ORIGINAL ARTICLE

CONTENTS

Profile of antibiotic resistance of the main infectious contaminants on the wound surface of wounded men in the Russian-Ukrainian war

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ABSTRACT
Aim: To study the spectrum of antibiotic resistance of causative agents of wound infection in wounded men at the stage of specialized medical care.
Materials and Methods: Retrospective analysis of the results of culture of wound secretions on chromogenic media. The research involved 113 samples of biomaterial from 85 wounded. Sensitivity of antibiotics was studied by the Kirby-Bauer method.
Results: Analysis of the microflora of the wound surface made it possible to determine the dominance of gram-negative bacteria, they were isolated in 80% of cases. The microbial spectrum of gram-negative bacteria is represented by *Pseudomonas aeruginosa* – 31 isolates, *Acinetobacter baumannii* – 29 isolates, *Enterobacter aerogenes* – 21 isolates, *Proteus vulgaris* – 13 isolates, *Escherichia coli* – 7 isolates, *Enterobacter cloacae* – 5 isolates, *Klebsiella pneumoniae* – 7 isolates and gram-positive bacteria *Staphylococcus aureus* – 5 isolates, *Enterococcus fecalis* – 32 isolates. Among all tested isolates of gram-negative non-fermenting bacteria and enterobacteria, the highest sensitivity was observed to colomycin and polymyxin B, from 60 to 80%. When analyzing of antibiotic sensitivity of *Klebsiella pneumoniae and Acinetobacter baumannii,* it was found that these pathogens retain 80-100% of their actual antibiotic sensitivity to polymyxins.
Conclusions: Gram-negative strains isolated from wound infection are sensitive to antibiotics: 70% to polymyxins (colistin), 30% to aminoglycosides (amikacin), 24-16% to cephalosporins (cefoperazone-sulbactam/cefoperazone-avibactam).

KEY WORDS: combat trauma, microbiome, antibiotic resistance

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INTRODUCTION

Wounds resulting from combat operations with the use of modern weapons require the speed of decision-making related to the provision of medical care, as they are characterized by a specific course of the wound process and the addition of secondary purulent complications. Purulent-septic processes occur as a result of 75% of gunshot and shrapnel wounds. They require long-term intensive therapy, multi-stage exhausting treatment aimed at combating the consequences of injury, and are always accompanied by severe complications [1, 2]. The development of the inflammatory process as a trigger of an immune response directed simultaneously against the infectious agent and triggers several mechanisms of the pathological action on cells of human body. It was shown a significant influence of opportunistic and pathogenic microorganisms as part of associations or monoinfection on the morphofunctional state of spermatozoa [3].

The main role in the pathogenesis of the development of infectious complications in the early and late period of wounds is played by infectious agents, the dominance of which can change depending on the state of the immune system and the transportation of the wounded to different institutions for the purpose of receiving qualified medical care. This creates conditions for the uncontrolled transfer and spread of polyresistant strains of microorganisms inside and outside the hospitals.

Scientists also note a worldwide trend of dominance of certain types of microorganisms that contaminate combat wounds. According to the order of the Ministry of Health of Ukraine No. 403 from February 27, 2023 "On the approval of the Procedure for conducting enhanced epidemiological surveillance of antimicrobial resistance of microorganisms that cause purulent-inflammatory infections of wounds in the wounded as a result of hostilities" [4], it is important to carry out a bacteriological examination of samples of biological materials patients, isolation and identification of microorganisms, determination and assessment of the

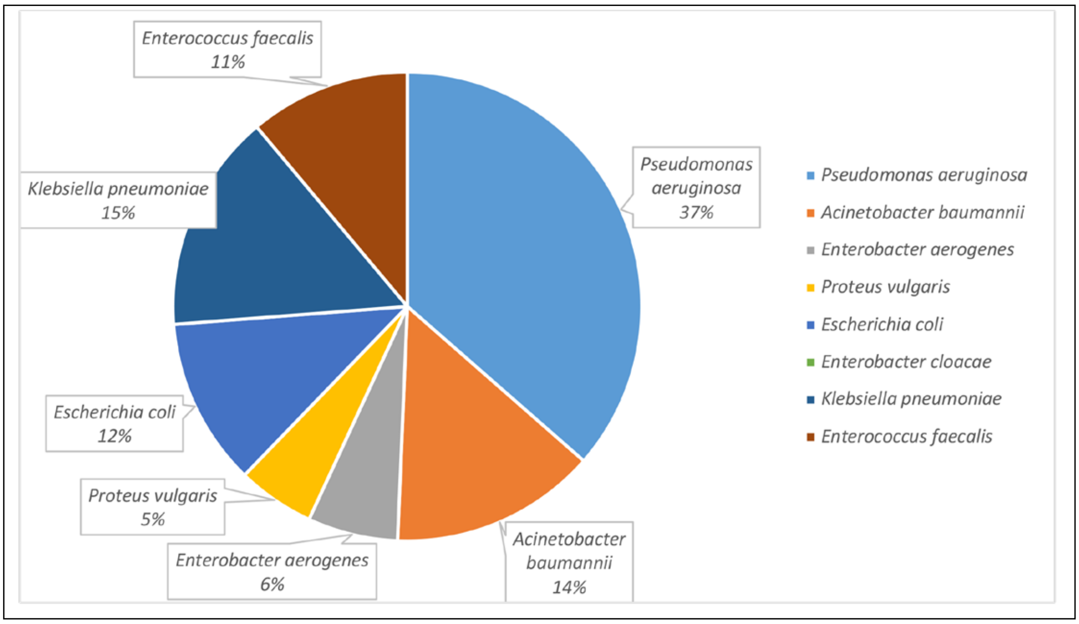

Fig. 1. Results of the study of the bacterial profile of combat injuries.

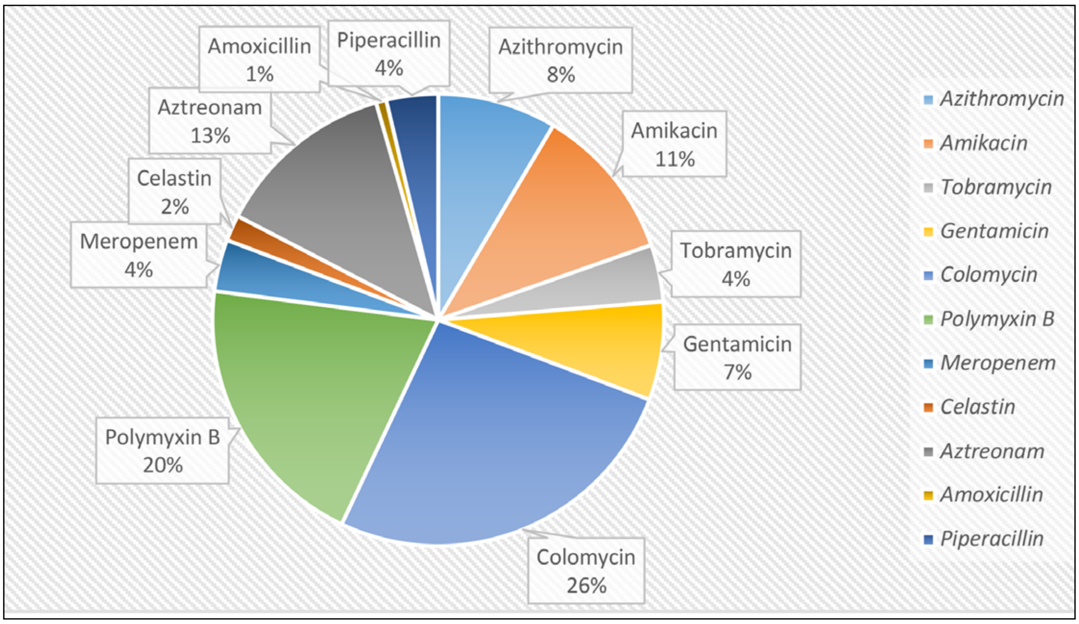

Fig. 2. Antibiotic sensitivity of gram-negative bacteria.

sensitivity of microorganisms to antimicrobial drugs, since the problem is characteristic of both the global and local levels. In everyday clinical practice, this means that infections caused by problematic polyresistant strains of microorganisms do not respond to traditional (protocol) treatment schemes. The wide distribution of methicillin-resistant staphylococci and enterobacteria producing beta-lactamases of a wide spectrum of action leads to the need to use new, more expensive antibacterial drugs. Irrational antibiotic therapy and, as a result, increased resistance of microorganisms prolong the duration of stay of the wounded in the hospital, lead to serious complications, and in some cases lead to the formation of scars. If purulent-septic complications are caused by polyresistant microorganisms, this increases the risk of mortality, and is also accompanied by a delay in the response to adequate antimicrobial therapy.

AIM

The aim is to study the spectrum of antibiotic resistance of causative agents of wound infection in wounded men at the stage of specialized medical care.

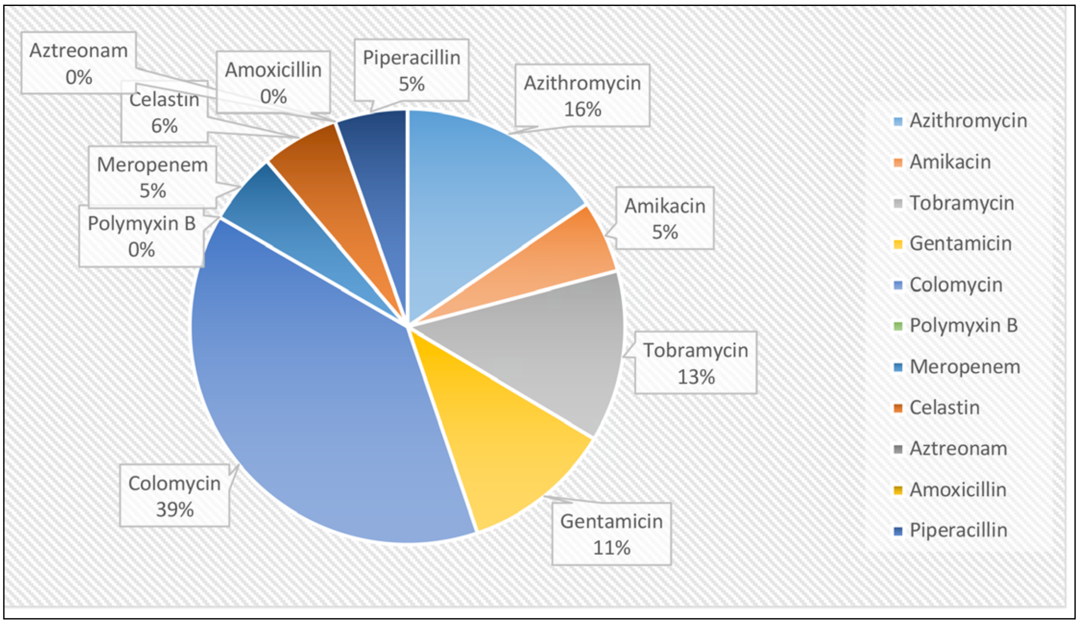

Fig. 3. Antibiotic sensitivity of *Klebsiella pneumoniae* (share of resistant strains, %).

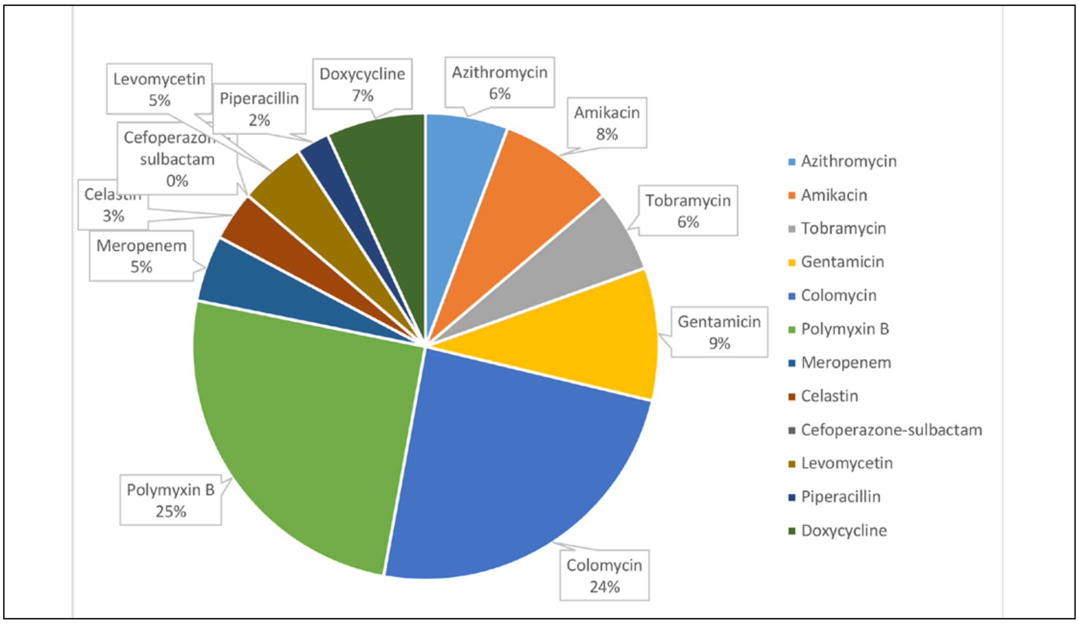

Fig. 4. Antibiotic sensitivity of *Acinetobacter baumannii*.

MATERIALS AND METHODS

Retrospective analysis of the results of culture of wound secretions on chromogenic media. The research involved 113 samples of biomaterial from 85 wounded. Sensitivity of antibiotics was studied by the Kirby-Bauer method.

RESULTS

The paper presents the results of microbiological analysis of the spectrum of pathogens isolated from combat wounds. Collection of material for bacteriological examination in the hospital was carried out in patients with landmine-explosive injuries, gunshot bullet injuries, shrapnel injuries, or injuries complicated by sepsis and multiple organ failure. It should be emphasized that, according to statistics, 90% of the material is taken against the background of previous antibiotic therapy. This often obscures the true picture of the microbiological landscape.

As a result of the conducted microbiological studies, 113 isolates of microorganisms were isolated. Analysis of the microflora made it possible to determine the dominance of gram-negative bacteria, they were isolated in 80% of cases. The microbial spectrum of gram-negative bacteria is represented by *Pseudomonas aeruginosa* – 31 isolates,

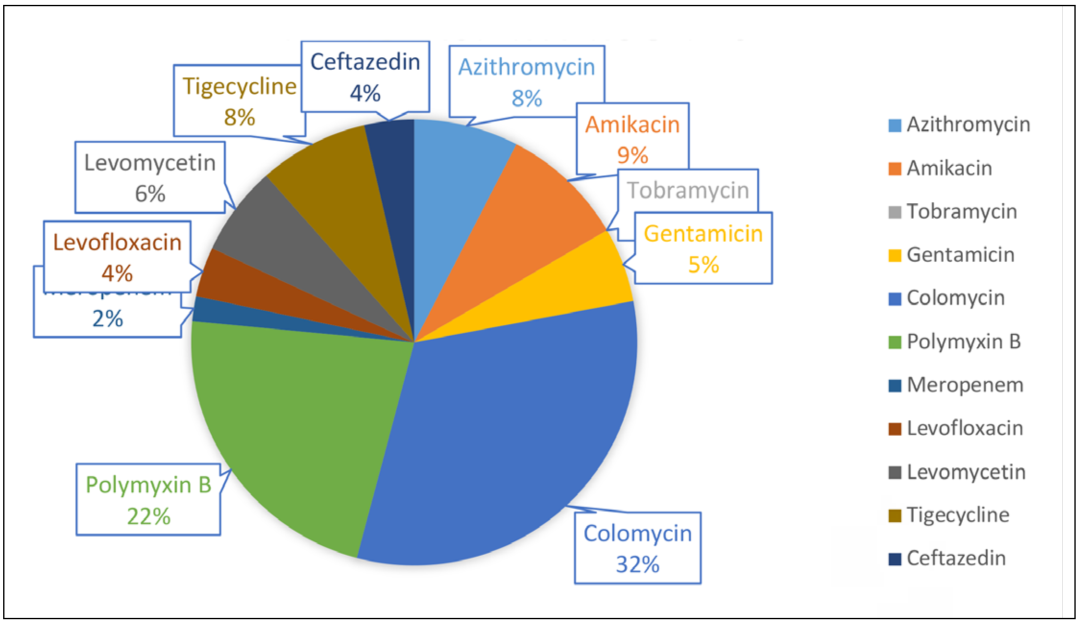

Fig. 5. Antibiotic sensitivity of *Enterobacter aerogenes*.

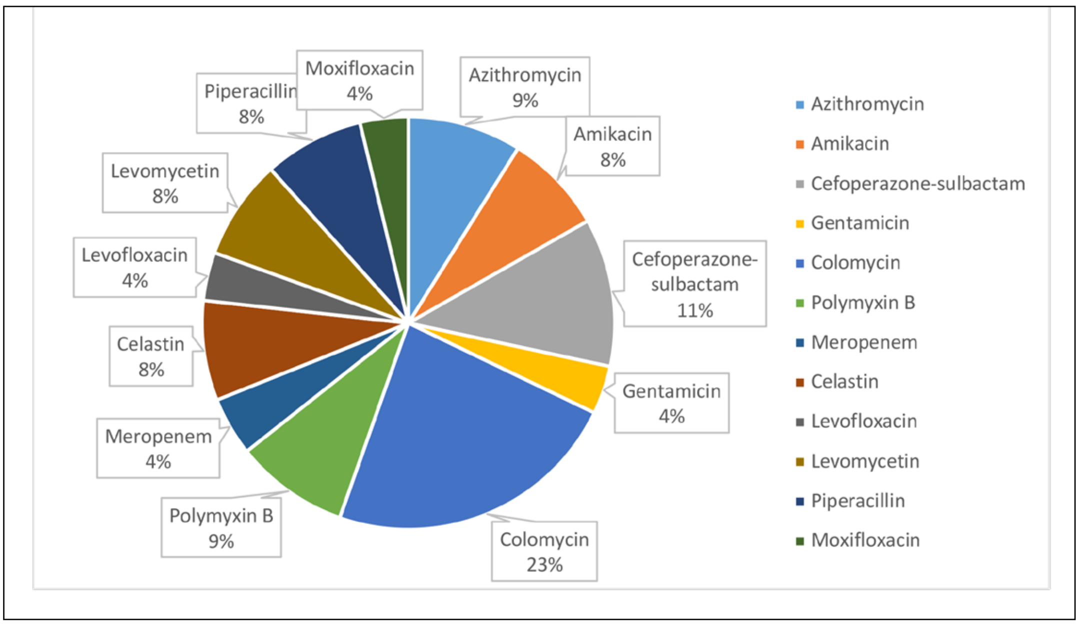

Fig. 6. Antibiotic sensitivity of *Escherichia coli*.

Acinetobacter baumannii – 29 isolates, *Enterobacter aerogenes* – 21 isolates, *Proteus vulgaris* – 13 isolates, *Escherichia coli* – 7 isolates, *Enterobacter cloacae* – 5 isolates, *Klebsiella pneumoniae* – 7 isolates and gram-positive *Staphylococcus bacteria aureus* – 5 isolates, *Enterococcus faecalis* – 32 isolates. (Fig. 1).

The analysis of the results of our research proved a high degree of resistance of the selected gram-negative microorganisms to most antibiotics, including carbapenems. Given that gram-negative bacteria are the dominant microbiome of combat wounds and in the development of infectious complications of gunshot wounds, their sensitivity to the most commonly used antibiotics was investigated and analyzed (Fig. 2). Thus, among all tested isolates of gram-negative non-fermenting bacteria and enterobacteria, the highest sensitivity was observed to colomycin and polymyxin B – from 60 to 80%. Also, these strains were resistant to fosfomycin, clindamycin, and rifampicin. Azithromycin, amikacin, tobramycin and gentamicin can be called the drugs of choice in the treatment of isolated gram-negative bacteria, since the average sensitivity to these drugs was from 20 to 50%.

When analyzing the antibiotic sensitivity of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, it was found that these pathogens retain 80-100% of their actual

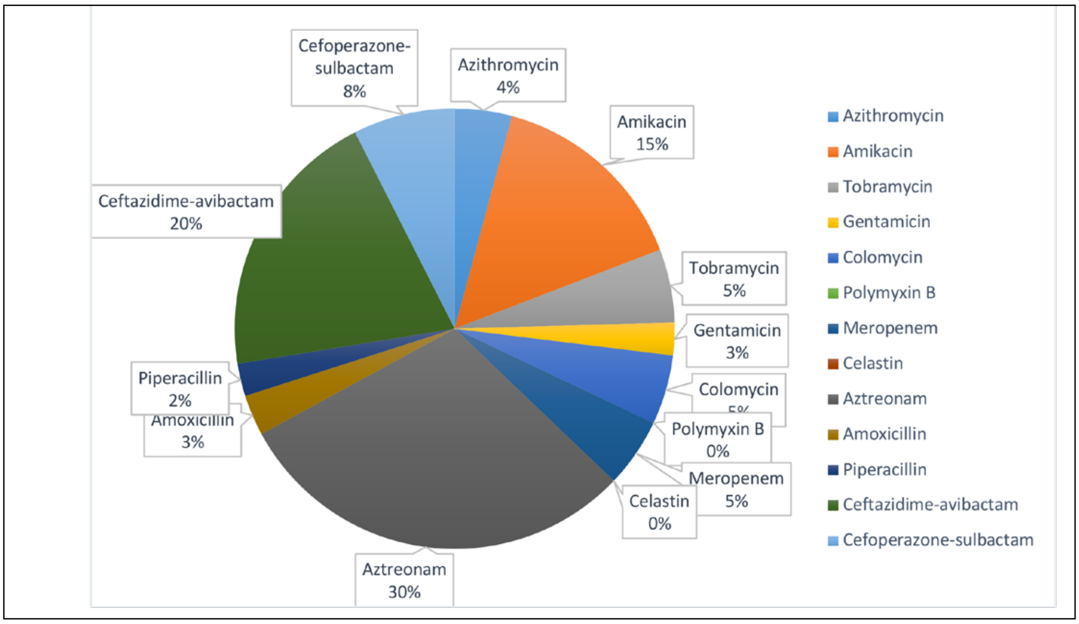

Fig. 7. Antibiotic sensitivity of *Proteus vulgaris*.

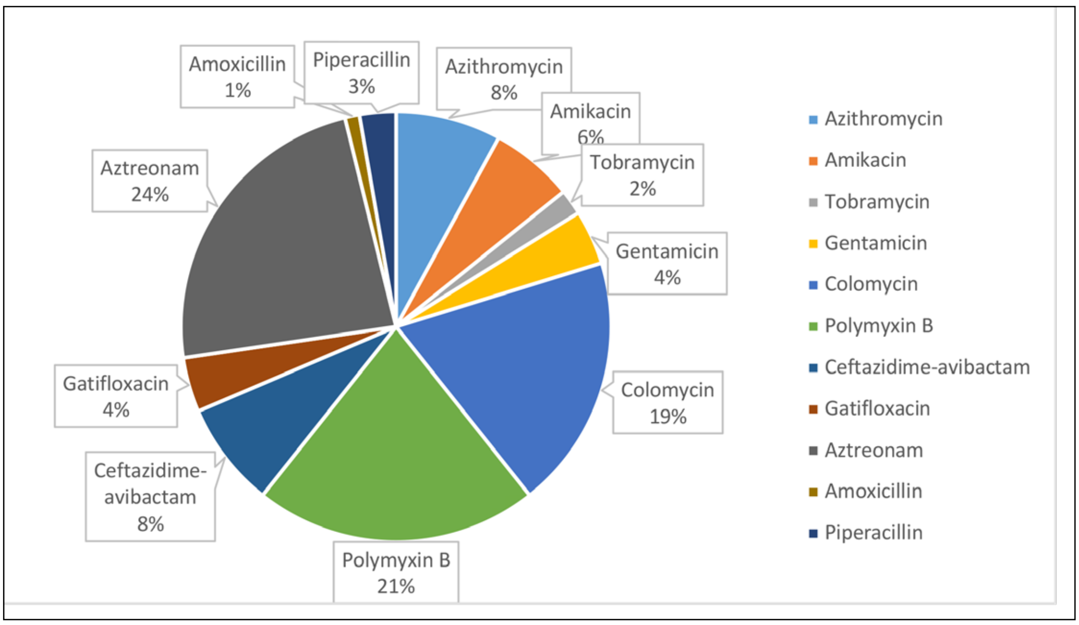

Fig. 8. Antibiotic sensitivity of *Pseudomonas aeruginosa*.

antibiotic sensitivity to polymyxins (Fig. 3, Fig. 4). Gentamicin and amikacin equally maintain moderate sensitivity at the level of 30% and are the drugs of choice for both pathogens. An interesting surprise was the moderate sensitivity of these pathogens to ceftriaxone at the level of 7-14%, although sensitivity to meropenem and celastin was also observed within the same limits.

It was found that all *Klebsiella* strains were resistant to this antibiotic as well. Resistance of *Acinetobacter baumannii* to piperacillin/tazobactam was at the level of 7%, and in *Klebsiella pneumoniae* – 14%.

The analysis of the antibioticogram showed a high sensitivity to polymyxins at the level of 65-90% (Fig. 5). And 100% resistance to cephalosporins, aztreonam, meropenem and piperacillin. The drugs of choice were azithromycin at the level of 21% and amikacin –25%.

When analyzing the antibiotic resistance of *Escherichia coli* (Fig. 6) isolated from patients with combat injuries, it was found that the bacteria have a high degree of resistance to almost all tested antibiotics, except for azithromycin and amikacin, the average sensitivity to which was from 20 to 30%.

The level of resistance to cephalosporins of the 3rd and 4th generation is 100%, since *Escherichia coli* is a producer of beta-lactamases of a wide spectrum of action and in the treatment only drugs that inhibit the most important beta-lactamases are required, therefore, in the analysis with cefoperazone-sulbactam with it turned out that the causative agent is indeed sensitive, as indicated by 43% of antibiotic sensitivity. Since, compared to other β-lactam antibiotics, cefoperazone-sulbactam is able to penetrate faster through the outer membrane of gram-negative bacteria and, in addition, to exert a pronounced postantibiotic effect on them, which lasts 7–10 hours.

Which is quite effective for combat injuries of the abdominal cavity when it is necessary to transport the patient over long distances. It should be noted that low sensitivity to aminoglycoside drugs is a natural retribution for their unjustified and widespread use and should serve as a signal to review their use in everyday clinical practice.

When studying the antibiotic sensitivity of 13 strains of *Proteus vulgaris*, there was a double feeling, on the one hand, it is generally accepted that the pathogen is very resistant to antibiotics due to the plasmids present in the bacterium, which makes it extremely difficult to treat. On the other hand, incorrect antibiotic therapy for *Proteus vulgaris*, before the patient entered our department, could lead to its dominance in the biofilms of the urogenital tract and, as a result, to the formation of struvite stones. Especially in patients with long-term use of catheters.

However, as practice has shown, *Proteus vulgaris* was moderately sensitive to the test antibiotics, but a high sensitivity to amikacin – 50%, aztreonam – 100%, cefoperazone – sulfbactam – 25%, and ceftazidime – aviabactam – 70% was observed (Fig. 7). However, unfortunately, 90% of resistance to fluoroquinolones and cephalosporins.

Analyzing the antibiotic sensitivity of *Pseudomonas aeruginosa*, its 100% resistance to doxycycline, clindamycin, ofloxacin, and levofloxacin was established. The same pattern is observed among cephalosporin preparations, but the sensitivity to ceftazidime-avibactam was at the level of 29%. However, positive points were also noted, in particular, when setting antibiotic sensitivity to polymyxins – 78% (Fig. 8).

DISCUSSION

It should be noted that in recent years, the specific weight of *Klebsiella* bacteria in the microbial spectrum of gunshot wound contaminants has increased, and this is a global issue because they produce various carbapenemases [5], which increases their resistance to certain types of antibiotics. To date, about 500 types of broad-spectrum beta-lactamases (BLRS) have been described, and this list is constantly being updated. As a rule, the genes that encode the synthesis of beta-lactamases are located on plasmids, which facilitates easy transfer between bacteria and, subsequently, the rapid spread of resistant pathogens.

Of particular concern among clinicians is the trend of increasing resistance of pathogens such as *Klebsiella pneumoniae* and *Acinetobacter baumannii*, which cause complications among patients with combat injuries and are also associated with concomitant pathology in soldiers. According to the analyzed data of bacteriological studies of combat injuries in the territory of Ukraine in the period 2014-2017, the share of these microorganisms isolated was 9.4% and was represented by the species *Klebsiella pneumoniae* and *Klebsiella oxytoca*, so during the full-scale war, pathogens were isolated in the wound contents of 1/3 of the examined [6, 7]. According to the EUCAST recommendations, when resistance to meropenem is detected, the establishment of sensitivity to piperacillin/tazobactam is a mandatory step.

Klebsiella aerogenes, formerly known as *Enterobacter aerogenes*, is a catalase-positive bacterium that causes opportunistic infections. Infections caused by this pathogen are generally susceptible to antibiotics, although complicated by induced resistance mechanisms, particularly beta-lactamase. Often, strains of *Enterobacter aerogenes* quickly become resistant to standard antibiotics during treatment, which requires special supervision, especially if it concerns gastrointestinal tract injuries and urogenital tract diseases.

Escherichia coli is singled out as a leading pathogen among diseases, injuries and complications, especially if it is related to the organs of the abdominal cavity. Over the past 20 years, the frequent treatment of infections caused by *Escherichia coli* has been determined by the widespread and completely unfounded use of 3rd generation cephalosporins, in particular cefotoxime, ceftriaxone, and cefoperazone in clinical practice.

There are many factors that can cause a delay and/or inability to heal a chronic wound, namely: the patient's age, obesity, oxygen saturation of the wound, long-term use of non-steroidal anti-inflammatory drugs. However, infiltration by biofilms, including *Salmonella enterica*, *S. bongori*, *Staphylococcus epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*, is probably the biggest factor [8].

When treating wounds, it is important to consider not only antimicrobial efficacy, but also how they may affect the complex cellular and extracellular mechanisms involved in wound healing. Because modern combat trauma is characterized by the massiveness of affected tissues, a high degree of contamination, and simultaneous damage to various organs and systems [9-12].

CONCLUSIONS

1. Analysis of the microbiological structure of secretions from an abdominal wound cavities and areas of the pelvis showed that the inflammatory process is initiated mainly by Gram-negative bacteria, to a lesser extent by Gram-positive bacteria. Isolated strains have a significant resistance to antibacterial drugs (49%), which indicates the need for constant periodic monitoring of antimicrobial therapy.
2. Gram-negative strains isolated from wound infection are sensitive to antibiotics, only 70% to polymexins (colistins), 30% to aminoglycosides (amikacin), 24-16% – cephalosporins (cefoperazone-sulbactam / cefoperazone-avibactam).
3. The use of antibiotics in the treatment of combat wounds is a prerequisite for effective treatment of servicemen, shortening of periods of incapacity for work and quick return to the ranks.

REFERENCES

1. Khomenko IP, Gerasimenko OS, Yenin RV et al. Peculiarities of surgical treatment of gunshot wounds to the abdomen. The Ukrainian Journal of Clinical Surgery. 2018;9:71-74. doi:10.26779/2522-1396.2018.09.71. DOI
2. Khomenko IP, Tsema EA, Tertyshnyi SV, Shklyarevich PO. Dynamika mikrobnoho obsimeninnya vohnepal'noyi rany pry kompleksniy khirurhichniy obrobtsi. [Dynamics of microbial contamination of a gunshot wound during complex surgical treatment]. Khirurhiya Ukrayiny. 2018;1:7-13. (Ukrainian)
3. Melnyk OV, Vorobets MZ, Fafula RV et al. Urogenital infection as a factor of development of male infertility. Microbiological journal. 2023;85(2):93-112. doi:10.15407/microbiolj85.02.093. DOI
4. Fomina NS, Fomin OO, Kovalchuk VP et al. The microflora of a modern combat wound and its sensitivity to antibiotics – what's new? Part II. Ukraine Med Journal. 2023;5(157):1-4. doi: 10.32471/umj.1680-3051.157.247288. DOI
5. Kryshevskyi YuP, Horoshko V. Antibiotic resistance and sensitivity of microorganisms isolated in patients with abdominal injuries. Emergency medicine. 2020;16:56-64. doi: 10.22141/2224-0586.16.5.2020.212225. DOI
6. Fomina N, Sukmanska H, Kordon Yu, Trofimenko J. Improving the methods of local treatment in case of aphthous lesions on the oral mucosa. Vinnytsia National Medical University Bulletin. 2020;24(1):106–109. doi: 10.31393/reports-vnmedical-2020-24(1)-28. DOI
7. Trutyak IR, Fil AYu, Medzin VI, Trutyak RI. Surgical treatment of the consequences of modern combat trauma. Trauma. 2017;18(4):58-62. doi: 10.22141/1608-1706.4.18.2017.109345. DOI
8. Alves PJ, Barreto RT, Barrois BM et al. Update on the role of antiseptics in the management of chronic wounds with critical colonisation and/or biofilm. Int Wound J. 2021;18(3):342-358. doi: 10.1111/iwj.13537. DOI
9. Hrytsai MP, Poliachenko YuV, Tsokalo VM et al. Treatment tactics in case of infectious complications in patients with combat injuries of the musculoskeletal system (according to the clinic's own experience). Erra orthopaedica. 2023;1(116):46-57. doi:10.37647/2786-7595-2023-116-1-46-57. DOI
10. Burmeister DM, Johnson TR, Lai Z et al. The gut microbiome distinguishes mortality in trauma patients upon admission to the emergency department. J Trauma Acute Care Surg. 2020;88(5):579-587. doi: 10.1097/TA.0000000000002612. DOI
11. Khomenko IP, Korol SO, Khalik SV et al. Clinical and Epidemiological analysis of the structure of combat surgical injury during Antiterrorist operation. Joint Forces Operation. Ukrainian Journal of Military Medicine. 2021;2(2): 5-13. doi:10.46847/ujmm.2021.2(2)-005. DOI
12. Kok CR, Bram Z, Thissen JB et al. The military gear microbiome: risk factors surrounding the warfighter. Appl Environ Microbiol. 2024;90(1):e0117623. doi: 10.1128/aem.01176-23. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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