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Letter to the Editor

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Editor: Stefania Stefani

We read with great interest the papers from The Netherlands and Germany, which showed a significant increase in the levels of carbapenemase-producing organisms (CPOs) in the respective countries, from patients originating from Ukraine [1,2]. Likewise, since the start of the war in Ukraine, an increase in CPO levels has been detected in Denmark (personal communication, Henrik Hasman). A study on war injuries in Eastern Ukrainian military hospitals during 2014–2020 also showed high proportions of carbapenem-resistant bacteria [3], with 2020 surveillance data from Ukraine showing that >50% of invasive *Klebsiella pneumoniae* isolates were carbapenem resistant [4].

Since September 5, 2018, CPOs have been noticeable in Denmark. CPO detection was performed according to the guidelines from NordicAST Brytpunktstabeller (nordicast.org). All CPOs were whole-genome sequenced at the National Reference Laboratory. To prevent the spread of multidrug-resistant bacteria, the patients either coming directly from Ukrainian hospitals or as refugees have been tested for the presence of CPOs according to the Danish administrative order concerning CPOs [5].

From February 24, 2022, through January 23, 2023, 371 CPOs from 288 patients were obtained as part of the Danish national CPO surveillance program. Of the 371 CPOs, 77 were collected from 42 patients originating from Ukraine who came into contact with the Danish health care system. More than one isolate was collected from individual patients if the isolates belonged to different bacterial species and/or had different carbapenemases.

Genomic DNA was extracted (DNeasy Blood and Tissue Kit, Qiagen, Copenhagen, Denmark), with subsequent library construction (Nextera Kit, Illumina, Little Chesterford, UK), and whole-genome sequencing was performed (Nextseq, Illumina) according to the manufacturer's instructions to obtain paired-end reads (2 × 150 bp). The quality and quantity of the raw reads of all 77 isolates were assessed using the BIFROST QC pipeline (https://github.com/ssi-dk/bifrost) and assembled into draft genomes using the SKESA assembler version 2.2 (https://github.com/ncbi/SKESA). Detection of resistance genes and multilocus sequence typing (MLST), as well as species identification, were carried out using the Bifrost pipeline utilizing the MLST and ResFinder databases available at the CGE homepage (www.cge.food.dtu.dk).

SeqSphere+ software version 8.5.1 (Ridom, Münster, Germany) was used for cluster analyses (MLST and core genome MLST) of the *K. pneumoniae, Escherichia coli, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* species. Official core genome MLST schemes were available from www.cgMLST.org. The Danish sequence data from patients originating from Ukraine were compared with similar sequencing data from a recent Dutch study [2]. Furthermore, comparison was made to Danish CPO isolates from the same period, to investigate nosocomial spread in Danish hospitals.

The 77 CPOs from patients from Ukraine were isolated from screening samples (n = 41) and clinical samples (n = 36). The screening samples were from rectal swabs. Among the clinical samples, samples from urine (n = 8), cicatrices (n = 7) and wound swabs (n = 8) were the most prevalent.

Nine different bacterial species were obtained, comprising 38 *K. pneumoniae,* 12 *E. coli*, 1 *Citrobacter freundii*, 4 *Providencia stuartii*, 2 *Proteus mirabilis,* 2 *Morganella morganii*, 1 *Citrobacter amalonaticus*, 8 *A. baumannii*, and 9 *P. aeruginosa* isolates (Supplementary Table S1). An average of 1.8 CPO was identified per patient, ranging from one isolate (n = 26) up to nine isolates (n = 1) per patient. The isolates from the patient with nine CPOs were OXA-244-producing *M. morganii,* OXA-48-producing *K. pneumoniae,* NDM-1-producing *K. pneumoniae,* NDM-1/OXA-48-producing *E. coli,* NDM-1-producing *P. stuartii,* NDM-1-producing *C. amalonaticus,* OXA-48-producing *C. freundii,* OXA-23-producing *A. baumannii*, and NDM-1-producing *P. aeruginosa.* The patient was initially treated in Ukraine. After two months, he was admitted to a Danish hospital, where he underwent extensive surgery and received broad-spectrum antibiotics. In screening and clinical samples, nine different CPOs were cultured over several weeks (personal communication, Mikala Wang).

Carbapenemases of the NDM- and OXA-type, especially NDM-1 and OXA-48, were the most frequently detected, which is consistent with findings from other studies [1,2] (Supplementary Table S1). Eight *K. pneumoniae* core genome MLST clusters (KP1–KP8) contained isolates from the present study and a similar study from the Netherlands [2], indicating transfer of the same carbapenemase-producing *K. pneumoniae* from Ukraine to Northern Europe (Table 1). One *A. baumannii* cluster (AB1) consisted of both Danish and Dutch isolates of Ukrainian origin. This indicates transfer of the same *A. baumannii* strain from Ukraine to both Denmark and the Netherlands. One *A. baumannii* cluster (AB1) and two *P. aeruginosa* clusters (PA1–PA2) contained only Danish isolates from Ukrainian patients, indicating transfer of the same isolates from Ukraine to Denmark (Table 1). None of the Danish CPOs of Ukrainian origin clustered with CPOs identified via Danish national CPO surveillance from the same period. This indicated that no nosocomial spread of CPOs occurred from the patients originating from Ukraine, in contrast with German hospitals [1].

In conclusion, the patients originating from Ukraine were colonised and/or infected by many CPOs per patient. During the study period, 21% of the total number of CPOs in Denmark were from patients originating from Ukraine. The most frequently found species was *K. pneumoniae.* Additionally, NDM- or OXA-48 family carbapenemases predominated, in accordance with previous studies [1–3]. There was no indication for transmission at Danish hos-

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Table 1
Description of the 13 genetic clusters in relation to MLST, cgMLST, carbapenemases, and numbers of isolates obtained in Denmark from patients associated with the war in Ukraine from February 24, 2022, to January 23, 2023

Species	Cluster No.	MLST	cgMLST	Carbapenemase	Numbers of isolates[a]
Klebsiella pneumoniae					
	KP1	ST39	CT7737	NDM-1	1[b]
	KP2	ST147	CT1206	NDM-1	1[b]
	KP3	ST147	CT7682	NDM-1, OXA-48[c]	10[d]
	KP4	ST147	CT7787	NDM-1	1[b]
	KP5	ST147	CT7914	NDM-1	1[b]
	KP6	ST395	CT7510	OXA-48	3[b]
	KP7	ST395	CT7718	OXA-48	3[b]
	KP8	ST395	CT7738	NDM-1[e]	2[b]
	KP9	ST5859	CT7392	NDM-1, OXA-232	2
Acinetobacter baumannii					
	AB1	ST2	CT2783	OXA-23	3[b]
	AB2	ST78	CT5370	OXA-72	2
Pseudomonas aeruginosa					
	PA1	ST773	CT2193	NDM-1	3
	PA2	ST1047	CT1931	IMP-1[f], IMP-10[g]	4

MLST, multilocus sequence typing; cgMLST, core genome multilocus sequence typing.
[a] Numbers of Danish isolates in the clusters from patients associated with Ukraine.
[b] Clustered with one *Klebsiella pneumoniae* isolate from a Dutch study of patients from Ukraine [2].
[c] One isolate was only positive for OXA-48.
[d] Clustered with three *Klebsiella pneumoniae* isolates from a Dutch study of patients from Ukraine [2].
[e] One isolate also produced OXA-48.
[f] One isolate was only positive for IMP-1.
[g] One isolate was only positive for IMP-10.

pitals, in contrast with German hospitals [1]. The study provides the first insight into CPO occurrence in Ukrainian patients in Denmark and one of the first insights in Northern Europe.

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Competing interests: None declared.

Ethical approval: Not required.

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Sequence information: Sequences are stored here: ENA PRJEB60743.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.06.002.

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