

SCREENING STUDIES OF ANTIMICROBIAL EFFICACY OF ANTISEPTICS AS ONE OF THE WAYS TO PREVENT NOSOCOMIAL INFECTIONS IN DENTISTRY

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ABSTRACT

INTRODUCTION: Over the last decades, the problem of formation and circulation of micro-organisms with resistance to antimicrobials has been steadily increasing. Hospital-acquired infections (HAI) are one of the most pressing problems in medicine, urgency of which is associated with high rates of morbidity, mortality, and significant socio-economic losses.

OBJECTIVES: The aim of the study was to investigate antimicrobial activity of commercial disinfectants on typical and clinical isolates of bacteria of genus *Staphylococcus*.

MATERIAL AND METHODS: Clinical base for isolation of bacteria was the University Dental Clinic of Uzhhorod National University, and evaluation of antimicrobial activity of disinfectants was conducted in microbiological laboratory of the Department of Genetics, Plant Physiology and Microbiology of Uzhhorod National University, Ukraine. Sensitivity of micro-organisms to disinfectants was determined using standard method of diffusion into agar (well diameter, 8 mm).

RESULTS: Previous studies have shown that *in vitro* experiments, the highest anti-staphylococcal effect was observed using Ecobriz antiseptic, AHD 2000, and Lysoformin 3000. It should be noted that sensitivity of coagulase-negative staphylococci was statistically significantly higher than that of coagulase-positive. Thus, the growth retardation zone under the action of AHD 2000 on clinical strain of *Staphylococcus aureus* was 19.0 ± 0.30 mm and on *S. haemolyticus* it was 23.9 ± 1.0 mm. This trend was characteristic for other antiseptics. The lowest antimicrobial activity was detected by using Chlorhexidine. Antimicrobial action of ethanol at applied dose was not observed. The highest activity among staphylococci was detected using antiseptics on *S. hominis* isolates.

CONCLUSIONS: Experiments established antimicrobial activity of all antiseptics except Dezoderm and Etasept. The highest level of activity against Gram-positive, Gram-negative bacteria, and microscopic fungi was found in Bacylol. High levels of antimicrobial activity were detected using AHD 2000, Lysoformin 3000, Ecobriz antiseptic, and Famidez, with higher activity of antiseptics against bacteria of genus *Staphylococcus*.

KEY WORDS: antiseptics, disinfection, nosocomial infection.

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INTRODUCTION

Over the last decades, the problem of formation and circulation of micro-organisms with resistance to antimicrobials has been steadily increasing. Hospital-acquired infections (HAI) are one of the most pressing problems in medicine, urgency of which is associated with high rates of morbidity, mortality, and significant socio-economic losses [1]. For the treatment and prophylactic dental profile institutions, the problem of HAI is most urgent since microbiota associations with the oral cavity include conditionally pathogenic micro-organisms. In recent decades, scientists have found a decrease in the effectiveness of antiseptics [2]. Resistance of micro-organisms to antimicrobial drugs is associated with ability of a micro-organism to acquire resistance through horizontal transfer of genes (plasmids, transposons), and with biofilm-forming properties of hospital strains. Moreover, a study by Gajadhar *et al.* showed that disinfectants themselves can be contaminated with micro-organisms [3]. This further indicates a high-rate of growth of antimicrobial resistance.

To date, there is significant number of antiseptic drugs on the market [4]. Modern antiseptics must be effective and non-toxic. In terms of COVID-19 pandemic and quarantine measures with permanent use of antiseptics, their effectiveness may decrease dramatically.

In dental clinic, the source of HAI is the patient or the carrier, and factors of transmission include blood, saliva, pus, not disinfected dental equipment and medical instruments, towels, sinks, door handles, chairs, etc. In this regard, not only the patient but also medical staff are at risk of infection [5]. Under these conditions, there is a growing need for monitoring studies evaluating the effectiveness of antimicrobial agents to develop effective approaches in using antiseptics and disinfectants.

OBJECTIVES

The aim of the study was to investigate the antimicrobial activity of commercially available disinfectants on typical and clinical isolates of bacteria of genus *Staphylococcus*.

MATERIAL AND METHODS

The clinical base for isolation of bacteria was the University Dental Clinic of Uzhhorod National University (UzhNU). Evaluation of antimicrobial activity of disinfectants was conducted in microbiological laboratory of the Department of Genetics, Plant Physiology, and Microbiology of UzhNU, Ukraine. Sensitivity of micro-organisms to disinfectants was determined with standard method of diffusion into agar (well diameter, 8 mm) [6, 7]. Commercially available antiseptics were used in the study, composition of which is given in Table 1.

As test cultures, bacteria of genus *Staphylococcus*, *Staphylococcus aureus* ATCC 25923, clinical isolates of *S. aureus* bacteria were used, including methicillin-resistant, *S. epidermidis*, *S. haemolyticus*, *S. hominis* isolated from the mouth of people with inflammatory periodontal disease. As test cultures, bacteria and yeasts from American type culture collection were applied, including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Streptococcus pyogenes* ATCC 19615, and *Candida albicans* ATCC 885-653. Moreover, samples with clinical strains of bacteria and yeasts (*Staphylococcus aureus* bacteria, such as methicillin-resistant *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *Escherichia coli*, *Streptococcus pyogenes*, and *Candida albicans*), isolated from hands of dentists and oral cavities of patients suffering from inflammatory diseases of periodontium were used.

Inoculum of bacteria or microscopic fungi with 100 µl in saline containing 5 × 10⁸ CFU/ml (0.5 Mc-

TABLE 1. Antiseptics used in the study

Disinfectants	Manufacturer	Active substance, %
AHD 2000	Blanidas, Ukraine	N-propanol, 40%; isopropanol, 35%; alkyltrimethylbenzylammonium chloride, 0.15%; aromatic substances, water
Lyzoformin 3000	Lyzoform, Dr. Hans Rosemann GmbH, Germany	Glutaraldehyde, 9.5%; glyoxal, 7.5%; didecyldimethylammonium chloride, 9.6%
Hospisept gel	Blanidas, Ukraine	Sodium salt of dichloroisocyanuric acid, 85.5%
Chlorhexidine digluconate	Monpharm, Ukraine	Chlorhexidine bigluconate, 20%
Ecobriz	World of Disinfection, Russia	Alkyldimethylbenzylammonium, 0.15%; chloride + didecyldimethylammonium chloride; isopropyl alcohol (propanol-2), 60%
Famidez	DezoMark®, Ukraine	Propanol, 60.00-66.62%; 1.3-butenediol, 0.104-0.126%; moisturizing and softening skin additives, water (up to 100.0)
Bacyllol	BODE Chemie GmbH, Ukraine	1-propanol, 45.0%; 2-propanol, 25.0%; ethanol, 4.7%
Desoderm	DezoMark®, Ukraine	Isopropyl alcohol, 60.0-66.5%; 1.3-dibutyl alcohol, 0.104-0.126%
Etasept	Blanidas, Ukraine	Ethyl alcohol, 9.5-10.5%

Farland standard) were sown on the surface of Muller-Hinton agar. Wells were formed in the agar with a diameter of 6 mm, in which test drugs were introduced with 200 µl. They were incubated at $37 \pm 2^\circ\text{C}$ for 24 hours. Diameter of the growth retardation zones was measured in mm, including diameter of the well. Each measurement of antimicrobial activity was performed three times. Antibacterial properties were evaluated according to the following criteria: 10 mm – no growth retardation zone, indicating that micro-organisms are not sensitive to the specimen introduced into the well; from 10-15 mm, showing weak level of sensitivity; 15-25 mm – sample sensitive; more than 25 mm – high sensitivity.

Assessment of effectiveness of antiseptics was also performed by examining microbiota of the hands of dentists before and after using antiseptic. Application on the hands was performed by applying a 3 ml of antiseptic on dry skin of the hands, followed by rubbing it into the skin for at least 30 seconds.

Hands washing was done using a sterile cotton swab pre-soaked in sterile saline before and after treatment with an appropriate antiseptic. The study involved 48 dentists, and samples were sown on differential diagnostic media, including sabouraud dextrose agar (HiMedia) for the cultivation of genus *Candida* microscopic fungi on hemolytic microflora, such as bacteria of *Streptococcus* and *Neisseria* – on blood agar, *Enterobacteriaceae* – Endo medium, and bacteria of genus *Staphylococcus* were cultured on mannitol salt agar (Biolife Italiana; Italy), bacteria of genus *Enterococcus* agar (Biolife Italiana; Italy). Identification of micro-organisms was performed using biochemical test systems, i.e., ENTERO-test, STREPTO-test, STAPHYLO-test (Erba Lachema; Czech Republic).

Each antimicrobial assay was performed at least three times. Obtained data were expressed as mean \pm standard deviation ($\times \pm$ SD) of the three measurements. Tukey's test was applied for comparisons of means, and differences were considered significant at $p < 0.05$.

RESULTS

Previous studies have demonstrated that *in vitro* experiments, the highest anti-staphylococcal effect was observed using Ecobriz antiseptic, AHD 2000, and Lysoformin 3000. It should be noted that the sensitivity of coagulase-negative staphylococci was statistically significantly higher than that of coagulase-positive (Table 2).

Therefore, the growth retardation zone while using AHD 2000 on clinical strain of *Staphylococcus aureus* was 19.0 ± 0.30 mm, and on *S. haemolyticus* it was 23.9 ± 1.0 mm. This trend was characteristic for other antiseptics. The lowest antimicrobial activity was detected with the use of chlorhexidine. Antimicrobial action of ethanol at applied dose was not observed. The highest activity among staphylococci was detected using antiseptics on *S. hominis* isolates.

A total of 8,104 isolates of bacteria of *Staphylococcus* was isolated and identified, including *S. epidermidis*, *S. aureus*, *S. saprophyticus*, *S. haemolyticus*, *S. hominis*. *S. aureus* – 26 isolates, all of them lecithinase-positive. *S. epidermidis* (8,011 isolates) was most often isolated, and 66% of *S. epidermidis* isolates were characterized by hemolytic activity.

Bacteria of *Enterobacteriaceae* family were isolated in one case, represented by one species of *Enterobacter cloacae*. No microscopic fungi were detected in any of the samples. Saprophytic micro-organisms represented bacteria of genus *Micrococcus* spp., *Bacillus* spp., and *Actinomyces* spp.

In vitro experiments established antimicrobial activity of all drugs, except for Dezoderm and Etasept. The highest level of activity against Gram-positive, Gram-negative bacteria, and microscopic fungi was found using Bacylol. High levels of antimicrobial activity against isolates were detected using AHD 2000, Lysoformin 3000, Ecobriz, and Ramidez antiseptics, with high activity of disinfectants detected against *Staphylococcus* bacteria (Table 2). During application of disinfectants on doctor's hands, the activity of antiseptics were established, except for Etasept (Table 3).

DISCUSSION

Problem of circulation of antibiotic-resistant pathogens of nosocomial infections is relevant worldwide. According to Weiner *et al.*, 4,515 hospitals reported that at least one case of HAI occurring between 2011 and 2014, and 408,151 pathogenic micro-organisms were registered from 365,490 infected patients. Fifteen groups of pathogens accounted for 87% of reported pathogens, out of which, the most common were *Escherichia coli* (15%), *Staphylococcus aureus* (12%), *Klebsiella* species (8%), and coagulase-negative staphylococci (8%). In general, the proportion of isolates with common resistance phenotypes was higher among devices associated with HAI compared to infections at surgical site. Although the percentage of resistance for most phenotypes was similar to previous reports, there was an increase in the percentage of resistance among *Escherichia coli* pathogens, especially those associated with fluoroquinolone resistance. The authors emphasized the need for constant and careful monitoring of these data within HAI types [8].

Our results indicated that the hands are a significant source of opportunistic pathogens. At the same time, the constant use of antiseptics promotes the development of resistance of micro-organisms to antimicrobial drugs. Our research has shown a predominant contamination of hands with different species of *Staphylococcus* because the culture test was used in both museum and clinical strains of different species of this genus.

There are various methods for determining antimicrobial activity of substances, including disco-diffusion,

TABLE 2. Antimicrobial activity of disinfectants against typical and clinical isolates of micro-organisms; $\bar{x} \pm SD$, $n = 30$

Test culture	AHD 2000	Lysoformin 3000	Hospisept gel	Chlorhexidine bigluconate	Ecobriz antiseptic	Famidez	Bacylol	Dezoderm	Etasept
<i>Staphylococcus aureus</i> ATCC 25923	21.0 ± 0.50 ^b	20.00 ± 0.50 ^a	8.00 ± 0.30 ^b	11.33 ± 0.33 ^b	22.8 ± 0.50 ^a	22.00 ± 1.0 ^a	34.0 ± 1.1 ^b	0.0	0.0
<i>Staphylococcus aureus</i> (clinic)	19.0 ± 0.30 ^c	19.50 ± 0.58 ^a	8.50 ± 0.58 ^b	12.33 ± 0.33 ^a	21.8 ± 0.1 ^b	21.00 ± 1.0 ^b	34.50 ± 0.75 ^a	0.0	0.0
MR <i>Staphylococcus aureus</i> (clinic)	18.50 ± 0.50 ^c	19.0 ± 0.20 ^a	8.2 ± 0.50 ^b	12.5 ± 0.50 ^a	21.5 ± 1.0 ^b	20.50 ± 0.75 ^c	35.0 ± 0.5 ^a	0.0	0.0
<i>Staphylococcus epidermidis</i> (clinic)	23.5 ± 1.0 ^a	20.5 ± 0.50 ^a	10.3 ± 0.20 ^a	13.0 ± 0.30 ^a	23.5 ± 1.50 ^a	22.00 ± 0.5 ^a	34.0 ± 0.5 ^b	0.0	0.0
<i>Staphylococcus haemolyticus</i> (clinic)	23.9 ± 1.0 ^a	20.9 ± 1.0 ^a	10.6 ± 0.50 ^a	13.5 ± 0.20 ^a	23.4 ± 0.5 ^a	22.50 ± 0.25 ^a	34.20 ± 0.20 ^b	0.0	0.0
<i>Staphylococcus hominis</i> (clinic)	24.0 ± 1.5	20.5 ± 0.30 ^a	11.0 ± 0.10 ^a	13.6 ± 0.50 ^a	23.8 ± 1.2 ^a	21.50 ± 0.5 ^b	35.0 ± 0.55	0.0	0.0
<i>Escherichia coli</i> ATCC 25922	19.50 ± 0.50 ^c	19.40 ± 0.50 ^b	7.50 ± 0.15 ^c	10.50 ± 0.50 ^c	22.20 ± 0.3 ^b	19.50 ± 0.9 ^d	32.00 ± 2.0 ^b	0.0	0.0
<i>Escherichia coli</i> (clinic)	19.20 ± 1.0 ^c	18.90 ± 0.25 ^b	7.80 ± 0.20 ^c	10.20 ± 0.30 ^c	21.70 ± 0.40 ^b	18.90 ± 1.4 ^e	32.10 ± 1.5 ^c	0.0	0.0
<i>Streptococcus pyogenes</i> ATCC 19615	19.50 ± 0.80 ^c	20.10 ± 0.15 ^a	8.10 ± 0.10 ^b	11.30 ± 0.50 ^b	21.20 ± 0.65 ^b	17.50 ± 1.2 ^f	30.00 ± 1.2 ^d	0.0	0.0
<i>Streptococcus pyogenes</i> (clinic)	19.0 ± 0.75 ^c	19.60 ± 1.00	8.00 ± 0.10 ^b	10.50 ± 0.35 ^c	21.00 ± 0.7 ^b	17.00 ± 0.15 ^f	29.00 ± 1.3 ^d	0.0	0.0
<i>Candida albicans</i> ATCC 885-653	16.70 ± 0.9 ^d	17.80 ± 0.90 ^c	7.60 ± 0.50 ^c	10.20 ± 0.50 ^c	19.30 ± 0.80 ^c	18.50 ± 1.20 ^c	28.00 ± 0.9 ^e	0.0	0.0
<i>Candida albicans</i> (clinic)	16.0 ± 0.75 ^d	17.60 ± 0.85 ^c	7.50 ± 0.30 ^c	10.30 ± 0.50 ^c	19.00 ± 1.0 ^c	18.00 ± 1.50 ^c	27.70 ± 1.0 ^e	0.0	0.0

Data were statistically significant as compared with controls ($p < 0.05$); letters indicate significantly differing zone inhibition according to Tukey's test; 0 – no inhibition

TABLE 3. Microbial contamination of dentists' hands before and after antiseptic treatment; $\times \pm$ SD, $n = 48$, microbial count

Antiseptics	Before/after processing	Total microbial count	Total number of hemolytic micro-organisms	Bacteria of genus <i>Staphylococcus</i>	Coli-form bacteria
AHD 2000	Before	56.0 \pm 1.0	0.0	9.0 \pm 0.50, <i>Staphylococcus aureus</i> 19.0 \pm 1.0, <i>Staphylococcus epidermidis</i> 24.0 \pm 2.0, <i>Staphylococcus saprophyticus</i> 4.0 \pm 0.5, <i>Micrococcus luteus</i>	0.0
	After	1.0 \pm 0.5	0.0	0.0	0.0
Lysoformin 3000	Before	39.0 \pm 2.0	0.0	9.0 \pm 0.5, <i>Micrococcus luteus</i> 20.0 \pm 0.7, <i>Staphylococcus epidermidis</i> 10.0 \pm 1.0, <i>Staphylococcus saprophyticus</i>	0.0
	After	0.0	0.0	0.0	0.0
Hospisept gel	Before	80.0 \pm 2.5	6.0 \pm 1.0	13.0 \pm 2.0, <i>Staphylococcus aureus</i> 51.0 \pm 1.5, <i>Staphylococcus epidermidis</i>	0.0
	After	0.0	0.0	0.0	0.0
Chlorhexidine bigluconate	Before	68.00 \pm 0.1		27.0 \pm 0.25, <i>Staphylococcus aureus</i> 25.0 \pm 2.0, <i>Staphylococcus epidermidis</i> 16.0 \pm 0.25, <i>Bacillus</i> spp.	0.0
	After	0.0	0.0	0.0	0.0
Ecobriz	Before	25.0 \pm 1.5 $\times 10^2$	0.0	20.0 \pm 0.50, <i>Staphylococcus aureus</i> 5.0 \pm 1.0, <i>Staphylococcus saprophyticus</i>	0.0
	After	0.0	0.0	0.0	0.0
Famidez	Before	30.0 \pm 1.5	0.0	4.0 \pm 1.0, <i>Staphylococcus aureus</i> 21.0 \pm 0.5, <i>Staphylococcus epidermidis</i> 5.0 \pm 1.0, <i>Staphylococcus hominis</i>	0.0
	After	0.0	0.0	0.0	0.0
Bacylol	Before	57.0 \pm 2.0 $\times 10^2$	0.0	49.0 \pm 4.0 $\times 10^2$, <i>Staphylococcus epidermidis</i> 2.0 \pm 2.5, <i>Staphylococcus saprophyticus</i> 5.0 \pm 1.5, <i>Micrococcus luteus</i>	0.0
	After	0.0	0.0	0.0	0.0
Dezoderm	Before	3.0 \pm 1.5 $\times 10^3$	0.0	3.0 \pm 0.5 $\times 10^3$, <i>Staphylococcus epidermidis</i>	0.0
	After	0.0	0.0	0.0	0.0
Etasept	Before	152.0 \pm 3.5	0.0	8.0 \pm 0.5, <i>Staphylococcus haemolyticus</i> 24.0 \pm 3.0, <i>Staphylococcus saprophyticus</i>	120.0 \pm 3.0
	After	70.0 \pm 2.0 <i>Bacillus</i> spp.	1.0 \pm 0.50	0.0	50.0 \pm 2.0

a method of determining minimum inhibitory concentrations, and method of diffusion into agar [6]. The chosen method of diffusion into agar allowed to objectively assess bactericidal activity of an antiseptic by areas of growth retardation. This method used was fast and not expensive.

Analyzing data from Lin *et al.*, their meta-regression assessment suggested that pooled rate of *S. aureus* contamination was lower in studies conducted in developed countries (OR = 0.664; 95% CI: 0.509-0.867; $p = 0.004$). *S. aureus* and MRSA contamination statuses of high-touch items are worrisome, and should be considered even more. Developing country status is a risk factor for

S. aureus contamination [9]. Scientists from Oman reported that antibiotic resistance to erythromycin (48%) and clindamycin (29%) was relatively high, and 9.3% of HA-MRSA isolates were vancomycin-resistant (nasal carriage, 6.6%) [10].

El Sayed in a study confirmed association between the presence of antiseptic resistance genes and resistance to different antibiotics, which may be attributed to the presence of both groups of genes on the same plasmid or to selection of resistant strains [11].

Our work showed a high level of circulation of antibiotic-resistant isolates of micro-organisms in patients

with generalized periodontitis [12]. Prospects of developing antiseptics based on natural ingredients have also been established [13]. Htun reported that antiseptic exposures were associated with carriage of *qac* genes, whereas chlorhexidine exposure was related to reduced chlorhexidine susceptibility, requiring continued surveillance for the emergence of resistance [14].

Combination of antiseptics, such as EDTA (ethylenediaminetetraacetic acid) and proteases at low concentrations, revealed a synergistic effect leading to total eradication of dense biofilms of both *Pseudomonas aeruginosa* and *Staphylococcus aureus* [15].

Our results and data from other authors indicate the need for continuous monitoring of sensitivity of microorganisms to antiseptics in a particular clinic in order to use effective safe antiseptics as well as to prevent infection of patients and physicians.

CONCLUSIONS

Experiments have shown that the surface of doctors' hands before antiseptic treatment, is mostly contaminated with various species of *Staphylococcus* bacteria, which can be a source of infection for patients. Experiments established antimicrobial activity of all drugs, except for Dezoderm and Etasept. The highest level of activity against Gram-positive, Gram-negative bacteria, and microscopic fungi was found using Bacylol, with higher activity of antiseptics against bacteria of genus *Staphylococcus* than representatives of Gram-negative bacteria. The obtained results substantiate the prospects of studying the circulation of microorganisms within clinics. The study of hands' microbiota, personal use of doctors, and microorganisms circulating in dental offices, would examine the effectiveness of disinfectants and develop local protocols for disinfection within a particular clinic.

CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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