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Microbiological diagnostics: From traditional to molecular genetic methods: A literature review

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Abstract. The development of new and optimisation of known cultural and molecular genetic methods for accurate species identification of microorganisms is an urgent and practically necessary task that is receiving great attention from researchers. The purpose of this study was to analyse and systematise theoretical scientific data on methods of microbial identification and assess their main advantages and disadvantages. For this purpose, a systematic review of 53 randomised research papers published between 2018 and 2023 was conducted. The search for publications using the keywords "microbiome", "microbiological diagnostics", "identification of microorganisms", "sequencing", and "omics technologies" in the title or text of the research paper was carried out in Web of Science, Scopus, PubMed, and Google Scholar. The study provides generalised information on traditional and modern methods of microbial identification. It is established that the advantages of traditional diagnostic methods include the possibility of preserving the obtained microorganisms for further research, in particular, for determining their antibiotic sensitivity. Modern molecular

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genetic methods open up new possibilities for the accurate identification of microorganisms, including those that are difficult to incubate using traditional cultural methods. The use of these methods allows obtaining detailed data on the genetic structure and diversity of microorganisms, which is important in many fields, including microbiology, medicine, and ecology. However, molecular methods are more sensitive to contamination or errors in the sample collection and processing. Moreover, omics technologies (genomics, transcriptomics, proteomics, and metabolomics) open up new opportunities for studying cells at more complex levels of life organisation. In everyday clinical practice, the microbiological method of isolating microbial cultures remains the main one, as it is the only one that allows determining not only the cause of the infection but also the antibiotic sensitivity. However, cultural methods for studying fastidious microorganisms are quite limited. Thus, the results obtained contribute to the development of fast and accurate methods for identifying, classifying, and systematising microorganisms, which facilitates the processes of diagnosis and development of strategies for the control and treatment of infectious diseases in laboratories and clinical institutions

Keywords: microbiology; diagnostics; nutrient medium; identification; sequencing; omics technologies

INTRODUCTION

Microorganisms can affect a person's life both positively and negatively. People have learned to use microorganisms in various areas of their lives. They are closely related to medicine, biotechnology, nutrition, genetic engineering, etc. As noted by Y. Gao *et al.* [1], the ability of bacteria and various types of fungi to produce antimicrobial substances makes them potentially useful in drug production. From the earliest childhood, the human body is also inhabited by a diverse community of microorganisms, which consists of bacteria, fungi, viruses, archaea, and is called the microbiome [2]. The human microbiome is a collection of microbiocenoses that colonise all surfaces of the human body, including the skin, respiratory system, gastrointestinal tract, and genitourinary system. These microorganisms perform a number of functions necessary for maintaining the homeostasis of the human body [3].

However, certain genetic features of bacteria make them dangerous to human health [4]. Antimicrobial resistance, which develops as a result of uncontrolled use of antibiotics, excessive use in animal husbandry and the food industry, poor infection control in hospitals and clinics, and lack of hygiene [5], are recognised as serious international problems. This problem makes it impossible to successfully treat many common bacterial infections and causes a growing number of deaths. A. Salmanov *et al.* [6] suggested possible ways to develop a multidisciplinary approach based on the principles of the "unified health" concept to solve problems related to antibiotic resistance.

Effective treatment becomes difficult without laboratory diagnostics, as the lack of diagnostic tests makes it difficult to establish any diagnosis. It is the qualitative diagnosis that determines the correct decisions of the doctor regarding the treatment methods used, evaluating their effectiveness, and in many cases preventing the occurrence of the disease due to the detection of pathology at an early stage of development [7]. Rapid identification of microorganisms plays a key role in the fight against infections. This helps to accurately identify the types of microorganisms that cause diseases and determine their sensitivity to antimicrobials. However, without rapid and accurate identification of infectious agents, complications associated with the wrong choice of antibiotic therapy for the patient may occur.

The range of diagnostic approaches used in microbiology is extremely wide and ranges from traditional to molecular genetic methods. Each method has its own strengths and weaknesses. Microbiological methods remain fundamental for diagnosing various types of infections in many laboratories. However, the limitations of these methods are the long time to obtain results, the possibility of obtaining false-negative results due to the use of antibiotics, and limited sensitivity to the detection of fastidious microorganisms [8]. Molecular methods are more accurate and specific for rapid diagnosis of aetiological pathogens of infections. M. Shevchenko *et al.* [9] showed the effectiveness of an optimised polymerase chain reaction protocol for identifying *Staphylococcus* spp bacteria, including the stage of detection in agarose gel and testing on clinical isolates from dogs. R. Symonenko [10] concluded that the use of modern methods of molecular diagnostics in periodontal tissue diseases allows quickly detecting periodontopathogens even in small quantities and identifying clinically significant types of microorganisms. In addition, this diagnostic method provides effective screening of periodontal tissue diseases. The study by I. Trubka *et al.* [11] analysed the microbiome of periodontal pockets of patients with rapidly progressive aggressive periodontitis using a molecular genetic method (Multident-5 test system). This approach identified the deoxyribonucleic acid (DNA) of the most clinically significant periodontopathogens in a single biological sample, providing a qualitative and quantitative assessment of the results. However, it is worth noting that although molecular methods open up new opportunities in microbiological diagnostics, their application in many countries with limited resources is quite difficult due to the high cost of this method.

In order to obtain the most accurate identification, classification, and taxonomy of microorganisms, it is extremely important to choose the appropriate methods and to have a thorough understanding of the mechanisms of their mechanisms of action. V. Motronenko & T. Vlasiuk [12] emphasised the importance of correctly interpreting the results obtained using culture and molecular methods in the context of accurate and rapid diagnosis of resistant forms of tuberculosis, and for ensuring effective treatment and care of patients. Microorganisms play a key role both for the benefit and harm of humans, and their identification is of great importance for modern medicine and biotechnology. Therefore, the purpose of the study was to analyse and systematise theoretical scientific data on methods for identifying microorganisms and evaluating their main advantages and disadvantages.

For this purpose, a systematic review of 53 randomised research papers published between 2018 and 2023 was carried out. The criteria for inclusion in the study included scientific publications in Ukrainian and English. The process of selecting publications included the stages of screening and quality assessment, where each paper was carefully analysed with regard to its scientific content and methodological aspects. Using electronic databases such as Web of Science, Scopus, PubMed, and Google Scholar, the authors searched for publications using the keywords "microbiome", "microbiological diagnostics", "microbial identification", "sequencing", and "omics technologies" in the title or text of the paper. It is important to note that the included papers were analysed according to various criteria, such as the year of publication and the main results. This approach helps to systematically summarise the literature data and provides a deeper look at the selected publications for the study.

TRADITIONAL METHODS OF MICROBIAL IDENTIFICATION

Most methods of isolation and identification of microorganisms remain unchanged and are based on the use of specific nutrient media that allow studying their various properties. Microorganisms differ in their needs, and therefore, there is no single environment or set of conditions that would ensure the growth of all species.

Conventionally, Nutrient agar, a universal medium, is used for the cultivation of microorganisms. By their composition, such nutrient media are designed for the cultivation of a wide range of microorganisms. Preparation of blood agar (BA) by adding 5% sheep blood to Nutrient agar expands the possibilities for studying and culturing those microorganisms that may require specific components or conditions for growth [13]. Thus, by the nature of haemolysis on BA, primary identification of streptococci is carried out. To identify certain infectious agents, it is important to use more specific nutrient media. For example, differential diagnostic nutrient media allow determining differences in the metabolic activity of microorganisms using systems of biochemical indicators. Nutrient media are also divided into selective ones, which are characterised by selective growth of a certain type of microorganism due to the addition of antibiotics to the nutrient medium. Metronidazole is often added to the nutrient medium for selective isolation of anaerobic microorganisms.

Chromogenic media are widely used in microbiological practice to identify specific types of microorganisms. The fluorogenic substrates present in these media are hydrolysed under the influence of specific enzymes produced by each type of microorganism. Hydrolysis of the fluorogenic substrate can lead to a change in the colour of the medium, which allows rapid and efficient identification of species [14].

After isolation of a pure culture, the phenotypic properties of the microorganism under study are determined for further identification. One of the main tests is the Gram stain method, which distinguishes between gram-positive and gram-negative microorganisms. The basic biochemical tests in clinical microbiology are the determination of catalase, oxidase, indole H_2S, etc. Most modern biochemical test kits include a combination of a wide range of biochemical determinations that are combined in a single test panel. This facilitates and speeds up the microbial identification process, providing faster and more accurate results.

Although traditional methods are available and provide information on the diversity of microorganisms in the sample, both quantitatively and qualitatively, the time required for identification based on traditional methods is estimated at a minimum of 2 to 5 days [15]. In addition, traditional methods do not provide an opportunity to identify uncultivated microorganisms. However, the advantages of this method are the ability to preserve the resulting microorganisms and test their sensitivity to antibiotics for effective treatment of the patient.

Immunological methods. Such immunological diagnostic methods in microbiology as agglutination, serology, immunoblotting, enzyme-linked immunosorbent assay (ELISA), etc., are worthy of attention. The basic principle of immunological methods is to investigate the interaction between an antibody and an antigen. These methods help in the identification of various microorganisms [16], but their use is accompanied by a number of limitations and disadvantages. Despite the high specificity of the analysis, this can lead to incorrect results or underestimate the presence of a pathogen. Some immunological methods are expensive, which can be a limiting factor for many laboratories due to the use of expensive equipment and reagents. In addition, the presence of chronic or immunodeficiency conditions can affect the accuracy and reliability of this method [17]. The unfavourable aspects of these methods do not reduce their importance as tools for studying and diagnosing microorganisms. Properly used immunological methods remain key in determining the immune response and identifying pathogens.

Mass spectrometry. Improvements in the identification of microorganisms have arisen from the need to reduce the time, cost, and simplify the procedures required to accurately identify these microorganisms. Therefore, the rapid development of mass spectrometry (MS) has led to its widespread use in microbiology [18]. The high speed, reduced cost, and ease of use of MS greatly facilitated and accelerated the identification of microorganisms compared to traditional cultural methods. The main principle of MS is the analysis of ions based on their mass-to-charge ratio (m/c), which allows studying the molecular structure, mass, and concentration of substances in various types of samples [19]. The combination of MS with gas or liquid chromatography significantly expanded the possibilities of this powerful method [20]. This has contributed to a better understanding of biological systems, allowing the analysis of a wide range of biomolecules.

The basic principle of gas chromatography mass spectrometry (GC-MS) is that first, using gas chromatography, the substances of the test sample are separated according to their properties. Next, the separated components are analysed in a mass spectrometer, where individual components are identified by their chemical properties [19]. This method is usually applied to identify microorganisms based on their lipid structures. Thus, S.P. Putri *et al.* [21] investigated 11 undetectable pathogenic Corynebacteria using GC-MS based on mycolic acid analysis. The effectiveness of the GC-MS method has also been demonstrated on yeast-like fungi [22]. The liquid chromatography mass spectrometry (LC-MS) method is based on the separation

of substances in a sample using the liquid phase and the analysis of the mass and charge of ions in a mass spectrometer. At this stage, individual components are identified by their mass and chemical properties [20].

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is an advanced tool for the rapid and accurate identification and classification of bacteria [23], fungi [24], and viruses [25]. The principle of MALDI-TOF MS is to ionise microorganisms using laser radiation, which leads to the formation of ions. These ions are accelerated in a vacuum system by an electric field and move towards the mass spectrometer detector in a certain way. The detector registers their mass, and the obtained data are used to identify microorganisms [26]. Among other applications, MALDI-TOF MS is also used to identify various metabolites. For example, the study by J. Doellinger *et al.* [27], using MALDI-TOF MS, investigated enterotoxins produced by pathogenic strains of *Bacillus cereus*. MALDI-TOF MS is also used to quickly detect antibiotic resistance genes in bacteria [28], which allows improving treatment strategies for infections, especially those caused by polyresistant strains of microorganisms.

Bacterial identification using MALDI-TOF MS mainly focuses on improving methods for isolating and cleaning pathogens from clinical samples, expanding spectral libraries and updating software. With the development of technology, many MALDI-TOF MS-based microbial identification databases and systems have been licensed and put into clinical use. However, there is a need to further improve the antimicrobial resistance analysis based on MALDI-TOF MS to provide comprehensive clinical microbiological characterisation [29]. The application of MALDI-TOF MS for the analysis of small molecules is usually limited by the choice of a suitable matrix. Matrices used for large-molecule analysis are often not suitable for analysing low-molecular-weight compounds (m/c <1,000 Da) due to matrix background noise, ion suppression, and uneven crystallisation by traditional organic matrices.

MOLECULAR GENETIC METHODS FOR IDENTIFYING MICROORGANISMS

With the development of molecular biology, the possibilities for identifying microorganisms have significantly expanded. Many microbiological laboratories still use phenotypic and biochemical methods to identify microorganisms. However, the high specificity of molecular methods helps to accurately and quickly identify various cultivated and uncultivated species of microorganisms in any samples without the need to grow them on nutrient media in laboratories. Notably, the significant development of molecular methods helped to launch a large-scale study of the microbiome and its impact on human health in 2007, called the Human Microbiome project [30].

The polymerase chain reaction (PCR) method has become the gold standard in microbiological laboratories, which is used to identify microorganisms. The standard PCR method is based on the identification of bacterial DNA by increasing certain fragments of nucleic acids, that is, their amplification, followed by sequencing and comparing them with databases [31]. Due to the wide variety of microorganisms in biological samples, their identification by cultural methods becomes difficult due to the inability to isolate and identify uncultivated forms. PCR-based methods not only provide rapid identification, but also help identify microorganisms that are difficult to isolate in the laboratory [32]. However, despite its effectiveness, the PCR method has a number of disadvantages. The specificity of the search is that information can be obtained only about those microorganisms for which specific primers are used. PCR does not provide information about the viability of microorganisms, as it uses DNA or ribonucleic acid (RNA), which may be important in some cases. The lack of the possibility of preserving and further using microorganisms limits its use in some studies. In addition, it is worth noting that the method does not provide information about the sensitivity of microorganisms to antibiotics, which is an important aspect in clinical studies.

DNA microarray. The use of DNA microarrays allows simultaneously identifying and sequencing hundreds or thousands of genes in a single study. A DNA microarray is a DNA probe attached to a microplate. The operation of a DNA microarray is based on the phenomenon of hybridisation, during which labelled samples are analysed with fluorescent probes [33]. Unlike other molecular genetic methods, DNA microarrays have a larger coverage area and faster results. M.A. Campanero-Rhodes *et al.* [34] demonstrated the successful use of microarrays to detect type-specific immunoglobulin G (IgG) antibodies against *Streptococcus pneumoniae* capsule polysaccharide in serum (CPS). The results showed the potential of microarrays for simultaneous analysis of the interaction of multiple CPS using small volumes of serum, which can be useful for studying limited volumes of serum samples. In addition, according to Hu. Arengaowa *at al.* [35], the DNA microarray method has been successfully used in the food industry to detect food pathogens. Despite the successful use of the DNA microarray method, it has its drawbacks, which are expensive and the need for high-quality DNA samples. Contamination or poor sample quality can lead to erroneous results, which complicates the research process.

16S rRNA sequencing is one of the most sensitive methods for detecting microorganisms, which is widely used in clinical settings. It is based on the detection of the 16S rRNA gene, which is specific for each type of microorganism [36]. This method provides accurate identification of genera and species of microorganisms that do not meet any recognised biochemical profiles. A. Szymczak *et al.* [37] compared histological method, PCR, and 16S rRNA gene sequencing for identification of *Helicobacter pylori*. Using gastric antral biopsy, the researchers showed that 16S rRNA gene sequencing is the most sensitive method for detecting *H. pylori*. However, there are certain problems that can be encountered when using this method. One of them is that there are variations in the 16S rRNA gene among strains of the same species [38], which may affect the estimation of the abundance of these microorganisms.

Next-generation sequencing (NGS). Continuous improvement of DNA sequencing technology has significantly improved the capabilities of molecular genetic diagnostic methods. The main idea of using NGS is parallel sequencing of many fragments of DNA or RNA, which allows obtaining significant amounts of genetic information in a short period of time [39]. W. Gu *et al.* [40] developed a next-generation metagenomic sequencing test (mNGS) using cell-free

DNA from body fluids to identify pathogens. The results showed that rapid mNGS testing is an effective tool for diagnosing unknown infections. However, NGS research requires expensive equipment, timely and sustainable maintenance, and training of technical specialists, which can be a problem even for middle-income countries [41].

Whole-genome sequencing (WGS) is the most accurate method for identifying microorganisms and is an important tool in microbiology [42]. This method expands the possibilities for analysing genes associated with antibiotic resistance [43], identifying their pathogenicity [44], and other key aspects, making an important contribution to understanding microbial ecology. Despite these advantages, the method has its drawbacks. It is considered the most expensive and time-consuming; it does not provide information about the viability of microorganisms, since it is based on DNA/RNA analysis. In addition, it provides only a correlation of microorganisms, and identification problems are associated with the lack of complete databases of genetic material.

Despite determining the microbial composition of an individual, it is important to understand the functions that bacteria perform in this community, their relationships, and their impact on the host [45]. The rapid development of molecular biology and information technology methods has contributed to the use of various omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics [46]. Omics technologies open up new opportunities for cell research at more complex levels of life organisation.

Genomics is a set of methods that are based on the investigation of the structure and functions of the genome of the studied objects [47], whereas transcriptomics methods focus on studying the transcriptome of an organism, that is, the sum of all its RNA transcripts [48]. Genomics and transcriptomics are two related but different areas of research that help uncover and understand genetic information and gene activity in the body. Proteomics examines the structure and function of all proteins in the body [49]. It helps to uncover the role of proteins in signalling interaction, vital activity, and detection of diseases such as Alzheimer's disease [50]. Metabolomics represents the latest branch of omics technology and is extremely promising for medicine. The main task of metabolomics is to identify and quantify a wide range of metabolites [51]. Detection of specific metabolic changes can serve as an indicator of the development of various diseases, in particular, in diabetes mellitus [52] or in chronic obstructive pulmonary disease [53].

Investigation of the microbial composition of an organism is a key to understanding the relationship between microorganisms and their impact on the host. The use of omics technologies provides new opportunities for studying life systems at different levels of their organisation. The integration of the data obtained with the help of these technologies helps to solve problems related to various pathological conditions of humans and the environment.

CONCLUSIONS

The study analysed and systematised the available methods for identifying microorganisms in clinical diagnostics. Analysing an overview of up-to-date data, it can be concluded that in everyday clinical practice, the microbiological method of isolating microbial cultures remains the main one. This method not only determines the cause of infection, but also helps to determine sensitivity to antibacterial drugs, contributing to the effective treatment of various infections. However, cultural methods for studying fastidious microorganisms are quite long and complex. Immunological methods based on the interaction of antibodies with foreign antigens are widely used in microbiology to identify individual species and serotypes of microorganisms. Despite their speed and simplicity of research, it is important to consider some of the disadvantages of this method. Immunological tests may be vulnerable to changes in the spectrum of antigens or antibodies, which can lead to false results. In addition, the presence of chronic or immunodeficiency conditions can affect the accuracy and reliability of this method.

Compared to traditional methods of microbiological diagnostics, molecular biology methods are characterised by higher sensitivity and specificity. The high sensitivity of these methods allows detecting microorganisms even with a minimal number of them. In particular, the greater specificity of molecular methods helps to more accurately identify a specific type or strain of microorganism. However, molecular genetic methods for identifying microorganisms also have drawbacks. High cost, expensive equipment, and the need for specially trained personnel limit their availability for many laboratories. In addition, molecular genetic methods do not provide information about whether the microorganism is alive in the test sample.

Omics technologies (genomics, transcriptomics, proteomics, metabolomics) are a group of high-performance methods that study large amounts of biological information at various levels of molecular structure and function, which provides a deep understanding of complex biological systems. The disadvantage of omics technologies and molecular genetic methods is their high cost and the need for complex equipment and interpreting large amounts of data requires specialised skills and bioinformatic knowledge.

The results obtained contribute to the choice of a fast and accurate method for identifying, classifying, and systematising microorganisms in accordance with specific needs. In particular, the ability to quickly identify microorganisms in various infectious conditions can significantly speed up the choice of the optimal antibiotic therapy regimen for doctors and provide more effective treatment for patients. Further research may include the development of new methods that combine the benefits of microbiological, immunological, molecular genetic, and omics technologies to improve the accuracy, speed, and accessibility of infectious disease diagnostics.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Мікробіологічна діагностика: від традиційних до молекулярно-генетичних методів: огляд літератури

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Анотація. Розробка нових та оптимізація відомих культуральних та молекулярно-генетичних методів для точної видової ідентифікації мікроорганізмів є актуальним і практично необхідним завданням, якому приділяється велика увага дослідників. Метою даної роботи було проведення аналізу, систематизація теоретичних наукових даних щодо методів ідентифікації мікроорганізмів та оцінка їхніх основних переваг і недоліків. Для цього було здійснено систематичний огляд рандомізованих 53 наукових робіт, опублікованих в період з 2018 по 2023 роки. Пошук публікацій із використанням ключових термінів «мікробіом», «мікробіологічна діагностика», «ідентифікація мікроорганізмів», «секвенування» та «омікс-технології» у назві чи тексті науково-дослідницької роботи здійснювали в системах Web of Science, Scopus, PubMed та Google Scholar. У статті надано узагальнену інформацію щодо традиційних та сучасних методів ідентифікації мікроорганізмів. Встановлено, що до переваг традиційних методів діагностики належить можливість збереження отриманих мікроорганізмів для подальших досліджень, зокрема для визначення їх чутливості до антибіотиків. Сучасні молекулярно-генетичні методи відкривають нові можливості для точної ідентифікації мікроорганізмів, зокрема тих, які складно культивувати за допомогою традиційних культуральних методів. Використання цих технік дозволяє отримати детальні дані про генетичну структуру та різноманіття мікроорганізмів, що є важливим у багатьох галузях, включаючи мікробіологію, медицину та екологію. Проте, молекулярні методи більш чутливі до забруднень чи похибок у процесі збирання та обробки зразків. Варто також відмітити omics-технології (геноміка, транскриптоміка, протеоміка та метаболоміка), які відкривають нові можливості для дослідження клітин на більш складних рівнях організації життя. В повсякденній клінічній практиці основним залишається мікробіологічний метод виділення мікробних культур, оскільки тільки він дозволяє визначити не лише причину інфекції, але й чутливість до антибіотиків. У той же час культуральні методи дослідження вибагливих мікроорганізмів досить обмежені. Таким чином, отримані результати сприяють розробці швидкої та точної методики ідентифікації, класифікації та систематизації мікроорганізмів, що полегшує процеси діагностики та розробки стратегій контролю і лікування інфекційних захворювань у лабораторіях та клінічних установах

Ключові слова: мікробіологія; діагностика; поживне середовище; ідентифікація; секвенування; омікс-технології