# Microbiological profile and antimicrobial resistance of mine-blast wounds in Ukraine: a single-center study

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Infections resulting from combat wounds pose a significant challenge to modern medicine. Their unique polymicrobial nature, combined with massive tissue damage and the presence of foreign bodies, creates a favorable environment for the development of multidrug-resistant pathogens. The increasingly high level of antimicrobial resistance in the context of combat operations in Ukraine underscores the critical need for continuous epidemiological surveillance and adaptation of clinical protocols.

The aim of this study was to determine the species composition and antimicrobial resistance profile of causative agents of suppurative-inflammatory soft tissue diseases in patients with blast-related trauma who were treated at a tertiary medical facility.

Materials and methods. A prospective, single-center, observational study was conducted from January to May 2025 at the Kyiv Regional Clinical Hospital. A total of 276 wound samples were analyzed. The identification of microorganisms and testing of anti-biotic sensitivity were performed according to the EUCAST methods and interpretation.

**Results.** Of the 276 samples, 86.6 % were positive. A total of 171 clinical strains were isolated, of which 58.5 % were Gram-negative and 38.2 % were Gram-positive microorganisms, and 2.3 % were fungi. The dominant etiological agent was *Acinetobacter baumannii* (21.6 %), followed by *Klebsiella pneumoniae* (13.5 %) and *Proteus mirabilis* (7.0 %). In *A. baumannii*, extremely high levels of resistance to carbapenems (imipenem – 83.8 %, meropenem – 73.0 %) and fluoroquinolones (94.6 %) were observed, while high sensitivity to colistin (100.0 %) and tobramycin (73.0 %) was maintained.

**Conclusions.** The local results obtained confirm the nationwide trend of the dominance of multidrug-resistant Gram-negative pathogens in combat wound infections. The detected high level of resistance to broad-spectrum drugs indicates a critical need for the optimization of empirical antibiotic therapy and the strengthening of infection prevention and control measures.

# Keywords:

blast injuries, wounds, blastrelated injuries, wound, antimicrobial resistance, Acinetobacter baumannii, Klebsiella pneumoniae.

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# Мікробіологічний профіль та антимікробна резистентність мінно-вибухових ран в Україні: одноцентрове дослідження

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Інфекції, що виникають внаслідок бойових поранень, є складною проблемою сучасної медицини. Їхня унікальна полімікробна природа, поєднана з масивним ушкодженням тканин і наявністю чужорідних тіл, створює сприятливе середовище для розвитку мультирезистентних патогенів. Зростання рівня протимікробної резистентності в умовах бойових дій в Україні підтверджує критичну потребу в безперервному епідеміологічному нагляді й адаптації клінічних протоколів.

**Мета роботи** – визначення особливостей видового складу та профілю протимікробної резистентності збудників гнійно-запальних захворювань м'яких тканин у пацієнтів із мінно-вибуховою травмою, які перебували на лікуванні у третинному медичному закладі.

Матеріали і методи. Проспективне одноцентрове обсерваційне дослідження здійснили з січня до травня 2025 року на базі Комунального некомерційного підприємства Київської обласної ради «Київська обласна клінічна лікарня». Проаналізовано 276 зразків, взятих із ран. Ідентифікацію мікроорганізмів і тестування чутливості до антибіотиків здійснили, використавши методи й інтерпретацію, що рекомендовані EUCAST.

**Результати.** Із 276 зразків 86,6 % виявились позитивними. Виділено 171 клінічний штам, серед них 58,5 % — грамнегативні, 38,2 % — грампозитивні мікроорганізми, 2,3 % — гриби. Етіологічний агент, що домінував, — *Acinetobacter baumannii* (21,6 %); менше виявляли *Klebsiella pneumoniae* (13,5 %) та *Proteus mirabilis* (7,0 %). Для *A. baumannii* зафіксовано вкрай високий рівень резистентності до карбапенемів (іміпенем — 83,8 %, меропенем — 73,0 %) та фторхінолонів (94,6 %), але зберігалася висока чутливість до колістину (100,0 %) та тобраміцину (73,0 %).

**Висновки.** Отримані локальні результати підтверджують загальнонаціональну тенденцію до домінування мультирезистентних грамнегативних збудників в інфекціях бойових ран. Встановлено високий рівень резистентності до препаратів широкого спектра дії, що підтверджує критичну потребу в оптимізації емпіричної антибіотикотерапії та посиленні заходів з профілактики інфекцій та інфекційного контролю.

Ключові слова:

мінно-вибухові травми, рана, антимікробна резистентність, Acinetobacter baumannii, Klebsiella pneumoniae.

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The problem of antimicrobial resistance (AMR) is recognized as one of the most critical global threats to public health. Military conflicts, with their unique medical care challenges and high rates of antibiotic resistance, act as powerful catalysts for the accelerated spread of AMR [1,2]. Studies conducted in the context of the full-scale invasion of Ukraine have already demonstrated an extremely high level of resistance, particularly to carbapenems, among pathogens such as Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa [3]. Analysis of strains isolated from wounded patients has revealed a wide spectrum of resistance genes, including blaNDM-1, blaOXA-23, blaOXA-48, and blaOXA-72. A highly unusual co-production of NDM and KPC in Pseudomonas aeruginosa and Acinetobacter baumannii has also been shown. suggesting the rapid spread and evolution of resistance genes [4]. Moreover, some strains, such as K. pneumoniae, have been identified as not only pan-resistant but also hypervirulent, meaning they can cause severe infections despite their resistance [5,6]. This highlights the critical importance of continuous monitoring and the search for new methods for eliminating these pathogens.

In light of this, conducting local studies is of particular importance. Such research allows for the identification of regional features of the microbiological landscape and resistance profiles, which may differ from nationwide data. Correlating local data with national epidemiological surveillance data makes it possible to identify both unique regional challenges and systemic problems in the healthcare system.

### **Aim**

The aim of this study was to determine the species composition and antimicrobial resistance profile of causative agents of suppurative-inflammatory soft tissue diseases in patients with blast-related trauma who were treated at a tertiary medical facility.

# **Materials and methods**

This prospective, single-center observational study was conducted over a 5-month period, from January to May 2025, at the Kyiv Regional Clinical Hospital. This institution is one of the oldest and largest public medical facilities in Ukraine, providing multidisciplinary medical care to the Kyiv region population. A total of 276 wound samples obtained from patients in inpatient departments were included in the study.

The general characteristics of the study population were as follows: mean age – 38.6 ± 9.0 years (range: 19–59 years); mean weight  $-78.9 \pm 9.8$  kg (range: 51–105 kg); sex all patients were male; mean length of stay in an intensive care unit – 41.2 ± 32.8 days (range: 4–163 days); percentage of patients requiring mechanical ventilation – 7.3 %.

The wound surface was prepared for sample collection using the Levine technique (wound cleansing with sterile saline to clean and debride). Samples were collected aseptically by rotating a sterile swab for 5 seconds over a 1 cm<sup>2</sup> wound area. The samples were transported to the bacteriological laboratory immediately after collection.

Cultural examination of the wound samples was performed by inoculating them onto 5 % blood agar, mannitol-salt agar, and MacConkey agar, followed by aerobic

incubation at 37 °C for 18-24 hours. The identification of clinical bacterial isolates was carried out based on morphological and biochemical characteristics according to standard methods.

The susceptibility profile of pure bacterial isolates was determined using the Kirby-Bauer disk diffusion method. The testing was performed with 26 different antibiotics covering a wide range of classes. The interpretation of the growth inhibition zone measurements was done according to the EUCAST criteria.

The antibiotics used were: Amikacin (30 µg), Ampicillin (2 µg), Aztreonam (30 µg), Ceftazidime (10 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Colistin reference MIC from 0.25 mg/L to 16.00 mg/L. Ceftazidime / Avibactam (30/20 µg), Imipenem (10 µg), Cefepime (30 µg), Cefoxitin (30 μg), Gentamicin (10 μg), Erythromycin (15 μg), Linezolid (10 μg), Rifampicin (5 μg), Meropenem (10 μg), Nitrofurantoin (100 μg), Levofloxacin (5 μg), Ampicillin / Sulbactam (10/10 µg), Trimethoprim / Sulfamethoxazole (1.25/23.75 µg), Ticarcillin / Clavulanic acid (75/10 µg), Teicoplanin (30 μg), Tigecycline (15 μg), Tobramycin (10 μg), Piperacillin / Tazobactam (30/6 μg), and Vancomycin (5 μg).

Statistical processing of data regarding the species composition of microorganisms, their quantity, and antibiotic susceptibility was performed using the WHONET 5.6 software. To initially evaluate the reliability of the results, confidence intervals were computed for each "microorganism-antibiotic" combination. It was established that an interval length below 15 % reflected high precision, 15-30 % suggested moderate precision, and above 30 % denoted low precision, necessitating a larger sample size or a reassessment of the methodology.

For example, the confidence interval length for the susceptibility of Acinetobacter baumannii to ciprofloxacin was 18.6 % (moderate precision), while for the susceptibility of Klebsiella pneumoniae to amikacin it was 36.6 % (low precision). These calculations emphasize that conclusions regarding some "microorganism-antibiotic" combinations should be made with caution, and a larger sample size is needed to obtain more reliable data.

# Results

From the total of 276 samples examined, positive results were obtained in 86.6 % of cases. In total, 171 clinical strains corresponding to the main etiological agents of infections were isolated. Gram-negative microorganisms were dominant, accounting for 58.5 % (100 strains), while Gram-positive organisms accounted for 38.2 % (67 strains). Fungal microflora was also detected, making up 2.3 % (4 strains). Microbial associations of two and three cultures were found in 7.4 % and 0.7 % of cases, respectively.

The dominant etiological agent most frequently isolated from the biological materials of surgical wounds was A. baumannii, which accounted for 37 of the isolated strains (21.6 %). The next most common was K. pneumoniae with 23 isolates (13.5 %), followed by Proteus mirabilis (12 isolates, 7.0 %) and Pseudomonas aeruginosa (11 isolates, 6.4 %).

The resistance analysis of the 37 clinical strains of A. baumannii showed an extremely high resistance to most of the tested antibiotics. Specifically, 83.3 % of the strains

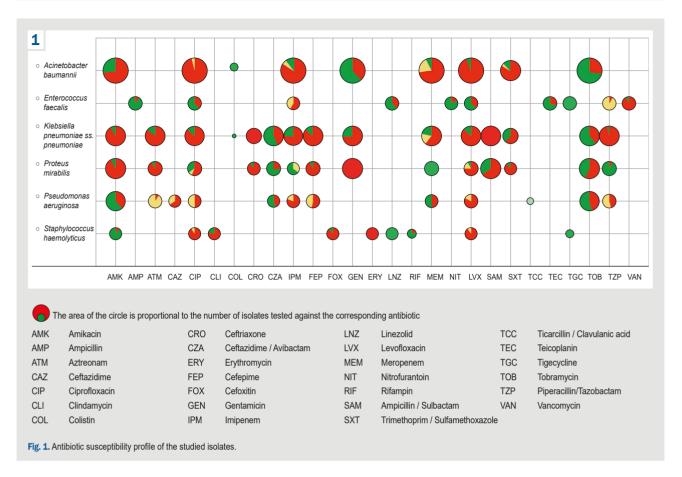


Table 1. Number of isolates tested with each antibiotic

Parameter	AMK	AMP	АТМ	CAZ	CIP	СП	СОГ	CRO	cza	ERY	FF.	FOX	GEN	IPM	LNZ	LVX	MEM	LIN LIN	RIF	SAM	SXT	TCC	TEC	TGC	TOB	TZP	VAN
Acinetobacter baumannii	37	-	-	-	37	-	4	-	-	-	-	-	37	37	-	37	37	-	-	-	24	-	-	-	37	-	-
Enterococcus faecalis	-	11	-	-	11	-	-	-	-	-	-	-	-	10	11	11	-	10	-	-	-	-	11	11	-	11	11
Klebsiella pneumoniae ss. pneumoniae	23	-	23	-	22	-	1	14	23	-	23	-	23	21	-	23	23	-	-	23	14	-	-	-	23	23	-
Proteus mirabilis	24	-	12		12	-	-	10	12	-	12	-	24	9	-	12	12	-	-	24	9	-	-	-	24	12	-
Pseudomonas aeruginosa	22	-	11	9	10	-	-	-	9	-	11	-	-	10	-	11	10	-	-	-	-	-	3	-	22	11	-
Staphylococcus haemolyticus	9	-	-		9	9	-	-	-	9		9	-	-	9	9	-	-	5	-	-	-	-	4	-	-	-

were resistant to Trimethoprim / Sulfamethoxazole. Resistance to carbapenems was also very high: 83.8 % of strains were resistant to imipenem, and 73.0 % were resistant to meropenem. The highest level of resistance was recorded for fluoroquinolones, with resistance to ciprofloxacin and levofloxacin at 94.6 %.

At the same time, *A. baumannii* showed significantly lower resistance to tobramycin (27.0 %) compared to amikacin (73.0 %) and gentamicin (37.8 %). Notably, no strains resistant to colistin were detected (0 % resistant strains) (*Fig. 1. Tables 1. 2*).

Other key pathogens also demonstrated significant resistance. For example, in *K. pneumoniae*, the resistance rate to Ceftazidime / Avibactam was 43.5 %. *Pseudomonas aeruginosa* showed high resistance to cefepime (54.5 %) and ciprofloxacin (50.0 %).

# **Discussion**

The results of this local study are generally in agreement with the national trend described in the analytical report from the Public Health Center of the Ministry of Health of Ukraine. In both cases, a dominance of multidrug-resistant Gram-negative bacteria was observed, particularly *Acinetobacter spp.* and *K. pneumoniae*. However, in our study, *A. baumannii* was the most common pathogen (21.6 % of all strains), whereas on a national scale, *S. aureus* ranked first (24.8 %), with *Acinetobacter spp.* (23.6 %) and *K. pneumoniae* (18.1 %) in second and third place, respectively.

This difference is likely related to the specific nature of the Kyiv Regional Clinical Hospital as a tertiary-level facility that provides specialized medical care. Patients admitted here often had established hospital-acquired infections

Table 2. Susceptibility rates (%) of isolates to antibiotics

Parameter	Acinetobacter baumannii			Enterococcus faecalis			Klebsiella pneumoniae ss. pneumoniae			Proteus mirabilis			Pseudomonas aeruginosa			Staphylococcus haemolyticus		
	%R	<b>%</b> I	%S	%R	<b>%</b> I	%S	%R	<b>%</b> I	%S	%R	<b>%</b> I	%S	%R	<b>%</b> I	%S	%R	<b>%</b> I	%S
AMK	73.0	0.0	27	-	_	-	73.9	0.0	26.1	91.7	0.0	8.3	36.4	0.0	63.6	11.1	0.0	88.9
AMP	-	-	-	9.1	0.0	90.9	-	-	-	-	-	-	-	-	-	-	-	-
ATM	-	-	-	-	-	-	82.6	0.0	17.4	91.7	0.0	8.3	9.1	90.9	0.0	-	-	-
CAZ	-	-	-	-	-	-	-	-	-	-	-	-	66.7	33.3	0.0	-	-	-
CIP	94.6	5.4	0.0	36.4	0.0	63.6	86.4	0.0	13.6	83.3	8.3	8.3	50.0	50.0	0.0	88.9	11.1	0.0
CLI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	77.8	0.0	22.2
COL	0.0	0.0	100.0	-	-	-	0.0	0.0	100.0	-	-	-	-	-	-	-	-	-
CRO	-	-	-	-	-	-	100.0	0.0	0.0	90.0	0.0	10.0	-	-	-	-	-	-
CZA	-	-	-	-	-	_	43.5	0.0	56.5	25.0	0.0	75.0	44.4	0.0	55.6	-	-	_
ERY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	0.0	0.0
FEP	-	-	-	-	-	-	87.0	0.0	13.0	91.7	0.0	8.3	54.5	45.5	0.0	-	-	-
FOX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	88.9	0.0	11.1
GEN	37.8	0.0	62.2	-	-	_	73.9	0.0	26.1	100.0	0.0	0.0	-	-	-	-	-	_
IPM	83.8	5.4	10.8	60.0	40.0	0.0	76.2	0.0	23.8	0.0	33.3	66.7	80.0	20.0	0.0	-	-	-
LNZ	_	-	-	36.4	0.0	63.6	-	-	_	-	-	_	-	-	-	0.0	0.0	100
LVX	94.6	0.0	5.4	36.4	0.0	63.6	87.0	0.0	13.0	75.0	16.7	8.3	81.8	18.2	0.0	88.9	11.1	0.0
MEM	73.0	18.9	8.1	-	-	-	60.9	17.4	21.7	0.0	0.0	100.0	50.0	-	50.0	-	-	-
NIT	-	-	-	20.0	0.0	80.0	-	-	-	-	-	-	-	-	-	-	-	-
RIF	_	-	-	-	-	_	-	-	-	-	-	_	-	-	-	200	0.0	80.0
SAM	-	-	-	-	-	-	100.0	0.0	0.0	66.7	0.0	33.3	-	-	-	-	-	-
SXT	83.3	4.2	12.5	-	-	_	64.3	0.0	35.7	88.9	0.0	11.1	-	-	-	-	-	_
TCC	-	-	-	-	-	_	-	-	-	-	-	-	0.0	100.0	0.0	-	-	-
TEC	-	-	-	27.3	0.0	72.7	-	-	-	-	-	-	-	-	-	-	-	_
TGC	-	-	-	0.0	0.0	100.0	-	-	-	-	-	-	-	-	-	0.0	0.0	100.0
TOB	27.0	0.0	73.0	-	-	-	39.1	0.0	60.9	58.3	0.0	41.7	45.5	0.0	54.5	-	-	_
TZP	-	-	-	9.1	90.9	0.0	95.7	0.0	4.3	8.3	0.0	91.7	45.5	54.5	0.0	-	-	-
VAN	-	-	-	90.9	0.0	9.1	-	-	-	-	-	-	-	-	-	-	-	_

caused by pathogens circulating within this specific hospital environment. This highlights that the microbial profile can differ significantly at various stages of medical evacuation, which requires the development of adapted clinical protocols for each level of care.

The national analytical report confirms that the length of hospital stay is a key factor in the spread of AMR. The highest number of positive samples was found in patients who had been wounded for over 72 hours and hospitalized for more than 48 hours. The report indicates a higher frequency of isolating monitored pathogens in patients after 48 hours of hospitalization, suggesting a relationship between longer hospital stays and pathogen detection. It is hypothesized that the initial contamination of the wound at the moment of injury, is often replaced by hospital-acquired, multidrug-resistant pathogens due to the selective pressure of antibiotics and breaches in infection control measures. Patients who are transferred between multiple medical facilities are exposed to an increased risk of infection with polyresistant strains circulating in these institutions [2].

Our results are consistent with the findings of other international studies on infectious complications of combat wounds in Ukraine, which also emphasize the problem of growing AMR. A study conducted in collaboration with laboratories at Lund University (Sweden) and the EU (EUCAST) has shown a high prevalence of multidrug-resistant Gram-negative bacterial infections among military trauma patients in Ukraine. Among 156 analyzed strains, 58 % were resistant to meropenem, including 76 % of *Klebsiella pneumoniae* strains and 73 % of *Acinetobacter* 

baumannii strains [7]. In our study, the level of resistance to meropenem was slightly lower: 73.0 % for *A. baumannii* and 60.9 % for *K. pneumoniae* according to the national report. However, this confirms the general trend of high carbapenem resistance in Ukraine.

In contrast to our study, where *Acinetobacter baumannii* was the most common pathogen (21.6 %), another study involving patients transferred from Ukraine to a Spanish hospital has found that Gram-negative rods and multidrug-resistant *S. aureus* were dominant. In that study, numerous resistance genes were identified, including NDM, KPC, OXA-48, VIM, IMP, and GES. Rare cases of NDM and KPC co-production in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were also detected, indicating a rapid evolution of resistance mechanisms [3].

Another important discovery completed by Swedish researchers concerns the phenomenon of "hypervirulence" in pan-resistant strains of *Klebsiella pneumoniae* isolated from wounded Ukrainian patients. Typically, bacteria lose virulence by expending energy to develop resistance. However, these strains had a full set of genes that made them both resistant to antibiotics and aggressive, capable of causing severe infections and rapid death in laboratory animals. This highlights that combating such pathogens is complicated not only by their resistance but also by their enhanced pathogenicity [8].

All this data confirms that combat wound infections in Ukraine differ from civilian injuries due to a high proportion of polymicrobial infections, the dominance of hospital-acquired multidrug-resistant pathogens (especially

Gram-negative bacteria), and the presence of complex genetic resistance mechanisms, which makes treatment extremely challenging.

## **Conclusions**

- 1. Findings from this study indicate that treating purulent-inflammatory infections in blast injury patients is complicated by the prevalence of highly resistant Gram-negative bacteria, notably Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Their resistance to common broad-spectrum antibiotics. including carbapenems and fluoroguinolones, limits the effectiveness of current empirical therapy.
- 2. Identifying a link between the length of hospitalization and the degree of resistance emphasizes that nosocomial infections are a major contributor to the rise of AMR. This challenge is significantly worsened by systemic failures in tracking diseases (epidemiological surveillance), the inappropriate use of antibiotics, and non-compliance with laboratory quality standards.

#### Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board. Permissions, that particularly cover this study, were approved by protocol No. 6, 22 July 2021, and No. 8, 26 September 2022, and No. 13, 15 December 2023 of the Commission on Ethics of Scientific Research, Experimental Development, and Scientific Works of Danylo Halytsky Lviv National Medical University, Ukraine. Written informed consent was obtained from the patient(s) for publication upon admission to the hospital.

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