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Original article

Characteristics of disinfectants activity against multidrug-resistant clinical isolates

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Abstract. *Background and aim:* health-care associated infections (HCAIs) significantly impair the quality and efficiency of medical care. Medical equipment's parts, which directly contact the patient's body, are contaminated with hospital strains and the patient's and medical staff's opportunistic microbiome and serve as fomites in the spreading of potential pathogens. Therefore, effective disinfection of medical equipment is one of the ways to decrease risks and the incidence of healthcare-associated infections (HCAIs). The susceptibility of hospital isolates to germicides, provided in medical practice, should be monitored for effective control of hospital infections. The aim of the research was to study the susceptibility to biocides with different chemical structures against multi-resistant clinical isolates' from hospital environment. *Methods:* in the research there was carried out the investigation of antimicrobial activity of biocides of different chemical origin (aqueous solutions of quaternary ammonium compounds, biguanides, oxidizers), widely used in medical practice as disinfectants for hospital environments, devices, and equipment. Multidrug-resistant clinical isolates with genetic markers of resistance, associated with respiratory infections or/and contaminated respiratory support equipment, were employed for comparative evaluation of ready-in-use germicides. Next microorganisms were used as test ones: clinical strains of methicillin-resistant *S. aureus* carrying *mec* A genes, *E.faecalis* carrying *aac(6')-aph(2"), lsa(A*) and tet(M) genes, *E.coli* carrying $bla_{\text{OXA-48}}$ gene, carbapenemase-producing *K. pneumoniae* carrying $bla_{\text{OXA-48}}$ gene, *A. baumannii* with $bla_{\text{OXA-72}}$ gene, metallobetalactamase-producing *P. aeruginosa* carrying NDM-1 gene, strains of enterobacteria and non-fermenting Gram-negative bacteria with a wide range of genes responsible for the production of aminotransferases that destroy aminoglycoside molecules. A quantitative suspension test was used to assess the activity of the tested biocides on some species of resistant microorganisms. The experimental study also includes antimicrobial efficacy testing of the disinfectant solutions, which was carried out on artificially contaminated with multidrug-resistant clinical isolates fragments of polymer tubes of the respiratory circuit. *Results:* Gram-negative and Gram-positive non-spore-forming bacteria demonstrated a variable resistance to the studied germicides. Utilizing the quantitative suspension method we found that antibiotic-resistant *S. aureus* and *E. faecalis* were sensitive to all studied biocides with their total killing effect within 3 min. Multi-resistant strains of the *Enterobacteriaceae* family showed higher level of resistance to both detergent and oxidizing types of biocides as the time, needed to kill bacterial cultures was 5- 10 min for chlorhexidine bigluconate (HB), hydrogen peroxide, 15 min for 0.2% solution of 1,3 - dichlor 5.5 - dimethylhydantoin (DMH) solution and reached 30 min in case of polyhexamethyleneguanidine phosphate (PGMG). The time required to reduce by 5 logs the number of viable *Klebsiella* cells was less than 10 min in solutions of H_2O_2, HB, and DMH, and less than 5 min in 0,05% solution dexamethoxine (DN), but 20 min for PGMG. Disinfecting effect against *P. aeruginosa* was registered after longer exposition time (15 min for DN, 20 min for hydrogen peroxide, 30 min for HB, 45 min for PGMG, DMH). Artificially

contaminated with tested isolates respiratory circuit tube pieces were disinfected after 80 sec-33 min' exposure to biocide solutions depending on type of colonizer and chemical structure of germicide. The *S. aureus* and *E. faecalis* isolates were destroyed by most biocides in up to 2 minutes on tube samples, except DMH solution. Investigated isolates of enteric bacteria as well as *A. baumannii* were killed in 5-6 minutes by DN and H_2O_2, but other biocides disinfected samples in 16-32 minutes. Complete inactivation of *P. aeruginosa* cells on solid phase took at least 11 minutes when DN was applied and at most 41 minutes when PGMG was used. *Conclusions*: The obtained results demonstrate that antibiotic-resistant clinical isolates of common causes of HCAI remain susceptible to such widely-used biocides as non-alcoholic quaternary ammonium compounds, biguanides, oxidizers, chlorine-containing compounds. Gram-positive cocci could be effectively destroyed or declined to minimal number on contaminated surfaces in 3 minutes by investigated biocides, while enterobacteria and non-ferments were inhibited or killed after 3-10 times longer exposure depending on microorganism type and used biocide. Best antimicrobial activity against all clinical isolates was demonstrated by 0,05% DN, 0,05% HB, and 3% hydrogen peroxide. Regarding to results the recommendations for aseptic measures in ICU can be done according to chemical structure of used biocide. (www.actabiomedica.it)

Key words: health-care-associated infections, multidrug antibiotic resistance, sensitivity to antiseptics and disinfectants

Introduction

Health-care associated infections (HCAIs) have become a global emergent socio-economic problem. According to the World Health Organization (WHO), in countries with low medical care, the frequency of such complications exceeds 25% of all inpatients. The effective management of HCAI depends on the quality of means, methods, and modes of disinfection measures in the hospital environment (1,2).

By mortality data, hospital-acquired pneumonia (HAP) takes second place in the structure of HCAI in critically ill patients in countries with mandatory registration of this pathology in healthcare institutions. The etiological structure of HAP includes a wide range of bacterial species of both endogenous and exogenous origin, among which *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis,* coagulase-negative staphylococci dominate as the cause of early HAP. Late HAP, which arises over the 6th day of hospitalization, is quite often caused by Gram-negative and Gram-positive bacteria with increased pathogenic potential and multidrug resistance, namely: *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter* spp., *Enterococcus* spp., which belong to the critical priority group for which new antibiotics are needed (3, 4, 5). Patients in intensive care units (ICU) are at risk of respiratory nosocomial infections (17-22%), and in the case of providing invasive respiratory support, the risk increases from 18% to 60%, depending on the duration of mechanical lung ventilation, patient age, nosology of the disease, the profile of the department and many others reasons (6,7).

In the current conditions of the pandemic spread of the SARS-CoV-2 virus and the growing number of ICU inpatients that need respiratory support, the problem of prevention of respiratory opportunistic and nosocomial infections is exacerbated by new challenges. The severe course of the underlying disease is often complicated by bacterial co-infections, the etiological agents of which can be hospital isolates with multiple antibiotic resistance (8-11).

The parts of medical equipment for invasive and non-invasive respiratory support often become a reservoir of potentially dangerous microorganisms. Opportunistic microorganisms are isolated from face masks, nasal catheters, endotracheal tubes, humidifier fluid, the inner and outer surface of the respiratory circuit, the connector, etc. (12,13). Such bacteria are able not only to adhere but also form biofilms on abiotic surfaces, which plays not the last role in the formation of

exogenous infection sources in the hospital environment. It is shown that biofilms begin forming on the inhalation tube's surface several hours after mechanical ventilation has been started. Being located within the structure of the mature biofilm, microorganisms are protected from the antimicrobial host defence mechanisms and antibiotics by the glycocalyx layer, in addition, a certain part of the bacterial consortium is in a state of low metabolic activity, which allows it to avoid the harmful effects of antimicrobial substances (14-17).

In the current epidemiological situation, it is important to consider that human coronaviruses, falling on plastic surfaces, from which most elements of medical equipment are made and which come into contact with the patient's body, retain their infectious properties for up to 9 days (18).

Sterilization and disinfection are aseptic measures, which are provided in the hospital environment. They allow to prevent the spreading of the hospital isolates, which can colonize hospital equipment and medical devices and cause healthcare-associated infections (HCAI), including HAP. Such chemical groups of biocides as aldehydes, phenols, chlorine-containing compounds, and alcohols are highly effective and used for disinfection and pre-sterilization antimicrobial treatment. However, the local and general toxic effects of disinfectants on the patient's body should be considered, when biocides are planned to use on contact medical devices when medical care is provided (19-21).

Some biocides from the group of oxidizing agents, quaternary ammonium compounds, and biguanide derivatives are increasingly used in the antimicrobial protection of medical devices, which are needed to provide respiratory support. Compared to phenol derivatives, aldehydes, and alcohols they have a less irritating and toxic effect on the living tissues. In addition, most of the latter do not have a destructive effect on the constructive materials of medical equipment (22-24). However, the antimicrobial effectiveness of some of these compounds has recently been found to have defects that threaten the formation of unexpected reservoirs of nosocomial pathogens. Especially since there are more and more frequent reports of the spread of hospital strains resistant not only to antibiotics but also to antiseptics and disinfectants (25-27)

Control of drug-resistant bacteria isolates in medical institutions remains an urgent problem of medicine and requires additional research into the ready-in-use disinfectants, studying the effectiveness of new means to update and improve practical guidelines on measures, which are aimed at the management of infections associated with health care.

Our work aimed to compare the antibacterial activity of non-alcohol biocides with different chemical structures, which are used in medical practice for the disinfection of medical devices, including those used in the process of providing respiratory support, against clinical strains with multiple drug resistance to antibiotics.

Materials and methods

The following solutions and chemical compounds known for use as disinfectants were used in the study: 0.05% aqueous solution of chlorhexidine bigluconate (HB); 0.1% aqueous solution of polyhexamethyleneguanidine phosphate (PGMG); 0.05% aqueous solution of decamethoxine ([1,10-decanediaminium,N,N,N',N'-tetramethyl-N,N-bis((2-methyl-5-(1-methylethyl)cyclohexyl)methyl)- dichloride] – a cationic surface-active antiseptic of bis-quaternary ammonium compounds (DN); 3% hydrogen peroxide solution; 0.2% aqueous solution of a commercial disinfectant, which contains chlorine-containing biocide 1,3 - dichlor 5.5 - dimethylhydantoin in the amount of 21.5-24% by weight (content of active chlorine is not less than 14%), (DMH) as the main active ingredient.

The microorganisms were isolated from the details of respiratory support equipment and the ICU inpatients with hospital respiratory infections in medical institutions of Ukraine (the total number of isolates was 157) within 2018-2022. The hospital isolates of the most common species, causing HCAI, namely: *S. aureus, Enterococcus faecalis, Escherichia coli, K. pneumoniae, P. aeruginosa,* and *A. baumannii*, were investigated for susceptibility to antimicrobials. Antibiotic susceptibility of clinical isolates was detected by the disc diffusion method (28) and evaluated according to the EUCAST recommendations (2021). For the experiment there were selected only isolates, which were assigned to the multidrug-resistant (MDR) group

after being detected by SOLiD system of generation sequencing as carries of resistance genes and compared by BLAST technology their nucleotide sequences with data of GenBank® of National Center for Biotechnology Information (USA) (29,30). Finally, 42 isolates of six main species were chosen for investigation.

Every tested isolate of *S. aureus* was resistant to penicillin, ampicillin, ceftriaxone, erythromycin, and tetracycline, but susceptible to vancomycin, linezolid, rifampicin, three of them were resistant additionally to ciprofloxacin and levofloxacin. Three isolates were resistant to oxacillin and identified as methicillin-resistant *S. aureus*. Isolates were carries of 2-5 resistance genes, which allowed to predict resistance to aminoglycosides (*aac(6')-aph(2")*), β-lactams (*mecA, bla Z*), macrolides (*erm(C)*), tetracyclines (*tet(K)*), and quinolones (*norA*).

Every tested isolate of *E. faecalis* was resistant to oxacillin, ceftriaxone, clindamycin, tetracycline, but susceptible to vancomycin, linezolid, ampicillin-clavulanic acid, some of them demonstrated resistance to ciprofloxacin, levofloxacin, erythromycin, trimethoprim-sulfamethoxazole, gentamycin, rifampicin. *E. faecalis* isolates were carries of 2-6 resistance genes, which allowed to predict resistance to aminoglycosides (*aac(6')-aph(2"), ant(6)-Ia, and aph(3')-III*), macrolides (*erm(B) and lsa(A))*, and tetracyclines (*tet(M), tet(O), dfrG*).

Every tested isolate of *E. coli* was resistant to ampicillin, ceftriaxone, cefazolin, erythromycin, gentamycin, some of them keep susceptibility to ampicillin-clavulanic acid, imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin, tobramycin, tetracycline, trimethoprim-sulfamethoxazole. *E. coli* isolates were carries of 2-7 resistance genes, which allowed to predict resistance to aminoglycosides (*aac(6')Ib-cr), ant(2")-Ia, and aph(6)-Id*), β-lactams (*bla OXA-48, bla SHV-11, bla SHV-36, bla TEM-1B*), sulfonamides (*sul2*).

Every tested isolate of *K. pneumoniae* was resistant to ampicillin, ampicillin-clavulanic acid, ampicillin-sulbactam, ceftriaxone, cefazolin, ceftazidime, cefepime, erythromycin, ciprofloxacin, levofloxacin, some of them were susceptible to imipenem, meropenem, amikacin, tobramycin, tetracycline, trimethoprim-sulfamethoxazole. *K. pneumoniae* isolates were carries of 4-16 resistance genes, which allowed to predict resistance to aminoglycosides (*aac(6')Ib-cr), ant(3")-Ia, ant(2")-Ia, and aph(6)-Id*), β-lactams (*bla OXA-1, bla OXA-48, bla SHV-11, bla SHV-36, bla CTX-M-15, bla TEM-1B*), sulfonamides (*sul1, sul2*), trimethoprim (*dfrA1*4),and low level resistance to quinolones (*oqxA, oqxB*).

Chosen for testing isolates of *P. aeruginosa* were resistant to ampicillin-clavulanic acid, ampicillin-sulbactam, ceftriaxone, ceftazidime, levofloxacin, tetracycline, erythromycin, trimethoprim-sulfamethoxazole. Every isolate remained susceptible to polymyxin. Susceptibility to imipenem, meropenem, piperacillin-tazobactam, amikacin, tobramycin varied in tested strains. *P. aeruginosa* isolates were carries of 5-13 resistance genes, which allowed to predict resistance to aminoglycosides (*aac(6')-Ib, aacA4, aadA1, aadB, aph(3')Ic, aph(3')VIa, aph(3')VIb, and aph(6)-Id*), β-lactams (*bla OXA-10, bla OXA-50, bla PAO, bla IPM-34, bla SHV-36, bla CTX-M-15, bla TEM-1B*), and quinolones (*qnrVC*I).

Isolates of *A. baumannii* were resistant to ampicillin, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, levofloxacin, gentamycin, and trimethoprim-sulfamethoxazole. Two of them were susceptible to ampicillin-clavulonic acid, ampicillin-sulbactam, imipenem, meropenem, tobramycin, amikacin; every tested isolate was susceptible to polymyxin. *A. baumannii* isolates were carries of 2-7 resistance genes, which allowed to predict resistance to aminoglycosides (*aac(3')-Ia, aac(6')-Ib, ant(3")-Ia, ant(2")-Ia, aph(3')VIa, aph(3')VI*b, and *aph(6)-Id*) and β-lactams (*bla ADC-25, bla GES-11, bla OXA-100, bla OXA-66*).

Average exposure time needed for total microorganism inactivation was defined, for each bacterial species to test, by inoculation of a bacterial suspension, in number 5×108 CFU/ml in a germicide solution (in volume correlation 1:10). Probes of 0.2 ml were taken off from suspensions every next minute, both for Gram-negative and Gram-positive isolates tested, and added to neutralizer solution (1.8 ml). Subsequently, 0.5 ml of the mixture was re-inoculated in 4.5 ml of liquid medium (tryptone-soy broth). Depending on the chemical structure of tested germicide we used next neutralizers: 0.5% solution of fresh egg yolk for surface-active disinfectants (HB, PGMG, DN), and 0,5% sodium thiosulfate for oxidizing germicides (H_2O_2, DMH). After overnight incubation,

bactericidal activity of the biocide solution was detected by minimal exposure after which bacterial growth had not been apparent in the culture medium.

The activity of disinfectants was studied according to standard methods of antiseptic and disinfectant solutions research by the requirements of European standards (31).

A quantitative suspension test was used to assess the specific effect of the biocides on isolates of different species. The test was performed on 7 clinical isolates of the same species.

Bacterial suspension was prepared with a control of bacterial concentration regarding to 5 units by McFarland standard employing densitometer DEN-1 indicator, which corresponded $1,5\times10^9$ CFU/ml. To evaluate the antimicrobial activity, the initial suspension was triple diluted to get a concentration of 5×10^8 CFU/ml, which was added in the amount of 1 ml to 9 ml of the germicide solution, kept for a certain period (from 5 to 60 min with the interval of 5 min, depending on detected bactericidal exposure for certain species), then added 1 ml of the mixture in 9 ml of appropriate neutralizer solution, prepared a dilution of the mixture in an isotonic solution of 10^{-1}, 10^{-2} and 10^{-3}.

Dilution of the mixture in 0.5 ml was inoculated on a nutrient medium (tryptone-soy agar) with the pour-plate technique. The inoculated plates were incubated for 20-24 hours and the number of grown colonies was counted. Recalculation of the average number of viable microorganisms in 1 ml (CFU/ml) was carried out considering the number of colonies that grew on the surface of the nutrient medium and dilutions of the initial bacterial suspension during the research according to the formula:

$$N_{avg} = 2 \times \frac{(N_1 x\ 10 + N_2 x\ 10^2 + N_3 x\ 10^3)}{3} \times 10^2, \text{ where}$$

N_{avg} – the average amount of CFU in 1 ml; N_1, N_2, N_3 – the number of colonies that grew on the nutrient medium after inoculating a certain dilution of the mixture.

At the same time, initial bacterial suspension (control) in 1 ml was added to 9 ml of an isotonic solution, and then tenfold successive dilutions of the suspension were prepared. Dilutions 10^{-6}, and 10^{-7} were inoculated on nutrient agar for counting. After daily incubation, the number of grown colonies on the plates was counted and the arithmetic mean value of the control CFU/ml was calculated considering dilutions and the amount of inoculate. The control count was used as the primary bacterial load to compare with data, which we got after biocides action at different exposure.

The obtained average values of the number of microorganisms were transformed into decimal logarithms (log) to compare the reduction of microorganisms' number after contact with a certain disinfectant for a definite period. The data for 7 isolates depending on the species were processed with statistical one-way analysis of variance (ANOVA) in SPSS to compare all results means to determine whether there was statistical evidence of the activity of all studied disinfectants against all studied species of MDR bacteria.

Disinfecting activity on the contaminated surface was studied on artificially contaminated with tested isolates fragments of polymer tubes 1 cm long, which are parts of the respiratory circuit. Sterile fragments of polymer tubes were immersed in a bacterial suspension in the concentration of 5×10^8 CFU/ml, prepared from the daily culture of the tested strain for 60 min, and then dried in a thermostat for 30 min. Contaminated samples were placed in a disinfectant solution every next minute for a period 1-5 minutes for Gram-positive isolates and 5-60 minutes for Gram-negative ones regarding to results of qualitative assay.

After exposure, the treated tubes were rinsed in a sterile saline solution, placed in a neutralizer solution (10 ml of 0.5% solution of fresh egg yolk for surface-active disinfectants, and 10 ml of 0,5% sodium thiosulfate for oxidizing germicides) for 5 min to inactivate the residual disinfectant action, and transferred to a liquid nutrient medium (tryptone-soy broth) for further daily incubation with to control the effectiveness of disinfection. The absence of growth signs in the nutrient medium indicated the destruction of microorganisms and effective disinfection.

Results

The quantitative suspension method was used to evaluate the effectiveness of biocides, find effective

Table 1. Disinfecting exposure time in suspension test for different biocides.

	Biocide				
	0.05% HB (1)	**0.1% PGMG (2)**	**0.05% DN (3)**	**3% H_2O_2 (4)**	**0.2% DMH (5)**
Type of Bacteria	**Exposure to the Complete Death of Bacteria (min.)**				
S. aureus	1.29 ± 0.49 □4;□5	1.14 ± 0.38 □4;□5	1.14 ± 0.38 □4;□5	2.71 ± 0.49 ●5;□1;□2;□3	3.29 ± 0.49 ●4;□1;□2;□3
E. faecalis	3.29 ± 0.49 ●3;□4	3.14 ± 0.38 ●3;□4	2.57 ± 0.53 ●1;●2;●5;Δ4	1.43 ± 0.53 Δ3;□1;□2;□5	3.14 ± 0.38 ●3;□4
E. coli	10.71 ± 0.95 □2;□3;□5	29.29 ± 0.95 □1;□3;□4;□5	4.57 ± 0.53 □1;□2;□4;□5	10.43 ± 0.79 □2;□3;□5	15.00 ± 0.82 □1;□2;□3;□4
K. pneumoniae	13.86 ± 0.69 □2;□3;□4;□5	39.14 ± 1.21 □1;□3;□4;□5	5.57 ± 0.79 □1;□2;□4;□5	16.14 ± 1.21 □1;□2;□3;□5	19.71 ± 1.25 □1;□2;□3;□4
P. aeruginosa	31.43 ± 1.62 □2;□3;□4;□5	44.0 ± 1,15 □1;□3;□4	10,86 ± 0,9 □1;□2;□4;□5	30.29 ± 0.76 □2;□3;□5	43.0 ± 1,73 □1;□3;□4
A. baumannii	14.00 ± 0.82 □2;□3;□4;□5	29.43 ± 0.79 Δ5;□1;□3;□4	5.71 ± 0.76 □1;□2;□4;□5	19.29 ± 0.95 □1;□2;□3;□5	31.57 ± 1.51 Δ2;□1;□3;□4

Notes: ● 1 – 0.05% chlorhexidine bigluconate (HB); 2 – 0.1% polyhexamethyleneguanidine phosphate (PGMG); 3 – 0.05% decamethoxine (DN); 4 – 3% hydrogen peroxide ; 5 – 0.2% 1,3 - dichlor 5.5 - dimethylhydantoin (DMH); ● – $p<0.05$; Δ – $p<0.01$; □ – $p<0.001$ (the difference between tested disinfectants).

modes of object disinfection, and allowed to study the dynamics of the microbial population death in the antimicrobial solutions and determine the time of exposure needed for the destruction of all microbial cells, inoculated into the biocide solution. There were found the following data, characterizes the exposures of disinfection effect of the solutions of the studied biocides against polyresistant to antibiotic strains of six bacteria species, as the most frequent pathogens of HCAI. At the same time, differences between clinical isolates in sensitivity to biocides were not significant (Table 1).

Experimental data, presented in Table 1, indicate that polyresistant to antibiotics clinical strains of Gram-positive cocci were sensitive to the action of biocides. For the complete death of *S. aureus* and *E. faecalis* cells, contact exposure above 3 minutes was sufficient both with solutions of detergent biocides and biocides with an oxidizing mechanism of action.

Multi-resistant strains of the *Enterobacteriaceae* family generally showed a significantly higher level of resistance to biocide solutions than Gram-positive cocci. At the same time, among guanidine derivatives, the 0.05% chlorhexidine solution had an advantage compared to the 0.1% PGMG solution. After all, the time of exposure, needed to kill all the *E. coli* isolates in the PGMG solution reached 29.29 ± 0.95 min, which was three times longer to cause the same effect in the HB solution ($p<0.001$). The death of *E. coli* in a 3% solution of hydrogen peroxide and a 0.05% solution of HB was registered after 10 minutes' exposure, and in a 0.2% solution of chlorine-containing DMH – after 15 minutes' one ($p<0.001$). Clinical isolates of *K. pneumoniae* were more resistant to the above-mentioned biocides, compared to *E. coli.* Although the difference was insignificant and the exposure to kill *Klebsiella* was greater by 5-10 min ($p>0.05$). There was found the best effect of 0.05% solution of the quaternary ammonium compound decamethoxine on enterobacteria, in the result the effective exposure of which effect on the cells of both types of bacteria was about 5 minutes.

Representatives of the non-fermenting Gram-negative bacteria demonstrated the highest resistance to biocides. The death of *P. aeruginosa* cells in solutions of PGMG and the chlorine-containing preparation DMH was observed alike only after 44.0±1,15 min and 43.0±1,73 min of impact exposition respectively without any significant difference ($p>0.05$). It took 30 min to destroy pseudomonads with a solution of HB.

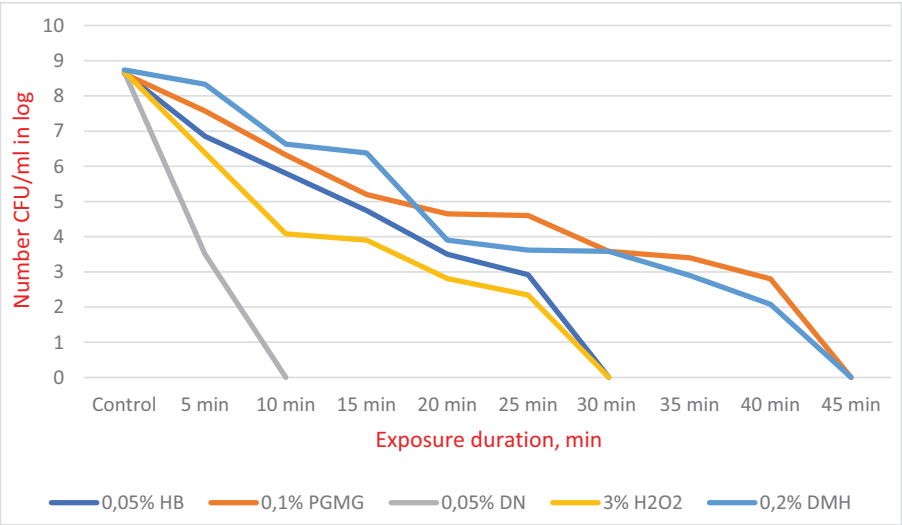

Figure 1. The dynamics of the quantity decrease of *P. aeruginosa* depending on the duration of contact with various disinfectant solutions.

The action of quaternary ammonium derivative DN on the cultures of *P. aeruginosa* was much more effective than hydrogen peroxide, – killing exposure was 10,86 ± 0,9 min and 30.29 ± 0.76 min, relatively ($p<0.001$). It is worth noting that the period of destruction of pseudomonad cells in all tested disinfectant solutions was the longest compared to all other types of bacteria used in the experiment. Other representatives of non-fermenting Gram-negative bacteria such as *A. baumannii* were close to those of enterobacteria in terms of most indicators of the death rate in the tested disinfectant solutions.

Using the quantitative suspension test, the effectiveness of disinfectants can be evaluated by the rate of reduction of the microbial population in the solution, calculating it in decimal logarithms (logs) [24]. In Figures 1 and 2 the rate of microorganism suspension reduction, which showed the highest tolerance to the influence of the studied biocides.

The reductions of the population of pseudomonads till 4 logs in 0.05% HB solution took less than 20 minutes and 10 minutes in 3% H2O2. At the same time, the total destruction of cells in those biocides occurred in the same period after 30 min ($p>0.05$). While using a 0.1% PGMG solution and 0.2% DMH solution it took above 30 minutes to reach the same effect in the number of *P. aeruginosa* cells with their total killing after 45 minutes. The fastest destruction of 4 logs of those microorganisms occurred in 0.05% DN solution (above 5 min) in comparison with others ($p<0.001$).

The rate of reduction of *Klebsiella* suspension in disinfectant solutions was significantly higher compared to pseudomonads ($p<0.001$). The contact exposure time to reach complete inactivation of viable cells was 15 min for 3% H2O2 and 0.05% HB and 20 minutes for 0.2% DMH solutions. In DN solution time needed was 5 min ($p<0.001$). And in the PGMG solution, this data was significantly higher than others and reached 40 min of impact exposure ($p<0.001$).

To approximate the results of the experimental research to the conditions of practical use of disinfectant solutions, we conducted a series of experiments to determine the speed of the respiratory circuit polymer tube fragments disinfection, artificially contaminated with cultures of various bacteria, by the method of immersion in disinfectant solutions. The obtained results are summarized in Table 2.

Analysis of the data in Table 2 shows that the results of research by immersion method of artificially contaminated test objects generally repeat the patterns noted during research using the suspension method, shown in Table 1. It should only be noted that the

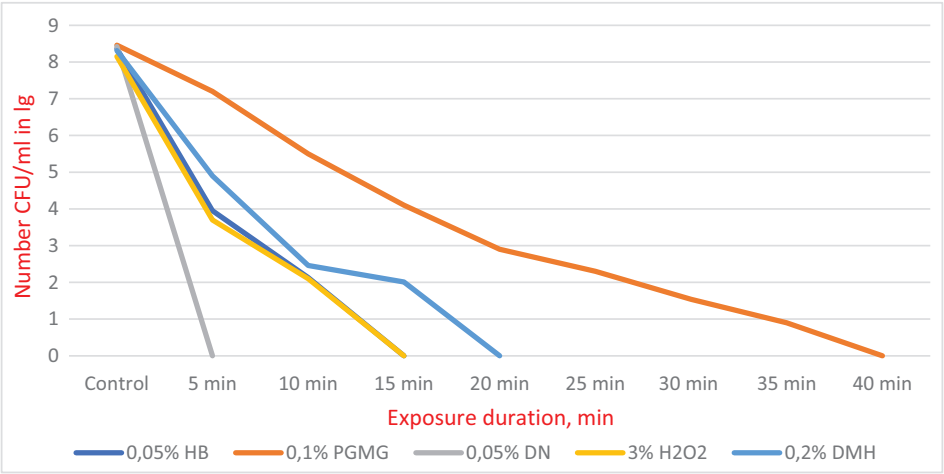

Figure 2. The dynamics of the quantity decrease of *K. pneumoniae* depending on the duration of contact with various disinfectant solutions.

Table 2. Disinfecting effect of disinfectant solutions on artificially contaminated polymer test objects by the immersion method.

Type of Bacteria	Biocide				
	0.05% HB (1)	**0.1% PGMG (2)**	**0.05% DN (3)**	**3% H_2O_2 (4)**	**0.2% DMH (5)**
	Decontamination Exposure (min)				
S. aureus	1.29 ± 0.49 □4;□5	1.43 ± 0.53 Δ4;□5	1.14 ± 0.38 □4;□5	2.57 ± 0.53 ●5;Δ2;□1;□3	3.14 ± 0.38 ●4;□1;□2;□3
E. faecalis	1.14 ± 0.38 □5	1.29 ± 0.49 □5	1.14 ± 0.38 □5	1.43 ± 0.53 □5	3.29 ± 0.49 □1;□2;□3;□4
E. coli	10.29 ± 0.49 □2;□3;□4	20.86 ± 0.90 □1;□3;□4;□5	4.71 ± 0.49 ●4;□1;□2;□5	5.43 ± 0.53 ●3;□1;□2;□5	10.86 ± 0.90 □2;□3;□4
K. pneumoniae	14.86 ± 0.38 ●5;□2;□3;□4	31.00 ± 0.82 □1;□3;□4;□5	5.71 ± 0.76 □1;□2;□5	5.86 ± 0.90 □1;□2;□5	15.86 ± 0.90 ●1;□2;□3;□4
P. aeruginosa	21.57 ± 1.27 □2;□3;□4;□5	31.14 ± 0.90 □1;□3;□4;□5	10.71 ± 0.49 □1;□2;□4;□5	15.00 ± 0.82 □1;□2;□3;□5	40.86 ± 0.69 □1;□2;□3;□4
A. baumannii	15.14 ± 0.38 ●4;□2;□3;□5	30.29 ± 0.49 □1;□3;□4	5.14 ± 0.38 □1;□2;□4;□5	15.86 ± 0.69 ●1;□2;□3;□5	30.71 ± 0.49 □1;□3;□4

Notes: 1 – 0.05% chlorhexidine bigluconate (HB); 2 – 0.1% polyhexamethyleneguanidine phosphate (PGMG); 3 – 0.05% decamethoxine (DN); 4 – 3% hydrogen peroxide ; 5 – 0.2% 1,3 - dichlor 5.5 - dimethylhydantoin (DMH); ● – $p<0.05$; Δ – $p<0.01$; □ – $p<0.001$; (the difference between tested disinfectants).

exposures of disinfection of test objects in most cases are somewhat smaller than those of complete bacterial cell death when using the suspension method. Thus, if the death of all *P. aeruginosa* cells in 0.05% HB in the suspension test was achieved after 30 min, then the disinfection of test objects contaminated with the same type of bacteria occurred in 21.57±1.27 min. This can be explained by the difference in the microbial load because, during the period of polymer test objects with microorganisms' artificial contamination, the number of bacterial cells adhering to the surface is relatively small.

Discussion

The main requirements for disinfectants are high antimicrobial activity, a wide spectrum of antimicrobial

activity, a quick inactivating effect on the most frequent contaminants of equipment and products, the absence of toxic effects in the case of drug residues entering the human body during the use of disinfected products (32, 33). Regularly using such means for sanitizing medical devices that come into contact with the patient's body is the key to avoiding spreading dangerous pathogens inside hospitals (34).

We have conducted a study of the disinfectant efficacy of a list of drugs that have undergone all necessary levels of expertise for compliance with requirements and in many countries have practical use. The peculiarity of our study is that in determining the indicators of disinfection activity of biocides using standard methods, instead of recommended by European standards museum reference bacteria, we used clinical polyresistant to antibiotics strains of the most common in the present-day pathogens associated with the provision of medical care. Among them, there were methicillin-resistant *S. aureus* carriers of *mec* A, carbapenemase-producing strains of *K. pneumoniae* as carriers of the genes *bla*OXA-48, *A. baumannii* – *bla*OXA-72, *P. aeruginosa*, carrying genes responsible for the production of aminotransferases, which could destroy the molecules of the aminoglycoside series antibiotics (35, 36).

The results of experimental studies of non-absorbent solutions of five different chemical structure biocidal substances showed their biocidal effects against all the clinical isolates of different bacteria used in this study. It should be noted, that in the study there were used solutions with only one concentration of each substance within the range of concentrations used in practice according to manufacturers' instructions. At the same time, the selected concentrations were approximate to the minimum or average of the recommended range.

In analysing the obtained results, attention was drawn to a significant difference in the sensitivity to the action of the Gram-positive and Gram-negative bacteria tested solutions. Antibiotic-resistant strains of Gram-positive non-spore-forming bacteria showed a high level of sensitivity to the action of disinfectant solutions and died no more than 3 min after the exposure to biocides according to the testing results. The exposure of the studied biocides' solutions, providing bactericidal effect on Gram-negative bacteria, was higher and varied against different species of tested microorganisms.

Hydrogen peroxide is known to have certain limitations in its use as a disinfectant due to its rapid neutralization by organic material and its ability to damage metal structural elements of medical equipment. Meanwhile, solutions of this compound with an oxidizing mechanism of action on microorganisms are admitted by most authors as an effective medicine for the struggle of a wide range of pathogens of HCAI (37, 38). The results obtained in our study show a sufficiently high level of disinfecting activity of a 3% hydrogen peroxide solution against polyresistant antibiotic clinical strains of bacteria. Population reduction by 5 logs CFU/ml of all used in the research strains of pseudomonads, which obtained the highest level of resistance to the studied disinfectants, occurred during no more than 20 min. However, for the complete disinfection of the artificially contaminated cultures of the pseudomonads polymer test sample, there was enough to immerse one in a solution for 15 min.

Non-fermenting Gram-negative bacilli *P. aeruginosa* and *A. baumannii* are known for their high resistance to biocides as biguanides and quaternary ammonium compounds (39). The data we obtained confirm their properties, because, for the reduction of the population of *P. aeruginosa* by 5 logs CFU/ml in a 0.1% solution of PGMG in a suspension test, an exposure of more than 30 min was required, but total inactivation happened 45 minutes after bacterial inoculation in this biocide. However, solutions with a wide range of PGMG concentrations from 0.1% to 4% are used as antiseptics and disinfectants. Therefore, it is impossible to deny the practical effectiveness of this disinfectant considering our research data for its minimum concentration in this range.

The 0.05% HB solution showed a higher level of disinfecting activity than the 0.1% PGMG solution ($p<0.001$). Thus, the killing exposure for *P. aeruginosa* was significantly shorter and equal to 30 minutes ($p<0.001$), while the reduction time by 5 logs CFU/ml was the same (20 minutes). The same exposure was sufficient for completely eliminating pseudomonads from artificially contaminated fragments of respiratory circuit tubes by their injection in a solution of HB. Although, the high disinfecting activity of HB solutions of a much higher concentration (0.5%) was previously reported (40).

The highest level of disinfecting activity among the solutions we tested was found in a 0.05% solution of the quaternary ammonium compound decamethoxine. A 5-minute exposure was enough to inactivate multidrug-resistant bacterial strains of *E. coli*, *A. baumannii*, and *K. pneumoniae*. Decontamination of polymer samples artificially contaminated with pseudomonads was complete after 10 min of exposition in the solution, and in case of infection with acinetobacteria or klebsiellae, after 5 min. The obtained results indicated the promising use of this drug to disinfect medical equipment, including that used to provide respiratory support. Moreover, that biocide was known to have a virucidal effect against complex viruses, including coronaviruses, and is low toxic for the human body (41).

A commercial disinfectant solution based on the chlorine-containing compound 1,3-dichloro-5,5-dimethylhydantoin provided a reduction in the number of *P. aeruginosa* by 5 log CFU/ml in the quantitative suspension test after 30 minutes' exposure, and disinfection of polymer objects artificially contaminated with the corresponding type of bacteria after 40 minutes' exposure. According to the manufacturer's instructions, exposing polymer medical products to 0.2% solution of this biocide is recommended for 30-60 min. The recommended regime may be ineffective, as in our research we used bacterial plankton forms for studying disinfectant efficacy. In practical use, there may be incidence of biofilm formation, for the eradication of which much higher concentrations and longer biocide exposition is required.

Conclusion

Multidrug-resistant clinical isolates of common causes of HCAI show a sufficiently high level of sensitivity to non-alcoholic solutions of biocides such as quaternary ammonium compounds, biguanides, oxidizers, chlorine-containing compounds, which are used in medical practice nowadays. However, it is necessary to monitor routinely the level of such sensitivity and the effectiveness of medical equipment disinfection recommended modes due to the possibility of the resistant isolates' appearance.

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