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

T. V. Sklyar*, K. V. Lavrentieva*, O. M. Rudas*, O. V. Bilotserkivska*, N. V. Kurahina*, M. G. Papiashvili**, O. A. Lykholat***

*Oles Honchar Dnipro National University, Dnipro, Ukraine
**Independent laboratory Invitro LLC, Dnipro, Ukraine
***University of Customs and Finance, Dnipro, Ukraine

The strategy of use of combination therapy of antibiotic 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 – cefotaxin. 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 monopreparations 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 microbial isolates. Use of combinations of antibacterial preparations was found to show the synergic effect of antibiotics (ceftriaxone, ofloxacin, gentamicin) and antiseptics (chlorhexidine, decesan), 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.
As with the intraspecies division of antibiotic-resistant isolates, 75.0% of strains of *Staphylococcus aureus* were characterized by resistance to cefoxitin, ceftriaxone, cefuroxime, amoxicillin and 62.5% – to ciprofloxacin, oxacillin and most aminoglycosides (Table 1). 60.0% of clinical isolates of *Staphylococcus epidermidis* were resistant to cefoxitin, ceftriaxone, 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*): 66.7% of the cultures were resistant to cefuroxime, chloramphenicol, cefotaxime, ciprofloxacin, oxacillin and most aminoglycosides (Table 1). 60.0% of clinical isolates of *Staphylococcus epidermidis* were resistant to cefoxitin, ceftriaxone, 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 *P. aeruginosa* and *Klebsiella pneumoniae*). The highest number of antibiotic-resistant isolates occurred among *P. aeruginosa*: 66.7% of the cultures were resistant to cefoxitin, cefotaxime, ciprofloxacin, gentamicin and 75.0% – to ceftriaxone, cefuroxime, cefotaxime, norfloxacin and kanamycin and 75.0% – to cefoxitin; 65.3% of strains of *P. vulgaris* – to cefoxitin, ceftriaxone, ciprofloxacin, kanamycin and gentamicin; 71.4% of cultures of *P. mirabilis* – to cefotaxime and ofloxacin. The highest number of antibiotic-resistant isolates occurred among *P. aeruginosa*: 66.7% of the cultures were resistant to cefoxitin, ofloxacin and norfloxacin; 75.0% – to cefotaxime and gentamicin; 83.3% – aminoglycoside antibiotics; 91.7% – to cefotaxime. All isolated cultures of

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.

Materials and methods

The study was performed on the basis of the Microbiology Department of PJC Nezalezhna Laboratorija Invitro (Dnipro) and the Department of Microbiology, Virulon 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).

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, ceftriaxone, 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 *P. aeruginosa* 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, ceftriaxone, gentamicin and 71.4% to ceftriaxone; 62.5% of isolates of *K. pneumoniae* – to ceftriaxone, cefotaxime, ofloxacin and norfloxacin; 71.4% of cultures of *P. mirabilis* – to ceftriaxone and ofloxacin. The highest number of antibiotic-resistant isolates occurred among *P. aeruginosa*: 66.7% of the cultures were resistant to cefoxitin, ofloxacin and norfloxacin; 75.0% – to cefotaxime and gentamicin; 83.3% – aminoglycoside antibiotics; 91.7% – to cefotaxime. 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, penem, tetracycline, ceftazidime, cefoxitin, oxacillin and gentamicin; poly-resistant strain 7 – to ofloxacin, gentamicin and ampicillin (Table 2).

### Table 1

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

### Table 2

| Strain | Number of strains in phenotype group | MDR | XDR | PDR |
|--------|-------------------------------------|-----|-----|-----|
| *S. aureus*, n = 8 | 2 | 1 | 1 |
| *S. epidermidis*, n = 10 | 1 | 1 | 0 |
| *E. coli*, n = 14 | 3 | 2 | 1 |
| *K. pneumoniae*, n = 8 | 2 | 1 | 0 |
| *P. vulgaris*, n = 8 | 1 | 1 | 1 |
| *P. mirabilis*, n = 7 | 2 | 1 | 0 |
| *P. aeruginosa*, 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 ceftazidime, gentamicin, tetracycline, ofloxacin, oxacillin and amoxicillin/clavulanat, multi-resistant strain 8 to ceftazidime, 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 ceftazidime, ofloxacin, gentamicin, tetracycline, meropenem, ampicillin. Multidrug-resistant strains 1 and 7 were characterized by resistance to ceftazidime, 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 ceftazidime, ofloxacin, gentamicin, tetracycline, ampicillin; XDR – 1 to ceftazidime, ofloxacin, tetracycline, gentamicin, amoxicillin/clavulanat. MDR-strains of *E. coli* 3 and 10 were resistant to ceftazidime, 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 cefazidine, and extensive drug-resistant strain 6 to ceftazidime, gentamicin, meropenem, tetracycline, ofloxacin and oxacillin. Multidrug-resistant strains 2 and 8 exhibited high resistance to ceftazidime, 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: ceftazidime, 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 *P. aeruginosa*, pandrug-resistant strain 9 – exceeded the normative value by 2 and 4 times; 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 *P. aeruginosa* 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 ceftazidime, 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 ceftazidime 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, against which MIC of ceftriaxone exceeded the norm 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 62.5 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 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

| Strains          | MIC of antibiotic, µg/mL | MIC of antiseptic, µg/mL |
|------------------|--------------------------|--------------------------|
|                  | ceftriaxone | ofloxacin | gentamicin | decasan | chlorhexidine |
| S. aureus        | 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          |
| S. epidermidis   | 256.0        | 32.0       | 64.0       | 100.0   | 31.3          |
| 5 (XDR)          | 64.0         | 16.0       | 32.0       | 3.0     | 31.3          |
| 7 (MDR)          | 64.0         | 8.0        | 16.0       | 3.0     | 16.0          |
| K. pneumoniae    | 256.0        | 64.0       | 32.0       | 3.0     | 31.3          |
| 6 (XDR)          | 128.0        | 32.0       | 64.0       | 50.8    | 8.0           |
| 7 (MDR)          | 64.0         | 8.0        | 16.0       | 3.0     | 31.3          |
| P. vulgaris      | 256.0        | 64.0       | 64.0       | 100.0   | 62.5          |
| 1 (PDR)          | 128.0        | 32.0       | 32.0       | 3.0     | 250.0         |
| 8 (MDR)          | 64.0         | 16.0       | 32.0       | 6.3     | 63.0          |
| P. mirabilis     | 256.0        | 32.0       | 64.0       | 100.0   | 125.0         |
| 3 (XDR)          | 128.0        | 64.0       | 32.0       | 16.0    | 100.0         |
| 7 (MDR)          | 128.0        | 8.0        | 16.0       | 6.3     | 250.0         |
| E. coli          | 256.0        | 64.0       | 32.0       | 6.3     | 125.0         |
| 2 (PDR)          | 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         |
| P. aeruginosa    | 256.0        | 4.0        | 8.0        | 30.0    | 125.0         |
| 9 (PDR)          | 128.0        | 4.0        | 4.0        | 25.0    | 125.0         |
| 6 (XDR)          | 128.0        | 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 63.0 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 31.2, 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 625.0 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, 9 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 antisepsics 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 gentamicins against strain 7 of K. pneumoniae (by 0.75 times), and also ceftriaxone, ofloxacin or gentamicin against strains of P. vulgares 8, P. mirabilis 7, E. coli 3, 4, 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. vulgares 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 antisepsics.

Table 4

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

| Strains | MIC of antibiotic/antisepsic, µg/mL |
|---------|-----------------------------------|
| S. aureus | CF/DC  | OF/DC  | GM/DC  | CF/CH  | OF/CH  | GM/CH  |
| 1 (FDR) | 128/0.15 | 16/0.15 | 32/0.15 | 128/0.625 | 16/0.625 | 32/0.625 |
| 4 (XDR) | 32/0.50 | 8/0.50 | 64/0.50 | 32/0.625 | 8/0.625 | 16/0.625 |
| 5 (MDR) | 16/0.15 | 2/0.31 | 4/0.31 | 16/0.25 | 2/0.25 | 4/0.25 |
| 7 (MDR) | 8/0.25 | 2/0.25 | 4/0.25 | 8/0.15 | 2/0.15 | 4/0.15 |
| S. epidermidis | CF/DC  | OF/DC  | GM/DC  | CF/CH  | OF/CH  | GM/CH  |
| 1 (FDR) | 128/0.15 | 16/0.15 | 32/0.15 | 128/0.625 | 16/0.625 | 32/0.625 |
| 6 (XDR) | 64/0.15 | 32/0.15 | 8/0.40 | 64/0.40 | 8/0.40 | 16/0.40 |
| K. pneumoniae | CF/DC  | OF/DC  | GM/DC  | CF/CH  | OF/CH  | GM/CH  |
| 4 (XDR) | 32/0.50 | 8/0.50 | 16/0.50 | 32/0.625 | 8/0.625 | 16/0.625 |
| 7 (MDR) | 32/0.40 | 8/0.40 | 16/0.40 | 32/0.625 | 8/0.625 | 16/0.625 |
| P. vulgaris | CF/DC  | OF/DC  | GM/DC  | CF/CH  | OF/CH  | GM/CH  |
| 1 (FDR) | 128/0.25 | 16/0.25 | 32/0.25 | 128/0.625 | 16/0.625 | 32/0.625 |
| 6 (XDR) | 32/0.15 | 16/0.15 | 32/0.15 | 32/0.625 | 8/0.625 | 16/0.625 |
| P. mirabilis | CF/DC  | OF/DC  | GM/DC  | CF/CH  | OF/CH  | GM/CH  |
| 1 (FDR) | 16/0.25 | 8/0.25 | 4/0.25 | 16/0.625 | 8/0.625 | 16/0.625 |
| 7 (MDR) | 16/0.40 | 8/0.40 | 4/0.31 | 16/0.15 | 8/0.15 | 16/0.15 |
| E. coli | CF/DC  | OF/DC  | GM/DC  | CF/CH  | OF/CH  | GM/CH  |
| 1 (FDR) | 32/0.63 | 16/0.63 | 32/0.63 | 32/0.625 | 16/0.625 | 32/0.625 |
| 11 (MDR) | 32/0.60 | 16/0.60 | 32/0.60 | 32/0.625 | 16/0.625 | 32/0.625 |
| P. aeruginosa | CF/DC  | OF/DC  | GM/DC  | CF/CH  | OF/CH  | GM/CH  |
| 3 (MDR) | 8/0.63 | 0.5/6.3 | 0.5/6.3 | 8/0.31 | 0.5/3.1 | 0.5/3.1 |
| 8 (MDR) | 16/0.63 | 0.5/6.3 | 0.5/6.3 | 8/0.31 | 0.5/3.1 | 0.5/3.1 |

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 (Campou 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; Mohariki et al., 2018). In 2017, the report of EARS- costly for treatment and people’s inability to work, increase in mortality rate (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 antisepsics, 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 (Tangdón, 2014; Thrwates et al., 2018; Schmid et al., 2019). Therefore, Garimella et al. (2018) performed a series of experiments on the effect of antibiotics: ampicillin, fosfomycin 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. Djaflao 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 antisepsics (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 (Dopce et al., 2020). The report by Fabry et al. (2014) described the synergic effect of antisepc 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 cultures 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, oxacillin and norfloxacine; 75.0% – to ceftriaxone and gentamicin; 83.3% – aminoglycoside antibiotics; 91.7% – to cefoxitin. 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 cefoxitin, ceftriaxone, oxacillin, tetracycline, ampicillin and 62.5% – to ciprofloxacin, cefoxitin and most aminoglycosides. A total of 60.0% of each clinical isolate of S. epidermidis was resistant to cefoxitin, 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, oxacillin and gentamycin) and antisepsics (decasian and chlorhexidine) in the form of monopreparations 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 oxacillin – 1.0–64.0 µg/mL; MIC of gentamycin – 1.0–128.0 µg/mL; MIC of decasian –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, oxacillin and gentamycin) and antisepsics (chlorhexidine, decasian) took place while using them in combinations, expressing in simultaneous decrease in MIC of each of the tested preparations by 2–9 times, compared with their isolated application. Such positive effect of combined use of antibiotics and antisepsics opens broad prospects for treatment of wounds, surgical inflammatory processes, nosocomial infections caused by antibiotic-poly-resistant strains of microorganisms.

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