ORIGINAL ARTICLE

CONTENTS

Healthcare associated infections in patients with combat wounds and antimicrobial resistance of the responsible pathogens in Ukraine: results of a multicenter study (2022-2024)

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ABSTRACT

Aim: Aim this study was to estimate the prevalence and incidence of HAI in patients with combat wounds, and determine phenotypic and genotypic aspects of antimicrobial resistance of the responsible pathogens in Ukraine.

Materials and Methods: A multicenter observational cohort study based on HAIs and antimicrobial resistance surveillance data in Ukraine. Between June 21, 2022 and December 31, 2024, patients (aged 20-74 years) with combat wounds were admitted to civilian hospitals which are located in Kharkiv, Dnipro, Kherson, Zaporizhzhia, Odessa, and Kyiv, Ukraine. The criteria for HAIs were adapted from the CDC/NHSN. Antimicrobial susceptibility test used Kirby – Bauer disc diffusion antibiotic test according to the EUCAST.

Results: Among 7,324 patients with combat wound, 5,022 (68.6%) HAIs were observed. The most frequently reported HAI types were surgical site infections (27.3%), bone and joint infections (25.6%), skin and soft tissue infections (15.7%), bloodstream infections (9.7%), central nervous system infections (7.9%), and pneumonia (5.3%). In total, 88.9% isolates from patients with combat wounds were found to be MDROs. A significant number of the MDROs isolated from patients with HAIs had β-lactamase genes, including extended-spectrum β-lactamase (ESBL) (53.1%), OXA-type (32.9%), AmpC-type (35.7%), KPC-type (31.8%), and metallo-β-lactamases (51.4%) including IMP-type (18.5%), VIM-type (29.6%), and NDM-1 (34.7%).

Conclusions: This study found a high prevalence of HAI in patients with combat wounds caused by MDROs, varying depending on the bacterial species, and antimicrobial group. The majority of MDRO isolates from patients with HAI carried β-lactamase genes.

KEY WORDS: healthcare-associated infections, multidrug-resistant organisms, β-lactamase genes, combat wound, Eastern Ukraine military conflict, Ukraine

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INTRODUCTION

The ongiong military conflict in Ukraine has placed extraordinary pressure on medical infrastructure due to the increase in the number of hospitalized patients. This situation is observed primarily in the east of Ukraine, where active military actions continue. In addition, both soldiers and civilians also suffer combat injuries in other regions of Ukraine.

Combat wounds are considered contaminated. Infectious complications are a frequent occurrence associated with combat-related trauma. Therefore, most injured patients receive a prophylactic dose of antibi-

otics upon hospitalization. However, despite antibiotic prophylaxis, some in patients with war injuries after surgical treatment develop infections. According to the literature, approximately 34-57% of patients at hospitals developed a combat-related wound infection [1, 2]. Currently, there is no national network for prospective surveillance for combat- related infections in patients evacuated to civil Ukrainian hospitals.

It is reported that a significant morbidity of combat-related infections in Iraq, Afghanistan, and Vietnam were associated with multidrug-resistant organisms (MDROs) [1, 3]. There is also concern regarding potential adverse outcomes associated with MDROs of the combat-related infectious complications in Ukraine. The Eastern Ukraine military conflict has disrupted the normal functioning of healthcare and poses a potential risk for the increased spread of prevalence MDROs in Ukrainian hospitals. There are already numerous health issues regarding the spread of diseases associated with MDROs in Ukraine. Antimicrobial resistance (AMR) spread in Ukrainian hospitals is a national concern [4, 5] and may have implications for other country [6, 7]. It has been highlighted in recent studies that has increase in antibiotic-resistant pathogens since the start of the war [6, 8, 9].

Due to high morbidity a combat-related wound infection caused by MDROs, early diagnosis and treatment of these infections. Currently, in Ukrainian hospitals, efforts to improve infection control (IC) training and to begin local HAI and AMR surveillance have been implemented. However, finance and personnel resources are limited in Ukraine. These creating difficulties implementing surveillance and establishing effective IC measures for MDROs prevention. Previous reports of combat-related HAIs in Ukraine have been limited to AMR of the responsible pathogens these infections in military personnel admitted only to military hospitals [10, 11].

AIM

The aim of this study was to estimate the prevalence and incidence of HAI in patients with combat wounds, and determine phenotypic and genotypic aspects of antimicrobial resistance of the responsible pathogens in Ukraine.

MATERIALS AND METHODS

STUDY DESIGN, SETTING AND PATIENTS

A multicenter observational cohort study based on HAIs and antimicrobial resistance surveillance data in Ukraine. This study was initiated in June 2022 as combat operations were increasing in Ukraine. Between June 21, 2022 and December 31, 2024, 7,324 patients (aged 20-74 years) with combat wounds were admitted to hospitals which are located in the Eastern, Southern and Central regions (Kharkiv, Dnipro, Kherson, Zaporizhzhia, Odessa, and Kyiv) of Ukraine. Of which 6,782 (92.6%) were military personnel that has combat wound in military conflict and 542 (7.4%) were combat trauma or injured outside of the active war theaters. All wounded military personnel and civilians with war trauma or injuries was medevac'd to local hospitals.

DEFINITION

An HAI cases was defined as an infection arising >48 h after surgical or other medical procedures. In this study the criteria for specific HAI site were adapted from the Centers for Disease Control and Prevention's and National Healthcare Safety Network's (CDC/NHSN) definitions [12].

DATA COLLECTION

We developed a special questionnaire for collecting infection-related data from all patients with trauma or injuries, admitted to participating hospitals. We developed a special questionnaire for collecting infection-related data from all patients, admitted to participating hospitals. In this study was collected data from medical records, including gender, age (years), microbiological investigations, invasive procedures, procedure/treatment, day of admission to the ICU, surgical interventions, previous hospitalization, antibiotics usage, and culture and sensitivity of the clinical isolates. Specimens from all patients were also collected and retained in a microbiological isolate repository. All patients were given the opportunity to enroll in a prospective follow-up cohort study. Follow-up of each patient (besides the military personnel) with trauma or injuries after surgical treatment was continued for one month, and for some infections for up to 90 days.

MICROBIOLOGICAL ANALYSIS

In this study, species identification isolated strains and was performed antibiotic susceptibility testing was performed by using automated microbiology testing (Vitek-2; bioMe´rieux, France). Some antimicrobial susceptibility test used Kirby – Bauer disc diffusion antibiotic test according to the protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://eucast.org). A bacterial isolate is considered resistant to antibiotics when tested and interpreted as resistant in accordance with the EUCAST clinical breakpoint criteria. All Multidrug resistance or-

Table 1. Distribution of healthcare-associated infections (HAI) detected in patients with combat wound in Ukraine, 2022-2024 (n=5,022)

Type of HAI	n	%	95% CI
Surgical site infections	1,372	27,3	26.8 – 27.8
Bone and joint infections	1,287	25,6	25.1 – 26.1
Skin and soft tissue infections	786	15,7	15.3 – 16.1
Bloodstream infections	487	9,7	9.4 – 10.1
Central nervous system infections	396	7,9	7.6 – 8.2
Pneumonia	267	5,3	5.1 – 5.6
Urinary tract infections	204	4,1	3.9 – 4.3
Eye, ear, nose, throat, or mouth infections	83	1,7	1.6 – 1.9
Lower respiratory infection, other than pneumonia	54	1,1	1.0 – 1.2
Cardiovascular system infections	52	1,0	0.9 – 1.1
Clinical sepsis	34	0,7	0.6 – 0.8

CI, Confidence Interval
Source: compiled by the authors of this study

ganisms (MDROs) identified in this study were collected for further molecular-based investigations.

MOLECULAR ANALYSIS

All phenotypically multidrug resistant (MDR) strains isolated from patients with trauma or injuries, were analysed for the presence of the b-lactamase genes using polymerase chain reaction (PCR). DNA was extracted from a single colony of each isolate using QIAmp® DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Confirmation of isolates as non-susceptible to β-lactamases was done by the Carba NP test, the EDTA double-disc synergy test (DDST), and PCR for bla_{VIM-}, bla_{IMP-} and bla_{NDM-}like genes [13-16]. Gram-negative isolates as ESBLs were detected by DDST with aztreonam and amoxicillin plus clavulanate discs [17]. In this study, selected β-lactamase genes, namely bla_{CTX-M-}, bla_{SHV-}, bla_{TEM-}, bla_{OXA-1-} and $bla_{OXA-48-}$like genes, and other genes were detected by PCR as described previously [18]. Cefoxitin-resistant *Staphylococcus aureus* (MRSA) was tested for the presence of the *mec*A gene using PCR, as previously described.

ETHICS

Ethical clearance for this study was obtained from the Ethics Board of the Ukrainian Association of Infection Control and Antimicrobial Resistance. Patients agreed to participate in this study. Participants' data were anonymised prior to the analysis.

STATISTICAL ANALYSIS

All the data entered into the electronic database and analyzed. IBM SPSS and Microsoft Excel (Microsoft Office 2016 Redmond, WA, USA) were used in the statistical analysis of the collected material. Statistical analysis presents descriptive statistics for the characteristics of units, patients, and types of HAI. A descriptive analysis was performed by calculating the number and percentage for each value. Pearson's chi-square test was performed to check the matching performance between the case and comparison groups and compare the differences between groups for categorical variables. In this study, the level of significance was $p < 0.05$.

RESULTS

PREVALENCE AND INCIDENCE OF HAI, AND PATIENT CHARACTERISTICS

During the study period (2022-2024), 5,022 (68.6%) of 7,324 patients with combat wound were found to have HAIs. HAIs cases in military personnel and civilians with combat wound were 68,7% (4657/6782) and 67,3 (365/542), respectively. Of the total cases of HAIs in patients with combat wound, 27.3% [95% confidence interval (CI), 26.8-27.8] were surgical site infections (SSI), 25.6% (95% CI, 25.1-26.1) bone and joint infections (BJ), 15.7% (95% CI, 15.3-16.1) skin and soft tissue infections (SST), 9.7% (95% CI, 9.4-10.1) bloodstream infections (BSI), 7.9% (95% CI, 7.6-8.2) central nervous system infections (CNS), 5.3% (95% CI, 5.1-5.6) pneumonia (PNEU), 4.1% (95% CI, 3.9-4.3) urinary tract infections (UTI), 1.7% (95% CI, 1.6-1.9) Eye, ear, nose, throat, or mouth infections (EENT), and 2.8% other infections (clinical sepsis, lower respiratory infection, other than pneumonia, and cardiovascular system infections) (Table 1).

Among patients with HAIs a mild combat injury was diagnosed in 15.5% of the wounded, severe – in 72.4%,

Table 2. Distribution of pathogens isolated from patients with combat wound infection in Ukraine, 2022-2024

Microorganism	Organisms reported, n (%)								
	All HAI (n=17,286)	SSI (n=1,895)	BJ (n=3,243)	SST (n=3,006)	BSI (n=2,091)	CNS (n=1,956)	PNEU (n=1,838)	UTI (n=2,311)	Other HAI (n=946)
Gram-positive cocci	4,039 (23.4)	531 (13.2)	827 (20.5)	583 (14.4)	666 (16.5)	441 (10.9)	275 (6.8)	532 (13.2)	184 (4.6)
Staphylococcus aureus	881 (21.8)	147 (16.7)	252 (28.6)	117 (13.3)	127 (14.4)	78 (8.9)	37 (4.2)	95 (10.8)	28 (3.2)
CoNS	751 (18.6)	17 (2.3)	39 (5.2)	19 (2.5)	289 (38.5)	118 (15.7)	9 (1.2)	211 (28.1)	49 (6.5)
Enterococcus faecalis	1,037 (25.7)	212 (20.4)	298 (28.7)	311 (30.0)	57 (5.5)	57 (5.5)	27 (2.6)	56 (5.4)	19 (1.8)
Enterococcus faecium	878 (21.7)	129 (14.7)	189 (21.5)	97 (11.0)	132 (15.0)	117 (13.3)	64 (7.3)	89 (10.1)	61 (6.9)
Streptococcus pneumoniae	356 (8.8)	9 (2.5)	27 (7.6)	18 (5.1)	39 (11.0)	58 (16.3)	127 (35.7)	69 (19.4)	9 (2.5)
Other Gram-positive cocci	136 (3.4)	17 (12.5)	22 (16.2)	21 (15.4)	22 (16.2)	13 (9.6)	11 (8.1)	12 (8.8)	18 (13.2)
Gram-negative bacilli	11,894 (68.8)	1,215 (10.2)	2,137 (18.0)	2,232 (18.8)	1,243 (10.5)	1,281 (10.8)	1,446 (12.2)	1,674 (14.1)	666 (5.6)
Enterobacteriales	7,395 (62.2)	757 (10.2)	1,324 (17.9)	1,583 (21.4)	669 (9.0)	648 (8.8)	748 (10.1)	1,227 (16.6)	439 (5.9)
Escherichia coli	2,239 (18.8)	327 (14.6)	578 (25.8)	757 (33.8)	112 (5.0)	94 (4.2)	77 (3.4)	197 (8.8)	97 (4.3)
Enterobacter cloacae	511 (4.3)	97 (19.0)	124 (24.3)	177 (34.6)	23 (4.5)	31 (6.1)	12 (2.3)	29 (5.7)	18 (3.5)
Enterobacter aerogenes	549 (4.6)	88 (16.0)	162 (29.5)	192 (35.0)	19 (3.5)	33 (6.0)	14 (2.6)	27 (4.9)	14 (2.6)
Citrobacter spp.	277 (2.3)	24 (8.7)	77 (27.8)	84 (30.3)	18 (6.5)	28 (10.1)	9 (3.2)	18 (6.5)	19 (6.9)
Klebsiella pneumonia	993 (8.3)	37 (3.7)	71 (7.2)	89 (9.0)	198 (19.9)	135 (13.0)	297 (29.9)	89 (9.0)	77 (7.8)
Klebsiella oxytoca	588 (4.9)	24 (4.1)	38 (6.5)	41 (7.0)	112 (19.0)	117 (19.9)	187 (31.8)	44 (7.5)	25 (4.3)
Proteus vulgaris	377 (3.2)	3 (0.8)	22 (5.8)	18 (4.8)	12 (3.2)	8 (2.1)	7 (1.9)	254 (67.4)	53 (14.1)
Proteus mirabilis	527 (4.4)	14 (2.7)	48 (9.1)	58 (11.0)	18 (3.4)	16 (3.0)	12 (2.3)	312 (59.2)	49 (9.3)
Proteus morganii	279 (2.3)	3 (1.1)	21 (7.5)	18 (6.5)	13 (4.7)	13 (4.7)	8 (2.9)	157 (56.3)	46 (16.5)
Providencia rettgeri	143 (1.2)	2 (1.4)	17 (11.9)	19 (13.3)	6 (4.2)	18 (12.6)	14 (9.8)	49 (34.3)	18 (12.6)
Serratia marcescens	849 (7.1)	137 (16.1)	159 (18.7)	122 (14.4)	127 (15.0)	141 (16.6)	108 (12.7)	41 (4.8)	14 (1.6)
Other Enterobacteriales	63 (0.5)	1 (1.6)	7 (11.1)	8 (12.7)	11 (17.5)	14 (22.2)	3 (4.8)	10 (15.9)	9 (14.3)
Non-fermenting Gram (-) bacteria	4,499 (37.8)	458 (10.2)	813 (18.1)	649 (14.4)	574 (12.8)	633 (14.1)	698 (15.5)	447 (9.9)	227 (5.0)
Acinetobacter lwoffii	512 (4.3)	18 (3.5)	42 (8.2)	29 (5.7)	106 (20.7)	98 (19.1)	139 (27.1)	41 (8.0)	39 (7.6)
Acinetobacter baumannii	987 (8.3)	29 (2.9)	127 (12.9)	84 (8.5)	174 (17.6)	179 (18.1)	202 (20.5)	105 (10.6)	87 (8.8)
Pseudomonas aeruginosa	1,988 (16.7)	299 (15.0)	438 (22.0)	397 (20.0)	148 (7.4)	238 (12.0)	205 (10.3)	197 (9.9)	66 (3.3)
Stenotrophomonas maltophilia	981 (8.2)	112 (11.4)	203 (20.7)	137 (14.0)	142 (14.5)	112 (11.4)	147 (15.0)	97 (9.9)	31 (3.2)

Table 2. Contt.

Other Pseudomonadaceae	31 (0.3)	0 (0)	3 (9.7)	2 (6.5)	4 (12.9)	6 (19.4)	5 (16.1)	7 (22.6)	4 (12.9)
Fungi	1,353 (7.8)	149 (11.0)	279 (20.6)	191 (14.1)	182 (13.5)	234 (17.3)	117 (8.6)	105 (7.8)	96 (7.1)
Candida spp.	1,158 (85.6)	132 (11.4)	211 (18.2)	137 (11.8)	175 (15.1)	212 (18.3)	112 (9.7)	92 (7.9)	87 (7.5)
Aspergillus spp.	164 (12.1)	16 (9.8)	61 (37.2)	49 (29.9)	1 (0.6)	18 (11.0)	2 (1.2)	9 (5.5)	8 (4.9)
Other fungi	31 (2.3)	1 (3.2)	7 (22.6)	5 (16.1)	6 (19.4)	4 (12.9)	3 (9.7)	4 (12.9)	1 (3.2)

HAI, Healthcare-associated infections; SSI, Surgical site infection; BJ, Bone and joint infection, SST, Skin and soft tissue; BSI, Bloodstream infection; CNS, Central nervous system; PNEU, Pneumonia; UTI, Urinary tract infection
Source: compiled by the authors of this study

extremely severe – in 12.1%. The most common combat wounds were shrapnel wounds (78.1%), bullet wounds (9.4%), explosive injury (8.4%), and burns (3.1%). Isolated combat wound was diagnosed in only 4.3% of observations. Most often, a combat injury was combined with injuries to the limbs (68.7%) and chest (51.4%). Head injuries and fractured among patients' bones were in 9.5% and 8.9%, respectively. Thoraco-abdominal wounds among patients with HAIs were found in 12.3% of the wounded. Most combat wounds (93.1%) in patients were classified as contaminated or considered to be dirty or infected. No combat wounds that belong to class 1 wounds were found. Combat wounds classified as class 2 wounds were found in 6.3% of patients. In this study, 22.9% of HAIs cases developed due as contaminated combat wound, and 77.1% from inadequate treatment of traumatic wounds, gross purulence, and evident infections, and MDROs found in combat wound in military personnel and civilians.

The incidence of HAIs was highest among patients with combat wounds admitted to intensive care units (ICUs). The most common HAI types in the ICU among patients with combat wound were respiratory infections and BSIs, while SSIs were the most common infection type in surgery and traumatology departments. In this study the strongest independent associations with HAIs were observed for intubation, urinary and vascular catheters. Most cases of pneumonia and UTI among patients with combat wounds were device-associated, and of BSIs were central-line-associated. During the study period, an increase in the incidence of HAI was observed ($P < 0.0012$), which was significantly associated with an increase in the incidence of ventilator-associated pneumonia (17.9%), catheter-associated bloodstream infections (15.3%), and SSI (11.4%). The prevalence of HAI in patients with combat wound varied widely within the Ukraine. An increase in the incidence of HAIs was observed in southern (Odesa, Dnipro) and eastern region (Kharkiv) of Ukraine.

ANTIMICROBIAL RESISTANCE OF RESPONSIBLE PATHOGENS

During study period in total, 17,286 organisms were isolated from 5,022 patients with HAIs (Table 2). Among 5,022 patients with HAIs, 874 (17.4%) had only monomicrobial infections and 4,148 (82.6%) had polymicrobial infections. A higher proportion of patients with monomicrobial HAIs had only one combat wound with an infected wound and associated microbiology data compared to polymicrobial patients with infections ($p<0.009$). Patients with polymicrobial HAIs had higher injury severity ($p<0.007$) and a greater number were admitted to the ICU ($p<0.012$). The most common bacterial isolates during study period were *Escherichia coli* (13%), *Pseudomonas aeruginosa* (11.5%), *Enterococcus faecalis* (6%), *Klebsiella pneumonia* (5.7%), *Acinetobacter baumannii* (5.7%), *Stenotrophomonas maltophilia* (5.7%), *Staphylococcus aureus* (5.1%), *Enterococcus faecium* (5.1%), and *Serratia marcescens* (4.9%). Gram-positive bacteria were the most common causes of BJ, SST, SSIs and BSIs, and Gram-negative bacteria were the most common causes of pneumonia, UTIs, and CNS infections. (Table 2).

In this study, antimicrobial susceptibility testing data were available for all pathogens causing HAI in patients with combat wound infections. Among the gram-positive bacteria, that 44.6% and 17.3% of coagulase-negative staphylococci (CoNS) isolates were b-lactam (oxacillin) – and glycopeptide (teicoplanin)-resistant, respectively. Meticillin resistance of *S. aureus* (MRSA) and vancomycin resistance of enterococci was found in 43.9% and in 18.1% strains, respectively. MRSA is based on cefoxitin. Data from molecular confirmation tests (detection of *mec*A gene by polymerase chain reaction or positive PBP2A-agglutination test) are prioritized over phenotypic antibiotic susceptibility-testing results. Third-generation cephalosporins (cefotaxime or ceftazidime) resistance was found in 81.6% of *K. pneumoniae*

Table 3. Distribution of pathogens isolated from patients with combat wound infection and antimicrobial resistance phenotype by bacterial species and antimicrobial group/agent, Ukraine, 2022-2024

Pathogen	Antimicrobial group/agent	AMR, n/%
Escherichia coli	Aminopenicillin (amoxicillin/ampicillin) resistance	1,721 (76.9)
	Third-generation cephalosporin (cefotaxime/ceftriaxone/ceftazidime) resistance	1,133 (51.6)
	Carbapenem (imipenem/meropenem) resistance	499 (22.3)
	Fluoroquinolone (ciprofloxacin/levofloxacin/ofloxacin) resistance	1,032 (46.1)
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	636 (28.4)
	Combined resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides	385 (17.2)
Klebsiella pneumoniae	Third-generation cephalosporin (cefotaxime/ceftriaxone/ceftazidime) resistance	810 (81.6)
	Carbapenem (imipenem/meropenem) resistance	594 (59.8)
	Fluoroquinolone (ciprofloxacin/levofloxacin/ofloxacin) resistance	787 (79.3)
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	723 (72.8)
	Combined resistance to fluoroquinolones, third-generation cephalosporins, and aminoglycosides	642 (64.7)
Pseudomonas aeruginosa	Piperacillin-tazobactam resistance	1,068 (53.7)
	Ceftazidime resistance	1,019 (51.3)
	Carbapenem (imipenem/meropenem) resistance	1,440 (72.4)
	Fluoroquinolone (ciprofloxacin/levofloxacin) resistance	1,388 (69.8)
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	1,318 (66.3)
	Combined resistance to ≥3 antimicrobial groups (among piperacillin-tazobactam, ceftazidime, carbapenems, fluoroquinolones, and aminoglycosides)	1,215 (61.1)
Acinetobacter baumannii	Carbapenem (imipenem/meropenem) resistance	811 (81.2)
	Fluoroquinolone (ciprofloxacin/levofloxacin) resistance	915 (92.7)
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	887 (89.9)
	Combined resistance to carbapenems, fluoroquinolones, and aminoglycosidesc	748 (75.8)
Staphylococcus aureus	Meticillin-resistant S. aureus	387 (43.9)
Streptococcus pneumoniae	Penicillin non-wild-type	38 (10.7)
	Macrolide (azithromycin/clarithromycin/erythromycin) resistance	35 (9.8)
	Combined penicillin non-wild-type and resistance to macrolidesc	31 (8.7)
Enterococcus faecalis	High-level gentamicin resistance	659 (63.5)
Enterococcus faecium	Vancomycin resistance	159 (18.1)

Source: compiled by the authors of this study

and in 51.6% of *E coli* isolates. Carbapenem resistance was reported in 59.8% of *K. pneumoniae* and in 72.4% of *P aeruginosa*, and in 81.2% of *A. baumannii* isolates. Distribution of pathogens isolated from patients with combat wound infection and antimicrobial resistance phenotype by bacterial species and antimicrobial group/agent, are presented in Table 3.

MOLECULAR CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE GENES

In present study, of the 17,286 strains isolated from patients with combat wound infection, 88.9% were found to be MDROs, predominantly, *A. baumannii* (75.8%), *K. pneumoniae* (64.7%), *P. aeruginosa* (61.1%), *S. aureus* (43.9%), *E. coli* (17.2%), *E. faecium* (18.1%), *S. maltophilia* (22.5%), *Enterobacter* spp. (17.9%), *S. marcescens* (19.3%), *P. mirabilis* (14.8%), CoNS (17.3%), *Citrobacter* spp. (11.8%), and other species (5.2%).

In this study, by PCR amplification, 43.7% of the MDROs showed the presence of β-lactamase genes, including AmpC, bla_{KPC}, bla_{NDM-1}, bla_{CTX-M}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV}, bla_{SIM}, bla_{TEM}, bla_{IMP-1}, bla_{VIM-1}, and bla_{VIM-2}. Overall, a significant number of the MDROs isolated from patients with combat

Table 4. Distribution of β-lactamase genes among multidrug-resistant organisms (MDROs) isolated from patients with combat wound infection in Ukraine, 2022-2024

Pathogen	Antibiotic resistance genes carried by isolates
Klebsiella pneumoniae	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{VIM-2}
Escherichia coli	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{VIM-2}
Pseudomonas aeruginosa	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{IMP-1}, bla_{VIM-2}
Enterobacter aerogenes	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{VIM-2}
Enterobacter cloacae	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{VIM-1}
Serratia marcescens	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-23}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{VIM-2}
Proteus mirabilis	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-23}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{VIM-1}
Acinetobacter baumannii	AmpC, bla_{CTX-M}, bla_{KPC}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV-1}, bla_{TEM-1}, bla_{SIM-1}, bla_{VIM-2}

Source: compiled by the authors of this study

wound infections had β-lactamase genes, including extended-spectrum β-lactamase (ESBL) (53.1%), OXA-type (32.9%), AmpC-type (35.7%), KPC-type (31.8%), and metallo-β-lactamases (51.4%) including IMP-type (18.5%), VIM-type (29.6%), and NDM-1 (34.7%). Among ESBLs bla_{TEM} was the commonest genotype (43.6 %), followed by $bla S_{HV}$, and bla_{CTX-M}. Most ESBL genes were identified in *K. pneumoniae* (78.2%), *E. coli* (51.2%), *E. cloacae* (48.3%), *P. mirabilis* (46.8%), *S.marcescens* (47.9%), and *E. aerogenes* (35.7%). The OXA-type ESBLs were identified in *P.aeruginosa* (44.2%) and *A. baumannii* (35.1%). AmpC-type β-lactamases were isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria (36.8%), including *E. aerogenes*, *A. baumannii*, *E. cloacae*, *S. marcescens*, *P. mirabilis, K. pneumoniae, P. aeruginosa*, and *E. coli*. In this study, Verona integron-encoded metallo-β-lactamase (VIM) and KPC-type carbapenemases were detected in 29.3% and 36.7% of isolates, respectively. In this study, OXA type β-lactamases and New Delhi metallo-β-lactamase (NDM-1) were detected in 41.1% and 32.7% of isolates, respectively. Characteristics of β-lactamase genes among MDROs isolated from patients with combat wound infection is presented in Table 4.

DISCUSSION

This study is based on multicentre prospective surveillance data for HAI and AMR of responsible pathogens in Ukraine. The aims of this study were to estimate the prevalence and incidence of HAIs in patients with combat wounds, and to determine phenotypic and genotypic aspects of antimicrobial resistance of the responsible pathogens in Ukraine. Our study expands upon the previous reports on HAI [4,5, 19,20] and is the first study to publish of prevalence and incidence of HAIs, and frequent pathogens and/or characterization of the phenotypic and genotypic mechanisms of responsible pathogens of HAI isolated from patients with combat wound in Ukraine.

In this study, the most common combat wounds were shrapnel wounds, bullet wounds, explosive injury, and burns. Most often, a combat injury was combined with injuries to the limbs, head injuries and fractured among patients' bones. This survey identified a high prevalence of HAI (68.6%) in patients with combat wound infections. It is well known that combat wounds are often characterized by high contamination rates, significant soft tissue damage, and the potential for severe complications like infection. A previous study reported that approximately 34% of the patients with combat wound at US hospitals developed a trauma-related infection during their initial hospitalization with skin and soft-tissue infections being predominant. Among this study cohort, 38% developed a new trauma-related infection with the incident infection being diagnosed following hospital discharge [1, 21]. On an infection level in patients with wounds, the majority were SST (45%) followed by PNEU (14%) and BSI (14%) [1]. In our study, of the all cases of HAIs in patients with combat wound, 27.3% were SSI, 25.6% BJ, 15.7% SST, 9.7% BSI, 7.9% CNS, 5.3% PNEU, 4.1% UTI, 1.7% EENT, and 2.8% other infections (clinical

sepsis, lower respiratory infection, other than pneumonia, and cardiovascular system infections). This study showed that combat wounds are much more complex because of higher contamination, mostly resulting from the environment where the wound occurred. Therefore, rapid wound healing time or surgical closure is indicated because of risk of infection [22].

The surgical wound classification was created to represent the bacterial load in a surgical field [23]. According to this classification, each class has a postoperative risk of a SSI with scores of 1% to 5% (Class 1, wounds are categorized as clean wounds), 3% to 11% (Class 2, wounds are categorized as clean-contaminated), 10% to 17% (Class 3, classified as contaminated), and more than 27% (Class 4, considered to be dirty or infected), respectively [24]. In our study, most combat wounds (93.1%) in patients with HAI were classified as contaminated or considered to be dirty or infected. No combat wounds that belong to class 1 wounds were found. Combat wounds classified as class 2 wounds were found in 6.3% of patients. In this study, 22.9% of HAIs cases developed due as contaminated combat wound, and 77.1% from inadequate treatment of traumatic wounds, gross purulence, and evident infections, and MDROs found in combat wound in military personnel and civilians.

According to the literature, during wars in Iraq and Afghanistan, improved survivability in severe trauma corresponded with a rise in the proportion of trauma-related infections, including those associated with MDROs [1]. It has been highlighted in recent studies that there has been an increase in antibiotic-resistant pathogens since the start of the Eastern Ukraine military conflict [6]. This was also observed in conflicts in Iraq [25], Syria [26], Congo [27] and Gaza [28]. AMR is a problem with no borders, especially in a war context. Nevertheless, limited research was conducted on the potential impacts of the Eastern Ukraine military conflict on AMR problem (AMR formation in microorganisms and transmission). Migration indirectly contributes to AMR formation and spread worldwide. Several publications highlighted gaps in such services as infection control, caused by limited resources and personnel, are exacerbating the transmission of MDROs in Ukraine [4, 5, 19, 20]. Therefore, healthcare networks in Europe now consider prior hospitalization in Ukraine to be a critical risk factor for colonization of MDROs [6, 29,30]. Previous reports from Eastern Ukraine military conflict zone have noted the emergence of multidrug-resistant (MDR) *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacterales* infections during hospitalization in Ukraine [10]. Those strains encompassed a variety of clonal lineages, with many carrying carbapenemases, extended-spectrum β-lactamases (ESBLs) [10]. Mc Gann PT, et al [11] reported that blood and surveillance cultures from an injured service member from Ukraine grew *A. baumannii*, *K.pneumoniae*, *E.faecium*, and three distinct *Pseudomonas aeruginosa* strains. Isolates were non-susceptible to most antibiotics and carried an array of β-lactamase genes, including carbapenemases (bla_{IMP-1}, bla_{NDM-1}, bla_{OXA-23}, bla_{OXA-48}, bla_{OXA-72}) [11].

In our study, many combat wound infections are polymicrobial. Among military personnel and civilians with severe combat trauma who survived, there was a corresponding rise in infectious complications caused MDROs. Previous studies (2021) have shown that in 85.1% isolates from patients were found to be MDROs [4]. In present study, 88.9% strains isolated from patients with combat wound infection were found to be MDROs, predominantly, *A. baumannii* (75.8%), *K. pneumoniae* (64.7%), *P. aeruginosa* (61.1%), *S. aureus* (43.9%), *E. coli* (17.2%), *E. faecium* (18.1%), *S. maltophilia* (22.5%), *Enterobacter* spp. (17.9%), *S. marcescens* (19.3%), *P.mirabilis* (14.8%), CoNS (17.3%), *Citrobacter* spp. (11.8%), and other species (5.2%). By PCR amplification, 43.7% of the MDROs showed the presence of β-lactamase genes, including AmpC, bla_{KPC}, bla_{NDM-1}, bla_{CTX-M}, bla_{OXA-1}, bla_{OXA-10}, bla_{OXA-20}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-30}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{SHV}, bla_{SIM}, bla_{TEM}, bla_{IMP-1}, bla_{VIM-1}, and bla_{VIM-2}. Overall, a significant number of the MDROs isolated from patients with combat wound infections had β-lactamase genes, including extended-spectrum β-lactamase (ESBL) (53.1%), OXA-type (32.9%), AmpC-type (35.7%), KPC-type (31.8%), and metallo-β-lactamases (51.4%) including IMP-type (18.5%), VIM-type (29.6%), and NDM-1 (34.7%). Previous studies have shown that in Ukrainian hospitals many hightouch surfaces in hospital wards and healthcare workers (HCWs) were contaminated with MDROs, including strains with similar AMR phenotypes and genotypes [4, 5]. Our results are consistent with those of previous studies on MDROs and presence of β-lactamase genes, including ESBL, OXA-type, AmpC-type, KPC-type, and metallo-β-lactamases including IMP-type, VIM-type, and NDM-1. This confirms that MDROs isolated from patients with combat wound infections in Ukrainian hospitals are spread principally by transmission between HCWs and patients, and between HCWs nd the hospital environment.

STRENGTH AND LIMITATIONS

One strength of our study was that it was a prospective multi-centre observational cohort study, based on HAI and AMR surveillance data for patients with combat wound. Also, this was the first study of phenotypic

and genotypic characterization of antibiotic resistance in MDROs isolated from patients with combat wound infection in Ukrainian civil hospitals. Limitations of the study included that it was conducted in civilian hospitals for adult patients with combat wound only, and the prevalence of HAI and dissemination of MDROs in Ukrainian children's hospitals was not investigated.

CONCLUSIONS

This study found a high prevalence of HAI in patients with combat wounds caused by MDROs, varying depending on the bacterial species, and antimicrobial group. The majority of MDRO isolates from patients with HAI carried β-lactamase genes. The prevalence of MDROs in Ukrainian hospitals continues to increase, while infection control gaps in healthcare settings facilitate their transmission between patients. Although military conflict in Ukraine is ongoing, analysis of infection-related data remains critical to optimizing current clinical practice guidelines with the overall goal of improving outcomes for combat-wounded military personnel and civilians. There is a need to sustain HAIs and MDROs isolated from patients with combat wounds research to improve care readiness (i.e., prevention and treatment of infections) for military conflicts.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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