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Case Report

Occurrence, resistance patterns, and management of carbapenemase-producing bacteria in war-wounded refugees from Ukraine

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ABSTRACT

We analyzed consecutive clinical cases of infections due to carbapenemase-producing gram-negative bacteria detected in war-wounded patients from Ukraine who were treated at one university medical center in southwest Germany between June and December 2022. The isolates of multiresistant gram-negative bacteria were subjected to a thorough microbiological characterization and whole genome sequencing (WGS). We identified five war-wounded Ukrainian patients who developed infections with New Delhi metallo-β-lactamase 1-positive *Klebsiella pneumoniae*. Two isolates also carried OXA-48 carbapenemases. The bacteria were resistant to novel antibiotics, such as ceftazidime/avibactam and cefiderocol. The used treatment strategies included combinations of ceftazidime/avibactam + aztreonam, colistin, or tigecycline. WGS suggested transmission during primary care in Ukraine. We conclude that there is an urgent need for thorough surveillance of multiresistant pathogens in patients from war zones.

Introduction

Antimicrobial resistance (AMR) was associated with an estimated 4.95 million deaths worldwide in 2019 alone, and carbapenem-resistant gram-negative bacteria were among the major contributors to this enormous disease burden [1]. All-age death rates were the highest in sub-Saharan Africa, followed by South Asia and Eastern Europe. The war in Ukraine has led to significant migration movements, with ≥7.8 million refugees across Europe until December 2022 [2]. We report AMR patterns in war-wounded patients from Ukraine.

Case descriptions

Patient P1

A male patient aged 34 years experienced a femoral shaft fracture after an explosion. Swabs from an inserted external fixator grew *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Both pathogens were highly resistant to antibiotics, including carbapenems, ceftazidime/avibactam, and cefiderocol. A New Delhi metallo-β-lactamase (NDM-1) was detected in the *K. pneumoniae* strain. Both pathogens remained susceptible to colistin, whereas tigecycline showed a comparatively low minimal inhibitory concentration (MIC) of 1.5 mg/L (Table 1). The patient developed an infection and was successfully treated with colistin plus tigecycline.

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Table 1
Resistance testing results and carbapenemases detected in gram-negative bacteria isolated from war-wounded patients from Ukraine in a university medical center in southwest Germany, June to November 2022.

Patient	Bacterium	Carbapenemase	Resistance to several antimicrobials (minimal inhibitory concentration, expressed as mg/L)										
			MER	IMI	ERT	TIG	AMI	GEN	TOB	COL	CEF/AVI	CEF/TAZ	CEFI	
													Disk diffusion	Microdilution	Epsilometry
P1.1	*Klebsiella pneumoniae*	NDM-1	R (>32)	R (>32)	R (>32)	IE (=1.5)	R (>256)	R (>1024)	R (>256)	S (0.25)	R (>8)	R (>256)	R (9 mm)	R (64)	R (3)
P1.2	*Pseudomonas aeruginosa*	None	R (>32)	R	–	–	R (>256)	–	R (>256)	S (=0.5)	R (>256)	R (>256)	R (20 mm)	–	–
P2.1	*Klebsiella pneumoniae*	NDM-1	R (>32)	I (=6)	R (>32)	IE (=1.5)	R (>32)	S (=1)	R (=24)	S (=0.25)	R (>8)	R (>256)	ATU (18 mm)	S (2)	S (0.36)
P2.2	*Pseudomonas aeruginosa*	None	R (>32)	I (=4)	–	–	R (=48)	–	R (>256)	S (=1)	R (>256)	R (>256)	S (25 mm)	–	–
P3.1	*Klebsiella pneumoniae*	NDM-1	R (>32)	R (>32)	–	IE (=0.75)	S (=8)	R (=64)	R (=64)	S (=2.0)	R (>8)	R (>256)	R (10 mm)	R (4)	R (12)
P4.1	*Providencia stuartii*	NDM-1	S (=2)	I (=6)	–	IE (=3)	R (>256)	R (>1024)	R (>256)	R (>16)	R (>8)	R (>256)	S (26 mm)	S (1)	S (0.047)
P4.2	*Klebsiella pneumoniae*	NDM-1 & OXA-48	R (>32)	R (>32)	–	IE (=0.5)	R (>256)	R (>1024)	R (>256)	S (=0.25)	R (>8)	R (>256)	R (14 mm)	R (8)	S (1.5)
P5.1	*Klebsiella pneumoniae*	NDM-1 & OXA-48	R (>32)	R (>32)	–	IE (=0.75)	R (>256)	R (>1024)	R (>256)	S (=0.25)	R (>8)	R (>256)	R (9 mm)	R (4)	S (1.5)
P5.2	*Providencia stuartii*	NDM-1	S (=2)	I (=6)	–	IE (=3)	–	–	R (>256)	R (>16)	–	R (>256)	–	–	–

AMI, amikacin; ATU, Area of technical uncertainty according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) definition; CEF/AVI, ceftazidime/avibactam; CEF/TAZ, ceftolozane/tazobactam; CEFI, cefiderocol; COL, colistin; ERT, ertapenem; GEN, gentamicin; IMI, imipenem; MER, meropenem; TOB, tobramycin; TIG, tigecyclin.
All carbapenem-resistant isolates reported here were also uniformly resistant to penicillins, cephalosporins (first to fourth generation), and fluoroquinolones.
Interpretation of testing results: S, Sensitive (standard exposure); I, Sensitive (increased exposure); R, resistant; IE, insufficient evidence (no established clinical breakpoint); –, not done.
Resistance testing was performed on a MicroScan WalkAway system. Carbapenem resistance was confirmed by epsilometry. Resistance testing for colistin was performed using broth microdilution, whereas epsilometry was applied to ceftazidime/avibactam and ceftolozane/tazobactam. For cefiderocol testing, we performed broth microdilution, epsilometry (except for *P. aeruginosa*), and agar disk diffusion.

Patient P2

A male patient aged 43 years had complex fractures of the tibia and humerus, which were caused by gunshots and blast injuries. The patient developed fever, and a highly resistant NDM-1-positive *K. pneumoniae* was detected in the blood cultures and wound swabs. Furthermore, a multiresistant *P. aeruginosa* strain and *Enterococcus faecalis* were recovered. The patient was successfully treated with combined colistin and high-dose imipenem (MIC of the isolate: 6 mg/L). The infection improved, but the patient required further wound debridement and vacuum-assisted closure therapy.

Patient P3

A female patient aged 58 years had severe wound infection after having undergone unilateral below-knee amputation. Wound smears revealed an NDM-1-positive *K. pneumoniae* strain. The antimicrobial treatment comprised colistin and tigecycline (MIC 0.75 mg/L). The patient's course improved and she was finally discharged to a rehabilitation center.

Patient P4

A female patient aged 64 years was admitted for severe blast injuries of the chest and above-elbow amputation with wound infection. *K. pneumoniae* grew in wound smears, which were positive for OXA-48 and NDM-1. Initial therapy included tigecycline (MIC 0.75 mg/L) and colistin. Colistin was discontinued due to acute kidney failure. Further smears grew NDM-positive *Providencia stuartii* strains. After surgical and antimicrobial treatment, the patient's condition improved and she was discharged.

Patient P5

A male patient aged 56 years was admitted for thoracic blast injuries and extensive soft tissue damage of one leg after gunshot injury. Combined femoral and tibial fractures showed signs of infection (osteitis) and maggot infestation. The patient developed a systemic infection with an OXA-48 and NDM-1-positive *K. pneumoniae* strain, which was isolated from the blood cultures and wounds. Initial therapy consisted of tigecycline and colistin. Furthermore, an NDM-positive *P. stuartii* grew in the wound swabs. Because the patient's condition did not improve, treatment was switched to ceftazidime/avibactam in combination with aztreonam. Later, the clinical course was complicated by candidemia and the patient underwent amputation of the infected leg due to persistent infection.

Microbiological characterization and whole genome sequencing (WGS) analysis

Details on the microbiological methods [3], sequencing techniques, and analysis models can be found in Supplement 1. Seven gene multilocus sequence typing with mlst (v2.22.1) suggested at least three different strains by predicting three different sequences types (STs) for *K. pneumoniae* isolates: ST395 (P3.1), ST147 (P2.1), and ST23 (P1.1, P4.2, and P5.1) (Supplementary Figure 1A). The three STs shared only one house-keeping gene, whereas ST147 and ST23 shared two house-keeping genes. Altogether, 25.484 single-nucleotide variants (SNVs) were shared by all three ST23 isolates, whereas 359 SNVs were unique to P1.1, 253 SNVs were unique to P4.2, and 231 SNVs were only detected for P5.1. Hence, the shared ST23, in combination with an increased SNV agreement, suggest a common epidemiological background and clonality of the *K. pneumoniae* isolates of patients 1, 4, and 5.

Resistance detection with ABRicate (v1.0.1) revealed a wide range of different resistance genes (Supplementary Table 1). Regarding carbapenem resistance, the NDM-1 coding gene *blaNDM-1* was found in all analyzed isolates. The gene coding for OXA-48 was detected in P4.2 and P5. The *blaNDM-1* plasmids of P2.1 and P3.1 were identical, as well as the plasmids isolated from P4.2 and P5, for which even chromosomal comparison showed high similarities, thus suggesting at least partial transmission between patients. Details on the plasmid genes are displayed in Supplementary Figure 1B.

Regarding the resistance against ceftazidime/avibactam and cefiderocol, respectively, neither bla_{KPC-2} nor bla_{NDM-35} could be detected.

Discussion

The ongoing war in Ukraine has a profound negative impact on the country's health care system, including the fight against infectious diseases. Due to the high number of Ukrainian migrants having fled the country, specific challenges have also arisen for public health and appropriate surveillance measures in other countries [4,5]. Indeed, a French practice guideline has put forth a host of recommendations to health care providers who care for migrants from Ukraine, which prioritize communicable diseases, vaccination catch-up, and psychological sequelae, etc. [6]. After anecdotal reports of "how war is spreading drug resistant superbugs across Ukraine and beyond", [7] recent genomic surveillance data highlighted the considerable challenges arising from carbapenemase-producing gram-negative bacteria [8]. Here, we report on a series of Ukrainian patients with contaminated wounds who were found to be colonized and/or infected with NDM-1- and NDM-1/OXA-48-positive *Enterobacterales* and nonfermentative bacteria. WGS findings and the rapid detection of pathogens in swabs taken on admission to our hospital suggest a previously established colonization with these bacteria.

The epidemiology of carbapenemases found in hospitalized patients varies considerably across Europe, with the highest rates being reported from southern and southeastern Europe. In Germany, OXA-48 was the most commonly detected carbapenemase in 2021, followed in descending order by VIM-1, KPC-2, and NDM-1 [9]. Of note, bacterial strains carrying more than one carbapenemase were a rarity (<5%). Several international guidance documents have been published on the treatment of infections caused by carbapenem-resistant gram-negative bacilli, which recommend the preferred use of ceftazidime/avibactam, if susceptible *in vitro*. For metallo-β-lactamases, such as NDM-1, the use of cefiderocol monotherapy or the combination of ceftazidime/avibactam plus aztreonam is conditionally recommended [10,11]. Although these recommendations are supported by compelling susceptibility data, none of the carbapenemase-producing strains in our investigation was susceptible to ceftazidime/avibactam, and cefiderocol was resistant in four of the six tested isolates. Of note, cefiderocol susceptibility is notoriously difficult to test [12], and we also observed some discrepancy depending on the testing method. The combination of ceftazidime/avibactam and aztreonam restores activity against NDM-1-producing *K. pneumoniae* and other *Enterobacterales*, but its routine use outside clinical studies is currently hampered by the unavailability of intravenous aztreonam in some European countries and the absence of a licensed fixed dose combination of these compounds. Furthermore, this combination seems much less promising in NDM-1-producing *P. aeruginosa* isolates [13].

Our WGS data suggest a high relatedness of the different carbapenemase-producing strains, which point to one or multiple common origins, *e.g.*, in the field hospital, where the patients had received emergency medical care before being transferred abroad. Our investigation is limited by the absence of environmental samples from the field hospital so that the exact transmission pathways cannot be reconstructed.

Conclusion

There is an urgent need for a thorough surveillance of multiresistant gram-negative bacteria in patients from Ukraine with war-related wounds in Europe and elsewhere. These pathogens should be subjected to a broad antimicrobial susceptibility testing because previously unknown rates of resistance to 'last-line' and novel antibiotics are to be expected.

Declaration of competing interest

Fabian K. Berger has received consultant fees from MSD and Pfizer (pertaining both to *Clostridioides difficile*). B.C. Gärtner has received honoraria from Pfizer, outside the submitted work. S.L. Becker has received speaker fees and advisory board participation fees from Pfizer (pertaining to ceftazidime/avibactam) and Shionogi (pertaining to cefiderocol). All other authors have no competing interests to declare.

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Ethical statement

Written informed consent was obtained from the patients reported in this manuscript.

Author contributions

Patient treatment: TF, NV, VP, TP. Microbiological diagnostics: FKB, FA, SR, SS, BCG, SLB. WGS and data analysis: GPS, JR, AK. Drafting the manuscript: FKB, GPS, JR, SLB. All authors have read and approved the final version of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.04.394.

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