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Original article

A current view on the phenotypic antibiotic resistance of leading pathogens in wounded patients during the war in Ukraine

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Abstract. *Background and aim:* The purpose of this study was to determine the effectiveness of antibacterial drugs through cluster analysis of antibiotic sensitivity data from clinical isolates of *Acinetobacter* and *Pseudomonas* spp., obtained from soft tissue wounds and burn injuries. *Methods:* The cohort included 76 clinical isolates of *Acinetobacter* and *Pseudomonas* spp. obtained from 141 patients (male – 95,04 %; female – 4,96 %) with combined injuries of soft tissues and burns. The mean age of patients was 38,57±10,90 years. The final identification of the obtained isolates was carried using MALDI-ToF (Bruker). The sensitivity of clinical isolates to antibacterial drugs was determined using the standard Kirby-Bauer method. Extended antimicrobial susceptibility testing was performed to measure the minimal inhibitory concentrations of antibiotics against the studied. A hierarchical cluster analysis was performed using the Ward method. *Results:* The high level of resistance formation was observed among representatives of this *Acinetobacter* genus. We found the highest level of resistance in *P. aeruginosa* to ciprofloxacin. The lowest proportions of resistant *Pseudomonas* spp. were observed for meropenem and tobramycin. Cluster analysis of susceptibility data for *P. aeruginosa* isolates resulted in the division of antibiotics into two clusters that differ significantly from each other. The isolated *A. baumannii* strains showed susceptible to a higher number of antibacterial drugs compared to *P. aeruginosa. Conclusions:* Clinical isolates of *A. baumannii* and *P. aeruginosa* obtained from patients with combined soft tissue injuries and burns in Ukraine in 2022 exhibit a high level of multidrug resistance. Cluster analysis results indicate that only tobramycin potentially retains efficacy against *A. baumannii* isolates. Clinical isolates of *P. aeruginosa* demonstrate susceptible to tobramycin and meropenem. (www.actabiomedica.it)

Key words: *Acinetobacter, Pseudomonas,* antibiotics, sensitivity, resistance, multidrug resistance

Introduction

Antimicrobial resistance has been declared by the WHO as one of the 10 global public health threats facing humanity (1). Since the onset of the full-scale Russian war against Ukraine, which started in February 2022, the incidence of infectious complications among the wounded has significantly increased within the country. Consequently, the demand for effective antimicrobial therapy and the judicious prescription of antibiotics has arisen, accompanied by a shortage of these drugs due to reduced pharmaceutical capacity and disrupted logistics chains during the times of war (2). The experience gained from the war in Iraq and combat operations during the long-lasting anti-terrorist operation in eastern Ukraine, commencing in

2014, confirms a direct correlation between military operations and the development of multidrug resistance among the primary pathogens responsible for wound infections (3, 4). Therefore, the potential global burden of antimicrobial resistance resulting from the Russian-Ukrainian war is a matter of concern within the global scientific community (5).

Acinetobacter and *Pseudomonas* spp. have a large arsenal of pathogenicity factors and the presence of mobile genetic determinants. Theses bacterial species can form biofilms on the surfaces of the human body and artificial medical devices, as well as rapidly acquire multiple antibiotic resistance due to a number of other mechanisms (4, 6, 7). According to the literature data, the representatives of these genera of bacteria cause the vast majority of complications of wound infections (8, 9). Determination of antimicrobial susceptibility of dominant pathogens found in wounds, such as *Acinetobacter* spp. and *Pseudomonas* spp., is highly important in wartime.

The frequency of infected wound development and the acute need for the use of antimicrobial agents are increasing, leading to dynamic changes in pathogen drug resistance patterns. However, a clear summary of antibiotic resistance among dominant microorganisms, which could contribute to revising the infections treatment tactics (3, 4), is not available due to the fragmented nature of the data. To address this issue, multivariate statistical research methods can be applied to collect and organize data based on the principle of homogeneity, using the frequency of isolating antibiotic-sensitive isolates. Ward's method can be employed to classify antibiotics based on the frequency of microorganisms retaining sensitivity to them. This classification will help identify the drugs that remain effective in combating infections (10).

The purpose of this study was to determine the effectiveness of antibacterial drugs through cluster analysis of antibiotic sensitivity data from clinical isolates of *Acinetobacter* and *Pseudomonas* spp., obtained from soft tissue wounds and burn injuries.

Materials and methods

The cohort included 76 clinical isolates of *Acinetobacter* and *Pseudomonas* spp. obtained from 141 patients (male – 95,04 %; female – 4,96 %) with combined injuries of soft tissues and burns. The mean age (M±SD) of patients was 38,57±10,90 years (median – 36 years). These patients were treated in 12 medical institutions of Ukraine providing tertiary care during 2022.

The material for research was collected using sterile probe-tampons, which were transferred to Aimes transport medium. The isolates were cultivated under aerobic conditions using meat-peptone, blood agar and thioglycol nutrient medium at a temperature of 37°C. The final identification of the obtained isolates was carried in the Riesbeck laboratory using MALDI-ToF (Bruker), followed by determination of their sensitivity to antimicrobial drugs at the EUCAST Development Laboratory (Växjö, Sweden) (11).

In the first stage of the study, the sensitivity of clinical isolates of Gram-negative bacteria to antibacterial drugs was determined using the standard Kirby-Bauer disc diffusion method according to the EUCAST methodology (12). The microorganisms were tested for sensitivity to piperacillin-tazobactam, ceftazidime, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin and trimethoprim-sulfamethoxazole. The isolates were categorized as sensitive (S), resistant (R) and sensitive under increased exposure (I), according to the tables of the limit indicators of the diameters of the growth retardation zones of microorganisms in the presence of the antibiotic (EUCAST Version 13.0, valid from 2023-01-01). Subsequently, extended antimicrobial susceptibility testing was performed to measure the minimal inhibitory concentrations (MIC) of antibiotics against the studied clinical strains, using the double serial dilution method according to ISO 20776-1 standard.

Written informed consent was obtained from each patient after providing a detailed explanation of the aims and protocol of the study. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki on Ethical Principles for Medical Research Involving Humans (13). It was approved by the commission on Biomedical Ethics Committee of Poltava State Medical University (protocol No. 210, November 23, 2022) and the Bioethics Committee of National Pirogov Memorial Medical University, Vinnytsya (protocol No. 11, November 10, 2022).

Statistical analysis

The normality of the data distribution was assessed using the Shapiro-Wilk test. Hypothesis testing was conducted using a two-sided approach. The data are expressed as mean (SD) and median (minimum-maximum), numbers and percentages (*n*, %). A significance level of $P < 0.05$ was considered statistically significant.

The hierarchical cluster analysis was performed using the Ward method to unite antibiotics to which the studied microorganisms retained sensitivity into the groups (clusters) (10). The method aims to combine statistically close spaced clusters and create small clusters. The distance between clusters was determined by incrementing the sum of squared distances of objects to the centres of the clusters resulting from their association. Analyses of variance methods were employed to estimate the distances between clusters. At each step of the algorithm, two clusters were merged if it led to a minimal increase in the objective function, which is the intra-group sum of squares. The conclusion was made about their similarity based on the distance between the clusters: as closer the clusters are, as more similar the clusters are to each other. Statistical analysis was performed using the standard IBM SPSS Statistics software version 22.0.

Results

As a result of the study, *Acinetobacter baumannii* (*n*=52) and *Pseudomonas aeruginosa* (*n*=24) were identified from patients with combined injuries.

Analyzing the susceptibility results of *Acinetobacter* spp. to antimicrobial agents recommended by EUCAST for testing, a high level of resistance formation was observed among representatives of this genus (Figure 1). Specifically, *A. baumannii* showed the highest level of resistance of to ciprofloxacin reaching 98.1% (51/52). Moreover, the proportion of resistant isolates of this species to the antibiotics used in the study exceeded 67.3% (35/52), also indicating the resistance of *A. baumannii* to tobramycin. It is worth noting that the proportion of *A. baumannii* resistant to carbapenems was 60.0% higher than the proportion of isolates sensitive to them.

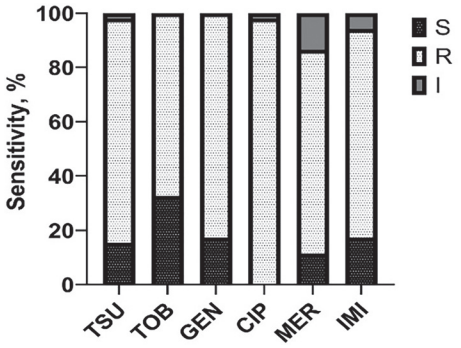

Figure 1. Antibiotic susceptibility of *A. baumannii* isolates (*n*=52). Abbreviations: S: sensitive, R: resistant, I: sensitive with increased exposure; TSU: trimethoprim-sulfamethoxazole; TOB: tobramycin; GEN: gentamicin; CIP: ciprofloxacin; MER: Meropenem; IMI: Imipenem.

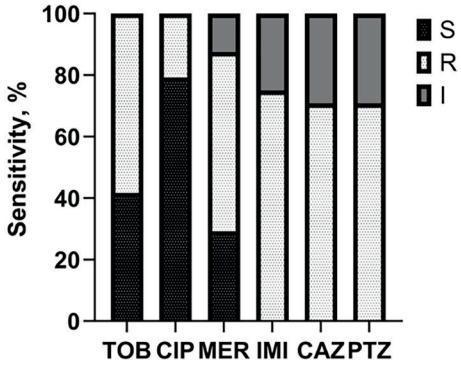

Figure 2. Sensitivity of *P. aeruginosa* isolates (*n*=24) to indicated antibiotics. Abbreviations: S: sensitive, R: resistant, I: sensitive with increased exposure; PTZ: piperacillin-tazobactam; CAZ: ceftazidime; IMI: imipenem; MER: meropenem; CIP: ciprofloxacin; TOB: tobramycin.

We found the highest level of resistance in *P. aeruginosa* to ciprofloxacin at 79.2% (19/24), with no susceptible isolates detected (Figure 2). The lowest proportions of resistant *Pseudomonas* spp. were observed for meropenem and tobramycin at 58.3% (14/24). Interestingly, 29.2% (10/24) of *P. aeruginosa* isolates were susceptible to meropenem, and 41.7% (14/24) were susceptible to tobramycin. It is noteworthy that no isolates were found to retain susceptibility to imipenem, ceftazidime and piperacillin-tazobactam.

The cluster analysis of *Acinetobacter* spp. susceptibility data resulted in the following findings. In the first stage, Cluster Ia included imipenem, gentamicin,

and trimethoprim-sulfamethoxazole, with susceptibility ranging from 15.4% to 17.3% (Figure 3). Cluster IIa consisted of meropenem, which had a a proportion of susceptible *A. baumannii* of 11.5%. This prevented it from being included in the previous cluster of antibiotics with slightly higher efficacy. Ciprofloxacin susceptibility entered Cluster IIIa and tobramycin Cluster IVa. These antibiotics represented the lowest and highest percentages of susceptible *Acinetobacter* spp. isolates, respectively. During the second and third steps of the cluster analysis, meropenem (IIa) and ciprofloxacin (IIIa) joined Cluster Ia, confirming the statistical homogeneity of the data on the susceptibility of *A. baumannii* to the antibiotics included in our analyses.

The data on antibiotic susceptibility of *Pseudomonas* spp. were subjected to cluster analysis, resulting in two main clusters identified at the first stage (Figure 4). Cluster Ia comprised imipenem, ciprofloxacin, ceftazidime, and piperacillin-tazobactam, all of which demonstrated complete effectiveness against *P. aeruginosa*. Meropenem and tobramycin, with susceptible rates of 29.2% and 41.7% among *Pseudomonas* spp. isolates, respectively, were assigned to Cluster IIa. Notably, Cluster IIa merged with the previous cluster during subsequent stages of the analysis, albeit with a significant merging distance, indicating a low statistical relationship between them.

Discussion

The development and introduction of new drugs, which potentially have antimicrobial properties, are considered to be a promising direction in the fight against antimicrobial resistance. However, in 2019 it was recognized that only about 15% of antibiotics in clinical research can really be considered innovative (1, 14). In addition, for many countries of the world, a factor that aggravates this problem is limited access to quality antimicrobial drugs (1).

Our results demonstrate the development of multiple antibiotic resistance among *A. baumannii*

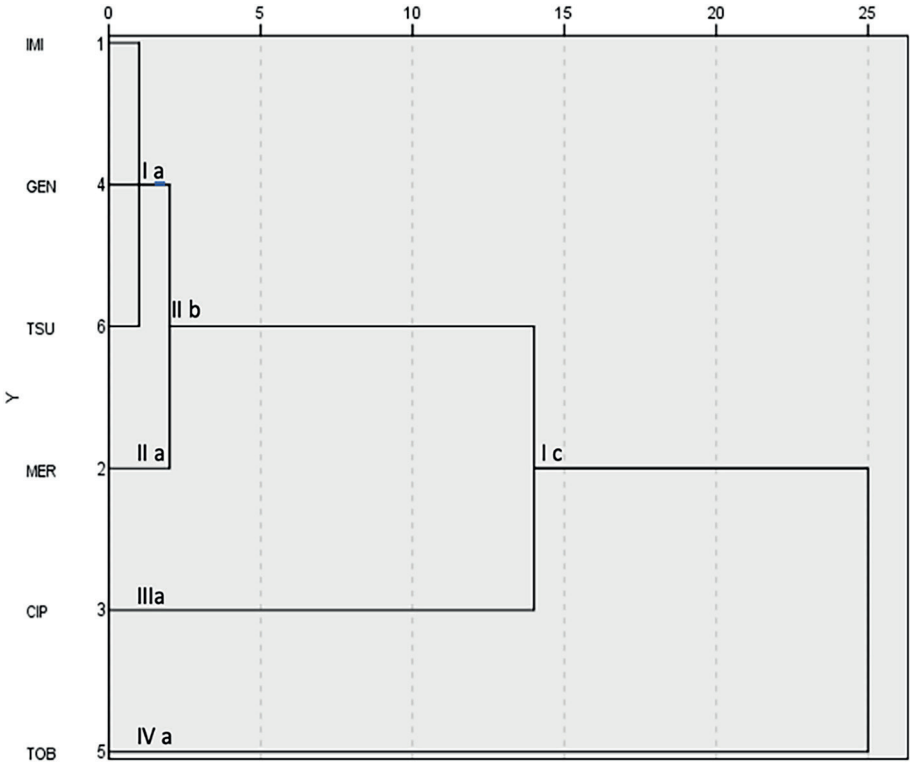

Figure 3. Dendrogram of cluster analysis using Ward's method depicting antibiotics according to their efficacy against *A. baumannii* isolates (*n*=52).

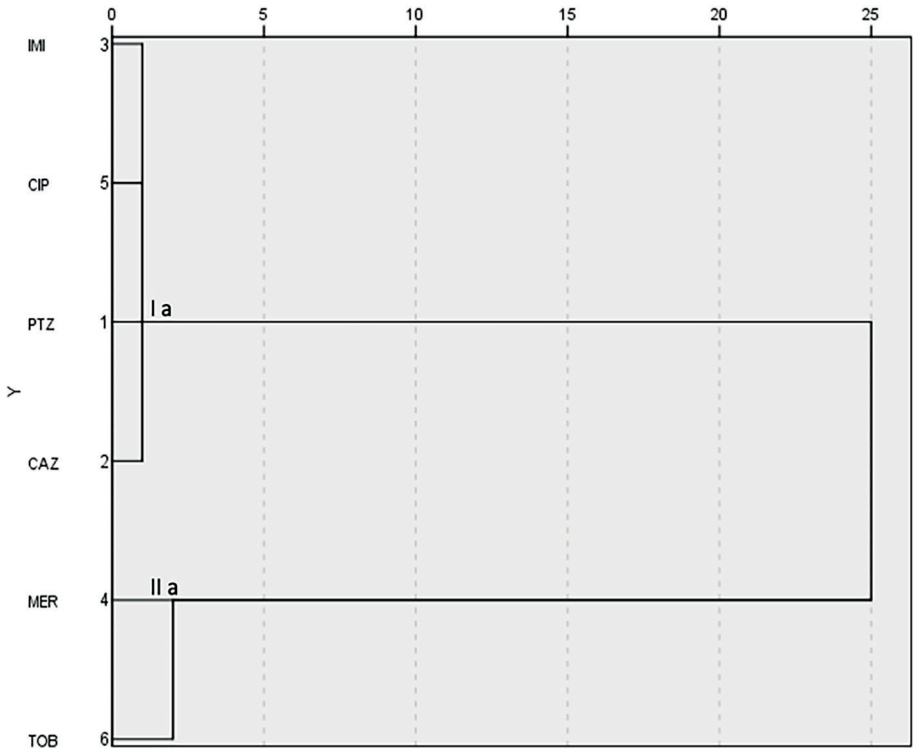

Figure 4. Dendrogram of cluster analysis using Ward's method depicting antibiotics according to their efficacy against *P. aeruginosa* isolates (*n*=24).

and *P. aeruginosa* isolates isolated from patients with combined soft tissue injuries in tertiary care facilities in Ukraine during 2022. Developing countries, including Ukraine, are generally characterized by rapid rates of antibiotic resistance in microorganisms. This is primarily attributed to the lack of mechanisms for regulating and monitoring the resistance development of at the state level, inadequate quality control of available antibiotics, and the widespread availability and inappropriate clinical use of antibacterial drugs (15). However, the situation in Ukraine has become more complex since the beginning of the Maidan Revolution in 2014, followed by the outbreak of the military conflict in the eastern part of the country, leading to a full-scale war. Indeed, literature data indicate a direct link between the development of multidrug resistance in microorganisms and natural disasters, military operations, and increased migration within the country (16 - 18).

Data from a retrospective multicentre microbiological study conducted in four military hospitals in Ukraine during 2014-2020 revealed the prevalence of *A. baumannii, P. aeruginosa and Klebsiella pneumoniae* as pathogens in infected surgical wounds of wounded individuals. Moreover, the resistance of *A. baumannii and P. aeruginosa* to carbapenems was found to be 95% and 55.6%, respectively, due to the presence of β-lactamases of classes A, D and carbapenemases carrying the *armA*, *rmtB* genes (19). Furthermore, in Germany Next Generation Sequencing was performed on *A. baumannii* isolates obtained from wounded individuals in eastern Ukraine in 2014. That study revealed that the majority of isolates had acquired resistance genes to carbapenems, including $bla_{\text{OXA-23}}$, $bla_{\text{OXA-72}}$ and $bla_{\text{GES-12}}$. In addition, by determining multilocus sequences, the authors were able to compare the isolates obtained from injured patients with international strains of *A. baumannii*, thereby establishing the emergence of numerous local clonal lineages (20, 21).

Simultaneously, a multicentre, blinded study evaluating the efficacy of antibacterial drugs against infections caused by resistant Gram-negative pathogens

reported positive results with piperacillin-tazobactam (22). However, our data demonstrate a resistance level of 70% among *P. aeruginosa* isolates towards this antibacterial drug. It is worth noting that a similar decreasing trend in the effectiveness of piperacillin-tazobactam against resistant isolates of *A. baumannii* and *P. aeruginosa* has been confirmed by studies conducted in Eastern European countries (23, 24).

Considering the statistical clustering data obtained in our study, the situation regarding antibacterial drugs that remain effective against *A. baumannii and P. aeruginosa* is a major concern. Cluster analysis of susceptibility data for *P. aeruginosa* isolates resulted in the division of antibiotics into two clusters with a significant dissimilarity distance, highlighting meropenem and tobramycin as promising treatment options for infections caused by multidrug-resistant *P. aeruginosa* isolates. On the other hand, although the isolated *A. baumannii* strains showed susceptible to a higher number of antibacterial drugs compared to *P. aeruginosa*, tobramycin was the only effective drug against them. This finding indirectly suggests a discouraging trend of imipenem, gentamicin, meropenem, and trimethoprim-sulfamethoxazole completely losing their effectiveness against *A. baumannii*, as indicated by their statistical similar to the zero-susceptibility data for ciprofloxacin.

Conclusion

Clinical isolates of *A. baumannii* and *P. aeruginosa* obtained from patients with combined soft tissue injuries and burns in Ukraine in 2022 exhibit a high level of multidrug resistance. The average level of resistance to carbapenems among *A. baumannii* isolates was 75%, while *P. aeruginosa* isolates showed a resistance level of 67%.

Cluster analysis results indicate that only tobramycin potentially retains efficacy against *A. baumannii* isolates. Moreover, clinical isolates of *P. aeruginosa* demonstrate susceptible to tobramycin and meropenem.

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Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: KR, ON and VK conceived the study; the project administration in Ukraine was provided by VK, ON and DD and in laboratories in Sweden by KR; KR, ON and DD designed the study and methodology; ON, HN, DD, LB and RC were involved in collecting microbial cultures and their shipping to the laboratories; ON, MF, BL carried out prior isolation an microbiological study of clinical strains; ON, MF, and LB performed the antibacterial activity determination; KR carried out final identification of the obtained isolates; HN, LB and RC were involved in the review of the literature; MF and HN participated in the data analysis and preparation of the article for submitting, ON, MF, BL wrote the manuscript, VK, KR and DD provided critical manuscript revisions for valuable intellectual content reviewed the draft manuscript. All authors contributed to the interpretation of the results, the revision of the draft manuscript, and the approval of the final version.

References

1. Antimicrobial resistance. Key facts [document on the Internet]. WHO; 2021 [cited 2023 May 27]. Available from: https://www.who.int/news-room/fact-sheets/detail / antimicrobial-resistance
2. Disruption of access to medicines and medical devices in Ukraine, February–June 2022. Copenhagen: WHO Regional Office for Europe; 2022. Licence: CC BY-NC-SA 3.0 IGO.
3. Calhoun JH, Murray CK, Manring MM. Multidrug-resistant organisms in military wounds from Iraq and Afghanistan. Clin Orthop Relat Res. 2008;466(6):1356-62. doi: 10.1007/s11999-008-0212-9.
4. Gautham M. The double crisis of antimicrobial resistance in humanitarian settings. Antimicrobial resistance centre [Internet]. 2022 April 26 [cited 2023 May 27]. Available from: https://www.lshtm.ac.uk/research/centres/amr/news /341576/double-crisis-
5. Nazarchuk O, Dmyrtriiev D, Babina Y, Faustova M, Burkot V. Research of the activity of local anesthetics and antiseptics regarding clinical isolates of Acinetobacter baumannii as pathogens of postoperative infectious complications. Acta Biomed. 2022;93(1):e2022003. doi: 10.23750 /abm.v93i1.11842.

6. Shaprynskyi V, Nazarchuk O, Faustova M, et al. Some aspects of infectious complications in patients with surgical diseases. [Internet] Multycentr Trials. Lek Obzor. 2020;69 (7-8):257-60. Available from: https://www.researchgate .net/publication/342946513 Some aspects of infectious complications in patients with surgical diseases - Niektore aspekty infekcnych komplikacii u pacientov s chirurgickymi chorobami Multicentricka studia
7. Ibrahim S, Al-Saryi N, Al-Kadmy IMS, Aziz SN. Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals. Mol Biol Rep. 2021;(10):6987-98. doi: 10.1007/s11033-021-06690-6.
8. Eastridge BJ, Jenkins D, Flaherty S, Schiller H, Holcomb JB. Trauma system development in a theater of war: Experiences from Operation Iraqi Freedom and Operation Enduring Freedom. J Trauma. 2006 Dec;61(6):1366-72; discussion 1372-3. doi: 10.1097/01.ta.0000245894.78941.90. PMID: 17159678.
9. Michalopoulos A, Falagas ME. Treatment of Acinetobacter infections. Expert Opin Pharmacother. 2010;11(5):779-88. doi: 10.1517/14656561003596350.
10. Murtagh F, & Legendre P. Ward's hierarchical agglomerative clustering method: which algorithms implement Ward's criterion? J Classif. 2014;31(3): 274-295. https://doi.org/10.1007/s00357-014-9161-z.
11. Ljungquist O, Nazarchuk O, Kahlmeter G, et al. Highly multidrug-resistant Gram-negative bacterial infections in war victims in Ukraine, 2022. Lancet Infect Dis. 2023;23(7):784-6. doi: 10.1016/S1473-3099(23)00291-8.
12. European Committee on Antimicrobial Susceptibility Testing. European antimicrobial breakpoints. Basel: EUCAST (2022) https://eucast.org/clinical_breakpoints/ [Accessed January 10, 2023].
13. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013; 310(20):2191-4. doi: 10.1001/jama.2013.281053.
14. Global antimicrobial resistance and use surveillance system (GLASS) report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.
15. Chokshi A, Sifri Z, Cennimo D, Horng H. Global Contributors to Antibiotic Resistance. J Glob Infect Dis. 2019;11(1):36-42. doi: 10.4103/jgid.jgid_110_18. Erratum in: J Glob Infect Dis. 2019;11(3):131.
16. Hrabák J, Študentová V, Adámková V, et al. Report on a transborder spread of carbapenemase-producing bacteria by a patient injured during Euromaidan, Ukraine. New Microbes New Infect. 2015;8:28-30. doi: 10.1016/j.nmni .2015.09.005.
17. Haque U, Naeem A, Wang S, et al. The human toll and humanitarian crisis of the Russia-Ukraine war: the first 162 days. BMJ Glob Health. 2022;7(9):e009550. doi: 10.1136/bmjgh-2022-009550.
18. Loban' G, Faustova M, Dobrovolska O, Tkachenko P. War in Ukraine: incursion of antimicrobial resistance. Ir J Med Sci. 2023:1–3. doi: 10.1007/s11845-023-03401-x.
19. Kondratiuk V, Jones BT, Kovalchuk V, et al. Phenotypic and genotypic characterization of antibiotic resistance in military hospital-associated bacteria from war injuries in the Eastern Ukraine conflict between 2014 and 2020. J Hosp Infect. 2021;112:69-76. doi: 10.1016/j.jhin.2021.03.020.
20. Higgins PG, Hagen RM, Podbielski A, Frickmann H, Warnke P. Molecular Epidemiology of Carbapenem-Resistant Acinetobacter baumannii Isolated from War-Injured Patients from the Eastern Ukraine. Antibiotics (Basel). 2020;9(9):579. doi: 10.3390/antibiotics9090579.
21. Nahaichuk VI, Nazarchuk OA, Osadchuk NI, Dmytriiev DV, Nazarchuk HH. The analytical prognosis of the susceptibility to aminoglycosides and doxycycline in acinetobacter baumannii isolated from burns of intensive care unit patients. Wiad Lek. 2018;71(3 pt 2):705-9. PMID: 29783252.
22. Titov I, Wunderink RG, Roquilly A, et al. A Randomized, Double-blind, Multicenter Trial Comparing Efficacy and Safety of Imipenem/Cilastatin/Relebactam Versus Piperacillin/Tazobactam in Adults With Hospital-acquired or Ventilator-associated Bacterial Pneumonia (RESTORE-IMI 2 Study). Clin Infect Dis. 2021;73(11):e4539-e48. doi: 10.1093 /cid/ciaa803.
23. AlAmri AM, AlQurayan AM, Sebastian T, AlNimr AM. Molecular Surveillance of Multidrug-Resistant Acinetobacter baumannii. Curr Microbiol. 2020;77(3):335-42. doi: 10.1007/s00284-019-01836-z. Epub 2019 Dec 12. PMID: 31832843.
24. Keskin H, Tekeli A, Dolapci İ, Öcal D. Klinik örneklerden izole edilen Acinetobacter baumannii suşlarında beta-laktamaz kaynaklı direncin moleküler karakterizasyonu [Molecular characterization of beta-lactamase-associated resistance in Acinetobacter baumannii strains isolated from clinical samples].[Turkish]. Mikrobiyol Bul. 2014;48(3): 365-76. PMID: 25052103.

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