Journal of Hospital Infection 112 (2021) 69–76

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Phenotypic and genotypic characterization of antibiotic resistance in military hospital-associated bacteria from war injuries in the Eastern Ukraine conflict between 2014 and 2020

V. Kondratiuk [a,*], B.T. Jones [b], V. Kovalchuk [c], I. Kovalenko [c], V. Ganiuk [d], O. Kondratiuk [e], A. Frantsishko [f]

[a] *Department of Emergency and Military Medicine, National Pirogov Memorial Medical University, Vinnytsia, Ukraine*
[b] *Walter Reed Army Institute of Research, Multidrug-Resistant Organism Repository and Surveillance Network, Silver Spring, MD, USA*
[c] *Department of Microbiology, National Pirogov Memorial Medical University, Vinnytsia, Ukraine*
[d] *Intensive Care Unit (For Surgical Patients), National Military Medical Clinical Center 'Main Military Clinical Hospital', Kyiv, Ukraine*
[e] *Military Medical Clinical Center of Central Region, Ambulatory Clinic, Vinnytsia, Ukraine*
[f] *Laboratory Department (Microbiological) Clinic of Laboratory Diagnostics, National Military Medical Center "Main Military Clinical Hospital", Kyiv, Ukraine*

ARTICLE INFO

Article history:
Received 13 December 2020
Accepted 21 March 2021
Available online 28 March 2021

Keywords:
Military
Ukraine
Whole-genome sequencing
Phenotype
Bacterial multiple drug resistance

SUMMARY

Background: Infections from the recent conflict in Ukraine have been poorly investigated.
Aim: To describe the phenotypic and genotypic mechanisms of antibiotic resistance in pathogens associated with war injuries in the Ukraine conflict.
Methods: This report describes a retrospective multi-centre microbiological survey conducted in four Ukrainian military hospitals between 2014 and 2020. The phenotypes of 813 organisms obtained from 1061 tests of 162 patients were analysed. Fifty-two isolates underwent whole-genome sequencing.
Findings: Resistance was highest in *Acinetobacter baumannii*, with 92.5% ((48/52) 95% confidence interval (CI) 81.8–97.9) resistant to fluoroquinolones, 83.0% ((43/52) 95% CI 70.2–91.9) resistant to aminoglycosides, and 67.9% ((37/52) 95% CI 53.7–80.1) resistant to carbapenems. In contrast, resistance to carbapenems was 55.6% ((30/52) 95% CI 41.4–69.1) in *Pseudomonas aeruginosa*, 42.9% in *Escherichia coli* ((12/28) 95% CI 24.5–62.8), and 32.8% in *Klebsiella pneumoniae* ((20/34) 95% CI 21.3–46.0). Multi-drug-resistant strains harboured an abundance of antibiotic resistance genes. *K. pneumoniae* co-produced class A and D β-lactamases, in one case with *bla*NDM-1 and *rmtC* 16S rRNA methyltransferase. *A. baumannii* carried class A and D β-lactamases but not metallo-β-lactamases; in four isolates, carbapenemases were present with the RmtASE gene *arm*A. *P. aeruginosa* harboured a wide range of class A and D β-lactamases along with metallo-β-

* Corresponding author. Address: Department of Emergency and Military Medicine, National Pirogov Memorial Medical University, Pyrohova St, 56, Vinnytsia, 21018, Ukraine. Tel.: +380 432 570 360.
E-mail address: kondratuk.slava@vnmu.edu.ua (V. Kondratiuk).

https://doi.org/10.1016/j.jhin.2021.03.020

lactamases, as well as the *Rmt*B4 RmtASE gene. Gram-positive cocci were generally sensitive to the tested antibiotics.

Conclusion: The incidence of resistance among the studied pathogens was higher than that in Ukrainian civilian hospitals and European countries. The discovery of *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* co-producing carbapenemases and RmtASEs is of particular importance, and hospitals should be vigilant for their emergence.

Introduction

The monitoring of antimicrobial resistance (AMR) has become a political priority declared on an international level, with its importance being demonstrated in large international surveillance initiatives [1].

Infections caused by resistant micro-organisms are commonly reported in war-wounded patients worldwide due to a combination of factors, which lead to the emergence of AMR. Multi-national responses to armed conflicts, the involvement of medical systems from allied countries in the treatment of war-injured soldiers, and long-distance evacuations increase the global spread of resistant bacteria [2]. Predominantly, the epidemiology and antibiotic resistance profiles of the species were comprehensively reported only from military conflicts in Iraq and Afghanistan a decade ago [3]. Infections in war-injured patients from the recent conflict in Eastern Ukraine have been poorly investigated, with a few studies providing insight into the genetic repertoire of *Acinetobacter* spp [4,5]. The few available studies performed in Ukrainian civilian hospitals have shed light on the spread of micro-organisms with a multi-drug-resistant (MDR) phenotype in the region [6]; however, they do not investigate micro-organisms in modern war.

The aim of this study was to analyse the phenotypic profile and delineate the molecular mechanisms of antibiotic-resistant bacteria that cause infectious complications due to war injuries sustained in the conflict in Eastern Ukraine.

Methods

A retrospective microbiological survey was performed between 2014 and 2020 in four Ukrainian military hospitals assigned for definitive medical care (NATO ROLE III—IV) and located in different regions throughout Ukraine. Patients enrolled in the study were adult servicemen who presented with hospital-acquired infections (HAIs), met the Centers for Disease Control (CDC) criteria and were related to the treatment of war injuries sustained in the conflict in Eastern Ukraine. Only pathogens prioritized by the World Health Organization (WHO), which were cultured from such HAIs, were included in the survey. Strains that were isolated consistently from the same infection source were deduplicated and excluded [7]. For the final analysis, consideration was given to the bacterium—antimicrobial combinations used by the European Centre for Disease Prevention and Control. The antimicrobial susceptibility test (AST) was performed by the disk diffusion method in accordance with Ukrainian national EUCAST-harmonized guidance in hospital laboratories.

Determination of antibiotic resistance genes

Fifty-two clinical isolates were forwarded to the multi-drug-resistant repository and surveillance network (MRSN) at the Walter Reed Army Institute of Research, USA, where they underwent additional identification by whole-genome sequencing (WGS) and AST. The raw sequencing reads generated in this project were then submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Accession numbers are provided in the Supplementary Data. Multi-locus sequence typing (MLST) was derived from the genome assemblies of all isolates using the pubMLST website (https://pubmlst.org/) and assigned the strains to different sequence types (STs) according to the Oxford and Pasteur seven-locus MLST schemes.

Ethics clearance

Clearance for the study was obtained from the ethics committee of the National Pirogov Memorial Medical University, Vinnytsia (minutes no. 1 from 17th January 2019).

Statistical analysis

Statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Categorical variables are expressed as percentages and were compared by the χ^2 two-tailed test. Incidence proportions of pooled bacterium-antimicrobial combinations and observed binary probabilities were calculated, which established the corresponding 95% confidence intervals and the *P*-values <0.05.

Results

A total of 1061 microbiology results with 813 organisms from 162 different patients were eligible for this study. Only 250 deduplicated bacterial isolates were selected for further analysis. The predominant micro-organisms appeared to be non-fermenting Gram-negative rods that belonged to *Acinetobacter* spp. and *Pseudomonas aeruginosa*, followed by a variety of *Enterobacterales* sourced in 81.2% of the wounds, (described in detail in the Supplementary Data).

Resistance phenotypes

As shown in Table I, the highest portion of isolates resistant to the studied antimicrobial group combinations was found in the *Acinetobacter* species. The most prominent level of resistance among these was to fluoroquinolones (92.5%), followed by aminoglycosides (83.0%) and carbapenems (67.9%). Approximately 74.1% of the *P. aeruginosa* strains were resistant

Table I
Total number of isolates tested (n)* and percentage resistance (%) per phenotype

	S. aureus		*E. species*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*		*Acinetobacter* spp	
	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI
AMX	11 (36.4)	10.9–69.2	34 (24.3)	11.78–41.2	27 (82.1)	63.1–93.9						
AMP			34 (27.0)	13.8–44.1	27 (85.7)	67.3–95.9	33 (47.5)	34.6–60.7	52 (74.1)	60.3–85.1		
AMC	11 (18.2)	2.3–51.8	34 (24.3)	11.8–41.2	27 (82.2)	63.1–93.9	34 (49.2)	36.1–62.3			53 (96.2)	87.1–99.5
SAM					27 (75.0)	55.1–89.3	33 (37.7)	25.6–51.0				
CFP					26 (50.0)	30.6–69.4	34 (52.5)	39.3–65.4			51 (39.6)	25.8–54.7
CTX							34 (52.5)	39.3–65.4			48 (98.1)	89.6–99.9
CAZ					27 (67.9)	47.6–84.1	34 (47.5)	34.6–60.7	51 (72.2)	58.6–83.5		
CRO					27 (57.1)	37.2–75.5	34 (31.1)	19.9–44.3	52 (92.6)	82.1–97.9		
FEP					27 (64.3)	44.1–81.4	34 (47.5)	34.6–60.7	52 (79.6)	66.5–89.4	52 (90.4)	78.9–96.8
PIP									52 (64.8)	50.6–77.3		
TZP					27 (35.7)	18.6–55.9	33 (19.7)	10.6–31.8	23 (42.6)	29.2–56.8		
PPS											48 (41.5)	28.1–55.9
CIP	11 (27.3)	6.0–60.9	33 (43.2)	27.1–60.5	27 (67.9)	47.7–84.1	34 (45.9)	33.1–59.2	52 (64.8)	50.6–77.3	52 (92.3)	81.5–97.9
OFX					26 (53.6)	33.9–72.5	34 (44.3)	31.5–57.6				
NOR	11 (54.5)	23.4–83.3										
LVX	11 (36.4)	10.9–69.2	34 (32.4)	18.0–49.8	27 (53.6)	33.9–72.5	34 (45.9)	33.1–59.2	52 (59.3)	45.1–72.4	52 (82.7)	69.7–91.8
MFX			34 (35.1)	20.2–52.5								
IPM					27 (39.3)	21.5–59.4			52 (50.0)	36.1–63.9	53 (54.7)	40.4–68.4
MEM					27 (39.3)	21.5–59.4	34 (29.5)	18.5–42.6	52 (46.3)	32.6–60.4	53 (64.2)	49.8–76.9
ETP					27 (32.1)	15.9–52.4	34 (32.8)	21.3–46.1				
GEN	11 (27.3)	6.0–60.9			27 (71.4)	51.3–86.8	34 (36.1)	24.2–49.4	52 (66.7)	52.5–78.9	51 (78.4)	64.7–88.7
GEH			34 (83.8)	67.9–93.8								
NET												
TOB									52 (53.7)	39.6–67.4	53 (60.4)	46.1–73.5
AMK			34 (67.6)	50.2–81.9	27 (46.4)	27.5–66.1	34 (32.8)	21.3–46.1	52 (48.1)	34.3–62.2	53 (67.9)	53.7–80.1
COL					27 (7.1)	0.9–23.5	34 (3.3)	0.4–11.4	52 (5.6)	1.2–15.4	53 (7.5)	2.1–18.2
TCG					27 (7.1)	0.9–23.5						
FOM					27 (17.9	6.1–36.9	34 (24.6)	14.5–37.3				
SXT									48 (88.9)	77.4–95.8		
ATM									46 (61.1)	46.9–74.1		
TEC			34 (10.8)	3.0–25.4								
PEN	11 (45.5)	16.7–76.6										
FOX	11 (18.2)	22.3–51.8										
OXA	11 (27.3)	6.1–60.9										
VAN	2 (0)	–	34 (5.4)	0.7–18.2								
RIF	11 (27.3)	6.0–60.9										

(*continued on next page*)

Table I (*continued*)

	S. aureus		*E. species*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*		*Acinetobacter* spp	
	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI	Tested (n)/ Resistant (%)	95% CI
LNZ	11 (0)	0—28.5	34 (0,0)	0—9.5								
DAP	11 (9.1)	0.2—41.3										
ERY	11 (36.4)	10.9—69.2										
LNM	11 (36.4)	10.9—69.3										

AMC, amoxicillin-clavulanic acid; AMK, amikacin; AMP, ampicillin; AMX, amoxicillin; ATM, aztreonam; CAZ, ceftazidime; CFP, cefoperazone + sulbactam; CI, confidence intervals; CIP, ciprofloxacin; COL, colistin; CRO, ceftriaxone; CTX, cefotaxime; DAP, daptomycin; ERY, erythromycin; ETP, ertapenem; FEP, cefepime; FOM, fosfomycin; FOX, cefoxitin; GEH, gentamicin-high; GEN, gentamicin; IPM, imipenem; LNM, lincomycin; LNZ, linezolid; LVX, levofloxacin; MEM, meropenem; MFX, moxifloxacin; NET, netilmicin; NOR, norfloxacin; OFX, ofloxacin; OXA, oxacillin; PEN, penicillin; PIP, piperacillin; PPS, sulbactam piperacillin; RIF, rifampin; SAM, ampicillin-sulbactam; SXT, trimethoprim-sulfamethoxazole; TCG, tigecycline; TEC, teicoplanin; TOB, tobramycin; TZP, piperacillin/tazobactam; VAN, vancomycin.

to both ceftazidime and aminoglycosides. Aminoglycosides, fluoroquinolones and third-generation cephalosporins were less effective than carbapenems against *Escherichia coli* and *Klebsiella pneumoniae*. Remarkably, isolates co-resistant to imipenem, meropenem, and ertapenem were recorded among both *E. coli* and *K. pneumoniae* species. The lowest percentage of resistance was detected for colistin, which constituted less than 10%, regardless of the genus. Among *Enterococcus* spp., resistance to gentamicin was significant and reached 83.8%, while only 5.4% of clinical *Enterococcus* spp. isolates were found to be resistant to vancomycin. Almost one-third (27.3%) of *Staphylococcus aureus* was found to be not susceptible to oxacillin.

As demonstrated in Figure 1, the proportions of MDR strains among the clinical isolates of *E. coli*, *K. pneumoniae*, *and P. aeruginosa were approximately 80%, followed by MDR Acinetobacter species, whose portion comprised just below 60%.* Figure 2 depicts multiple antimicrobial resistances to different groups of antibiotics among the studied bacteria. The co-resistance to fluoroquinolones, aminoglycosides, and carbapenems confers an MDR profile in 49.1% of the *Acinetobacter strains. A few strains demonstrated simultaneous resistance to four or five antibacterial groups, mostly because of the combination of the latter pattern with resistance to colistin and/or ampicillin-sulbactam. Combined resistance of P. aeruginosa was caused mostly by resistance to four antibacterial classes. In* 16.7% of MDR, *P. aeruginosa* isolates were additionally resistant to piperacillin/tazobactam. The highest portion of isolates co-resistant to five different antimicrobial groups was among *E. coli* and accounted for a total of 37.0%. *K. pneumoniae* rarely tested as resistant to five classes of antibiotics. However, co-resistance to the combination of third-generation cephalosporins + fluoroquinolones + aminoglycosides + carbapenems was found in 26.2% of strains.

Identified molecular resistance mechanisms

Fifty-two isolates subjected to WGS were identified as follows: 13 strains of *Acinetobacter baumannii*, 16 strains of *P. aeruginosa*, 6 strains of *K. pneumoniae*, three strains of *E. coli*, six isolates of *Enterobacter hormaechei*, six isolates of *Enterococcus faecalis*, and two isolates of *S. aureus*. Detailed descriptions of the genes retrieved from the genome and the *in silico* MLST assignment are listed in the Supplementary Data.

The carbapenem resistance of 13 *A. baumannii* isolates was correlated with the presence of OXA beta-lactamases: *bla*OXA-23, *bla*OXA-24, and *bla*OXA-72. *In silico* MLST assigned 13 strains to five different sequence types (STs). *P. aeruginosa* isolates contained a wide range of extended-spectrum beta lactamases (ESBLs), carbapenemase genes such as *bla*NDM-1, *bla*IMP-1, *bla*VIM-2, and an array of genes encoding resistance to aminoglycosides. *In silico* MLST assigned 16 *P. aeruginosa* strains to six different sequence types, with one strain assigned to ST-218, which is a relatively rare sequence type to be a common source of infection. The most potent resistance genes sequenced in the genome of *K. pneumoniae* were carbapenemase *bla*OXA-48, *blaNDM-1*, the prolific ESBL gene *bla*CTX-M-15, *bla*SHV, *bla*TEM and *rmtC* 16S methyltransferase, which makes all relevant aminoglycosides ineffective. Three *E. coli* isolates revealed the presence of 16 different AbR genes, including *aac(6′)-Ib-cr5*, which confers resistance to aminoglycosides, a range of ESBLs encoded by *bla*EC, *bla*OXA-*1*,

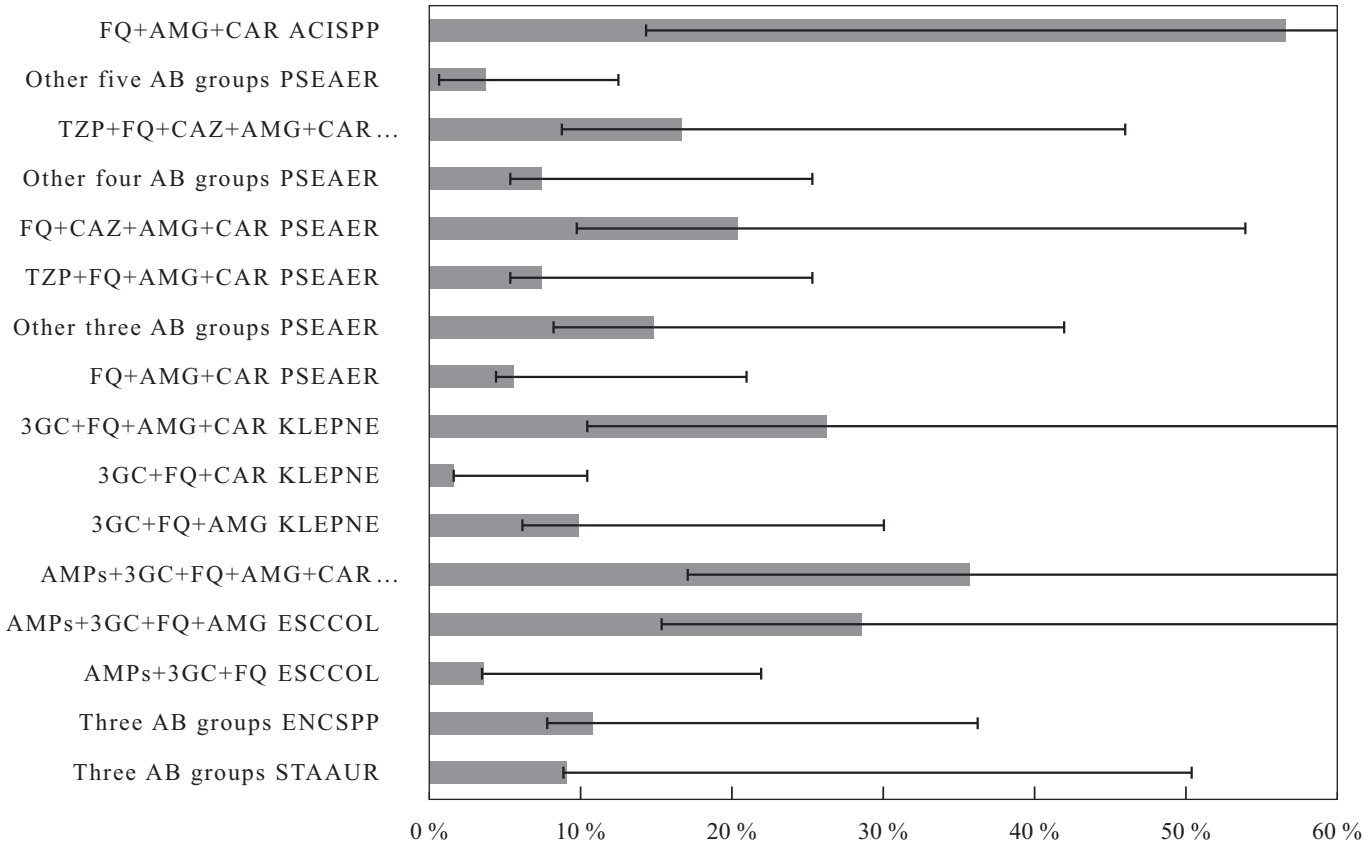

Figure 1. Incidence proportions of co-resistant phenotypes among isolates causing hospital-acquired infections due to war injuries. 3GC, third-generation cephalosporins; AB, antimicrobial; ACISPP, *Acinetobacter* species; AMG, aminoglycosides; AMPs, aminopeptillins; CAR, carbapenems; CAZ, ceftazidime; ENCSPP, *Enterococcus* species; ESCCOL, *Escherichia coli*; FQ, fluoroquinolones; KLEPNE, *Klebsiella pneumoniae*; PSEAER, *Pseudomonas aeruginosa*; STAAUR, *Staphylococcus aureus*; T-bars, 95% confidence intervals; TZP, piperacillin/tazobactam.

*bla*CTX-M-14, and *bla*CTX-M-15. Two of three isolates of *E. coli* belonged to ST-167. One of two studied strains of *S. aureus* harboured the *mecA* gene, along with the macrolide resistance gene *erm(C)*. All studied *E. faecalis* were found to be vancomycin-sensitive. All *Enterococcus* isolates belonged to novel ST and likely represent distinct *E. faecalis* strains that are currently circulating within Ukraine.

Remarkably, the *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii* strains harboured dangerous gene combinations such as carbapenemases together with other genes, which makes them non-susceptible to all clinically relevant aminoglycosides. The list of alarming combinations of potent resistance genes to various classes of antibiotics is presented in Table II. In some cases, resistance revealed in AST can be explained by point mutations in the corresponding genes. The whole-genome sequences of the investigated micro-organisms were deposited in GenBank, and the accession numbers can be found in the Supplementary Data.

Discussion

The pathogens causing warfare-associated infections during the military conflict in Eastern Ukraine predominantly demonstrated multi-drug-resistant phenotypes. This was confirmed by both antibiotic susceptibility tests and by sequencing antibiotic resistance genes. Widespread isolation of such micro-organisms in all surveyed hospitals reflects a dangerous inclination to the emergence and dissemination of resistant isolates not only in the home country but also globally, considering the evacuations and treatments offered by other countries.

A major strength of our study is that the isolates were cultured in four Ukrainian military hospitals from different geographical regions. The inclusion criteria were confined to hospital-acquired infections after a combat injury caused only by indicative micro-organisms according to EARS-Net surveillance. Deduplication of analysed strains makes the results more objective. The AbR genes were mined by the precise WGS method, unveiling all mechanisms behind the multiple drug resistance of clinical isolates.

We found that *Acinetobacter* species demonstrated such a high resistance to fluoroquinolones and aminoglycosides that they must be considered absolutely ineffective. Notably, all *Acinetobacter* spp. with the MDR phenotype, which accounted for 60% of all *Acinetobacter* strains, were resistant to carbapenems. In a multi-centre study in civilian Ukrainian hospitals between 2014 and 2016, the prevalence of carbapenem resistance in *A. baumannii* (as high as 63.2%) was reported, which is similar to military hospitals. However, *Acinetobacter* strains that were cultured in civilian facilities did not

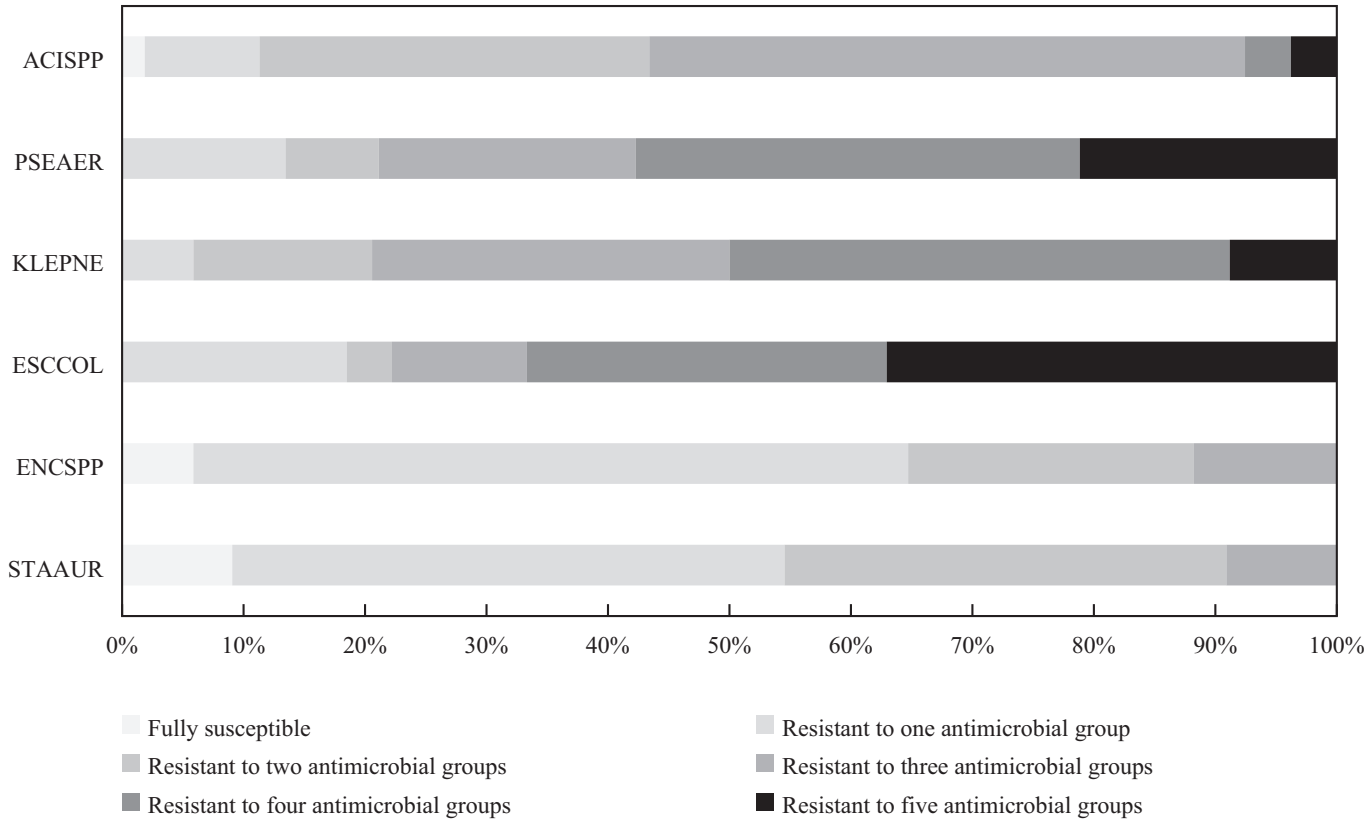

Figure 2. Distribution of isolates with co-resistance. ACISPP, *Acinetobacter* species; ENCSPP, *Enterococcus* species; ESCCOL, *Escherichia coli*; KLEPNE, *Klebsiella pneumoniae*; PSEAER, *Pseudomonas aeruginosa*; STAAUR, *Staphylococcus aureus*.

demonstrate total non-susceptibility to any of the other antibacterial classes [6]. The highest resistance level to fluoroquinolones and aminoglycosides among *Acinetobacter* spp. was recorded at approximately 90% in Croatia, Greece and Lithuania [1]. The percentage of MDR phenotypes we found among the clinical isolates of *E. coli, K. pneumoniae*, and *P. aeruginosa* was noticeably higher than that for *Acinetobacter* spp. Additionally, *K. pneumoniae* and *E. coli* isolates derived their MDR phenotypes from co-resistance to four or even five classes of antibiotics and were less likely to combine resistance with any of the carbapenems. Our findings were in agreement those of with another study, which demonstrated that *K. pneumoniae* strains are characterized by multi-antibiotic-resistance profiles that involve mostly β-lactams, along with other non-β-lactams, and fewer that included carbapenems [8]. Moreover, resistance rates seen in *E. coli* to cephalosporins (71.4%) from war-related infections were pointedly higher than those among *E. coli* from HAIs in civilian hospitals, where the highest resistance rate to third-generation cephalosporins was 32.1% [9]. The MDR phenotype of *P. aeruginosa* was formed mostly due to combinations among antipseudomonal cephalosporins, fluoroquinolones and aminoglycosides. The resistance of *P. aeruginosa* isolates from our collection was significantly higher than that reported in a similar study, which investigated strains originating from the intensive care unit only [10]. In contrast to civilian trauma care protocols, antibiotic prophylaxis is the first-line treatment for war injuries because they are assumed to be primarily contaminated. Lack of battlefield medicine experience in Ukrainian surgeons and the absence of antibiotic stewardship programmes has led to the unrestricted administration of antibiotics. In our opinion, the combination of core differences in the treatment of war trauma in concert with the liberal prescription of antibiotics has resulted in a disparity in patterns of resistance of causative pathogens in military and civilian hospitals. Interestingly, Gram-positive cocci sustained susceptibility to a wide range of antibiotics.

Phenotypic AST performed in Ukraine by the disc-diffusion method and in MRSN on commercial automated panels were largely in keeping with the analysis of the antibiotic resistance genes. The common finding is that all isolates, regardless of genus, harboured resistance genes that were linked together and conferred resistance to antibiotics from various classes, which explains their multi-drug-resistant phenotype. The presence of combinations of extremely dangerous resistance mechanisms was previously described [11]. In our study, two *A. baumannii* isolates carried the potent β-lactamases *bla*OXA-23, *bla*OXA-24, *bla*OXA-72, and *bla*CTX-M-115, along with the *arm*A gene, which crucially limits the effect of aminoglycosides. Furthermore, we isolated *P. aeruginosa* strains that were resistant to aminoglycosides due to the presence of the *Rmt*B4 16S gene along with metallo-β-lactamases of different classes, which is a rare but dangerous combination [12].

The TEM, SHV, and CTX-M genes encoding ESBL enzymes were prevalent among *Enterobacterales;* moreover, among them, the co-production of carbapenemase OXA-48 and EBSL CTX-M-15 was detected. A combination of metallo-β-lactamase *bla*NDM-1 and *rmtC* 16S methyltransferase, encoding

Table II
Alarming combinations of antibiotic resistance genes carried by isolates

Pathogen	Isolate ID	MLST of isolates	Gene co-production	Predicted resistance phenotype
P. aeruginosa	3314	773	*rmt*B4	16S Methyltransferase — all relevant aminoglycosides
			*bla*NMD-1	β-Lactams: carbapenems
	594, 593,	235	*bla*VIM-2	β-Lactams: carbapenems
	617, 635,		*Bla*OXA	β-Lactams: carbapenems
	and 618		*aac(6′)II*	Aminoglycosides: streptomycin, tobramycin, low-level amikacin, fluoroquinolones
A. baumannii	3504, 583-1	78	*ArmA*	16S Methyltransferase — all relevant aminoglycosides
			blaOXA-72	β-Lactams: carbapenems
			*bla*CTX-M-115	β-Lactams: extended-spectrum cephalosporins, monobactams
	133	1	*Arm*A	16S Methyltransferase — all relevant aminoglycosides
			blaOXA-23	β-Lactams: carbapenems
	136	78	*ArmA*	16S Methyltransferase — all relevant aminoglycosides
			*bla*TXM-124	ß-lactams: extended-spectrum cephalosporins, monobactams
K. pneumoniae	160	23	*Rmt*C	16S Methyltransferase — all relevant aminoglycosides
			aac(6′)Ib-cr	Aminoglycosides: tobramycin, low-level amikacin, fluoroquinolones
			*bla*CTX-M-55	β-Lactams: extended-spectrum cephalosporins, monobactams
			*bla*SHV-1	β-Lactams: penicillins, early cephalosporins
			*bla*OXA-1	β-Lactams: carbapenems
			*bla*OXA-48	β-Lactams: carbapenems
			*bla*NMD-1	β-Lactams: carbapenems
	086, 140	23, 395	*aac(6′)Ib-cr*	β-Lactams: extended-spectrum cephalosporins, monobactams
			*bla*OXA-48	β-Lactams: carbapenems
			blaCTX-M-15	β-Lactams: extended-spectrum cephalosporins, monobactams
	608	395	*bla*OXA-48	β-Lactams: carbapenems
			blaCTX-M-15	β-lactams: extended spectrum cephalosporins, monobactams
E. coli	3273, 3272,	167, 167,	*blaCTX-M-15*	β-lactams: extended spectrum cephalosporins, monobactams
	3281	131	*aac(6′)-Ib-cr5*	Aminoglycosides: tobramycin, low-level amikacin, fluoroquinolones
S. aureus	072	8	*mecA*	β-lactams: oxacillin and methicillin
			aac(6′)-aph(2″)	Aminoglycosides: gentamicin, tobramycin, and kanamycin

MLST, multi-locus sequence typing.

resistance to aminoglycosides, was sequenced in a single genome of a *K. pneumoniae* isolate, which reflects clonal dissemination and horizontal gene transfer.

Regarding the different STs found, strains from the same clusters showed similar susceptibility patterns. Some strains of different genera belonged to globally dispersed ST notorious for harbouring multi-drug-resistant strains, which was reflected in this study. Remarkably, strains sourced at different time intervals and in geographically separated hospitals were assigned to the same sequence types or even demonstrated clonal identity, suggesting transmission during treatment end evacuation through the country, which was also noted in another smaller study [4].

One limitation of this study is that the AST in Ukraine was performed by disc diffusion tests and not by dilution testing methods, which potentially can bring different susceptibility to the assessment of certain antibiotics and which fall out of EUCAST recommendations. AST performed in MDRS was performed according to the CLSI standard, which contains some variations from the EUCAST technique used in Ukraine labs. However, all results were concordant. The WGS results of the 21 Ukrainian isolates currently under submission to the GenBank database, along with their accession numbers, do not appear in Supplementary File 2, although all genes were retrieved from the genome listed in Supplementary File 3.

In conclusion, the antimicrobial resistance rates and the proportion of MDR strains related to HAIs due to combat injuries are much higher than those in Ukrainian civilian hospitals and most European countries. MDR phenotypes comprise resistance to the main classes of antibiotics targeting the causative pathogens. The detected coexistence of genes that code several classes of carbapenemases, along with potent enzymes that deactivate clinically significant aminoglycosides, in the individual genomes of *P. aeruginosa, A. baumannii* and *K. pneumoniae* is an alarming trend in emerging high-risk superbugs. The striking similarities revealed by MLST typing between strains, cultured in geographically distant hospitals, proved that such superbugs can spread and establish a firm hold in the military hospital setting.

Author contributions

V.K. conceived of the presented idea, plotted the design of the study, analysed the data, and drafted the manuscript. B.T.J. performed identification by whole genome sequencing, raw sequencing reads, multi-locus sequence typing, and submission to GenBank. V.P.K. coordinated all work related to the study and critically reviewed the manuscript. I.K. performed data analysis, quality control of primary identification and

antimicrobial susceptibility tests and organized the transportation of isolates. V.G. collected clinical isolates and patient data, processed the data, performed the analysis, and designed the figures. O.K. performed antimicrobial susceptibility tests, analysed coresistance, aided in interpreting the results, and worked on the manuscript. A.F. performed the identification of micro-organisms and antimicrobial susceptibility tests and contributed to the interpretation of the results. All authors read and approved the final manuscript.

Acknowledgements

Research was performed as a part of science program of Department of Microbiology, National Pirogov Memorial Medical University, Vinnytsia, Ukraine. All found for tools, materials and equipment were sourced from there. The number of science program is provided in section funds. This is typical model of science funding in Ukraine.

Conflict of interest statement
All authors state no conflicts of interests.

Funding sources
This study was performed within the framework of the research work of the Department of Microbiology of National Pirogov Memorial Medical University, Vinnytsya 'Study of the biological properties of micro-organisms, classified by the World Health Organization as "priority pathogens", that pose a threat to human health and the development of a means to combat these pathogens' (National registration No. 0117U006903).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2021.03.020.

References

[1] European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2018. annual report of the European antimicrobial resistance surveillance network (EARS-Net) [Internet]. Stockholm, Sweden: European Centre for Disease Prevention and Control; 2019. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2018.pdf [last accessed December 22, 2020].

[2] Calhoun JH, Murray CK, Manring MM. Multidrug-resistant organisms in military wounds from Iraq and Afghanistan. Clin Orthop Relat Res 2008;466:1356—62.

[3] Murray CK, Yun HC, Griffith ME, Thompson B, Crouch HK, Monson LS, et al. Recovery of multidrug-resistant bacteria from combat personnel evacuated from Iraq and Afghanistan at a single military treatment facility. Mil Med 2009;174:598—604.

[4] Granzer H, Hagen RM, Warnke P, Bock W, Baumann T, Schwarz NG, et al. Molecular epidemiology of carbapenem-resistant Acinetobacter Baumannii complex isolates from patients that were injured during the Eastern Ukrainian conflict. Eur J Microbiol Immunol (Bp) 2016;6:109—17.

[5] 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:E579.

[6] Salmanov AG, Vdovychenko SY, Litus OI, Litus VI, Bisyuk YA, Bondarenko TM, et al. Prevalence of health care-associated infections and antimicrobial resistance of the responsible pathogens in Ukraine: results of a multicenter study (2014e2016). Am J Infect Control 2019;47:E15e20.

[7] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309—32.

[8] Avgoulea K, Di Pilato V, Zarkotou O, Sennati S, Polit L, Cannatelli A, et al. Characterization of extensively drug-resistant or pandrug-resistant sequence type 147 and 101 OXA-48-producing Klebsiella pneumoniae causing bloodstream infections in patients in an intensive care unit. Antimicrob Agents Chemother 2018;62. e02457-17.

[9] Salmanov A, Vozianov S, Kryzhevsky V, Litus O, Drozdova A, Vlasenko I. Prevalence of healthcare-associated infections and antimicrobial resistance in acute care hospitals in Kyiv, Ukraine. J Hosp Infect 2019;102:431—7.

[10] Yang X, Lai Y, Li C, Yang J, Jia M, Sheng J. Molecular epidemiology of Pseudomonas aeruginosa isolated from lower respiratory tract of ICU patients. Braz J Biol 2020;81:6.

[11] Wong PH, von Krosigk M, Roscoe DL, Lau TT, Yousefi M, Bowie WR. Antimicrobial co-resistance patterns of gram-negative bacilli isolated from bloodstream infections: a longitudinal epidemiological study from 2002—2011. BMC Infect Dis 2014;14:393.

[12] Murugan N, Malathi J, Therese KL, Madhavan HN. Application of six multiplex PCR's among 200 clinical isolates of Pseudomonas aeruginosa for the detection of 20 drug resistance encoding genes. Kaohsiung J Med Sci 2018;34:79—88.