THE RESEARCH OF ANTIMICROBIAL ACTIVITY OF ANTISEPTICS AGAINST MULTIDRUG RESISTANT *KLEBSIELLA PNEUMONIAE* ISOLATED FROM INFECTED WOUNDS OF PATIENTS WITH COMBAT TRAUMA

Výskum antimikrobiálnej aktivity antiseptík proti multirezistentnej *Klebsiella pneumoniae* izolovanej z rán pacientov s bojovou traumou

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Abstract
The aim of this work was to study the antimicrobial activity of antiseptics against planktonic bacterial forms and biofilm formation of multidrug-resistant (MDR) clinical strains of *K. pneumoniae* isolated from infected wounds of patients with combat trauma during the war in Ukraine.
Materials and methods. The activity of antiseptics was determined against clinical antibiotic-resistant strains of *K. pneumoniae* (n=34). Clinical strains were isolated from patients with infectious complications of combat burn and shrapnel wounds of different localization, received during war conflict in Ukraine in the period since 24 of February to April of 2022.
Results. Determining the activity of antiseptics against *K. pneumoniae* in planktonic form, high efficiency of the main surfactant active antiseptics was revealed. The highest activity against clinical strains of *K. pneumoniae* among the studied antiseptics from the group of quaternary ammonium compounds (QAC) was found in decamethoxine, miramistinand octenidine. IAA (index of antiseptic activity) values were highest (IAA > 4) for the antiseptics decamethoxine (0.1 %), octenidine (0.1%), and polyhexanide (0.1 %).
Conclusions. Determination of anti-biofilm forming activity showed that sub-bacteriostatic concentrations of chlorhexidine, decamethoxin, miramistin, octenidine, polyhexanide and povidone-iodineinhibited the formation of *K. pneumoniae* biofilms, but decamethoxine, polyhexanide and povidone-iodine had exhibited the most pronounced effect.

Abstrakt
Cieľom tejto práce bolo zistiť antimikrobiálnu aktivitu antiseptík proti planktónnym bakteriálnym formám a tvorbu biofilmu multirezistentných (MDR) klinických kmeňov *K. pneumoniae* izolovaných z infikovaných rán pacientov s bojovou traumou počas vojny na Ukrajine.
Materiály a metódy. Aktivita antiseptík bola stanovená proti klinicky rezistentným kmeňom *K. pneumoniae* (n = 34). Klinické kmene boli izolované od pacientov s infekčnými komplikáciami bojových popálenín a šrapnelových rán rôznej lokalizácie získaných počas vojnového konfliktu na Ukrajine v období od 24. februára do apríla 2022.
Výsledky. Stanovením aktivity antiseptík proti *K. pneumoniae* v planktónovej forme sa zistila vysoká účinnosť hlavných povrchovo aktívnych antiseptík. Najvyššiu aktivitu proti klinickým kmeňom *K. pneumoniae* spomedzi študovaných antiseptík zo skupiny kvartérnych amóniových zlúčenín (QAC) mali dekametoxín, miramistín a oktenidín. Hodnoty IAA (index antiseptickej aktivity) boli najvyššie (IAA > 4) pre antiseptiká dekametoxín (0,1 %), oktenidín (0,1 %) a polyhexanid (0,1 %).
Závery. Stanovenie aktivity tvorby biofilmu ukázalo, že subbakteriostatické koncentrácie chlórhexidínu, dekametoxínu, miramistínu, oktenidínu, polyhexanidu a povidón-jódu inhibovali tvorbu biofilmov *K. pneumoniae*, ale dekametoxín, polyhexanid a povidón-jód vykazovali najvýraznejší účinok. Na základe analýzy všetkých vykonaných štúdií roztoky 0,1 % a 0,02 % dekametoxínu, 0,01 % miramistínu, 0,05 % chlórhexidínu, 0,1 % oktenidínu, 0,1 % polyhexanidu, 10 % povidón-jódu sú

Based on the analysis of all conducted studies, 0.1 % and 0.02 % decamethoxine, 0.01 % miramistin, 0.05 % chlorhexidine, 0.1 % octenidine, 0.1 % polyhexanide, 10 % povidone-iodine are the effective agents against MDR clinical isolates of *K. pneumoniae (Tab. 3, Fig. 2, Ref. 35). Text in PDF www.lekarsky.herba.sk.*
KEY WORDS: Klebsiella, antiseptics, infection, combat wound, resistance, biofilms, antibiofilm-forming activity.
Lek Obz 2024, 73 (1): 8 – 14

účinné proti MDR klinickým izolátom *K. pneumónie (tab. 3, obr. 2, lit., 35). Text in PDF www.lekarsky.herba.sk.*
KĽÚČOVÉ SLOVÁ: Klebsiella, antiseptiká, infekcia, bojová rana, rezistencia, biofilmy, antibiofilmotvorná aktivita.

Lek Obz 2024, 73 (1): 8 – 14

Introduction

Healthcare-associated infections (HCAIs) are a threat to patient safety and cause substantial medical and economic burden in acute care and long-term care facilities. Infection complications, accompanying combat traumatic injuries including superficial and deep incisional surgical site infections, are among the most frequent HCAIs. Other skin and soft tissue infections associated with healthcare settings include vascular access site infections, infected burns and traumas, and decubitus ulcer infections (33).

Wounds are particularly predisposed to microbial colonization and considered at very high risk for multidrug resistant organism (MDRO) infections (14, 25, 26).

The antimicrobial resistance is a topic of global interest in the treatment of wound infections. For Ukraine today, this is especially relevant, since wound infections are major consequences of combat wounds.

WHO reports show that drug resistance in bacteria has been found in all regions of the world, accounting for approximately 50% of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginos*a infections that were resistant to most potent antibiotics (24, 32, 34).

The microbiology of war-related wound infections is variable with predominance of Gram-negative bacteria in later stages (27, 28, 31).

The World Health Organization has considered the antimicrobial resistance as top ten threats to global health and is working to increase the knowledge in this field, in order to both decrease the rate of microbial infections and to provide a more aware and appropriate use of antimicrobial drugs (21).

The use of antiseptics to prevent colonization and infection directly at the portal of entry is a vital step in preventing further infectious complications.

This work studied the antimicrobial activity of antiseptics against planktonic bacterial forms and biofilm formation of multidrug-resistant clinical strains of *K. pneumoniae* isolated from combat wounds with infectious and inflammatory complications.

Materials and methods

The activity of antiseptics was determined against clinical antibiotic-resistant strains of *K. pneumoniae* (n = 34). Clinical strains were isolated from patients with infectious complications of combat burn and shrapnel wounds of different localization, received during war conflict in Ukraine in the period since 24 of February to April of 2022. All patients underwent an antigen and PCR test for SARS-CoV-2, and at the time of surgical resolution, were SARS-CoV-2 negative, anti-epidemic measures were followed. Patients with suspected infection until receiving the PCR test were managed according to the protocol of the management of the disease of COVID-19 (6, 10, 11, 16). Pain management was also implemented taking into account possible post-covid complications (7–9, 12). A reference strain from the American Type Culture Collection (ATCC) *Klebsiella pneumoniae subsp. Pneumoniae* ATCC 700603 (extended-spectrum beta-lactamase positive) was used as a control. 26 isolates (76 % of the total) were identified as multidrug-resistant strains according to the definition criteria MDR, XDR and PDR in *K. pneumoniae*, proposed by European Centre for Disease Prevention and Control (ECDC), and Centers for Disease Control and Prevention (CDC).

Tested antiseptic substances were used in the form of pharmaceutical products available in Ukraine: povidone-iodineof initial concentration of 10 % and recommended working dilutions of 1: 5 (2 %) and 1:10 (1 %) (PVP-I, Betadine®, EGIS Pharmaceuticals PLC, Hungary), octenidine dihydrochloride 0.1 % (Octenisept farblos/incolore, Schulke&Mayr GmbH, Germany), polyhexanide solution 0.1 % (Prontosan, B Braun Medical, Germany), decamethoxine 0.1 % (was prepared from the substance powder of Decamethoxine, Yuria-Pharm, Ukraine), decamethoxine 0.02 % (Decasan, Yuria-Pharm, Ukraine) chlorhexidine digluconate 0.5 % (Chlorhexidine-Viola, FF, JSC, Ukraine), Miramistin 0.01 % (Miramistin, Darnitsa PrAT, Ukraine).

The study evaluated the antimicrobial activity of antiseptics, determining their minimum inhibitory (bacteriostatic) and bactericidal concentrations (MIC and MBC, respectively) against the reference and clinical strains of *K. pneumoniae*. The MIC of the antimicrobials was determined by the Standard macromethod of double serial dilutions (the in-tube dilution test), according to guidelines of Ukraine No. 167 dated April 5, 2007 and Standards for Antimicrobial Susceptibility Testing, in accordance to the Clinical and Laboratory Standards Institute guidelines (CLSI, USA) (5, 22). Three replicates were performed for each strain and antimicrobial compound.

Additionally, a comparative analysis of the antimicrobial efficacy of antiseptics by the index of antisep-

tic activity (IAA) was performed, differentiating bacteriostatic and bactericidal effects according to the method (1, 18): bacteriostatic index of antiseptic activity (BS IAA) was calculated as the ratio of the working concentration of the antiseptic to its minimum inhibitory concentration relative to the pathogen; and bactericidal index of antiseptic activity (BC IAA) was calculated as the ratio of the working concentration of the antiseptic to its minimum bactericidal concentration. IAA is an indicator that allows comparison of the antiseptic activity of drugs regardless of their working concentration. According to the method, the antiseptic was evaluated as active by IAA > 4, because under natural conditions, the activity of antiseptics is reduced by an average of 4 times.

For determining the biofilm-forming ability, the microtiter-plate Christensen test (quantitative crystal violet assay) was applied. The effect of antiseptics on biofilm formation (on immature biofilm) was assessed by reproducing biofilms with the addition (simultaneously with bacterial culture) of antiseptics at sub-inhibitory concentrations during 24 hours and subsequent spectrophotometric ODU (optical density units) assessment. Each of the 25 strains of *K. pneumoniae* and each antiseptic corresponded to its own sub-inhibitory concentration, which represented one third of the MIC.

Interpretation of the results was carried out according to the conventional methodology. Thus, the ability of microorganisms to form biofilms was assessed as low at optical density < 0.120, average – at optical density = 0.121 – 0.239 and high – at optical density > 0.240. The optical density for each isolate without the use of antiseptics was taken as a control against which the results were compared (4, 19).

To assess the degree of reliability of the obtained results, we used the variation-statistical method of analysis, calculating the arithmetic mean (M), the arithmetic mean error (m), the mean error (t), the reliability of the difference (p). Statistical processing was performed using a Microsoft Office Excel spreadsheet (V. 16.0.5056.1000, 2016) and Statistica software packages (v. 12.5.192.7, StatSoft Inc.). The differences were considered statistically significant at $p \leq 0.05$, insignificant at $p > 0.10$.

Results

At the first stage of the study, under the action of antiseptics on planktonic forms of clinical isolates of the wound pathogen *K. pneumoniae*, high efficiency of the main antiseptics from the surfactant group was revealed.

Quantitative indicators of the bacteriostatic and bactericidal action of the studied antiseptics in the form of a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC) are presented in Table 1. Coefficients of reliability of the difference between the minimum inhibitory concentrations of the studied antiseptics are shown in Table 2. Coefficients of reliability of the difference between the minimum bactericidal concentrations are presented in Table 3.

Minimum inhibitory concentrations were the lowest for decamethoxin (0.1 % and 0.02 %) and miramistin (0.01 %).

Effective inhibition of the growth of *K. pneumoniae* was observed with the use of decamethoxin (0.1 % and 0.02 %) at concentrations of 9.6 ± 0.75 μg/ml and 10.34 ± 0.85 μg/ml respectively; miramistin – at a concentration of 15.28 ± 2.16 μg/ml.The mean values of the minimum inhibitory concentrations for chlorhexidine 0.5 % (CHG), octenidine 0.1 % (OCT) and polyhexanide 0.1 % (PHMB) did not differ significantly from each other ($p > 0.05$) and were 21.62 ± 1.70 μg/ml; 22.37 ± 3.69 μg/ml and 21.63 ± 4.45 μg/ml respectively.

Table 1. Characteristics of susceptibility to antiseptics of clinical strains of *Klebsiella pneumoniae* isolated from patients with infectious complicationsof combat wounds, in μg/ml (M ± m).
Tabuľka 1. Charakteristika citlivosti klinických kmeňov *Klebsiella pneumoniae* izolovaných od pacientov s infekčnými komplikáciami bojových rán na antiseptiká v μg/ml (M ± m).

Antiseptics	*Klebsiella pneumoniae* (n = 25)			
	MIC *	p_1***	MBC **	p_2†
Decamethoxine 0.1 %	9.6±0.75	–	20.61±1.49	–
Decamethoxine 0.02 %	10.34±0.85	>0.05	21.15±1.15	>0.05
Miramistin 0.01 %	15.28±2.16	<0.05	26.37±3.36	>0.05
Chlorhexidine 0.05 %	21.62±1.70	<0.001	41.47±3.54	<0.001
Octenidine 0.1 %	22.37±3.69	<0.01	28.23±3.73	>0.05
Polyhexanide 0.1 %	21.63±4.45	<0.05	39.06±8.33	<0.05
Povidone-Iodine 10.0 %	5384.6±549.3	<0.001	7693.3±969.9	<0.001

Note: * MIC – minimum inhibitory concentration; ** MBC – minimum bactericidal concentration, *** p_1, – coefficients of reliability of the difference between the minimum inhibitory concentrations of the studied antiseptics in comparison with decamethoxine 0.1 %; † p_2, – coefficients of reliability of the difference between the minimum bactericidal concentrations of the studied antiseptics in comparison with decamethoxine 0.1 %.

The bacteriostatic effect of decamethoxineagainst clinical strains of *K. pneumoniae* significantly exceeded that of miramistin by 1.53 times ($p < 0.05$), chlorhexidine – by 2.17 times ($p < 0.001$), octenidine – by 2.24 times ($p < 0.01$), polyhexanide – by 2.17 times ($p < 0.05$).The minimum bacteriostatic concentrations of miramistin were significantly lower than those of chlorhexidine by 1.4 times ($p < 0.05$).

MBC values were equivalent for decamethoxin (0.1 % and 0.02 %), miramistin and octenidine ($p > 0.05$).Bactericidal properties were determined in the presence of concentrations that were 20.61 ± 1.49 μg/ml; 21.15 ± 1.15 μg/ml; 26.37 ± 3.36 μg/ml and 28.23 ± 3.73 μg/ml, respectively (Tabs. 1, 3).The highest values of the minimum bactericidal concentration were determined for chlorhexidine and polyhexanide (41.47 ± 3.54 μg/ml and 39.06 ± 8.33 μg/ml respectively), which is twice their bacteriostatic concentration.

Table 2. Coefficients of reliability of the difference between the minimum inhibitory concentrations of the studied antiseptics against clinical strains of *K. pneumoniae* (p_1).
Tabuľka 2. Koeficienty spoľahlivosti rozdielu medzi minimálnymi inhibičnými koncentráciami študovaných antiseptík proti klinickým kmeňom *K. pneumoniae* (p_1).

	MIC of DCM 0.1%	MIC of DCM 0.02%	MIC of CHG 0,05%	MIC of OCT 0.1 %	MIC of MRN 0.01 %	MIC of PHMB 0.1 %
MIC of Decamethoxine 0.1%	1	>0,05	<0,001	<0,01	<0,05	<0,05
MIC of Decamethoxine 0.02%	>0,05	1	<0,001	<0,01	<0,05	<0,05
MIC of Chlorhexidine 0,05%	<0,001	<0,001	1	>0,05	<0,05	>0,05
MIC of Octenidine 0.1 %	<0,01	<0,01	>0,05	1	>0,05	>0,05
MIC of Miramistin 0.01 %	<0,05	<0,05	<0,05	>0,05	1	>0,05
MIC of Polyhexanide 0.1 %	<0,05	<0,05	>0,05	>0,05	>0,05	1

Note: * MIC – minimum inhibitory concentration of antiseptics

Table 3. Coefficients of reliability of the difference between the minimum bactericidal concentrations of the studied antiseptics against clinical strains of *E. coli* (p_2).
Tabuľka 3. Koeficienty spoľahlivosti rozdielu medzi minimálnymi baktericídnymi koncentráciami študovaných antiseptík proti klinickým kmeňom *E. coli* (p_2).

	MBC of DCM 0.1%	MBC of DCM 0.02%	MBC of CHG 0,05%	MBC of OCT 0.1 %	MBC of MRN 0.01 %	MBC of PHMB 0.1 %
MBC of Decamethoxine 0.1%	1	>0,05	<0,001	>0,05	>0,05	<0,05
MBC of Decamethoxine 0.02%	>0,05	1	<0,001	>0,05	>0,05	<0,05
MBC of Chlorhexidine 0.1 %	<0,001	<0,001	1	<0,05	<0,01	>0,05
MBC of Octenidine 0.1 %	>0,05	>0,05	<0,05	1	>0,05	>0,05
MBC of Miramistin 0.01 %	>0,05	>0,05	<0,01	>0,05	1	>0,05
MBC of Polyhexanide 0.1 %	<0,05	<0,05	>0,05	>0,05	>0,05	1

Note: *MBC – minimum bactericidal concentration

The values of the minimum bactericidal concentrations of miramistin were significantly lower than those of chlorhexidine by 1.57 times (< 0.01).

The bactericidal activity of decamethoxin was significantly higher than that of chlorhexidine by 1.99 times ($p < 0.001$), polyhexanide – by 1.87 times ($p < 0.05$).

To summarize the above, it can be emphasized that among the quaternary ammonium antiseptics studied, clinical *K. pneumoniae* isolates were most susceptible to decamethoxin, miramistin and octenidine, and least susceptible to chlorhexidine and polyhexanide.

MICs of povidone-iodine against *K. pneumoniae* averaged 5384.62 ± 549.34 μg/ml, and bactericidal concentrations were 7693.31 ± 969.96 μg/ml.

We compared the activity of drugs that belong to the same chemical group of antiseptics (quaternary ammonium compounds). Povidone-iodine belongs to the halide-containing compounds. Its active substance is present in the initial solution in much higher concentrations and cannot be compared with the concentrations of quaternary ammonium compounds. In this case, it is possible to evaluate the activity of an antiseptic and compare it with other drugs using the antiseptic activity index.

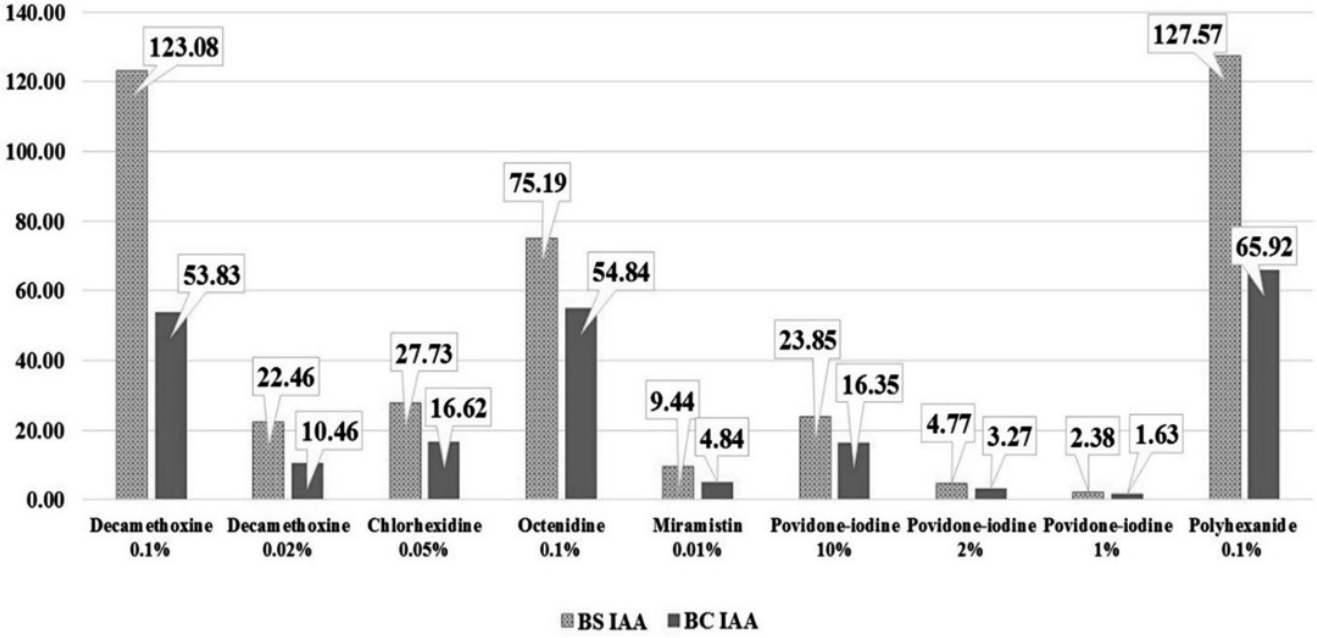
A comparative analysis of antiseptics was carried out using a differentiated indicator of IAA (index of antiseptic activity), calculating BS IAA (bacteriostatic index of antiseptic activity) and BC IAA (bactericidal index of antiseptic activity), which made it possible to assess the feasibility of using certain concentrations of the active substance in the initial working solution of the preparation. As povidone-iodine dilutions of 1:5 and 1:10 are recommended for use, such concentrations (2% and 1%) were also included in the comparative analysis as stock solutions of the drug (Fig. 1).

The bacteriostatic index of antiseptic activity (BS IAA) of decamethoxine 0.1 % was 123.08, and the bactericidal index of antiseptic activity (BC IAA) for decamethoxine 0.1 % was 53.83. The BS IAA and BC IAA for decamethoxine 0.02 % were 22.46 and 10.46 respectively. For chlorhexidine 0.05 %, BS IAA and BC IAA values of 27.73 and 16.62 were determined. The BS IAA and BC IAA values for octenidine 0.1 % were 75.19 and 54.84 respectively. For miramistin 0.01 %, the values of BS IAA and BC IAA were 9.44 and 4.84. For polyhexanide 0.1 %, BS IAA and BC IAA values of 127.57 and 65.92 were determined. The values of BS IAA and BC IAA for povidone-iodine 10 % were 23.85 and 16.35, for povidone-iodine 2 % – 4.77 and 3.27, for povidone-iodine 1 % – 2.38 and 1.63 respectively.

At the second stage of the study, we evaluated the effect of antiseptics in vitro on the biofilm-forming activity of K. pneumoniae.

All the tested strains were efficient biofilm-forming isolates. The multidrug resistant clinical isolates of *K. pneumoniae* were found to have medium biofilm-for-

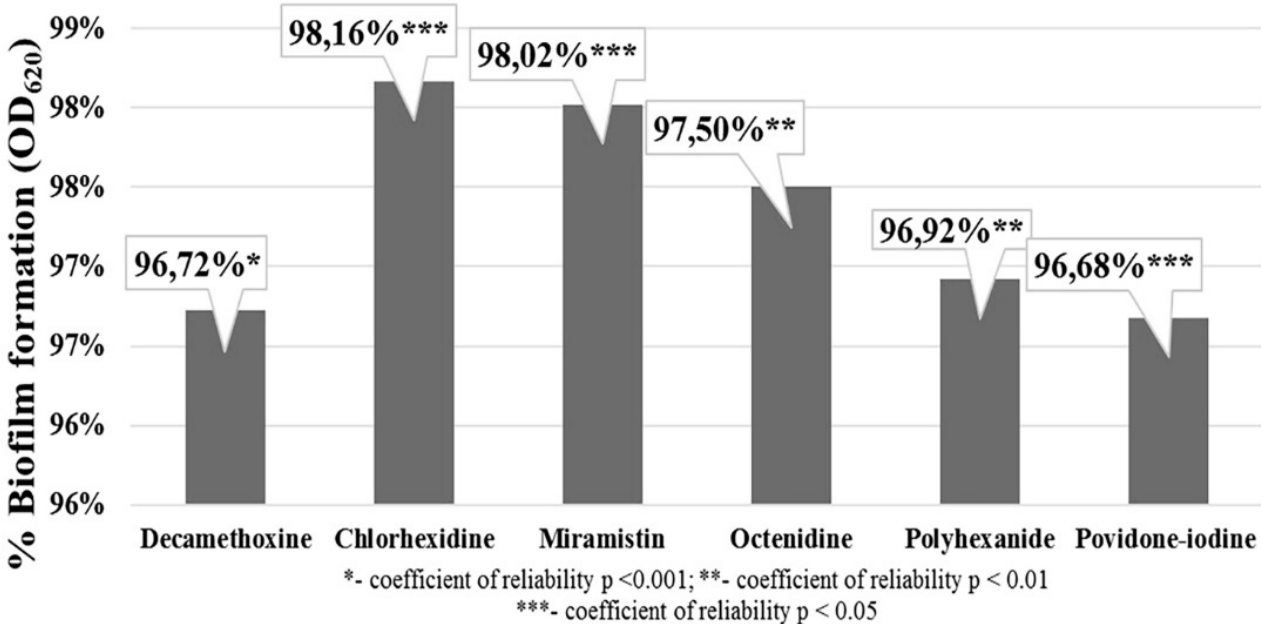
Figure 1. The average values of bacteriostatic (BS) **and bactericidal** (BC) **IAA in relation to clinical isolates of *K. pneumoniae*.**
Obrázok 1. Priemerné hodnoty bakteriostatickej (BS) **a baktericídnej** (BC) **IAA vo vzťahu ku klinickým izolátom *K. pneumoniae*.**



ming properties. The average value of the degree of the dye absorption by biofilms in the control wells was 0.212 ± 0.002 optical density units (ODU).

Determination of anti-biofilm forming activity showed that sub-bacteriostatic concentrations of all tested substances reliably inhibited the formation of *K. pneumoniae* biofilms within 24 hours. Under the effect of decamethoxine, the average value of the optical density of K. pneumoniae biofilms decreased 1.04 times ($p < 0.001$), compared with the control and amounted to 0.204 ± 0.001 ODU, in the presence of chlorhexidine andmiramistin – by 1.02 times ($p < 0.05$) and was 0.207 ± 0.001 ODU, in the presence of octenidine – by 1.03 times ($p < 0.01$) and was 0.206 ± 0.001 ODU, in the presence of povidone-iodine – by 1.04 times ($p < 0.01$) and was 0.204 ± 0.001 ODU and in the presence of polyhexanide by 1.03 times and was 0.205 ± 0.001 ODU. Results of these experiments are summarized in percentages of the biofilm-forming ability of *K. pneumoniae* isolates in the presence of antiseptics compared to the untreated control (Fig. 2).

Figure 2. Percentage indicator of biofilm-forming ability of *Klebsiella pneumoniae* (n = 25) **in the presence of antiseptics compared to the untreated control.**
Obrázok 2. Percentuálny indikátor schopnosti *Klebsiella pneumoniae* tvoriť biofilm (n = 25) **v prítomnosti antiseptík v porovnaní s neliečenou kontrolou.**



On evaluating the anti-biofilm effect of the mentioned antiseptics, decamethoxine, polyhexanide and povidone-iodine had exhibited the most pronounced effect on immature biofilms. The inhibitory effect was 96.72 %, 96.92 % and 96.68 % compared to the control (100 %). For chlorhexidine and miramistin this value was 98.16 % and 98.02 % respectively, for octenidine – 97.50 %.

Discussion

The drug resistance among the Gram-negative bacteria is present as a serious global problem.

Over the last few decades, *K. pneumoniae* has rapidly developed multidrug resistance worldwide, limiting therapeutic choices (23, 29, 30, 35).

The use of antiseptics as topical preparations to prevent further resistance is critical. In the past years, there is an increasing concern on the development of resistance not only to antibiotics but also to antiseptic agents, which are essential to limit the spread of multidrug-resistant pathogens in healthcare (13, 15).

Due to the increase in the prevalence of resistance determinants, many researchers report a decrease in susceptibility to all biocides (20).

Stephen Buxser reports an increase in resistance of *K. pneumoniae* to chlorhexidine of about 2.8 times in 50 years (3).

Bock et al found that not all chlorhexidine formulations kill MDR *K. pneumoniae* after the recommended exposure time. Activity, especially against chlorhexidine-adapted strains, depends on additional ingredients (2).

High MIC values of PVP-I, silver and silver nanoparticles reported for isolates of *K. pneumoniae* (13, 20).

Nevertheless, our study found a fairly high in vitro efficacy of the tested antiseptics which are commonly used. Based on the MIC and MBC values, it can be concluded that among the quaternary ammonium antiseptics studied, clinical *K. pneumoniae* isolates are most susceptible to decamethoxin, miramistin and octenidine, and least susceptible to chlorhexidine and polyhexanide.

There are currently no established guidelines to define antiseptic resistance breakpoints from the Clinical Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST). Therefore, to assess the feasibility of using certain concentrations of the active substance in the initial working solution of the drug, as well as for the comparative analysis of antiseptics, the IAA indicator is very important. Differentiation of bacteriostatic and bactericidal effect in the analysis of IAA is especially relevant at the present time, since the cidal effect of an antiseptic is more preferable to prevent the selection of resistant strains (17). For QAC and halogen compound antiseptics, as their IAA (BS and BC) was > 4, they were considered active.

IAA values were highest (IAA > 4) for the antiseptics decamethoxine (0.1 %), octenidine (0.1 %), and polyhexanide (0.1 %), which correlated with high concentrations of active substance in initial working solutions of these drugs. Conducting a comparative analysis of antiseptics using a differentiated indicator of IAA, it was found that the feasibility of using povidone-iodine at a concentration of 2 % is questionable as the BS IAA is above the threshold value, while the BC IAA is not, which can create selective conditions for the emergence of resistant strains. The effectiveness of povidone-iodine 1 % against multidrug-resistant *K. pneumoniae* was found insufficient since BS IAA and BC IAA were below the threshold.

Understanding the importance of microbial biofilms, we studied the effectiveness of antiseptic substances against bacteria in biofilms, namely their effect on biofilm formation (action on immature biofilm). Our study showed that sub-bacteriostatic concentrations of chlorhexidine, decamethoxin, miramistin, octenidine, polyhexanide and povidone-iodineinhibited the formation of *K. pneumoniae* biofilms, but decamethoxine, polyhexanide and povidone-iodine had exhibited the most pronounced effect.

Conclusions

Clinical strains of *K. pneumoniae* with antibiotic resistance were most susceptible to MIC and MBC of octenidine, decamethoxinandmiramistin. But IAA values were highest (IAA > 4) for the antiseptics decamethoxine (0.1 %), octenidine (0.1 %), and polyhexanide (0.1 %).

The effectiveness of povidone-iodine 2 % (BS IAA = 3.27) and 1 % (BS IAA = 2.38; BC IAA = 1.63, both < 4) against multidrug-resistant *K. pneumoniae* was found insufficient.

Decamethoxine, polyhexanide and povidone-iodine had exhibited the most pronounced effect on immature biofilms.

Based on the analysis of all conducted studies, 0.1 % and 0.02 % decamethoxin, 0.01 % miramistin, 0.05 % chlorhexidine, 0.1 % octenidine, 0.1 % polyhexanide, 10 % povidone-iodine are the effective agents against MDR clinical isolates of *K. pneumoniae*.*

*__Compliance with Ethics Requirements:__ The authors declare no conflict of interest regarding this article. The authors declare, that all the procedures and experiments of this research respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law.

__Conflict of interest:__ The authors declare no conflict of interest.

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Accepted for publication on July 26, 2023.

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