. TYPICAL ORGANISMS

Three stages are encountered: the active ameba, the inactive cyst, and the intermediate precyst. The ameboid trophozoite is the only form present in tissues. It is also found in fluid feces during amebic dysentery. Its size is 15-30 µm. The cytoplasm has two zones, a hyaline outer margin and a granular inner region that may contain red cells (pathognomonic) but ordinarily contains no bacteria. Iron-hematoxylin or Wheatley's trichrome staining shows the nuclear membrane to be lined by fine, regular granules of chromatin with a small central body (endosome or karyosome). Movement of trophozoites in fresh material is brisk and unidirectional. Pseudopodia are finger-like and broad.

Cysts are present only in the lumen of the colon and in mushy or formed feces. Subspherical cysts of pathogenic amebas range from 10 to 20 µm. Smaller cysts, from 10 µm ranging down to 3,5 µm, are considered nonpathogenic *Entamoeba hartmanni*. The cyst wall, 0,5 µm thick, is hyaline. The initial uninucleate cyst may contain a glycogen vacuole and chromatoidal bodies with characteristic rounded ends (in contrast to splinter chromatoidals in developing cysts of *Entamoeba coli*). Nuclear division within the cyst produces the final quadrinucleate cyst, during which time the chromatoid bodies and glycogen vacuoles disappear. Diagnosis in most cases rests on the characteristics of the cyst, since trophozoites usually appear only in diarrheic feces in active cases and survive for only a few hours, though they may be excellently preserved in polyvinyl alcohol (PVA) fixative. Stools may contain cysts with 1-4 nuclei depending on their degree of maturation.

B. CULTURE

Trophozoites are readily studied in cultures; both encystation and excystation can be controlled.

C. GROWTH REQUIREMENTS

Growth is most vigorous in various rich complex media or in cell culture under partial anaerobiosis at 37 °C and pH 7,0—with a mixed flora or at least a single oexisting species.

D. VARIATION

Variations in cyst size are due to nutritional differences or to the presence of the small nonpathogenic species, *E. hartmanni*. The invasive or pathogenic species is now considered a species distinct from the more common lumen-dwelling nonpathogenic commensal species, given an older name, *E. dispar*, with the name *E. histolytica* reserved for the pathogenic form. *E. dispar* and the related *E. moshkovskii* are distinct species though microscopically identical, based on isoenzyme and genetic analyses. There is currently no readily available way to distinguish them except by isoenzyme electrophoresis and DNA analysis. The presence of red cells in the trophozoites, amebic antibodies in the blood, or clinical indications must therefore be relied upon in the absence of the difficult and costly biochemical analyses.

Pathogenesis, Pathology, & Clinical Findings

The trophozoite emerges from the ingested cyst (metacyst) after activation of the excystation process in the stomach and duodenum. The metacyst divides rapidly, producing four amebulae (one for each cyst nucleus), each of which divides again to produce eight small trophozoites per infective cyst. These pass to the cecum and produce a population of lumen-dwelling trophozoites. The trophozoites multiply by binary fission. In the majority of infections, perhaps 90%, the infection remains luminal, and the trophozoites multiply as a bacteria-feeding colony, ultimately encyst, and pass out in the feces. These are presumed to be due to *E. dispar*, *E. moshkovskii*, or *E. hartmanni*.

Disease results when the trophozoites of *E. histolytica* invade the intestinal epithelium. Mucosal invasion with the aid of proteolytic enzymes occurs through the crypts of Lieberkehn, forming discrete ulcers with a pinhead-sized center and raised edges, from which mucus, necrotic cells, and amebas pass. Pathologic changes are always induced by trophozoites: *E. histolytica* cysts are not produced in tissues. The mucosal

surface between ulcers typically is normal. Amebas multiply and accumulate above the muscularis mucosae, often spreading laterally. Healing may occur spontaneously with little tissue erosion if regeneration proceeds more rapidly than destruction, or the amebic trophozoites may break through the muscularis into the submucosa. Rapid lateral spread of the multiplying amebas follows, undermining the mucosa and producing the characteristic «flask-shaped» ulcer of primary amebiasis: a small point of entry, leading via a narrow neck through the mucosa into an expanded necrotic area in the submucosa.

Bacterial invasion usually does not occur at this time, cellular reaction is limited, and damage is by lytic necrosis. Subsequent spread may coalesce colonies of amebas, undermining large areas of the mucosal surface. Trophozoites may penetrate the muscular coats and occasionally the serosa, leading to perforation into the peritoneal cavity. Subsequent enlargement of the necrotic area produces gross changes in the ulcer, which may develop shaggy overhanging edges, secondary bacterial invasion, and accumulation of neutrophilic leukocytes. Secondary intestinal lesions may develop as extensions from the primary lesion (usually in the cecum, appendix, or nearby portion of the ascending colon). The organisms may travel to the ileocecal valve and terminal ileum, producing a chronic infection. The sigmoid colon and rectum are favored sites for later lesions. An amebic inflammatory or granulomatous tumor-like mass (ameboma) may form on the intestinal wall, sometimes growing sufficiently large to block the lumen.

Factors that determine invasion of amebas include the following: the number of amebas ingested, the pathogenic capacity of the parasite strain, host factors such as gut motility and immune competence, and the presence of suitable enteric bacteria that enhance amebic growth. Correct and prompt identification of the *Entamoeba* species remains a critical problem. Until a rapid and reliable test is available to the diagnostic laboratory, confusion and needless treatment will continue. Trophozoites, especially with red cells in the cytoplasm, found in liquid or semiformed stools are pathognomonic. Formed stools usually contain cysts only, while patients with active disease and liquid stools (flecked with blood and mucous strands containing numerous amebas) usually pass trophozoites only. Symptoms vary greatly depending upon the site and intensity of lesions. Extreme abdominal tenderness, fulminating dysentery, dehydration, and incapacitation occur in serious disease. In less acute disease, onset of symptoms is usually gradual, and often includes episodes of diarrhea, abdominal cramps, nausea and vomiting, and an urgent desire to defecate. More frequently, there will be weeks of cramps and general discomfort, loss of appetite, and weight loss with general malaise.

Symptoms may develop within 4 days of exposure, may occur up to a year later, or may never occur.

Extraintestinal infection is metastatic and rarely occurs by direct extension from the bowel. By far the most common form is amebic hepatitis or liver abscess (4% or more of clinical infections), which is assumed to be due to microemboli, including trophozoites carried through the portal circulation. It is assumed that hepatic microembolism with trophozoites is a common accompaniment of bowel lesions but that these diffuse focal lesions rarely progress. A true amebic abscess is progressive, nonsuppurative (unless secondarily infected), and destructive without compression and formation of a wall. The contents are necrotic and bacteriologically sterile, active amebas being confined to the walls. A characteristic «anchovy paste» is produced in the abscess and seen on surgical drainage. More than half of patients with amebic liver abscess give no history of intestinal infection, and only one-eighth of them pass cysts in their stools. Rarely, amebic abscesses also occur elsewhere (eg, lung, brain, spleen, or draining through the body wall).

Any organ or tissue in contact with active trophozoites may become a site of invasion and abscess.

Diagnostic Laboratory Tests

A. SPECIMENS

1. Fluid feces:

a. Fresh and warm for immediate examination for trophozoites.

b. Preserved in polyvinyl alcohol (PVA) fixative or merthiolate-iodine-formalin (MIF) fixative for mailing to a diagnostic laboratory (in a waterproofed or double mailing tube, the inner one of metal).

c. After a saline purge (or high enema after saline purge) for cysts and trophozoites.

2. Formed feces for cysts.

3. Scrapings and biopsies obtained through a sigmoidoscope or (more commonly) colonoscope, most frequently found by colonoscopy.

4. Liver abscess aspirates collected from the edge of the abscess, not the necrotic center. Viscous aspirates should be treated with a liquefying enzyme such as streptodornase, then cultured or examined microscopically.

5. Blood for serologic tests and cell counts.

B. MICROSCOPIC EXAMINATION

If possible, always examine fresh warm feces for trophozoites if the patient is symptomatic and has diarrheic stools. Otherwise, stain smears with trichrome or iron-hematoxylin stain. The stools in amebic dysentery can usually be distinguished from those in bacillary dysentery. The former contain much fecal debris, small amounts of blood with strings of nontenacious mucus and degenerated red cells, few polymorphonuclear cells or macrophages, scattered Charcot-Leyden crystals, and trophozoites. Although considerable experience is required to distinguish *E. histolytica* from *E. coli*, it is necessary to do so because misdiagnosis often leads to unnecessary treatment, overtreatment, or a failure to treat. The problem of routinely distinguishing *E. histolytica* from *E. dispar* or *E. moshkovskii* remains acutely unresolved.

Differentiation of *E. histolytica* (H) and *E. coli* (C), the most common intestinal ameba other than *E. dispar*, can be made in stained smears as follows:

1. Trophozoites - The cytoplasm in H is glassy and finely granular and contains only red cells and spherical vacuoles. The cytoplasm in C is granular, with many bacterial and other inclusions and ellipsoid vacuoles. The nucleus of H has a very small central endosome and fine regular chromatin granules lining the periphery; the nucleus of C has a larger, eccentric endosome, and the peripheral chromatin is more coarsely beaded and less evenly distributed around the nuclear membrane. Moribund trophozoites and precysts of H and C are usually indistinguishable.

2. Cysts - Glycogen vacuoles disappear during successive divisions. Nuclei resemble those of the trophozoites. Rare cysts of H and C may have 8 and 16 nuclei, respectively.

Cysts of H in many preparations contain many uninucleate early cysts; these are rarely seen with C. Binucleate developing cysts of C often show the nuclei pushed to opposite sides of the cell wall by the large central glycogen vacuole.

Chromatoidal bodies in early cysts of H are blunt-ended bars; those of C are splinter-like and often occur in clusters.

C. CULTURE

Diagnostic cultures are made in a layer of fluid overlying a solid nutrient base in partial anaerobiosis. Dobell's diphasic and Cleveland-Collier media are most often used.

D. SEROLOGY

Serologic testing is primarily for extraintestinal amebiasis, when stools are often negative. Serodiagnosis, most commonly by IHA test, is considered sensitive and specific, though it cannot distinguish recent from past infections.

Serologic testing in intestinal infections is less reliable except where considerable tissue invasion has occurred. Commercially available preparations employ the latex agglutination technique (Serameba), Ouchterlony double diffusion (ParaTek), and counterelectrophoresis (Amoebogen). Positive responses to several tests are of value in supporting a tentative diagnosis in doubtful cases of extraintestinal amebiasis. Antiamebic antibodies occur only with *E. histolytica*, since the nonpathogenic species do not elicit a serologic response.

The *Enzymeba* test is based on the finding of histolysain (the major cysteine protease of the virulent form) in the intestine plus circulating antibodies to histolysain after tissue invasion. The test is a solid-phase enzyme immunoassay to detect histolysain in stools. This test is especially helpful in cases where cysts or trophozoites are not found microscopically. Another test to distinguish pathogenic from nonpathogenic strains in a stool specimen is an ELISA that uses monoclonal antibodies against the galactose adhesin, a pathogen-specific epitope of *E. histolytica*. Amebic antigen (Tech-Lab Test) in the stool is sensitive and specific for *E. histolytica* and generally does not respond to *E. dispar* or other nonpathogenic amebas.

E. RADIATION METHODS

Hepatic abscess, usually showing as an elevation of the right dome of the diaphragm, can be observed by ultrasonography, CT, MRI, or radioisotope scanning. The round or oval hepatic lesion is clearly and often abruptly demarcated from the surrounding normal tissue. Serologic tests in these cases are usually strongly positive.

Treatment

Asymptomatic (cyst-passing) amebiasis can be treated with iodoquinol (Yodoxin), or diloxanide furoate (Furamide), or paromomycin (Humatin).

Metronidazole (Flagyl) is probably a drug of choice for symptomatic amebiasis even though it is

mutagenic in bacteria. For mild to moderate intestinal disease, give metronidazole or tinidazole (Fasigyn) (an excellent drug of low toxicity but not available in the United States). For severe intestinal disease (amebic dysentery), give the regimen described above or, if the other regimens cannot be followed, dehydroemetine (or emetine). For hepatic or other extraintestinal involvement or for ameboma, give metronidazole or tinidazole or dehydroemetine (or emetine).

Epidemiology, Prevention, & Control

Cysts are usually ingested through contaminated water. In the tropics, contaminated vegetables and food are also important cyst sources; flies have been incriminated in areas of fecal pollution. Asymptomatic cyst passers are the main source of contamination and may be responsible for severe epidemic outbreaks where sewage leaks into the water supply or breakdown of sanitary discipline occurs (as in mental, geriatric, prison, or children's institutions). A high-carbohydrate, low-protein diet favors the development of amebic dysentery both in experimental animals and in known human cases. Control measures consist of improving environmental and food sanitation. Treatment of carriers is controversial, although it is agreed that they should be barred from food handling. Possible environmental contamination should be considered in the treatment decision for an asymptomatic cyst passer. No fully satisfactory and safe drug is yet available for chemoprophylaxis, and the mix of drugs required for therapy attests to the problems of treating amebiasis.

OTHER INTESTINAL AMEBAS

Entamoeba histolytica must be distinguished not only from *E. dispar* and *E. moshkovskii* but also from four other ameba-like organisms that are also intestinal parasites of humans:

(1) *Entamoeba coli*, which is very common;

(2) *Dientamoeba fragilis*, the only intestinal parasite other than *E. histolytica* that has been suspected of causing diarrhea and dyspepsia, but not by invasion;

(3) *Iodamoeba betschlii*;

(4) *Endolimax nana*.

To facilitate detection, cysts should be concentrated by zinc sulfate flotation or a similar technique. Unstained, trichrome- or iron-hematoxylin-stained, and iodine-stained preparations should be searched systematically.

Mixed infections, including both *E. histolytica* and *E. dispar*, may occur. Polyvinyl alcohol (PVA) fixation is especially valuable for preservation of trophozoites.

The presence of nonpathogenic amebas is strongly indicative of poor sanitation or of accidental fecal contamination - both warnings of possible exposure to pathogenic *E. histolytica* - or a possible pre-AIDS immunodeficient state.

BALANTIDIUM COLI

Balantidium coli, the cause of balantidiasis or balantidial dysentery, is the largest intestinal protozoan of humans. Morphologically similar ciliate parasites are found in swine and nonhuman primates.

Morphology & Identification

A. TYPICAL ORGANISMS

The trophozoite is a ciliated, oval organism, 60 x 45 µm or larger. Its motion is a characteristic combination of steady progression and rotation around the long axis. The cell wall is lined with spiral rows of cilia. The cytoplasm surrounds two contractile vacuoles, food particles and vacuoles, and two nuclei - a large, kidney-shaped macronucleus and a much smaller, spherical genetic micronucleus. When the organism encysts, it secretes a double-layered wall. The macronucleus, contractile vacuoles, and portions of the ciliated cell wall may be visible in the cyst, which ranges from 40 µm to 70 µm in diameter.

B. CULTURE

These organisms may be cultivated in many media used for cultivation of intestinal amebas.

Pathogenesis, Pathology, & Clinical Findings

When cysts are ingested by the new host, the cyst walls dissolve and the released trophozoites descend to the colon, where they feed on bacteria and fecal debris, multiply both sexually and asexually, and form cysts that pass in the feces. Most infections are apparently harmless.

However, rarely, the trophozoites invade the mucosa and submucosa of the large bowel and terminal ileum. As they multiply, abscesses and irregular ulcerations with overhanging margins are formed. The number

of lesions formed depends upon intensity of infection and degree of individual host susceptibility. Chronic recurrent diarrhea, alternating with constipation, is the most common clinical manifestation, but there may be bloody mucoid stools, tenesmus, and colic. Extreme cases may mimic severe intestinal amebiasis, and some have been fatal.

Diagnostic Laboratory Tests

The diagnosis of balantidial infection, whether symptomatic or not, depends upon laboratory detection of trophozoites in liquid stools or, more rarely, of cysts informed stools. Sigmoidoscopy may be useful for obtaining material directly from ulcerations for examination. Culturing is rarely necessary.

Immunity

Humans appear to have a high natural resistance to balantidial infection. Factors underlying individual susceptibility are not known.

Treatment

A course of oxytetracycline may be followed by iodoquinol or metronidazole if necessary.

Epidemiology

B. coli is found in humans throughout the world, particularly in the tropics, but it is a rare infection. Infection results from ingestion of viable cysts previously passed in the stools by humans and possibly by swine. Pig farmers and slaughterhouse workers are particularly at risk, though poor sanitation and crowding in jails, mental institutions, or encampments are associated with infection. In the swine-based cultures of Papua New Guinea, infection levels of 28% have been reported.

FREE-LIVING AMEBAS

Primary amebic meningoencephalitis occurs in Europe and North America from amebic invasion of the brain. The free-living soil amebas *Naegleria fowleri*, *Acanthamoeba castellanii*, *Balamuthia mandrillaris*, and possibly species of *Hartmanella* have been implicated. Most cases have developed in children who were swimming and diving in warm, soil-contaminated pools, either indoors or - usually - out-doors. The amebas, primarily *N. fowleri*, apparently enter via the nose and the cribriform plate of the ethmoid, passing directly into brain tissue, where they rapidly form nests of amebas that cause extensive hemorrhage and damage, chiefly in the basilar portions of the cerebrum and the cerebellum. In most cases, death ensues in less than a week.

Entry of *Acanthamoeba* into the central nervous system occurs from skin ulcers or traumatic penetration, such as keratitis from puncture of the corneal surface or ulceration from contaminated saline used with contact lenses. Chronic granulomatous disease from *Acanthamoeba* and *Balamuthia* may infect both immunocompetent and immunosuppressed humans and animals. Infection of the CNS from the skin lesion may occur weeks or months later. It is termed granulomatous amebic encephalitis to distinguish it from the explosive, rapid brain infection from *Naegleria* (primary amebic meningoencephalitis). Diagnosis is by microscopic examination of the cerebrospinal fluid, which contains the trophozoites and red cells but no bacteria.

Amebas can be readily cultured on nonnutrient agar plates seeded with *Escherichia coli*. These soil amebas are distinguished by a large, distinct nucleus; by the presence of contractile vacuoles and mitochondria (absent in *Entamoeba*); and by cysts that have a single nucleus and lack glycogen or chromatoidal bodies. *Acanthamoeba* may encyst in invaded tissues, whereas *Naegleria* does not. Treatment with amphotericin B has been successful in a few cases, chiefly in the rare instances when diagnosis can be made quickly.

BLOOD SPOROZOANS

THE PLASMODIA

Sporozoa of the genus *Plasmodium* are pigment-producing ameboid intracellular parasites of vertebrates, with one habitat in red cells and another in cells of other tissues. Transmission to humans is by the bloodsucking bite of female *Anopheles* mosquitoes of various species.

Morphology & Identification

A. TYPICAL ORGANISMS

Four species of plasmodia typically infect humans: *Plasmodium vivax*, *P. ovale*, *P. malariae*, and *P. falciparum*.

B. CULTURE

Human malaria parasites have been successfully cultivated in fluid media containing serum, erythrocytes,

inorganic salts, and various growth factors and amino acids. Continuous cultivation of the erythrocytic phase undergoing schizogony (asexual multiple division) has been achieved and is of critical importance in vaccine development.

C. GROWTH CHARACTERISTICS

In host red cells, the parasites convert hemoglobin to globin and hematin; the latter becomes modified into the characteristic malarial pigment. Globin is split by proteolytic enzymes and digested. Oxygen, dextrose, lactose, and erythrocytic protein are also utilized.

D. VARIATION

Variations of strains exist within each of the four species that infect humans. Variations have been detected in morphology, pathogenicity, enzyme characteristics, resistance to drug therapy, infectivity for mosquitoes, and vaccine development.

Pathogenesis, Pathology, & Clinical Findings

Human infection results from the bite of an infected female anopheles mosquito, in which the sporozoites, resulting from the sexual and subsequent sporogonic cycle of development in the mosquito, are injected into the human bloodstream. The sporozoites rapidly (usually within 1 hour) enter parenchymal cells of the liver, where the first stage of development in humans takes place (exoerythrocytic phase of the life cycle). Subsequently, numerous asexual progeny, the merozoites, rupture and leave the liver cells, enter the bloodstream, and invade erythrocytes. Parasites in the red cells multiply in a species-characteristic fashion, breaking out of their host cells synchronously. This is the erythrocytic cycle, with successive broods of merozoites appearing at 48-hour intervals (*P. vivax*, *P. ovale*, and *P. falciparum*) or every 72 hours (*P. malariae*). The incubation period includes the exoerythrocytic cycles (usually two) and at least one or two erythrocytic cycles. For *P. vivax* and *P. falciparum*, this period is usually 10-15 days, but it may be weeks or months. The incubation period of *P. malariae* averages about 28 days. There is no return of merozoites from red blood cells to liver cells. Without treatment, falciparum infection ordinarily will terminate spontaneously in less than 1 year unless it ends fatally.

The other three species continue to multiply in liver cells long after the initial bloodstream invasion, or there may be delayed multiplication in the liver. These exoerythrocytic cycles coexist with erythrocytic cycles and, in *P. vivax* and *P. ovale*, may persist as nongrowing resting forms, or hypnozoites, after the parasites have disappeared from the peripheral blood. Resurgence of an erythrocytic infection (relapse) occurs when merozoites from hypnozoites in the liver break out, are not phagocytosed in the bloodstream, and succeed in reestablishing a red cell infection (clinical malaria). Without treatment, *P. vivax* and *P. ovale* infections may persist as periodic relapses for up to 5 years. *P. malariae* infections lasting 40 years have been reported; this is thought to be a cryptic erythrocytic rather than an exoerythrocytic infection and is therefore termed a recrudescence to distinguish it from a relapse.

During the erythrocytic cycles, certain merozoites enter red cells and become differentiated as male or female gametocytes. The sexual cycle therefore begins in the vertebrate host, but for its continuation into the sporogonic phase, the gametocytes must be taken up and ingested by bloodsucking female *Anopheles*.

P. vivax, *P. malariae*, and *P. ovale* parasitemias are relatively low-grade, primarily because the parasites favor either young or old red cells but not both; *P. falciparum* invades red cells of all ages, including the erythropoietic stem cells in bone marrow, so parasitemia may be very high. *P. falciparum* also causes parasitized red cells to produce numerous projecting knobs that adhere to the endothelial lining of blood vessels, with resulting obstruction, thrombosis, and local ischemia. *P. falciparum* infections are therefore far more serious than the others, with a much higher rate of severe and frequently fatal complications (cerebral malaria, malarial hyperpyrexia, gastrointestinal disorders, algid malaria, blackwater fever). Consequently, correct and prompt diagnosis of falciparum malaria is imperative and may be lifesaving. *P. malariae* has been implicated in a nephrotic syndrome in children - «quartan nephrosis» - with a peak incidence at about age 5 years.

Periodic paroxysms of malaria are closely related to events in the bloodstream. An initial chill, lasting from 15 minutes to 1 hour, begins as a synchronously dividing generation of parasites rupture their host red cells and escape into the blood. Nausea, vomiting, and headache are common at this time. The succeeding febrile stage, lasting several hours, is characterized by a spiking fever that frequently reaches 40 °C or more. During this stage, the parasites presumably invade new red cells. The third, or sweating, stage concludes the episode. The fever subsides, and the patient falls asleep and later awakes feeling relatively well. In the early stages of infection, the cycles are frequently asynchronous and the fever pattern irregular; later, paroxysms

may recur at regular 48- or 72-hour intervals, although *P. falciparum* pyrexia may last 8 hours or longer and may exceed 41 °C. As the disease progresses, splenomegaly and, to a lesser extent, hepatomegaly appear. A normocytic anemia also develops, particularly in *P. falciparum* infections.

Diagnostic Laboratory Tests

A. SPECIMENS AND MICROSCOPIC EXAMINATION

The thick blood film stained with Giemsa's stain is the mainstay of malaria diagnosis. This preparation concentrates the parasites and permits detection even of mild infections. Examination of thin blood films stained with Giemsa's stain is necessary for species differentiation. Several antigen-capture tests, using chromatographic methods to detect a trophozoite-derived protein in lysed blood, can be used for rapid diagnosis without need for microscopic examination. They employ a dipstick or test strip with monoclonal antibodies against target parasite antigens. These rapid diagnostic tests (RDTs) can distinguish *P. falciparum* or all four species but not the other three species individually. Currently under intensive study, RDTs are potentially of great importance for diagnoses in the field, where facilities and personnel for microscopic diagnoses are not available.

B. OTHER LABORATORY FINDINGS

Normocytic anemia of variable severity may be detected.

During the paroxysms there may be transient leukocytosis; subsequently, leukopenia develops, with a relative increase in large mononuclear cells. Liver function tests may give abnormal results during attacks but revert to normal with treatment or spontaneous recovery. The presence of protein and casts in the urine of children with *P. malariae* is suggestive of quartan nephrosis. In severe *P. falciparum* infections, renal damage may cause oliguria and the appearance of casts, protein, and red cells in the urine.

Immunity

The mechanisms of immunity in malaria are still not clearly understood. An acquired strain-specific immunity has been observed that appears to depend upon the presence of a low-level parasitemia that somehow inhibits new infections or maintains the infection at a nonsymptomatic level. This so-called premunition, or concomitant immunity, is soon lost after the parasites disappear from the blood. Exoerythrocytic forms in the liver cannot alone support premunition, and they elicit no host inflammatory response. Hence, superinfection of the liver by homologous strains can continue to occur. Natural genetically determined partial immunity to malaria occurs in some populations, notably in Africa, where sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, and thalassemia provide some protection against lethal levels of falciparum infection. Most blacks in West Africa, where malaria is endemic, are totally resistant to *P. vivax* malaria because they lack the duffy antigen (FyFy), which acts as a receptor for *P. vivax*; in its absence, *P. vivax* cannot invade erythrocytes. *P. ovale* frequently replaces *P. vivax* in this region.

The gene responsible for the sporozoite coating antigen has been identified and cloned using monoclonal antibody and hybridoma techniques. An antisporozoite vaccine has been developed; its initial testing in humans was not successful, however. A synthetic tripeptide vaccine, SPf66, developed by Patarroyo and colleagues, has been tested in Colombia and found to be partially effective (< 50%). A complete prophylactic vaccine would have to be active against both sporozoites and merozoites of the target species, with an antigametocyticidal effect to curb transmission; this is still some years in the future in spite of intensive research efforts.

Treatment & Prevention

Chloroquine (Aralen) is the drug of choice for treatment of all susceptible forms of malaria during the acute attack; 1,5 g of chloroquine (base) is given over a 3-day period or 1,8 g over 4 days. In cases of falciparum malaria coma (cerebral or algid malaria), parenteral quinine dihydrochloride (no longer available in the United States) or quinidine gluconate should be used until oral therapy is possible. *P. vivax* strains resistant to chloroquine occur, but chloroquine is still the drug of choice for all malarias except chloroquine-resistant falciparum infection. Primaquine, an 8-aminoquinoline, eliminates the exoerythrocytic forms in the liver (potentially relapsing malaria), permitting a so-called radical cure.

Falciparum malaria does not remain in the liver after its erythrocytic phase, so a cure of the clinical form is a radical cure. Primaquine therapy should follow treatment for clinical malaria. Individuals deficient in glucose-6-phosphate dehydrogenase, frequently blacks or persons originally from the eastern Mediterranean, should be given a longer low-level course of primaquine (or none), owing to the possibility of hemolytic anemia.

Primaquine also has gametocyticidal activity against *P. falciparum* after a single dose (for adults) of 45

mg base.

Drug-resistant strains of *P. falciparum* (multiply resistant in some areas, particularly Southeast Asia) are now found in all endemic tropical regions except the Arabian peninsula, Central America, and the Caribbean region. These resistant strains should be treated with quinine sulfate plus a single dose of pyrimethamine-sulfadoxine (Fansidar), with quinine plus doxycycline or tetracycline, or with quinine plus clindamycin. Mefloquine (Lariam) and halofantrine (Halfan) are recommended alternative drugs for treatment of chloroquine-resistant *P. falciparum* malaria.

Malarial coma from chloroquine-resistant falciparum malaria should be treated with parenteral quinine or quinidine plus intravenous doxycycline or clindamycin. Oral therapy should replace parenteral treatment as soon as possible - as for non-chloroquine-resistant cerebral malaria.

Suppressive prophylaxis can be achieved with chloroquine phosphate or amodiaquine except in chloroquine-resistant falciparum areas. Mefloquine is now the chemoprophylactic drug of choice in areas of chloroquine resistance, though repeated reports of neurologic and sleep-disturbing side effects have caused some authorities to recommend against its continued use as a prophylactic (especially in the United Kingdom). Doxycycline, taken daily, can be used in areas of multiple drug resistance of *P. falciparum*, such as in Thailand near its western border with Myanmar (Burma) and its eastern border with Cambodia, though mefloquine has been shown to be an effective suppressant among pregnant women in the Thai-Burma border area. Alternatively, chloroquine can be taken for prophylaxis and pyrimethamine-sulfadoxine withheld for presumptive treatment (as a single dose of three tablets) in case of a malarial breakthrough. No drug regimen can ensure prevention of malaria.

Recently approved in the United States is the combination of proguanil (100 mg) and atovaquone (250 mg) (Malarone), both for prophylaxis and for treatment of multidrug-resistant falciparum malaria. It is not safe for use in pregnancy and is contraindicated for persons with hepatic or renal disorders. Though mefloquine is still considered the drug of choice for malaria prophylaxis in resistant areas, Malarone is increasingly being used in its place. Combination drugs are also increasingly favored for therapy in hope of delaying development of resistance. Besides Malarone, this would include atovaquone (500 mg) plus doxycycline (100 mg) as well as artesunate and other derivatives of artemisinin from the drug qinghaosu, derived from the Chinese plant Artemisia annuae, followed by mefloquine. Travelers should avoid mosquito bites, use diethyltoluamide (deet) repellent, and sleep under a mosquito net impregnated with pyrethrin (RID).

In pregnancy, continued prophylaxis with mefloquine (perhaps excepting the first trimester) or chloroquine (not pyrimethamine or a sulfonamide) is essential because of the danger of transplacental transmission of malarial agents to the fetus and the danger to a nonimmune mother.

Epidemiology & Control

Malaria today is generally limited to the tropics and subtropics, although outbreaks in Turkey attest to the capacity of this disease to reappear in areas cleared of the agent. Malaria in the temperate zones is relatively uncommon, although severe epidemic outbreaks may occur when the largely nonimmune populations of these areas are exposed; it is usually unstable and relatively easy to control or eradicate. Tropical malaria is usually more stable, difficult to control, and far harder to eradicate. In the tropics, malaria generally disappears at altitudes above 6000 feet. *P. vivax* and *P. falciparum*, the most common species, are found throughout the malaria belt. *P. malariae* is also broadly distributed but considerably less common. *P. ovale* is rare except in West Africa, where it seems to replace *P. vivax*. All forms of malaria can be transmitted transplacentally or by blood transfusion or by needles shared among addicts when one is infected. Such cases do not develop a liver or exoerythrocytic infection; thus, relapse does not occur. Natural infection (other than transplacental transmission) takes place only through the bite of an infected female anopheles mosquito.

Malaria control depends upon elimination of mosquito breeding places, personal protection against mosquitoes (screens, pyrethrin-treated netting, protective clothing with sleeves and long trousers, repellents), suppressive drugs for exposed persons, and adequate treatment of cases and carriers. Eradication requires prevention of biting contact between anopheles mosquitoes and humans long enough to prevent transmission, with elimination of all active cases by treatment and by spontaneous cure. The results of massive efforts in highly endemic tropical areas have been unsuccessful.

Costly eradication projects undertaken between 1955 and 1970 have been replaced with control programs specifically geared to the mosquito vector ecology and malaria epidemiology of each area. These programs must be continued as permanent public health responsibilities. A major WHO-led effort to «roll back

malaria» is now under way.

BABESIA MICROTI

Babesia species are widespread animal parasites, causing infectious jaundice of dogs and Texas cattle fever (redwater fever). Babesiosis, a red cell-infecting tick-borne piroplasmosis caused in the United States by *Babesia microti*, is a human disease reported in increasing numbers - over 300 cases - from Massachusetts, the primary focus being Nantucket Island. A case of babesiosis in a splenectomized soldier that was reported from California was attributed to *B. gibsoni*. European cases - about 30 have been reported - are attributed to *B. divergens*, recently reclassified as *Microbabesia divergens*, while *B. microti* was transferred to the family Theileriidae as *Theileria microti*. The earlier classification is retained here in view of the wide medical use of the term babesiosis. Recent outbreaks have been in healthy individuals with no record of splenectomy, corticosteroid therapy, or recurrent infection. The great majority of infections in immunologically intact individuals are asymptomatic, but in affected persons the illness develops 7-10 days after the tick bite and is characterized by malaise, anorexia, nausea, fatigue, fever, sweats, myalgia, arthralgia, and depression. *Babesia* may be mistaken in humans for *P. falciparum* in its ring form in red cells, though its «Maltese cross» form in the red cell without pigment or gametocytes is diagnostic. Human babesiosis is more severe in the elderly than in the young. Splenectomized individuals may develop progressive hemolytic anemia, jaundice, and renal insufficiency with prolonged parasitemia. Chloroquine provides clinical relief but is not curative. Good clinical results follow treatment with clindamycin intravenously or intramuscularly plus oral quinine. A 10-day course of azithromycin followed by atovaquone or quinine has also been effective.

OTHER SPOROZOANS

ISOSPORA

Isospora belli, a sporozoan of the human intestine, causes coccidiosis in humans. Numerous species of intestinal sporozoa or coccidia occur in other animals and cause some of the most economically important diseases of domestic mammals and fowl. *I. belli* is one of the few coccidia that multiply sexually in the human intestine - ie, in which humans are the definitive host.

Morphology & Identification

A. TYPICAL ORGANISMS

Only the elongated ovoid oocysts are known for *I. belli*.

Intestinal biopsies of patients with chronic isosporosis demonstrated both asexual schizogonic and oocyst-producing sexual phases. The oocyst of *I. belli* is 25-33&12-16 µm and often has an asymmetric cyst wall.

Cyclospora cayetanensis is a minute intestinal intracellular coccidian that produces two sporocysts, each with two sporozoites, in the intestinal epithelium. Infection is by oocysts, 8-10 µm, in food or water. Mixed infection with *Cryptosporidium* is common.

B. CULTURE

These parasites have not been cultivated.

Pathogenesis & Clinical Findings

I. belli inhabits the small intestine. Signs and symptoms of coccidiosis apparently are due to the invasion and multiplication of the parasites in the intestinal mucosa.

Oocysts are shed into the intestinal lumen and passed in the stools. Infections may be silent or symptomatic. About 1 week after ingestion of viable cysts, a low-grade fever, lassitude, and malaise may appear, followed soon by mild diarrhea and vague abdominal pain. The infection is usually self-limited after 1-2 weeks, but diarrhea, weight loss, and fever may last for 6 weeks to 6 months. Symptomatic coccidiosis is more common in children than in adults. Chronic infections occur in poorly nourished people living under unsanitary conditions where continued reinfection is more likely, and in immunosuppressed persons.

Diagnostic Laboratory Tests

Diagnosis rests upon detection of oocysts in fresh stool specimens. Stool concentration techniques are usually necessary.

Immunity

Immunity to the coccidia following active infection is well documented in animals, although data from

human infection are lacking. The many Coccidia species are notably host-specific.

Treatment

Treatment of mild cases consists of bed rest and a bland diet for a few days. Treatment for more severe and chronic cases is with trimethoprim-sulfamethoxazole. Patients allergic to sulfonamides (eg, some AIDS patients) may respond to daily pyrimethamine or ciprofloxacin. Immunosuppressed patients may have to be treated continuously.

Epidemiology

Human coccidiosis results from ingestion of cysts. It is usually sporadic and most common in the tropics and subtropics, although it occurs elsewhere, including the United States.

SARCOCYSTIS

Sarcocystis species are coccidia with a biphasic life cycle: an intestinal (sexual) stage in gut mucosal cells of carnivores, and an encysted tissue (asexual) stage in muscle or other cells of herbivores or other prey animals. Humans apparently serve as both intermediate and final hosts depending on the species of *Sarcocystis*. Human volunteers fed raw beef and pork with *Sarcocystis* cysts later passed isospora-like oocysts in their stools; similar results have been obtained with dogs and cats. Human sarcocystosis develops from ingestion of undercooked beef (*S. bovihumanis*) and pork (*S. suihumanis*).

Morphology & Identification

In the muscles, the parasites develop in elongated sarcocysts that range from less than 0,1 mm to several centimeters long. When the infective zoites (bradyzoites) are freed from a sarcocyst in ingested meat into the gut of a definitive (final) host, they invade the cells of the intestinal mucosa and enter a sexual stage to produce the oocysts. Typically, the oocysts separate into two sporocysts, each containing four sporozoites infective to the intermediate host (usually a herbivore). The sporocysts are discharged in the definitive host's feces. When ingested by an intermediate host, the sporocysts open in the gut, each releasing four sporozoites, which penetrate the gut wall, pass to tissue sites, and invade host cells. Each sporozoite then develops into a new sarcocyst with numerous bradyzoites.

Pathogenesis & Clinical Findings

Heavy sarcocystis infections may be fatal in some animals (eg, mice, sheep, swine). Extracts of the parasite contain sarcocystin, a toxin that is probably responsible for the pathogenic effects. Humans apparently can serve as an intermediate host (with tissue sarcocysts) as well as a definitive (or final) host, with oocysts forming in the intestinal mucosa. Fleeting subcutaneous swellings, eosinophilia, and heart failure have been attributed to sarcocystis. Sarcocysts have been found in the human heart, larynx, and tongue as well as in skeletal muscles of the extremities. This species of *Sarcocystis* is called «*S. lindemanni*», a group designation, in the absence of means to experimentally feed out the human tissue parasites to a dog or other predator for identification of the parasite species.

Diagnostic Laboratory Tests

The infection ordinarily causes no symptoms or signs in humans, though severe symptoms presumably would develop in immunosuppressed persons. A reliable CF test has been developed for detection of suspected infections.

Treatment

There is no known effective treatment.

Epidemiology

Intestinal infections in humans result from ingestion of intermediate host tissues: raw or poorly cooked infected lamb, beef, or other meats. Tissue infections in humans follow accidental ingestion of infective sporocysts, presumably from feces of dog or cat final hosts.

CRYPTOSPORIDIUM

Cryptosporidium species, typically *C. hominis*, can infect the intestine in immunocompromised persons (eg, those with AIDS) and cause severe intractable diarrhea.

The organisms are coccidia related to *Isospora*. They have long been known as parasites of rodents, fowl, rhesus monkeys, cattle, and other herbivores and have probably been an unrecognized cause of self-limited, mild gastroenteritis and diarrhea in humans.

Morphology & Identification

The parasites are minute (2-5 µm) intracellular spheres found in great numbers just under the outer membrane of the cells lining the stomach or intestine. Thus, they are intracellular but extracytoplasmic. The mature trophozoite (schizont) divides into eight arc-shaped merozoites, which are released from the parent cell to begin a new cycle. Oocysts measuring 4-5 µm and containing four sporozoites may be seen, but no sporocysts have been demonstrated. Oocysts, passed into feces in enormous numbers, are the infective agents.

Pathology & Clinical Findings

Cryptosporidium inhabits the brush border of mucosal epithelial cells of the gastrointestinal tract, especially the surface of villi of the lower small bowel. The prominent clinical feature of cryptosporidiosis is diarrhea, which is mild and self-limited (1-2 weeks) in normal persons but may be severe and prolonged in immunocompromised or very young or old individuals.

Diagnostic Laboratory Tests

Diagnosis depends on detection of oocysts in fresh stool samples. Stool concentration techniques using a modified acid-fast stain are usually necessary. Monoclonal antibody can detect low-level infections, and fluorescent microscopy with auramine staining is useful. ELISA tests are now available for detection of fecal antigen.

Treatment

Treatment is unnecessary for patients with normal immunity. For those receiving immunosuppressant drugs, cessation of immunosuppressants may be indicated; for those with AIDS or congenital immunodeficiency, only continuous supportive therapy is available. Spiramycin (Rovamycine), paromomycin (Humatin), nitazoxanide (Cryptaz), or combination therapy with azithromycin may be temporarily effective. A new drug, nitazoxanide, has been approved for use as an elixir for treatment of cryptosporidiosis in the United States.

Epidemiology & Control

Cryptosporidiosis is acquired from infected animal or human feces or from feces-contaminated food or water. Mild cases are common in farm workers. For those at high risk (immunosuppressed and very young or old persons), avoidance of animal feces and careful attention to sanitation are required. The organisms are widespread and probably infect asymptomatically a significant proportion of the human population.

Occasional outbreaks, such as the one that occurred in Milwaukee in early 1993, affecting over 400,000 people, can result from inadequate protection, treatment, or filtration of water supplies for large urban centers.

In this instance, cattle manure from large dairy farms apparently was the source of contamination of the water supply. The capacity of as few as 30 organisms to initiate an infection - and the ability of the parasit to complete its life cycle, including the sexual phase, within the same individual host - makes possible the fulminating infections frequently observed in immunosuppressed individuals.

TOXOPLASMA GONDII

Toxoplasma gondii is a coccidian protozoan of worldwide distribution that infects a wide range of animals and birds but does not appear to cause disease in them.

The normal final hosts are strictly the cat and its relatives in the family Felidae, the only hosts in which the oocyst-producing sexual stage of *Toxoplasma* can develop. Organisms (either sporozoites from oocysts or bradyzoites from tissue cysts) invade the mucosal cells of the cat's small intestine, where they form schizonts or gametocytes. After sexual fusion of the gametes, oocysts develop, exit from the host cell into the gut lumen of the cat, and pass out via the feces. These infective, resistant oocysts resemble those of *Isospora*.

Within each oocyst, two sporocysts form, and in about 48 hours, four sporozoites form within each sporocyst.

The oocyst with its eight sporozoites, when ingested, can either repeat its sexual cycle in a cat or - if ingested by certain birds or by a rodent or other mammal, including humans - can establish an infection in which it reproduces asexually. In the latter case, the oocyst opens in the human's or animal's duodenum and releases the eight sporozoites, which pass through the gut wall, circulate in the body, and invade various cells, especially macrophages, where they form trophozoites, multiply, break out, and spread the infection to lymph nodes and other organs. These rapidly multiplying crescentic cells (tachyzoites) initiate the acute stage of disease. Subsequently, they penetrate nerve cells, especially of the brain and eye, where they multiply slowly (as bradyzoites) to form quiescent tissue cysts, initiating the chronic stage of disease. The tissue cysts (formerly called pseudocysts) are infective when ingested by cats (resulting in the intestinal sexual stage and

oocyst production) or, when eaten by other animals, more tissue cysts are produced.

The organism in humans produces either congenital or postnatal toxoplasmosis. Congenital infection, which develops only when nonimmune mothers are infected during pregnancy, is usually of great severity; postnatal toxoplasmosis is usually much less severe. Most human infections are asymptomatic. However, fulminating fatal infections may develop in patients with AIDS, presumably by alteration of a chronic infection to an acute one. Varying degrees of disease may occur in immunosuppressed individuals, resulting in retinitis or chorioretinitis, encephalitis, pneumonitis, or various other conditions.

Morphology & Identification

A. TYPICAL ORGANISMS

The trophozoites are boat-shaped, thin-walled cells that are 4-7 x 2-4 µm within tissue cells and somewhat larger outside them. They stain lightly with Giemsa's stain; fixed cells often appear crescentic. Packed intracellular aggregates are occasionally seen. True cysts are found in the brain or certain other tissues. These cysts contain many thousands of sporelike bradyzoites, which can initiate a new infection in a mammal ingesting the cyst-bearing tissue.

B. CULTURE

T. gondii may be cultured only in the presence of living cells, in cell culture or eggs. Typical intracellular and extracellular organisms may be seen.

C. GROWTH REQUIREMENTS

Optimal growth is at about 37-39 °C in living cells.

D. VARIATIONS

There is considerable strain variation in infectivity and virulence, possibly related to the degree of adaptation to a particular host. All of these forms are thought to comprise a single species, *T. gondii*.

Pathogenesis, Pathology, & Clinical Findings

The tachyzoite directly destroys cells and has a predilection for parenchymal cells and those of the reticuloendothelial system. Humans are relatively resistant, but a low-grade lymph node infection resembling infectious mononucleosis may occur. When a tissue cyst ruptures, releasing numerous bradyzoites, a local hypersensitivity reaction may cause inflammation, blockage of blood vessels, and cell death near the damaged cyst. Congenital infection leads to stillbirths, chorioretinitis, intracerebral calcifications, psychomotor disturbances, and hydrocephaly or microcephaly. In these cases, the mother was infected for the first time during pregnancy. Prenatal toxoplasmosis is a major cause of blindness and other congenital defects. Infection during the first trimester generally results in stillbirth or major central nervous system anomalies. Second- and third-trimester infections induce less severe neurologic damage, though they are far more common. Clinical manifestations of these infections may be delayed until long after birth, even beyond childhood. Neurologic problems or learning difficulties may be caused by the long-delayed effects of late prenatal toxoplasmosis.

Diagnostic Laboratory Tests

A. SPECIMENS

Blood (buffy coat of heparinized sample), sputum, bone marrow, cerebrospinal fluid, and exudates; lymph node, tonsillar, and striated muscle biopsy material; and ventricular fluid (in neonatal infections) may be required.

B. MICROSCOPIC EXAMINATION

Smears and sections stained with Giemsa's or other special stains, such as the periodic acid-Schiff technique, may show the organisms. The densely packed cysts, chiefly in the brain or other parts of the central nervous system, suggest chronic infection.

C. ANIMAL INOCULATION

This is commonly used for definitive diagnosis. A variety of specimens are inoculated intraperitoneally into groups of mice that are free from infection. If no deaths occur, the mice are observed for about 6 weeks, and tail or heart blood is then tested for specific antibody. The diagnosis is confirmed by demonstration of cysts in the brains of the inoculated mice.

Infection of cells in tissue culture is also useful and rapid (3-6 days), and polymerase chain reaction will amplify *T. gondii* DNA, though this may require a regional laboratory experienced in the procedure.

D. SEROLOGY

The Sabin-Feldman dye test depends upon the appearance in 2-3 weeks of antibodies that will render the membrane of laboratory-cultured living *T. gondii* impermeable to alkaline methylene blue, so that organisms

are unstained in the presence of positive serum. It is being replaced by the IFA and ELISA tests. These tests do not expose technologists to the danger of living organisms, as is required for the dye tests. A CF test may be positive (1:8 titer) as early as 1 month after infection, but it is valueless in many chronic infections.

The IFA, direct agglutination, and toxoplasmin skin tests are routinely used for diagnostic purposes. Frenkel's intracutaneous test is useful for epidemiologic surveys.

Immunity

Some acquired immunity may develop in the course of infection. Antibody titers in mothers, as detected in either blood or milk, tend to fall within a few months.

Yet, the fact that prenatal infection is limited to infants born of mothers who were first exposed during their pregnancy strongly suggests that the presence of circulating antibodies is at least partially protective. Immune deficiency diseases (eg, AIDS), immunosuppressant drugs, or changes in host resistance may cause chronic infection with *Toxoplasma* to become a fulminating, acute toxoplasmosis.

Treatment

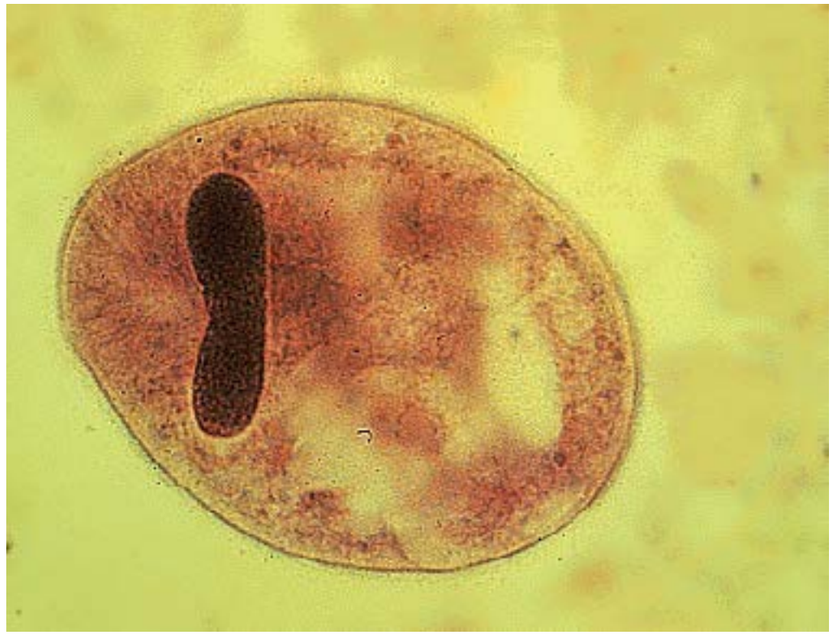
Acute infections can be treated with a combination of pyrimethamine and sulfadiazine or trisulfapyrimidines. Alternative drugs include spiramycin, clindamycin, trimethoprim-sulfamethoxazole, and various other sulfonamide drugs. For use in pregnancy, spiramycin (Rovamycine) is recommended, continued until delivery.

Epidemiology, Prevention, & Control

Transplacental infection of the fetus has long been recognized. Domestic cats have been incriminated in the transmission of the parasite to humans; the infection is transmitted by an isospora-like oocyst found only in the feces of cats and related animals. Rodents play a role in transmission, since they harbor in their tissues infective cysts that may be ingested by cats.

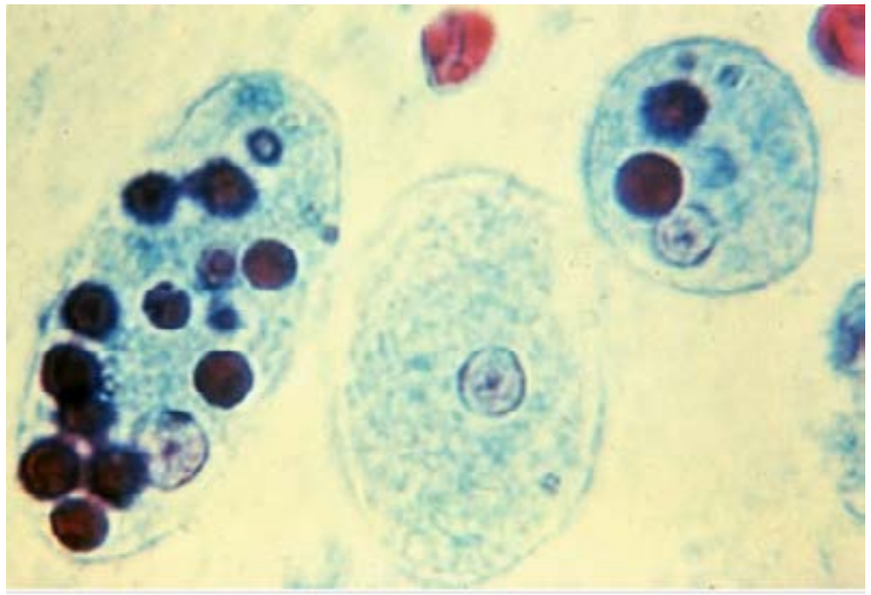
Avoidance of human contact with cat feces is clearly important in control, particularly for pregnant women with negative serologic tests. Since oocysts usually take 48 hours to become infective, daily changing of cat litter (and its safe disposal) can prevent transmission. However, pregnant women should avoid all contact with cats, particularly kittens. An equally important source of human exposure is raw or undercooked meat, in which infective tissue cysts are frequently found. Humans (and other mammals) can become infected either from oocysts in cat feces or from tissue cysts in raw or undercooked meat. Freezing meat at −20 °C for 48 hours or heating to 50 °C for 4-6 minutes will provide sterilization. Kitchen cleanliness, hand washing after touching raw meat, and avoidance of cats and cat litter are essential during pregnancy. Periodic serologic screening for IgG and IgM antibody to *Toxoplasma* is recommended.

17 Class – Illustrations

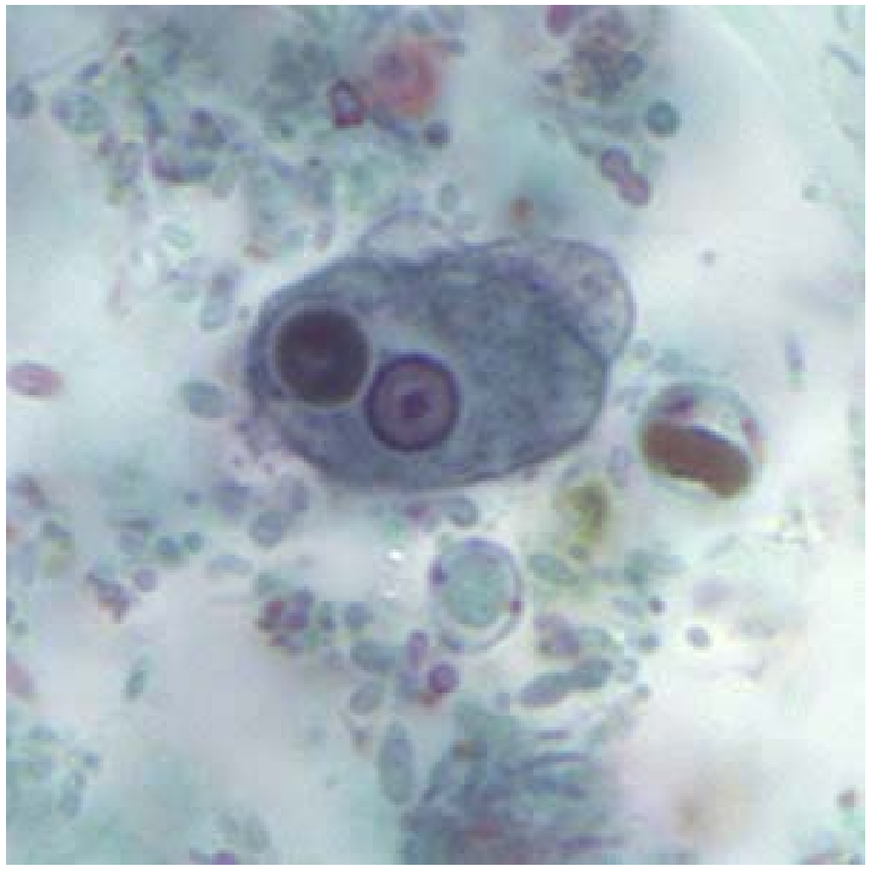


Balantidium coli trophozoite

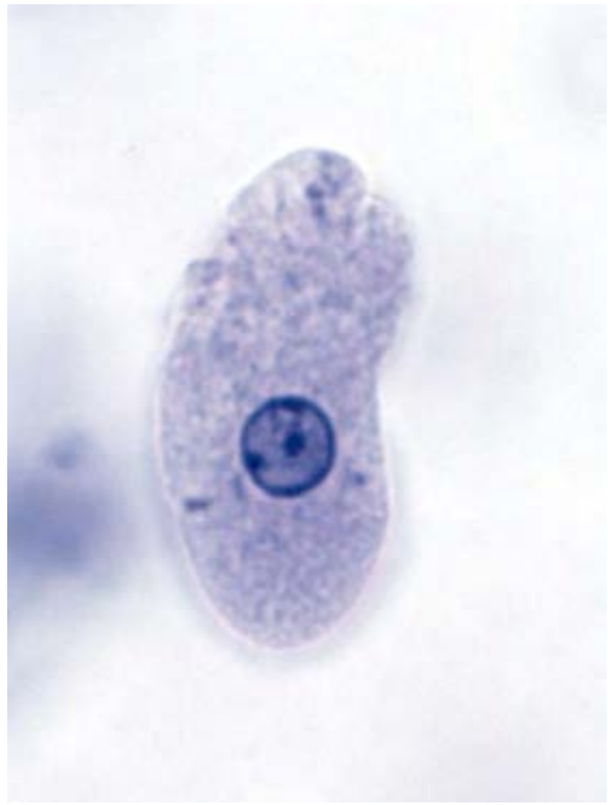
Balantidium coli as seen in a wet mount of a stool specimen. The organism is surrounded by cilia



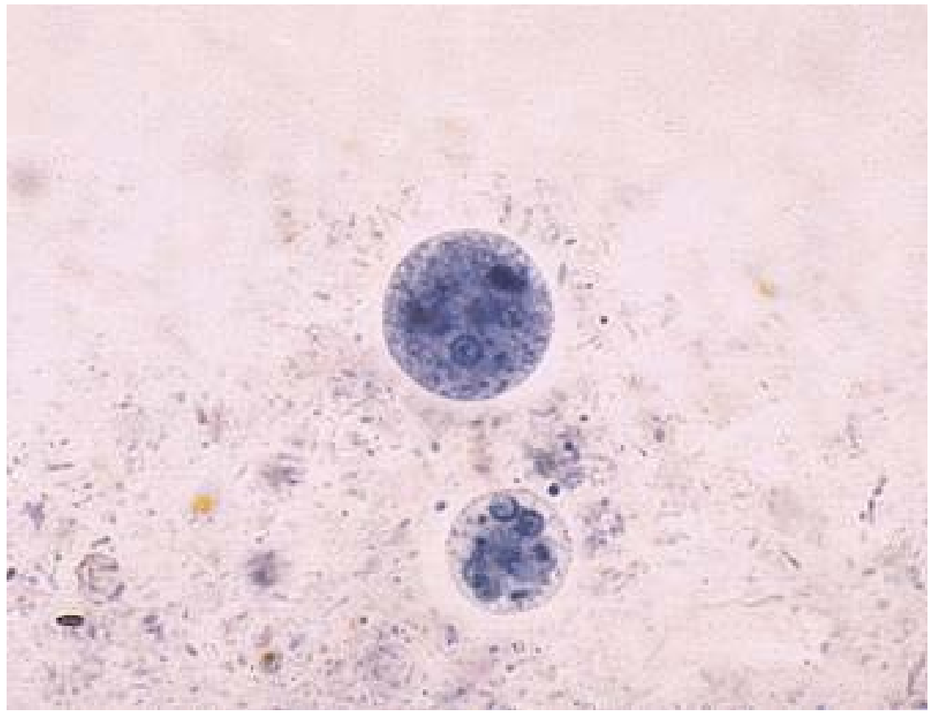
Trichrome stain of *Entamoeba histolytica* trophozoi



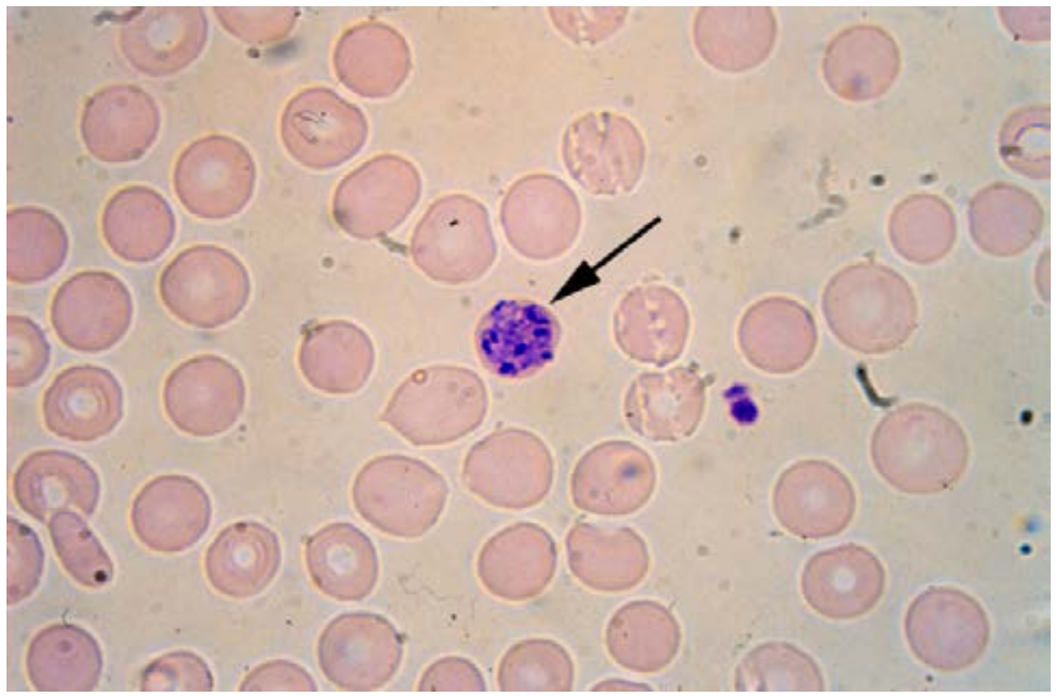
Trophozoites of *Entamoeba histolytica* with ingested erythrocytes stained with trichrome



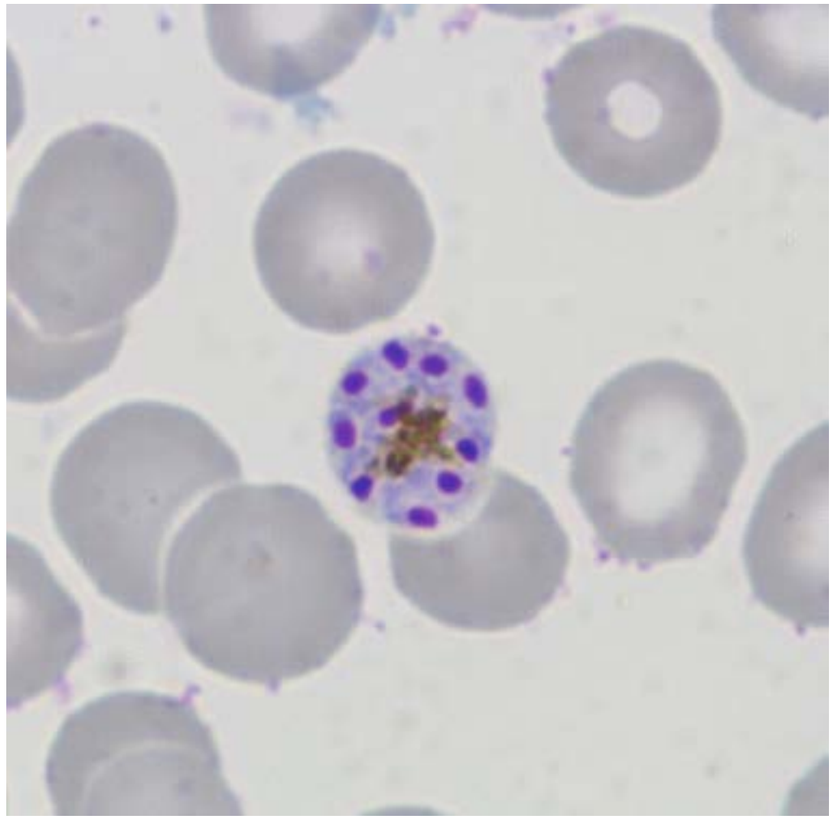
Entamoeba histolytica



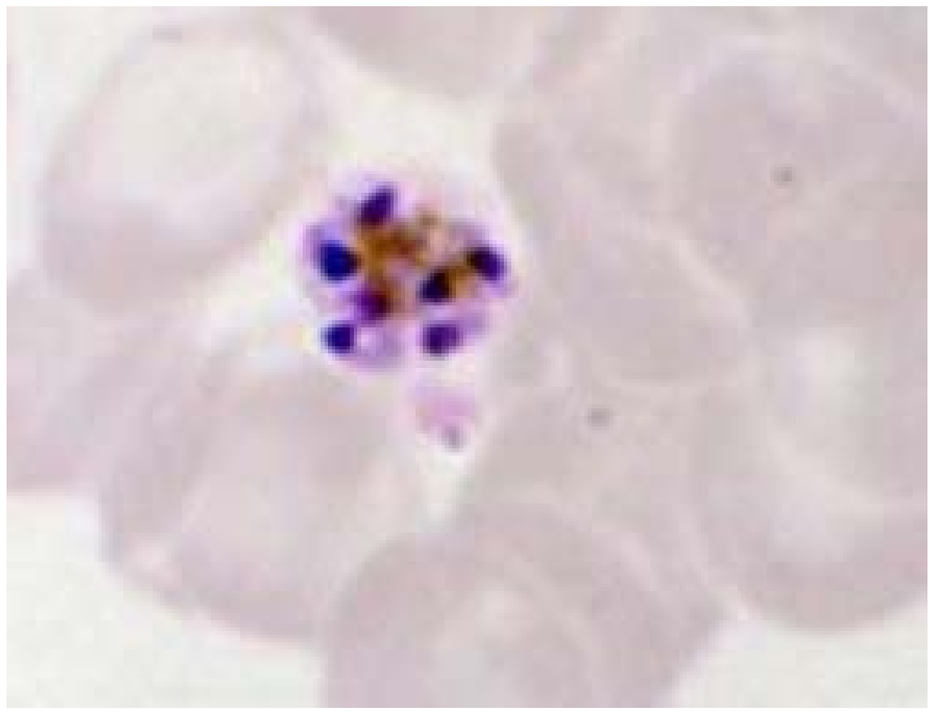
Entamoeba coli (trichrom)



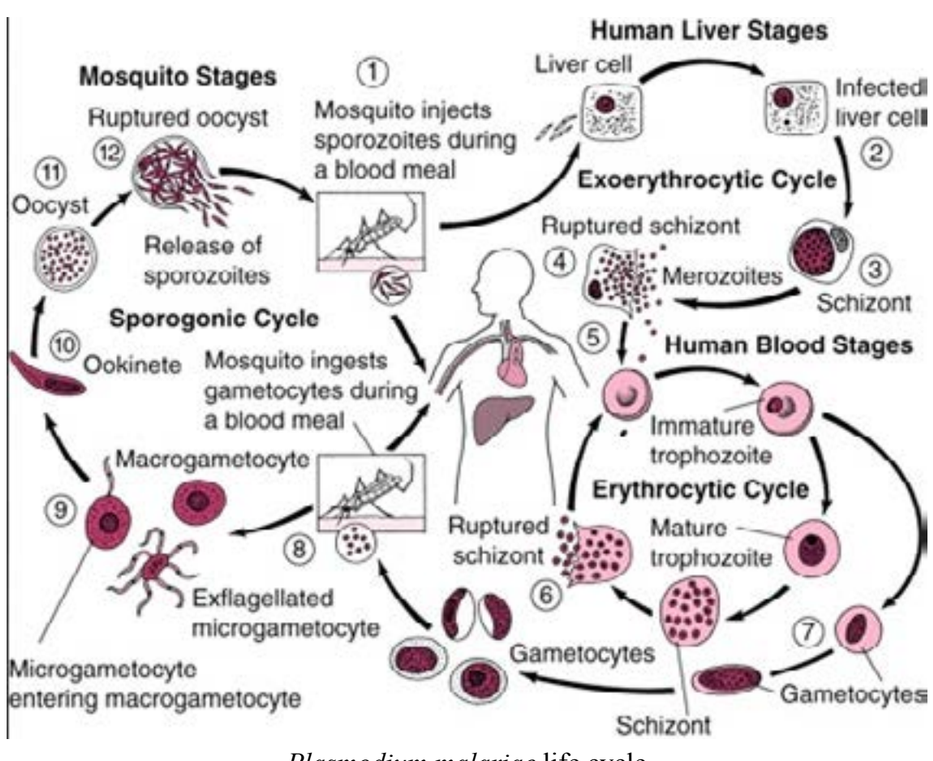
Plasmodium malariae



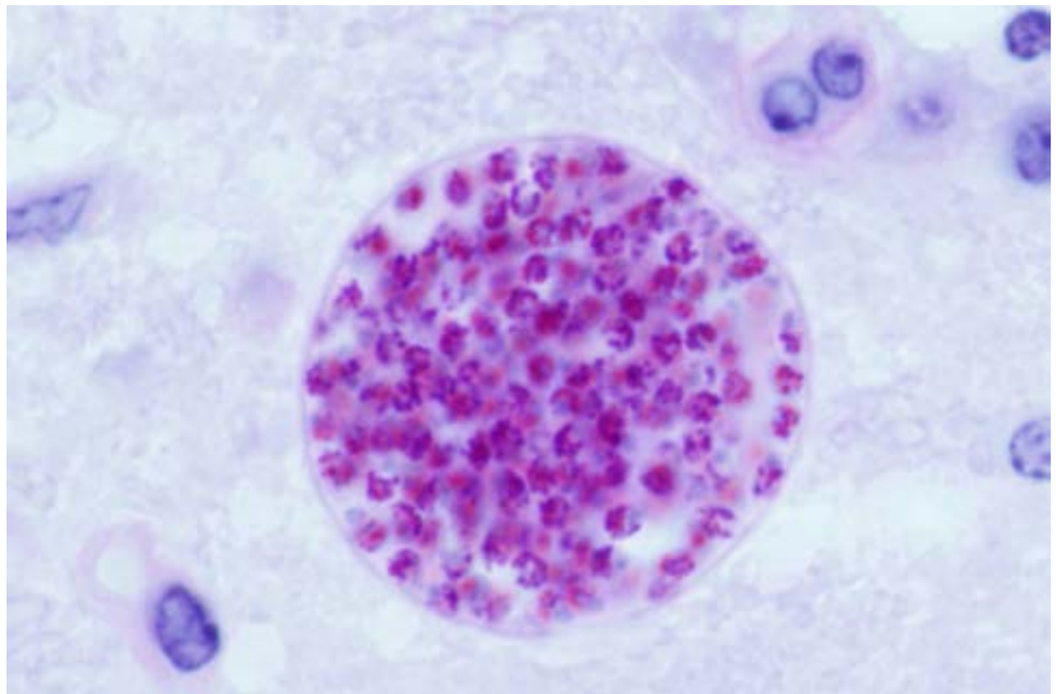
Plasmodium malariae schizont



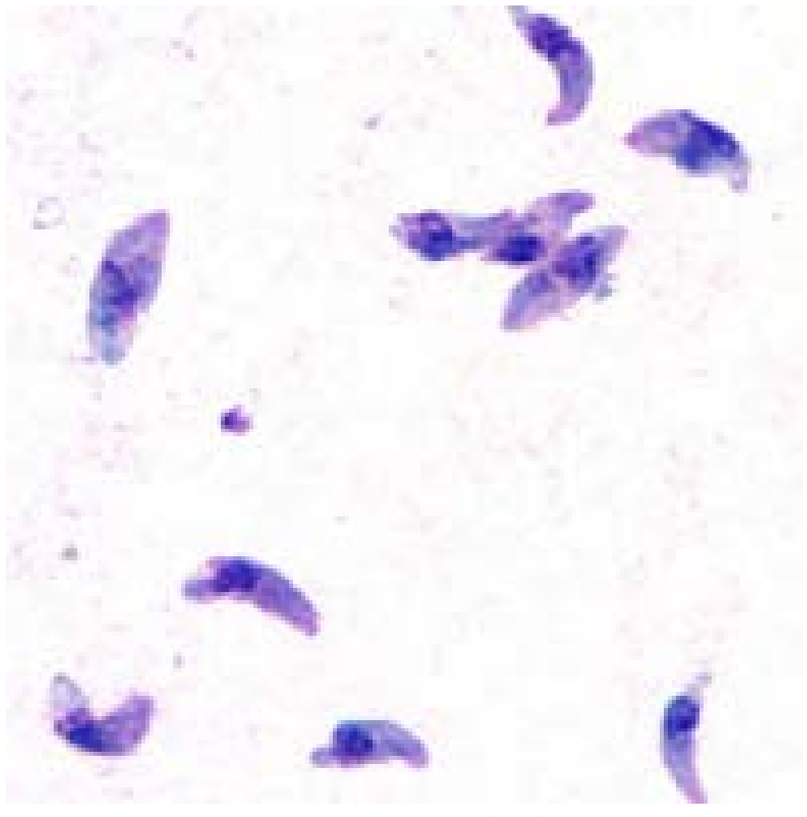
Plasmodium malariae schizont&merozoites



Plasmodium malariae life cycle



Toxoplasma gondii tissue cyst in a mouse brain, individual bradyzoites can be seen within



Toxoplasma gondii tachyzoites

18 Class - The repetition of examined material. Submodule-3

1. *Rickettsia*. The causative agent of typhus. Overview of morphological cultural characteristics, Weil-Felix reaction. Pathogenicity for animals and humans. Pathogenesis of the disease. Immunity. Brill-Zinsser disease. Laboratory diagnosis. Prevention.

2. *Rickettsia* pathogens of rat rickettsiosis. General characteristics of disease pathogenesis. Methods of laboratory diagnosis. Differentiation of epidemic and endemic typhus.

3. *Rickettsia* causative agents of spotted fever: north Asian, vesicular, Marseille. Laboratory diagnosis. Pathogenesis of the disease. Immunity, prevention.

4. *Rickettsia* as pathogens of the tsutsugamushi and paroxysmal fever. Pathogenesis of the disease. Laboratory diagnostic. Treatment and prevention.

5. Ku-fever *Rickettsia*. General characteristics and ecology. Pathogenesis of the disease, clinical forms. Laboratory diagnostics. Immunity. Prevention.

6. *Bartonellas*, general characteristics, pathogenesis of disease, laboratory diagnosis.

7. *Ehrlichia*. General characteristics of the pathogenesis of the disease, laboratory diagnosis.

8. Pathogenic *Chlamydia*. Cycles of development, general characteristics. Causative agents of trachoma, conjunctivitis new-borns (blenorea with inclusions). The causative agent of psittacosis and venereal Hodgkin's disease. Pathogenesis of the disease. Laboratory diagnostics. Prevention.

9. Pathogenic mycoplasmas. General characteristics of mycoplasma. Classification.

10. Opportunistic microorganisms, biological properties, etiologic role in the occurrence of opportunistic infections. Characteristics of the diseases that they cause.

11. Nosocomial infection, the conditions of their occurrence. Properties of the hospital bacteria ecovars, microbiological diagnosis of inflammatory, wounds, burn infections caused by hospital strains of bacteria.

12. Clinical Microbiology, object, objectives, methods, objects of research. Criteria for etiologic role of opportunistic microorganisms isolated from patients obtained material.

13. General characteristics of eukaryotes. Latin names of major humans infectious diseases.

14. Taxonomy and classification of fungi. Morphology, structure, cultivation. Toxicity. Pathogenesis of the disease in humans. Principles of laboratory diagnostic and treatment. Immunity.

15. Mild fungi. General characteristics. Morphology and ecology. Characteristics of the diseases that they cause. Laboratory diagnostics and treatment.

16. Superficial mycoses. General characteristics. Pathogenesis of the disease, clinical forms. Laboratory diagnostics. Treatment.

17. Deep and superficial candidiasis. Ecology of pathogens. Pathogenesis of the disease. Laboratory diagnostics. Treatment. Prevention.

18. Causative agents of the opportunistic fungal infections. General characteristics. Pathogenesis of the disease. Laboratory diagnostics, prevention and treatment.

19. Mycotoxicosis, general characteristics of pathogens, disease pathogenesis, clinical manifestations, laboratory diagnosis and treatment.

20. Causative agents of the histoplasmosis. General characteristics and ecology. Pathogenesis of the disease. Laboratory diagnostics. Treatment and prevention.

21. General characteristics of blastomycosis pathogens. Distribution. Symptoms. The pathogenesis of disease. Laboratory diagnostics. Prevention.

22. Chromomycosis. General characteristics and ecology of the pathogen. Pathogenesis and disease symptoms. Laboratory diagnostics. Treatment, prevention.

23. Sporotrichosis. General characteristics and ecology of the pathogen. Pathogenesis and disease symptoms. Laboratory diagnostics. Treatment, prevention.

24. Elementary, general characteristics. Development cycle. Classification.

25. *Leishmania*. General characteristics. The pathogenesis of cutaneous leishmaniasis form. Ecology of pathogens. Treatment. Prevention.

26. The causative agent of visceral leishmaniasis. Ecology. Pathogenesis of the disease. Laboratory diagnostics, treatment, prevention.

27. *Trypanosoma*. General characteristics. Ecology. Chagas disease. Pathogenesis of the "sleepy" disease. Laboratory diagnostics, treatment and prevention.

28. Trichomoniasis. Causative agents of the trichomoniasis. General characteristics. Ecology. Pathogenesis of the disease. Laboratory diagnostics and treatment.

29. *Giardia*. General characteristics. Pathogenesis of the disease, the source of infection. Laboratory diagnostics. Immunity. Prevention.

30. *Sarcodina*. Causative agents of the amebiasis. General characteristics. Stages of development in the body of living beings. Pathogenesis of the disease. Laboratory diagnostics. Treatment. Prevention.

31. *Plasmodium malaria.* The cycle of the malaria plasmodium. Pathogenicity for animals and humans. Pathogenesis of the disease in humans. Clinical forms of malaria. Immunity. Laboratory diagnostics. Treatment. Prevention

32. Toxoplasmosis. General characteristics of toxoplasmosis. Ecology. Pathogenicity to humans and animals. Pathogenesis of the disease in humans. Source of infection. Laboratory diagnosis. Treatment. Prevention.

33. Balantidias. Overview of morphological and cultural properties. Structure. Ecology. Pathogenesis of the disease. Laboratory diagnosis. Treatment.

34. Mild fungal infections. Pathogenic actinomycetes. General characteristics, types, properties and the environment. The diseases they cause. Laboratory diagnosis.

Content