

Efficiency of combined action of antimicrobial preparations against poly-resistant strains of conditionally-pathogenic bacteria isolated from wounds of surgery patients

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Article info

Received 16.07.2020

Received in revised form

20.08.2020

Accepted 21.08.2020

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Sklyar, T. V., Lavrentieva, K. V., Rudas, O. M., Bilotserkivska, O. V., Kurahina, N. V., Papiashvili, M. G., & Lykholat, O. A. (2020). Efficiency of combined action of antimicrobial preparations against poly-resistant strains of conditionally-pathogenic bacteria isolated from wounds of surgery patients. *Regulatory Mechanisms in Biosystems*, 11(3), 392–398. doi:10.15421/022060

The strategy of use of combination therapy of antibacterial preparations is being broadly introduced to clinical practice to fight bacterial infections caused by poly-resistant strains of microorganisms. From the wounds of surgery patients, we isolated 67 clinical strains of conditionally-pathogenic bacteria identified as *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis*, *Pseudomonas aeruginosa*. Using disk diffusion method, the isolated bacterial strains were found to be most resistant to penicillin preparations: ampicillin, oxacillin, amoxicillin/clavulanat; tetracycline and cephalosporin of the II generation – cefoxitin. The percentage of strains insusceptible to these antibacterial preparations accounted for 65.0%. The division of antibiotic-resistant cultures regarding phenotype groups according to the level of their antibiotic resistance allowed determination of 4 PDR-, 8 XDR- and 14 MDR-strains. During the studies on experimental determining of MIC of antibiotic and antiseptics in the condition of applying them as monoprparations against isolated bacterial cultures, we saw significant excess in the threshold values of MIC, and, first of all, regarding pandrug-resistant and extensive drug-resistant clinical microbial isolates. Use of combinations of antibacterial preparations was found to show the synergic effect of antibiotics (ceftriaxone, ofloxacin, gentamicin) and antiseptics (chlorhexidine, decasan), which is expressed in simultaneous decrease in MIC of each of the tested preparations by 2–8 times compared with their isolative application. Such combinatory approach regarding simultaneous application of antibacterial preparations may be considered as one of the most promising ways to combat poly-resistant clinical isolates of conditionally-pathogenic microorganisms and to offer a new strategic approach to prevention of spread of antibiotic resistance as a phenomenon in medical practice.

Keywords: antibiotics; antiseptics; multi-drug resistant strains; combinative effect.

Introduction

The most relevant problem of contemporary medical practice is the development and spread of resistance of infectious agents to antibacterial preparations, leading to loss of clinical significance of a number of medical preparations due to absolute therapeutic inefficacy, significant losses of medical resources, increase in expense of treating patients and duration of their stay in medical institutions (Velez & Sloand, 2016; Pervical et al., 2016; Rather et al., 2017; Aslam et al., 2018). According to recent data, 700 thousand patients die every year due to infections caused by antibiotic-resistant strains of microorganisms (Jenull et al., 2017; Tacconelli et al., 2018; Pachori et al., 2019).

In 2001, the WHO adopted and approved the fundamental document “Global strategy for containment of antimicrobial resistance” in which emergence and formation of antibiotic-resistant strains of microorganisms is recognized by representatives of countries of the EU and North America as a problem of international scale, and the main approaches to prevention of spread of resistant cultures were determined. In February 2017, the WHO for the first time published a list of bacteria with high level of resistance to the action of antibiotics, which pose the greatest threat to the health of people. Out of this list, to the group of critically high level of antibiotic-resistance, such species of microorganisms were identified as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*,

Acinetobacter baumannii, *Pseudomonas aeruginosa* and *Enterobacter* species (Botelho et al., 2019).

Before the broad introduction of antibacterial preparations to medical practice, microorganisms were characterized by a high extent of natural susceptibility to antibiotics. Uncontrolled mass and prolonged use of the latter contributed to the gradual development and spread of resistant pathogenic and conditionally-pathogenic microorganisms in the environment, antibiotic-resistance of which is related not only to biochemical (modification of target of action, change in penetrability of cytoplasmic membrane of microbial cell, efflux, inactivation of active agent), but also to genetic mechanisms. Bacteria can become antibiotic-resistant as a result of mutations and horizontal transfer of genes localized in mobile genetic elements, genomic islands, phages, plasmids (Kramer et al., 2016; Von Wintersdorff et al., 2016). Furthermore, microorganisms may have not only interstrain and intraspecies, but also inter-genus transfer of antibiotic-resistance genes, which would contribute to emergence of cultures with obtained resistance to antibiotics and development of multidrug-resistant variants (Campbell et al., 2018; Cheng et al., 2019).

Until recently, the most effective preparations against poly-resistant strains of bacteria were considered to be aminoglycoside (gentamicin, amikacin, netilmicin), carbapenems (imipenem, meropenem), cephalosporins (ceftazidime, cefepime), fluoroquinolones (ciprofloxacin, levofloxacin), penicillins with β -Lactamase inhibitors (piperacillin / tazobac-

tam), monobactams (aztreonam), phosphinous acids (fosfomicin) and polymyxins (colistin, polymyxin B) (Chellat et al., 2016; Bassetti et al., 2018; Marques et al., 2018). In recent years, there has been a significant increase in the resistance of clinical microbial isolates to preparations of, for example, fluoroquinolones and β -lactam antibiotics, imposing narrow limits on the therapeutic capabilities of contemporary medicine against agents of infectious diseases (Dalhoff, 2012; Agyekum et al., 2016; Anesi et al., 2016).

Apart from antibiotics, medical practice also applies antiseptics (Campbell et al., 2018; Palchykov et al., 2019; Zazharskyi et al., 2019). They are used in low concentrations for disinfection of skin and mucous membranes, surfaces of wounds. They include preparations of iodine, chlorine (halogen-containing substances), hydrogen peroxide (oxidant), miramistin, chlorhexidine (cationic detergents), calendula tincture, extract from eucalypt (preparations of plant origin), etc. (Williamson et al., 2017; Pachori et al., 2019). Antiseptics are prescribed mostly for external application (solutions of chlorhexidine bigluconate, hydrogen peroxide, ethyl alcohol, etc.), some preparations are used internally (furazidin, sodium hypochlorite and others) (Pachori et al., 2019).

Antiseptics are characterized by their broad range of action. They have no specific targets. Their antibacterial effect is associated with the processes of denaturalization of protein, damaging the penetrability of cytoplasmic membrane, inhibition of activity of enzymes of the microflora they affect on (Al-Talib et al., 2019). Recently, reports that constant use of antiseptics underlies increase in the number of strain pathogens resistant to them emerge more and more often (Matthew et al., 2017; Sweeney et al., 2018). At the same time, there are data that during their use in combination with other active substances, they, by contrast, increase the antibacterial effect of the latter (Williamson et al., 2017). Antiseptics are known to be capable of increasing susceptibility of antibiotic-resistant strains of microorganisms to penicillin, chloramphenicol, tetracycline, streptomycin, kanamycin, neomycin, novobiocin, erythromycin and cephalosporins, fluoroquinolones (Jenull et al., 2017; Pachori et al., 2019).

Regarding the relevance of the topic, the objective of the work was determining the efficiency of the combined effect of antimicrobial preparations on poly-resistant strains of conditionally pathogenic bacteria isolated from wounds of surgery patients.

Materials and methods

The study was performed on the basis of the Microbiology Department of PJZ Nezalezna Laboratoria Invitro (Dnipro) and the Department of Microbiology, Virulogy and Biotechnologies of the Dnipro National University. The objects of the study were clinical strains of conditionally pathogenic bacteria isolated from wounds of surgery patients: *Staphylococcus aureus* (8), *S. epidermidis* (10), *Escherichia coli* (14), *Klebsiella pneumoniae* (8), *Proteus vulgaris* (8), *Proteus mirabilis* (7), *Pseudomonas aeruginosa* (12).

Species of the isolated strains of enterobacteria and *Pseudomonas aeruginosa* were identified using test-kit API 20E (BioMerieux, France), staphylococci – using API Staph (BioMerieux, France).

Then, in the isolated cultures of conditionally pathogenic bacteria, we determined the extent of susceptibility to a number of antibiotic preparations of various pharmacological groups using disk diffusion method and method of minimum serial dilutions (ISO 20776-1:2006). For disk diffusion method we used standard disks with antibiotics (Hi Media, India). The spectrum of antibiotic preparations included: penicillins (ampicillin, oxacillin, amoxicillin/clavulanat acid), cephalosporins (cefotaxime, ceftriaxone, cefoxitin, cefuroxime, cefepime, ceftazidime), fluoroquinolones (ciprofloxacin, levofloxacin, ofloxacin, norfloxacin, gatifloxacin), aminoglycosides (amikacin, gentamicin, kanamycin, neomycin, netilmicin), tetracyclines (tetracycline), carbapenems (meropenem). The susceptibility of isolated bacterial strains to antibiotics was assessed according to the criteria CLSI/NCLLS, the extent of sensitivity was determined using the system SIR (EUCAST, 2015). As reference strains we used: *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853. Further, all the isolates, according to the generally accepted classification, were divided into phenotypic groups characterized by different extent of antibiotic-resistance (Magiorakos et al., 2012).

The reference method to determine susceptibility of isolated pathogens to antibiotics and antiseptics was the method of serial dilutions. It allows us also to determine minimum inhibitory concentration (MIC) of antibiotic / antiseptic or their combination against the tested strain. In the experiment we used two antiseptics most commonly applied in medical practice: decasan and chlorhexidine.

While using the methods of serial dilutions to prepare inocula, we used 24 h bacterial culture. Prior to inoculation to the medium, optical density of bacterial suspension of each test culture was adjusted to 0.5 according to the McFarland standard. From the main solutions of antibacterial preparations we prepared series of two-fold dilutions in test tubes to which we introduced the tested strains of microorganisms. At the same time, two controls were made. The inoculated test tubes were incubated in a thermostat at the temperature of 37 °C, during 18–24 h, and then we monitored changes in the turbidity of the medium in the test tube. MIC of antibacterial preparations or their combinations were determined according to the concentration of antibacterial preparation or their combination in the last test tube where no growth of the tested strain was observed (Potapov et al., 2016).

Results

During the study of antibiotic-resistant clinical strains of conditionally pathogenic bacteria isolated from wounds of surgery patients, using disk diffusion method, we determined that the cultures were most resistant to antibiotics of the penicillin group: ampicillin – 89.6%, oxacillin – 86.6%, amoxicillin/clavulanat – 73.1% of the cultures. A high level of resistance remained also to tetracycline – 82.0% and cephalosporin of the II generation – cefoxitin – 70.2%. To most antibiotics, the share of found resistant cultures was within 53.7–65.7%. Greater effectiveness against the strains of conditionally pathogenic bacteria was exerted by preparations of last generations. Therefore, cephalosporins ceftazidime (III) and cefepime (IV) inhibited growth of 56.7% and 62.7%, and fluoroquinolones levofloxacin and gatifloxacin (II) – respectively 58.2% and 62.7% of the isolates. The microbial cultures were found to be most susceptible to meropenem. Frequency of isolates resistant to this antibiotic did not exceed 35.8%.

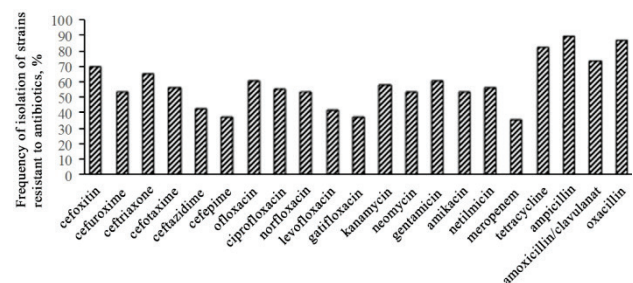


Fig. 1. Antibiotic-resistance of clinical strains of conditionally pathogenic bacteria isolated from wounds of surgery patients (n = 67)

As with the intraspecies division of antibiotic-resistant isolates, 75.0% of strains of *Staphylococcus aureus* were characterized by resistance to cefoxitin, ceftriaxone, ofloxacin, tetracycline, ampicillin and 62.5% – to ciprofloxacin, oxacillin and most aminoglycosides (Table 1). 60.0% of clinical isolates of *Staphylococcus epidermidis* were resistant to cefoxitin, cefotaxime, ciprofloxacin, tetracycline and ampicillin. Regarding representatives of Enterobacteriaceae family, the least efficient were antibiotics tetracycline (to which resistance was exhibited by 71.4% of isolates of *Escherichia coli*, 75.5% of *K. pneumoniae* and all isolated cultures of *Proteus*) and penicillin preparations (the share of resistant tested isolates of enterobacteria exceeded 71.4%). Moreover, 64.3% strains of *E. coli* were characterized by resistance to cefoxitin, cefotaxime, gentamicin and 71.4% to ceftriaxone; 62.5% of isolates of *K. pneumoniae* – to ceftriaxone, cefotaxime, norfloxacin and kanamycin and 75.0% – to cefoxitin; 62.5% of strains of *P. vulgaris* – to ceftriaxone, cefotaxime, ofloxacin, kanamycin and gentamicin; 71.4% of cultures of *P. mirabilis* – to cefoxitin and ofloxacin. The highest number of antibiotic-resistant isolates occurred among *P. aeruginosa*: 66.7% of the cultures were resistant to cefuroxime, ofloxacin and norfloxacin; 75.0% – to ceftriaxone and gentamicin; 83.3% – aminoglycoside antibiotics; 91.7% – to cefoxitin. All isolated cultures of

Pseudomonas aeruginosa were characterized by resistance to tetracycline and all tested penicillin preparations.

We determined that among antibiotic-resistant isolates, there occurred those which exhibited resistance to several antibiotics at the same time, thus, according to the classification generally accepted in medical practice, they were divided into corresponding phenotypic groups: MDR-strains – multidrug-resistance – insensitive to at least one preparation of three or more classes; XDR – extensive drug-resistance – insensitive to at least one preparation in all the tested classes of antibiotics except one-two classes;

PDR – pandrug-resistance – insensitive to all antibiotics of all the tested classes. Among the isolated strains of *S. aureus* we found 4 poly-resistant isolates, of which two were identified to MDR- and one of each XDR- and PDR-groups. Pandrug-resistant strain 1 was characterized by resistance to all antibacterial preparations except cefepime, extensive drug-resistant strain 4 – to tetracycline, gentamicin, ofloxacin, ceftriaxone, ampicillin; polyresistant strain 5 – tetracycline, ceftriaxone, oxacillin and gentamicin; poly-resistant strain 7 – ofloxacin, gentamicin and ampicillin (Table 2).

Table 1

Frequency of isolation of antibiotic-resistant isolates from the contents of wounds of surgery patients (absolute number of / % of the number of strains within species)

Antibiotic	<i>S. aureus</i> , n=8	<i>S. epidermidis</i> , n=10	<i>E. coli</i> , n=14	<i>K. pneumoniae</i> , n=8	<i>P. vulgaris</i> , n=8	<i>P. mirabilis</i> , n=7	<i>P. aeruginosa</i> , n=12
Cefoxitin	6/75.0	6/60.0	9/64.3	6/75.0	4/50.0	5/71.4	11/91.7
Cefuroxime	4/50.0	5/50.0	8/57.1	3/37.5	4/50.0	4/57.1	8/66.7
Ceftriaxone	6/75.0	5/50.0	10/71.4	5/62.5	5/62.5	4/57.1	9/75.0
Cefotaxime	4/50.0	6/60.0	9/64.3	5/62.5	5/62.5	2/28.6	7/58.3
Ceftazidime	3/37.5	4/40.0	7/50.0	3/37.5	4/50.0	3/42.9	5/41.7
Cefepime	2/25.0	3/30.0	6/42.9	2/25.0	3/37.5	3/42.9	6/50.0
Ofloxacin	6/75.0	5/50.0	8/57.1	4/50.0	5/62.5	5/71.4	8/66.7
Ciprofloxacin	5/62.5	6/60.0	7/50.0	4/50.0	4/50.0	4/57.1	7/58.3
Norfloxacin	4/50.0	5/50.0	6/42.9	5/62.5	4/50.0	4/57.1	8/66.7
Levofloxacin	3/37.5	5/50.0	5/35.7	4/50.0	3/37.5	2/28.6	6/50.0
Gatifloxacin	2/25.0	4/40.0	4/28.6	3/37.5	3/37.5	3/42.9	6/50.0
Kanamycin	4/50.0	5/50.0	6/42.9	5/62.5	5/62.5	4/57.1	10/83.3
Neomycin	3/37.5	3/30.0	8/57.1	4/50.0	4/50.0	4/57.1	10/83.3
Gentamicin	5/62.5	5/50.0	9/64.3	4/50.0	5/62.5	4/57.1	9/75.0
Amikacin	5/62.5	5/50.0	6/42.9	4/50.0	3/37.5	3/42.9	10/83.3
Netilmicin	5/62.5	4/40.0	7/50.0	4/50.0	4/50.0	4/57.1	10/83.3
Meropenem	2/25.0	4/40.0	5/35.7	2/25.0	3/37.5	3/42.9	5/41.7
Tetracycline	6/75.0	6/60.0	10/71.4	6/75.0	8/100.0	7/100.0	12/100.0
Ampicillin	6/75.0	6/60.0	13/92.9	8/100.0	8/100.0	7/100.0	12/100.0
Amoxicillin/clavulanat	4/50.0	3/30.0	11/78.6	7/87.5	6/75.0	6/85.7	12/100.0
Oxacillin	5/62.5	5/50.0	13/92.9	8/100.0	8/100.0	7/100.0	12/100.0

Among the isolated strains of *S. epidermidis*, we found no pandrug-resistant ones. Only one strain 3, resistant to ofloxacin, tetracycline, ceftriaxone, cefoxitin, gentamicin and ampicillin, was identified to phenotype XDR; another one – 7 to MDR, with resistance to ceftriaxone, ofloxacin and gentamicin. Pandrug-resistant isolates were not found also among strains of *K. pneumoniae*. Two isolates – 4 and 7, which exhibited high resistance to such antibacterial preparations as ceftriaxone, gentamicin, ofloxacin, oxacillin and ampicillin, were identified to MDR-strains, and strain 6, which was resistant to gentamicin, ceftriaxone, ofloxacin, tetracycline and ampicillin, – to XDR.

Table 2

Classification to phenotypic groups of antibiotic-resistant isolates from wounds of surgery patients

Strain	Number of strains in phenotype group		
	MDR	XDR	PDR
<i>S. aureus</i> , n=8	2	1	1
<i>S. epidermidis</i> , n=10	1	1	0
<i>E. coli</i> , n=14	3	2	1
<i>K. pneumoniae</i> , n=8	2	1	0
<i>P. vulgaris</i> , n=8	1	1	1
<i>P. mirabilis</i> , n=7	2	1	0
<i>P. aeruginosa</i> , n=12	3	1	1

According to the obtained results presented in Table 2, among eight isolated cultures of *P. vulgaris*, one pandrug-, one extensive drug- and one multidrug-resistant isolate was detected. PDR-strain 1 was resistant to all antibacterial preparations except cefepime. Extensive drug-resistant strain 6 exerted resistance to ceftriaxone, gentamicin, tetracycline, ofloxacin, oxacillin and amoxicillin/clavulanat; multi-resistant strain 8 – to ceftriaxone, ofloxacin, gentamicin and ampicillin. Among eight isolated cultures of *P. mirabilis*, we determined one extensive drug- and two multidrug-resistant strains. Extensive drug-resistant strain 3 demonstrated resistance to ceftriaxone, ofloxacin, gentamicin, tetracycline, meropenem, ampicillin. Multidrug-resistant strains 1 and 7 were characterized by resistance to ceftriaxone, ofloxacin, gentamicin. The highest number of poly-resistant isolates was found among species of *E. coli* and *P. aeruginosa*: three of

MDR-, two XDR- and one PDR-strains for each of the species. Among isolates of *E. coli*, PDR-strain 2 was resistant to all the antibiotics except levofloxacin; XDR – 6 – to ceftriaxone, ofloxacin, gentamicin, tetracycline, ampicillin; XDR – 1 – ceftriaxone, ofloxacin, tetracycline, gentamicin, amoxicillin/clavulanat. MDR-strains of *E. coli* 3 and 10 were resistant to ceftriaxone, ofloxacin, gentamicin, and strain 13 – also to tetracycline. Among the isolated cultures of *P. aeruginosa*, pandrug-resistant strain 9 was characterized by resistance to all the tested antibiotics except ceftazidime, and extensive drug-resistant – 6 – to ceftriaxone, gentamicin, meropenem, tetracycline, ofloxacin and oxacillin. Multiresistant strains 2 and 8 exhibited high resistance to ceftriaxone, gentamicin and ofloxacin; and strain 12 – also to ciprofloxacin.

The results we obtained demonstrate that most poly-resistant clinical strains of conditionally pathogenic bacteria isolated from wounds of surgery patients were characterized by resistance to three antibiotics: ceftriaxone, ofloxacin and gentamicin. Therefore, in the next stage of the survey we determined the minimum inhibitory concentration (MIC) of particularly these antibacterial preparations against resistant-to-them PDR-, XDR-MDR-isolates (Table 3). For MDR-strains of *Staphylococcus aureus* (5 and 7), the values of MIC of ceftriaxone, ofloxacin and gentamicin coincided with the threshold values (respectively 64, 8 and 16 µg/mL), for XDR-strain 4 – twice exceeded the threshold values, and for PDR-strain 1 – exceeded the values by four times. As with antibiotic-resistant isolates of *Staphylococcus epidermidis*, for MDR-strain 7, the MIC of ceftriaxone corresponded to the threshold value, and MIC of ofloxacin and gentamicin – exceeded it two-fold. For XDR-strain 3 of *Staphylococcus epidermidis*, the experimental values of MIC of three tested antibiotics exceeded the corresponding threshold value by 4 times.

MIC values for ceftriaxone, ofloxacin and gentamicin for MDR-strain 4 of *K. pneumoniae* exceeded the normative value by 2 and 4 times; and for MDR-strain 7 coincided with them. The extremely-resistant strain *K. pneumoniae* 6 showed the high level of resistance, therefore values of MIC of all three tested antibiotics for this isolate were higher than the norm values by 8, 4 and 2 times. PDR-strain of *P. vulgaris* 1 was found to be highly resistant. MIC of ceftriaxone for this isolate equaled 256.0 µg/mL with the norm ≥ 64.0 µg/mL, MIC of ofloxacin and gentamicin – 64.0

µg/mL, exceeding the norm parameters respectively by 8 and 4 times. Values of MIC of ceftriaxone and ofloxacin for XDR-strain 6 exceeded the threshold values by 2 and 4 times, MIC of gentamicin coincided with the normative value. For MDR-strain 7, the MIC values of ceftriaxone and gentamicin did not exceed the threshold value, MIC of ofloxacin was 16.0 µg/mL, while the norm equals 8.0 µg/mL. Extensive drug-resistant strain 3 of *P. mirabilis* was characterized by high level of resistance to three tested antibiotics. Against this strain, MIC of ceftriaxone equaled 256.0 µg/mL, ofloxacin – 32.0 µg/mL, gentamicin – 64.0 µg/mL. As with multidrug-resistant isolates, excess of MIC value of ceftriaxone was recorded only in relation to MDR-isolate 7, and that of ofloxacin and gentamicin – for MDR-isolate 7. For MDR-strains of *E. coli* (3, 10 and 13), the MIC

values of ceftriaxone, gentamicin and ofloxacin coincided with the threshold values, for XDR-strains 6 and 11 – exceeded threshold values respectively by 2 and 4 times, and for PDR-strain 2 – by 4, 2 and 8 times.

For MDR-strains of *P. aeruginosa* (12, 8 and 3), the MIC values of ceftriaxone coincided with the threshold values, and in the case of gentamicin and ofloxacin were even lower than them. MIC value of gentamicin and ofloxacin against pan-resistant and extra-resistant isolates of *P. aeruginosa* were also lower than the threshold values of MIC of these antibiotics, by contrast to MIC of ceftriaxone: against XDR-strain 6, this parameter equaled 128.0 µg/mL, and for PDR-isolate 9 – even higher, – 256.0 µg/mL.

Table 3

Experimental values of minimum inhibitory concentration of antibiotics and antiseptics against poly-resistant isolates of conditionally pathogenic bacteria isolated from wounds of surgery patients

Strains	MIC of antibiotic, µg/mL			MIC of antiseptic, µg/mL		
	ceftriaxone	ofloxacin	gentamicin	deccasan	chlorhexidine	
<i>S. aureus</i>	1 (PDR)	256.0	32.0	64.0	3.0	125.0
	4 (XDR)	128.0	16.0	32.0	100.0	125.0
	5 (MDR)	64.0	8.0	16.0	6.3	8.0
	7 (MDR)	64.0	8.0	16.0	100.0	62.5
<i>S. epidermidis</i>	3 (XDR)	256.0	32.0	64.0	100.0	31.3
	7 (MDR)	64.0	16.0	32.0	3.0	31.3
<i>K. pneumoniae</i>	6 (XDR)	256.0	64.0	32.0	3.0	16.0
	4 (MDR)	128.0	32.0	64.0	50.8	8.0
	7 (MDR)	64.0	8.0	16.0	3.0	31.3
<i>P. vulgaris</i>	1 (PDR)	256.0	64.0	64.0	100.0	62.5
	6 (XDR)	128.0	32.0	32.0	3.0	250.0
	8 (MDR)	64.0	16.0	16.0	6.3	125.0
<i>P. mirabilis</i>	3 (XDR)	256.0	32.0	64.0	100.0	125.0
	1 (MDR)	64.0	16.0	32.0	100.0	16.0
	7 (MDR)	128.0	8.0	16.0	6.3	250.0
<i>E. coli</i>	2 (PDR)	256.0	64.0	32.0	6.3	125.0
	6 (XDR)	128.0	32.0	32.0	12.5	62.5
	11 (XDR)	128.0	32.0	32.0	6.3	4.0
	3 (MDR)	64.0	8.0	16.0	6.3	8.0
	10 (MDR)	64.0	8.0	16.0	12.5	250.0
	13 (MDR)	64.0	16.0	16.0	100.0	125.0
<i>P. aeruginosa</i>	9 (PDR)	256.0	4.0	8.0	50.0	125.0
	6 (XDR)	128.0	4.0	4.0	25.0	125.0
	3 (MDR)	64.0	1.0	1.0	25.0	62.5
	8 (MDR)	64.0	2.0	2.0	12.5	31.3
	12 (MDR)	64.0	2.0	2.0	25.0	250.0

Thus, the obtained results demonstrate that significant increase in the threshold values of MIC of the tested antibiotics were recorded against pandrug-resistant and extensive drug-resistant clinical isolates of bacteria from contents of wounds of surgery patients.

In the next stage of work the subject of interest was the experimental determining of MIC of decasan and chlorhexidine against poly-resistant isolates of the tested cultures of microorganisms (Table 3). Out of four tested strains of *Staphylococcus aureus*, the most resistant to the action of both antibiotics were XDR-strain 4 and MDR-strain 5. MIC of decasan and chlorhexidine against these isolates reached quite high values: respectively 100.0, 125.0 and 62.5 µg/mL. Among the isolates, the most susceptible to decasan was pan-resistant isolate 1, against which MIC of decasan exceeded 3.0; at the same time, MIC of chlorhexidine was high – 125.0 µg/mL. Decasan- and chlorhexidine-susceptibility was recorded for MDR-strain 5, in this case MIC of two antiseptics respectively equaled 6.3 and 8.0 µg/mL. MDR-strain 7 of *S. epidermidis* was also susceptible to decasan and chlorhexidine. Against this strain, MIC of decasan was 3.0 µg/mL, and that of chlorhexidine – 31.3 µg/mL. XDR-strain 3 was characterized by high level of resistance to antiseptics: MIC of chlorhexidine = 31.3 µg/mL, and MIC of decasan equaled even more – 100.0 µg/mL. Decasan was found to be an effective antiseptic also against poly-resistant strains of *Klebsiella*. Therefore, against MDR-strain 7 and XDR-strain 6, MIC of decasan = 3.0 µg/mL, unlike MDR-strain 4, for which MIC of decasan reached the value of 50.8 µg/mL. However, opposite results were obtained regarding minimum inhibitory concentration of decasan and chlorhexidine, equaling respectively 31.3, 16.0 and 8.0 µg/mL against tested poly-resistant strains of *K. pneumoniae*. High

bactericidal activity was exhibited by decasan towards XDR-isolate 6 (MIC = 3.0 µg/mL), MDR-isolate 8 of *P. vulgaris* and MDR-strain 7 of *P. mirabilis* (MIC = 6.3 µg/mL). It had low activity against other tested isolates of *Proteus* (MIC = 100.0 µg/mL). The highest resistance to chlorhexidine was observed for XDR-isolate 6 of *P. vulgaris* and MDR-isolate 1 of *P. mirabilis*. MIC of chlorhexidine against these isolates reached the value of 250.0 µg/mL. Somewhat lower (125.0 µg/mL) MIC of chlorhexidine was seen for MDR-strain 8 of *P. vulgaris* and XDR-strain 3 of *P. mirabilis*. The most sensitive isolate to the action of chlorhexidine was MDR-strain 1 of *P. mirabilis*. In this case MIC of chlorhexidine did not exceed 16.0 µg/mL. Practically all the poly-resistant isolates of *Escherichia coli* were susceptible to decasan except MDR-strain 13. Against it, MIC of decasan equaled 100.0 µg/mL. As with chlorhexidine, the lowest value of its MIC was determined only against two isolates of *E. coli*: XDR 11 and MDR 3. Other poly-resistant strains of *E. coli* were quite resistant to this antiseptic. Its MIC against these cultures exceeded the value of 62.5 µg/mL. High susceptibility to decasan was seen in all the tested isolates of *Pseudomonas aeruginosa*. Its MIC was within 12.5 to 50.0 µg/mL. All the cultures were resistant to chlorhexidine, as demonstrated by MIC of the preparation equaling higher than 31.3 µg/mL.

The final stage of our study was research on the combined effect of chlorhexidine and decasan with ceftriaxone, ofloxacin and gentamicin against the tested bacterial strains. According to the data of Table 4 and comparison of them to the data of Table 3, we can draw a conclusion that towards a larger amount of the studied cultures the bactericidal effect of antibiotics increased during their combined application with antiseptics, manifesting through decrease in MIC of the tested preparations.

The greatest decrease in MIC of antibiotics to its threshold values, below which the culture could be considered susceptible, was seen while using combination gentamicin + decasan or chlorhexidine against all polyresistant isolates of *Pseudomonas aeruginosa*, strains of *E. coli* 10 and 13, *P. mirabilis* 7, *K. pneumoniae* 7, *S. aureus* 5 and 7; ofloxacin + decasan or chlorhexidine against isolates of *P. aeruginosa* 6, 3, 8 and 12, *P. mirabilis* 7, *K. pneumoniae* 7, strains of *S. aureus* 5 and 7; ceftriaxone + decasan or chlorhexidine against strains of *P. aeruginosa* 3, 8, 12 and *S. aureus* 7. The greatest susceptibility to the combined action was observed in MDR-isolates of *P. aeruginosa* and *S. aureus*, the greatest resistance was shown by strains of *S. epidermidis*, *P. vulgaris* and *E. coli*.

During combined application of antiseptics and antibiotics, we observed a certain decrease in MIC against bacterial cultures, though not as

actively as in the case of antibiotic preparations (mostly twofold). Insignificant decrease in MIC was seen for decasan and its combination with gentamicin against strain 6 and ceftriaxone, ofloxacin or gentamicin against strain 7 of *K. pneumoniae* (by 0.75 times), and also ceftriaxone, ofloxacin or gentamicin against strains of *P. vulgaris* 8, *P. mirabilis* 7, *E. coli* 2, 3, 11 (by 1.5 times); chlorhexidine combined with ceftriaxone, ofloxacin and gentamicin against strains of *K. pneumoniae* 4, 7. It should be noted that in the following experimental variants: decasan + ceftriaxone, ofloxacin or gentamicin against strains of *S. aureus* 7, *P. mirabilis* 1, *E. coli* 13, *P. aeruginosa* 9, and also chlorhexidine + ceftriaxone, ofloxacin or gentamicin against strains of *S. aureus* 7, *P. vulgaris* 8, *E. coli* 2, *P. aeruginosa* 3 and 8, the value of MIC decreased to the threshold value, making the tested cultures susceptible to the effect of antiseptics.

Table 4

Pattern of combined effect of antibiotics and antiseptics on strains of poly-resistant bacteria isolated from wounds of surgery patients

Strains	MIC of antibiotic/antiseptic, µg/mL						
	CF/DC	OF/DC	GM/DC	CF/CH	OF/CH	GM/CH	
<i>S. aureus</i>	1 (PDR)	128.0/1.5	16.0/1.5	32.0/1.5	128.0/62.5	16.0/62.5	32.0/62.5
	4 (XDR)	32.0/50.0	8.0/50.0	16.0/50.0	32.0/62.5	8.0/62.5	16.0/31.3
	5 (MDR)	16.0/1.5	2.0/3.1	4.0/3.1	16.0/2.0	2.0/4.0	2.0/2.0
	7 (MDR)	8.0/25.0	2.0/25.0	4.0/25.0	16.0/15.7	1.0/15.6	2.0/15.6
<i>S. epidermidis</i>	3 (XDR)	128.0/50.0	16.0/50.0	32.0/50.0	128.0/15.7	16.0/15.6	32.0/15.6
	7 (MDR)	16.0/1.5	8.0/1.5	8.0/0.8	16.0/7.8	4.0/7.8	8.0/15.6
<i>K. pneumoniae</i>	6 (XDR)	64.0/1.5	32.0/1.5	8.0/4.0	64.0/4.0	8.0/2.0	8.0/4.0
	4 (MDR)	32.0/25.0	8.0/12.5	8.0/25.0	64.0/8.0	32.0/8.0	8.0/4.0
	7 (MDR)	32.0/4.0	4.0/4.0	8.0/4.0	16.0/31.3	2.0/31.3	4.0/31.3
<i>P. vulgaris</i>	1 (PDR)	64.0/25.0	16.0/25.0	16.0/25.0	64.0/16.0	32.0/31.3	32.0/31.3
	6 (XDR)	32.0/1.5	16.0/1.5	16.0/1.5	32.0/125.0	8.0/62.5	8.0/125.0
	8 (MDR)	32.0/4.0	8.0/4.0	8.0/4.0	16.0/31.3	8.0/62.5	8.0/31.3
<i>P. mirabilis</i>	3 (XDR)	64.0/25.0	4.0/25.0	16.0/25.0	64.0/62.5	8.0/62.5	32.0/62.5
	1 (MDR)	16.0/25.0	8.0/25.0	8.0/25.0	32.0/8.0	8.0/8.0	16.0/8.0
	7 (MDR)	64.0/4.0	4.0/4.0	8.0/4.0	32.0/62.5	2.0/62.5	4.0/62.5
<i>E. coli</i>	2 (PDR)	64.0/4.0	32.0/4.0	32.0/4.0	32.0/31.3	16.0/62.5	32.0/31.3
	6 (XDR)	32.0/6.3	16.0/6.3	32.0/6.3	32.0/31.3	8.0/16.0	16.0/31.3
	11 (XDR)	32.0/4.0	16.0/4.0	16.0/4.0	32.0/2.0	16.0/2.0	32.0/2.0
	3 (MDR)	32.0/4.0	4.0/4.0	8.0/4.0	32.0/4.0	4.0/4.0	8.0/2.0
	10 (MDR)	32.0/6.3	4.0/6.3	4.0/6.3	32.0/125.0	4.0/125.0	4.0/125.0
	13 (MDR)	16.0/25.0	4.0/50.0	2.0/25.0	16.0/62.5	8.0/62.5	4.0/31.3
<i>P. aeruginosa</i>	9 (PDR)	64.0/25.0	4.0/25.0	2.0/50.0	64.0/62.5	4.0/62.5	2.0/62.5
	6 (XDR)	32.0/12.5	1.0/12.5	1.0/12.5	32.0/62.5	1.0/62.5	1.0/62.5
	3 (MDR)	8.0/6.3	0.5/6.3	0.5/6.3	8.0/3.3	0.5/31.3	0.5/62.5
	8 (MDR)	16.0/6.3	0.5/6.3	0.5/3.1	8.0/7.8	0.5/3.9	0.5/7.8
	12 (MDR)	8.0/6.3	0.5/3.1	0.5/3.1	8.0/62.5	0.5/62.5	0.5/62.5

Note: CF/DC – combination of ceftriaxone and decasan; OF/DC – combination of ofloxacin and decasan; GM/DC – combination of gentamicin and decasan; CF/CH – combination of ceftriaxone and chlorhexidine; OF/CH – combination of ofloxacin and chlorhexidine; GM/CH – combination of gentamicin and chlorhexidine.

Discussion

Currently, resistance of conditionally pathogenic strains of bacteria to antibacterial preparations is one of the main global problems in the sphere of healthcare. Its solution requires a complex approach. The strategy of use of combined therapy with antibacterial preparations is being increasingly introduced into clinical practice to treat bacterial infections caused by poly-resistant strains of microorganisms (Garimella et al., 2020). A necessary condition for combining antibacterial preparations is the rationality of their combination (Campos et al., 2020).

Antibiotic-resistant strains of microorganisms are a common cause of nosocomial infection; increase in the period of hospitalization of patients, costs for treatment and people's inability to work, increase in mortality rate (Barnes et al., 2018; Mobarki et al., 2018). In 2017, the report of EARS-net noted that the indicator of resistance of *E. coli* to cephalosporins of the III generation has reached the level of 14.9% in the European countries over recent decades (Annual report, 2018). Besides cephalosporins of the III generation, resistant isolates of *E. coli* are often resistant to antibiotics of classes of fluoroquinolones and aminoglycosides (Chervet et al., 2018).

Researchers have empirically and finally determined that combination therapy leads to better outcomes than monotherapy (Rodríguez-Baño et al., 2018). The importance of combining antibacterial preparations, in particular antibiotics and antiseptics, over the recent years is being pointed out by many scientists (Noites et al., 2014; Hansen et al., 2018). The synergic effect of applying such combinations was seen against a number of

strains of multi-resistant Gram-negative bacteria (Tängdén, 2014; Thwaites et al., 2018; Schmid et al., 2019). Therefore, Garimella et al. (2018) performed a series of experiments on the effect of antibiotics: ampicillin, fosfomicin and ciprofloxacin both in isolated application and in combinations on the level of antibiotic-resistance of the clinical uropathogenic strain of *Escherichia coli* CFT073. The results of their study revealed that double and triple combinations of antibiotics significantly reduce antibiotic-resistance of *E. coli* of subpopulation CFT073. Djachenko et al. (2016) demonstrated that the greatest effect against PDR-strains of *P. aeruginosa* and *E. coli* is exhibited by combination of fluoroquinolones and cephalosporins (in the study – ciprofloxacin and ceftazidime) For 76.9% of isolates of *Pseudomonas aeruginosa* and 66.7% of strains of enterobacteria, such combination had a synergic pattern.

The results of our study indicate that against a higher number of clinical isolates, synergic action of antibiotics (ceftriaxone, ofloxacin and gentamicin) and antiseptics (chlorhexidine, decasan) takes place during their use in combination, expressing through simultaneous 2–8-fold decrease in MIC of each of two tested preparations compared with their isolated application. The data we obtained substantiate results reported by other scientists according to whom the simultaneous use of chlorhexidine and gentamicin (or penicillin and tetracycline) against strains of *S. aureus* caused increase in susceptibility of the staphylococci to the corresponding antibiotic (Dopcea et al., 2020). The report by Fabry et al. (2014) described the synergic effect of antiseptic combined with erythromycin, doxycycline and linezolid against clinical isolates of *S. aureus*.

Conclusions

In the study of the content isolated from wounds of surgery patients, we isolated 67 strains of conditionally pathogenic bacteria which were characterized by high level of resistance to antibiotics of various pharmacological groups. Eight isolates were identified to *Staphylococcus aureus*, 10 – to *Staphylococcus epidermidis*, 14 – *Escherichia coli*, 8 – *Klebsiella pneumoniae*, 8 – *Proteus vulgaris*, 7 – *Proteus mirabilis*, 12 – to *Pseudomonas aeruginosa*. The least effective antibiotics against the representatives of the Enterobacteriaceae family were tetracycline (resistance to which was displayed by 71.4% of isolates of *E. coli*, 75.5% of *K. pneumoniae* and all isolated cultures of *Proteus*) and penicillin preparations (the percentage of resistant tested isolates of enterobacteria exceeded 71.4%). A total of 66.7% of cultures of *P. aeruginosa* was resistant to cefuroxime, ofloxacin and norfloxacin; 75.0% – to ceftriaxone and gentamicin; 83.3% – aminoglycoside antibiotics; 91.7% – to ceftioxin. All the isolated cultures of *P. aeruginosa* were characterized by resistance to tetracycline and all the tested penicillin preparations. A total of 75.0% of strains of *S. aureus* was characterized by resistance to ceftioxin, ceftriaxone, ofloxacin, tetracycline, ampicillin and 62.5% – to ciprofloxacin, oxacillin and most aminoglycosides. A total of 60.0% of each clinical isolate of *S. epidermidis* was resistant to ceftioxin, cefotaxime, ciprofloxacin, tetracycline and ampicillin.

Among the antibiotic-resistant isolates, we found a number which exhibited resistance simultaneously to several antibiotics, therefore, according to the classification generally accepted in the medical practice, they were divided into the corresponding phenotypic groups: MDR-strains – multidrug-resistant; XDR – extensive drug-resistant; PDR – pandrug-resistant. The division of the cultures into the phenotypic groups according to the level of antibiotic-resistance allowed us to determine 4 poly-resistant isolates among the isolated strains of *S. aureus*, including two identified to MDR- and one to each of XDR- and PDR-strains; *S. epidermidis* – one XDR- and one MDR-isolate; *K. pneumoniae* – two MDR- and one XDR-isolate. Among eight isolated cultures of *P. vulgaris*, we found one pandrug-, one extensive drug- and one multidrug-resistant isolate, and among *P. mirabilis* – one extensive drug- and two multidrug-resistant strains. The highest number of poly-resistant isolates was found among *E. coli* and *P. aeruginosa*: three of each belonging to MDR-, two XDR- and one of each to PDR-strains.

According to use of antibiotics (ceftriaxone, ofloxacin and gentamicin) and antiseptics (deccasan and chlorhexidine) in the form of monoprparations against the tested cultures, we determined that minimum inhibitory concentration (MIC) of ceftriaxone, depending on the tested strain, ranged 64 to 256 µg/mL; MIC of ofloxacin – 1.0–64.0 µg/mL, MIC of gentamicin – 1.0–128.0 µg/mL, MIC of deccasan – 3.0 to 100.0 µg/mL; MIC of chlorhexidine – 4.0 to 250.0 µg/mL. Against most clinical isolates, synergic action of antibiotics (ceftriaxone, ofloxacin and gentamicin) and antiseptics (chlorhexidine, deccasan) took place while using them in combinations, expressing in simultaneous decrease in MIC of each of the tested preparations by 2–8 times, compared with their isolated application. Such positive effect of combined use of antibiotics and antiseptics opens broad prospects for treatment of wounds, surficial inflammatory processes, nosocomial infections caused by antibiotic-poly-resistant strains of microorganisms.

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