Antimicrobial Resistance and Infection Control

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Successful termination of a multi-year wastewater-associated outbreak of NDM-5-carrying *E. coli* in a hemato-oncological center

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Abstract

Background In May 2018, an outbreak of NDM-5-carrying *Escherichia coli* (NDM-5-EC) was detected at the hemato-oncology department of a tertiary care center in Austria. This report details the outbreak investigation, control measures and the whole genome sequencing (WGS) data of the outbreak isolates.

Methods A total of 15 isolates (seven clinical isolates from allogenic stem cell transplant (SCT) recipients and eight wastewater isolates recovered from patients' toilets) were analyzed by whole genome sequencing.

Results Genome based typing identified two clusters of the high risk clones ST167/CT12607 and ST617/CT2791. Long-read sequencing of selected isolates from both clusters identified two different plasmids, however with a highly similar genetic context of the $bla_{\text{NDM-5}}$ containing region. Genomic analysis revealed the presence of additional resistance genes, including $bla_{\text{CTX-M-15}}$, and $bla_{\text{OXA-1}}$, and virulence factors. Four patients were colonized with NDM-5-EC, two patients suffered bacteremia caused by the outbreak strain and two deaths were associated with an NDM-5-EC infection. The outbreak source was traced to toilet sewage pipes, which remained persistently contaminated despite extensive cleaning and disinfection. Successful eradication of NDM-5-EC from the installations required disassembly, hot water pressure washing of the sewage pipes and complete replacement of all movable parts. Additionally, colonized patients were instructed to use wheeled commodes instead of toilets, and a pre-admission screening strategy was implemented for all patients undergoing hematologic stem cell transplantation. The outbreak was successfully terminated in November 2020.

Conclusion NDM-5-EC, especially high-risk clones such as ST167 and ST617, can persist in hospital wastewater systems despite cleaning and disinfection efforts and can cause prolonged outbreaks. Therefore, a comprehensive bundle of interventions like the ones applied in our study is essential, especially in clinical settings with heavily immunosuppressed patients.

Keywords *Escherichia coli*, NDM-5, Austria, Whole genome sequencing, Outbreak, Hematooncology, Stem cell transplantation, Plasmid, Wastewater

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Background

The rapid emergence and dissemination of New Delhi metallo-β-lactamase (NDM-5) producing *Escherichia coli* (NDM-5-EC) poses a significant threat to public health (1). NDM-5, a variant of the NDM enzyme first identified in 2009 (2), confers resistance to a broad spectrum of β-lactam antibiotics, including carbapenems, which are considered last-resort treatments for multidrug-resistant bacterial infections. The NDM-5 carbapenemase has been increasingly reported in *Enterobacterales* worldwide (3–7) and NDM-5-EC has been implicated in numerous hospital outbreaks (4, 8). These outbreaks frequently involve high-risk clones of sequence types ST167, ST405, ST410, ST617 and ST648, which are known for their capacity to spread rapidly (9, 10). The genetic adaptability of $bla_{\text{NDM-5}}$, often carried on plasmids, facilitates its horizontal transfer between different bacterial species, thereby enhancing its dissemination potential (11).

In this study, we described a wastewater-associated outbreak of NDM-5-EC in a stem cell transplant unit at a tertiary care center in Austria. By integrating comprehensive genomic data with epidemiological investigations, we aimed to enhance our understanding of the mechanisms driving the spread of NDM-5-EC and inform effective strategies for controlling its dissemination in the hospital environment.

Methods

Department description

The affected department at a tertiary care center comprises a general oncology ward (16 rooms for 33 patients), a leukemia and autologous stem cell transplantation (SCT) ward (eight rooms for 12 patients), an allogenic SCT unit (five single rooms) and an outpatient clinic. Its bed occupancy rate ranges between 90 and 100%. The outbreak affected the SCT and the general oncology ward.

Outbreak description

In May 2018, a surveillance stool culture from a hemato-oncological patient (A) and a blood culture from another patient (B) were positive for an NDM-carrying *E. coli*. A third patient (C) had an NDM-carrying *E. coli* positive surveillance stool culture in the end of August 2018 followed by a positive blood culture in September 2018. Suspecting a reservoir in the sanitary installations, water samples were taken from sinks, shower drains and toilets in the affected patients' rooms. NDM-positive *E. coli* was first found in toilet wastewater in September 2018 and thereafter repeatedly in a total of six patient room toilets on two wards.

The fourth patient (D) had a positive surveillance stool culture in September 2018. A fifth patient (E) had a positive surveillance urine culture in November 2018. In July 2019, a positive surveillance stool culture from a sixth patient (F) was detected, and the seventh and last patient (G) had a positive surveillance urine culture in November 2019.

Case definition

A case was defined as any patient with NDM-EC identified from May 2018 onwards. An ongoing surveillance program (stool, urine, throat swab cultures) had shown no cases before this date. Cases were included in this study until the end of November 2020, when the outbreak was declared terminated.

Patients

Demographic and epidemiological data such as patient outcome (death/survival), stem cell transplant date, microbiology test results and other parameters were collected by chart review, anonymized and then analyzed using Microsoft Excel 2016.

Culture based screening of patients

All neutropenic patients were screened by throat swab, stool culture, urine culture and blood culture twice weekly with a focus on detection of VRE, MRSA and gram-negative bacteria using the following agars: Tryptone Soya Agar with 5% sheep blood, MacConkey Agar, Brilliance ESBL/Brilliance CRE BiPlate, Brilliance VRE (all Thermo Fisher Scientific, UK) and CHROMID MRSA (Biomérieux, France).

Environmental isolates

During the outbreak, environmental isolates were collected by plating 1 ml of toilet trap water on Tryptone Soya Agar with 5% sheep blood and on MacConkey Agar. Additionally, 10 ml toilet trap water was filtrated using the Microfil funnel and filter system (Merck Millipore, UK) with a pore size of 0.45 µm and the filters were placed on plates for culture. Only eight environmental isolates from random time points were archived.

Identification and susceptibility testing

Identification of presumptive *Enterobacterales* was done by Maldi TOF (Bruker Daltonics, Hilden, Germany) and susceptibility testing according to EUCAST was applied. Isolates meeting screening cut-off values for carbapenemase-producing *Enterobacterales* (12) and/or growing on the Brilliance ESBL/Brilliance CRE BiPlate were further characterized by PCR.

Bacterial isolates and carbapenemase gene PCR

These isolates were analyzed by conventional PCR with previously published primer sets covering the most prevalent ESBL and carbapenemase genes (13, 14).

The first clinical isolates from all seven patients (labelled A-G) and eight isolates (labelled 1–1 to 6–1) obtained from toilet wastewater of six different patient rooms (one toilet was sampled three times over one year) were included in the study. Isolates (n = 15) were retrieved from cryobanks (Mast, Reinfeld, Germany) and cultured on Tryptone Soya Agar with 5% sheep blood for whole genome sequencing (WGS).

DNA isolation, WGS and typing

DNA from overnight cultures was extracted using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA libraries were prepared using the Illumina DNA Prep M Tagmentation Kit combined with IDT for Illumina UD Indexes (Illumina, San Diego, USA) followed by whole-genome sequencing on the iSeq 100 system (Illumina, San Diego, USA) with 2 × 150 bp paired end reads. Bioinformatic analyses were performed with Ridom SeqSphere + software v9.0.8 (Ridom GmbH, Münster, Germany) (15). FastQC v0.11.9 was used for quality analysis of the sequences (16). Raw reads were assembled with SKESA v2.4.0 (17) and assembly remapping and polishing was performed with the BWA-MEM v0.7.15 algorithm (18).

NCBI AMRFinderPlus 3.11.2 was used for identification of resistance genes (19). Likewise, Warwick MLST (20) and cgMLST (Enterobase) (21) analyses were run on SeqSphere + and a minimum spanning tree (MST) was generated using the default threshold of ≤ 10 allelic differences (AD). Genomes were additionally analysed using VirulenceFinder-2.0 Server with default settings (Software version: 2.0.5 (2024–01-31), Database version: (2022–12-02)) (22).

For further investigation of the location of $bla_{\text{NDM-5}}$ in the bacterial genome, four selected isolates (patient C, patient F and two environmental isolates: room 2–1 and room 6–1) additionally underwent long-read sequencing with Oxford Nanopore Technologies. Genome libraries were prepared using the rapid barcoding kit SQK-RBK004 (ONT, Oxford, United Kingdom) according to the standard protocol and loaded on a R9.4.1 flowcell (FLO-MIN106D, ONT, Oxford, United Kingdom). Sequencing was performed on a MinION M1kB for 72 h. Quality check and fast basecalling were done with MinKNOW and Guppy v6.3.5 software. Hybrid assembly was performed using unicycler v. 0.5 with default parameters. For plasmid visualization and comparison DNA Features Viewer v. 3.1.2 was used (23). Plasmids were annotated for resistance genes using NCBI-AMRFinder version 3.12.8 (db version 2024–05-02.2) (24).

Results

Patients

A total of seven allo-SCT patients were affected by this outbreak, with their demographic and epidemiological characteristics summarized in Table 1.

Figure 1 shows the epidemiological curve of the outbreak by collection date of the first NDM-5-EC isolate from each patient. Most patients had multiple positive samples, typically collected from stool, urine or throat swabs as part of routine screening, four of them were considered colonized. Patient B and C had NDM-5-EC bacteremia and patient B and E did not survive the infection. Three additional patients died of unrelated causes during follow-up. Two patients are still alive and persistently colonized, as shown by screening of stool samples during follow-up until the time of this publication.

Isolate susceptibility data

All isolates were multidrug resistant according to the definition of the ECDC (25). Detailed results of antimicrobial susceptibility testing are presented in Table 2.

Outbreak control measures

When the movement of patients in the affected wards was tracked, it was found that patients A and B had consecutively occupied the same room (Room 1). A screening regimen for neutropenic patients (twice weekly rectal swab/stool sample, urine, throat swab) was already in place and was continued with special regard to NDM carrying *E. coli*. Additionally, a pre-admission screening strategy was implemented for all patients undergoing hematologic stem cell transplantation. Single-room care for colonized patients with strict contact precautions and increased frequency of cleaning and disinfection of patient-near surfaces especially in bathrooms was initiated after detection of the first case.

In September 2018, after detection of patient C and D (who had also occupied Room 1), a source in the patient room was suspected. Following reports describing hospital water as a reservoir for carbapenemase producing organisms causing infections (26), analysis of water samples revealed contamination of the pipes draining the toilets. Consequently, a vigorous cleaning routine was established: All affected toilets were intensively cleaned, and weekly application of 500 ml of household bleach (2.8 g sodium hypochlorite per 100 g solution) was initiated. Weekly, and later monthly, surveillance of wastewater from the affected toilets was implemented. Toilets were cleared for use only after three negative surveillance

Table 1 Characteristics of the seven allogenic stem cell transplant recipients affected by the outbreak (GvHD: graft versus host disease, SCT: stem cell transplant)

Patient ID	Sex	Age (years)	First detection of NDM5-EC	Detection of NDM5-EC days after/before SCT	Samples positive for NDM5-EC	Status	Outcome	Died days after first NDM5-EC detection	Association with rooms
A	m	68	2018–05-15	154	stool	colonized	died of unrelated causes	24	1, 2, 4
B	f	57	2018–05-20	66	blood culture, stool	infection	died (NMD-5-EC sepsis, mucormycosis, multi organ failure)	9	1
C	m	44	2018–08-24	-20	blood culture, stool, throat swab	infection	died of unrelated causes	70	3, 6
D	m	49	2018–09-19	21	stool	colonized	alive	no	1, 2, 6
E	f	31	2018–11-27	22	stool, urine	infection	died (assumed NDM-5-EC sepsis, GvHD, multi organ failure)	215	1, 3
F	f	61	2019–07-03	30	stool, urine, throat swab	colonized	alive	no	1, 6
G	m	50	2019–11-12	33	stool, urine	colonized	died of unrelated causes	91	4, 5

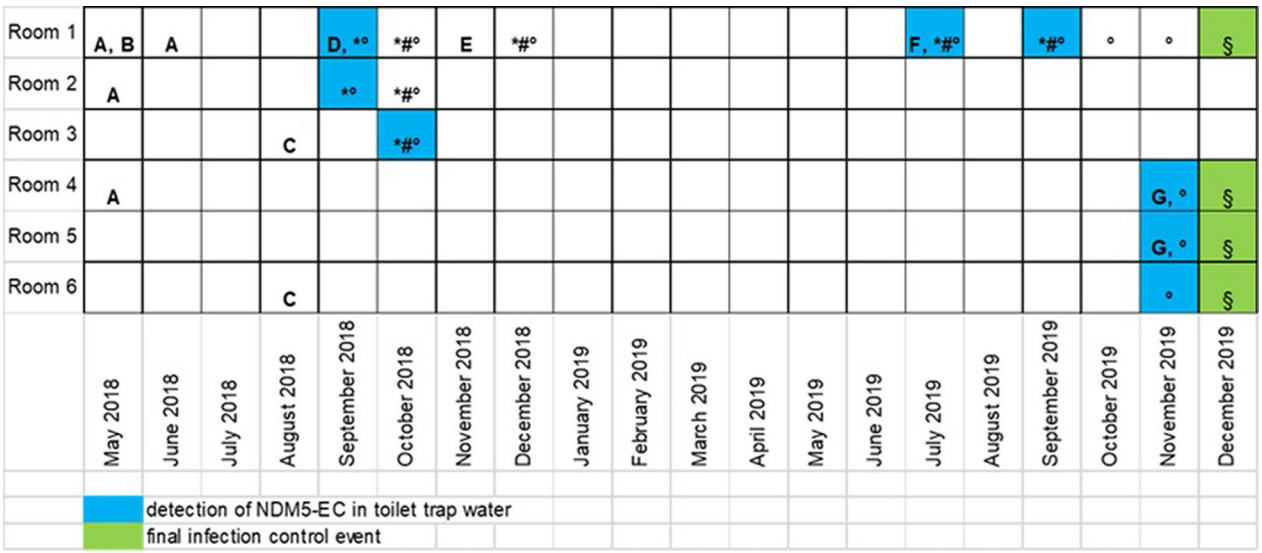

Fig. 1 Epidemiological curve representing the seven patients (A-G) with NDM-5-EC and their occupation of the affected rooms within the outbreak period from May 2018 to December 2019. Only first detections are shown. Additionally, sampling dates for the environmental isolates (denoted in blue) and infection control events are shown (*: disinfection of toilet bowl with bleach and UV light, increased frequency of cleaning and disinfection of patient-near surfaces, #: change of toilet rubber cuff, °: weekly application of bleach in toilet trap water until negative cultures, §: hot water pressure washing and change of movable parts)

cultures. However, due to the repeated reappearance of the outbreak strain, especially in Room 1, and ongoing transmission to patients, four of the affected toilets could only be released for use after the total renewal of all movable parts (toilet bowl, rubber seal, closet flange) and intense cleaning of the drainpipes with hot water pressure washing (Fig. 2).

Ultimately, colonized patients were discouraged from using the toilets and were instead advised to use wheeled commode chairs without connection to the hospital

Table 2 Antimicrobial susceptibility testing data according to EUCAST for all patient and environmental isolates. Minimum inhibitory concentrations are indicated in brackets. n.d.: not determined

Antibiotic	Patient A	Patient B	Patient C	Patient D	Patient E	Patient F	Patient G	Room 1–1	Room 1–2	Room 1–3	Room 2–1	Room 3–1	Room 4–1	Room 5–1	Room 6–1
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Amoxicillin/clavulanic acid	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Cefuroxime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Cefotaxime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ceftazidime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Cefepime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ceftolozane-Tazobactam	R	R	R	R	R	R	R	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ceftazidime-Avibactam	R	R	R	R	R	R	R	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Meropenem	R [16]	R [16]	R [16]	R [16]	R [16]	R [16]	I [8]	I	R	I	R	R	I	I	R
Gentamicin	S	S	R	S	S	I	S	S	S	S	R	S	S	S	R
Tobramycin	R	R	R	R	R	R	R	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Amikacin	S	S	S	S	S	I	S	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Fosfomycin	S [2]	S [1]	S [1]	S [0.5]	S [0.5]	S [4]	S [1]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Tigecyclin	S [0.25]	S [0.125]	S [0.5]	S [0.25]	S [0.125]	S [0.25]	S [0.25]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Trimethoprim-Sulfamethoxazole	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Colistin	S [0.25]	S [0.25]	S [0.25]	S [0.25]	S [0.25]	S [1]	S [1]	n.d	S [1]	n.d	n.d	n.d	n.d	n.d	S [1]

wastewater system for the total length of their hospital stay to prevent re-contamination of the installations.

The toilet installations were finally cleared from NDM-5-EC in June 2020.

WGS-based typing

Two different sequence types / cgMLST complex types were assigned to the outbreak isolates (ST617/CT12607 n = 12, ST167/CT2791 n = 3). A cgMLST analysis grouped the 15 isolates into two clusters (threshold: ≤ 10 AD) corresponding to the two STs, respectively (Fig. 3). The two clusters differed by 700 alleles and isolates within the clusters differed by a maximum of two and three alleles, respectively (Fig. 3). Table 3 provides an overview of assigned STs and cgMLST CTs for all isolates. They shared a common betalactam resistance gene pattern ($bla_{\text{NDM-5}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$) with variable detection of $bla_{\text{TEM-1}}$ and multiple resistance genes for other antibiotic classes (Table 3). Moreover, a wide array of virulence genes as well as genes associated with disinfectant tolerance and biofilm formation was detected (Table 3).

Analysis of the hybrid assemblies from isolates of both clusters (patient C (ST167/ CT2791), patient F (ST617/ CT12607), room 2–1 (ST617/ CT12607) and room 6–1 (ST167/ CT2791)) revealed the localization of $bla_{\text{NDM-5}}$ on IncFII type plasmids (Fig. 4, image generated with DNA Features Viewer). The plasmids were of different sizes: Plasmid 1 measured approximately 133–139 kb (patient C and room 6–1) and Plasmid 2 approximately 168 kb (patient F and room 2–1). Plasmids of similar size were highly identical over large collinear blocks (Fig. 4), while Plasmid 1 and Plasmid 2 were approximately 97% identical (74% coverage). All plasmids shared common identical blocks encoding $bla_{\text{NDM-5}}$ on a 13,801 bp long identical segment. The recombined regions around $bla_{\text{NDM-5}}$ were interspersed with multiple copies of the insertion sequence IS26.

Discussion

In this study, we report a wastewater-associated NDM-5-EC outbreak in an Austrian hospital, which led to colonization as well as infection of allogenic stem cell (allo-SCT) transplant recipients. This patient population is particularly vulnerable due to bone marrow suppression, impaired mucosal barriers and often prolonged hospitalization with exposure to broad-spectrum antibiotics (such as antipseudomonal cephalosporins and carbapenems) during therapy of neutropenic fever (27). Colonization with multidrug resistant bacteria such as NDM-5-EC has been shown to pose a risk for subsequent invasive infection in allo-SCT recipients and negatively impact survival (28). Therefore, prompt detection of colonized patients through screening is recommended to minimize onward transmission (27).

Hospitals are critical reservoirs for multidrug-resistant bacteria due to the high antimicrobial selection pressure, especially in wards treating heavily immunocompromised patients (29). Antibiotics excreted by patients are discharged into the hospital wastewater system, where they may reach high concentrations (30), exerting selection pressure on environmental bacteria and promoting the emergence and spread of multidrug-resistant clones (31). Several studies have investigated the role of toilets and sanitary installations in the transmission of carbapenemase-producing bacteria within hospital settings. A recent paper covered a prolonged outbreak of carbapenemase-producing *K. pneumoniae* and *P. aeruginosa*, finding that environmental and clinical isolates were closely related (29). An outbreak of NDM-producing ST167 *E. coli* was linked to a toilet in a Danish hospital where environmental samples from the toilet water trap revealed the presence of the outbreak isolate, indicating a possible source of infection (4). Similarly, in the burn center of Ghent University Hospital, an outbreak of OXA-48-producing *Klebsiella pneumoniae* was traced to toilet and drain water. The outbreak strain persisted in some rooms even after two months of daily disinfection with bleach, highlighting the potential of the strain to spread between rooms through common wastewater plumbing and the failure of disinfectants to prevent recolonization after discontinuation (32). This corresponds to our experience of continuing reappearance of NMD-5-EC in affected toilets until complete replacement of the affected installations, underscoring the importance of targeted infection control measures and environmental disinfection to mitigate the risk of pathogen transmission through hospital sanitary facilities, especially if they encode diverse persistence and resistance mechanisms.

Additionally, the characteristics of the outbreak strains possibly contributed to the extended duration of this outbreak. The initially obtained short-read sequencing data revealed two separate clusters, which could have been interpreted as two distinct outbreak events. However, due to the very low prevalence of NDM-5-EC in Austria (33), we decided to perform further analysis by Nanopore sequencing. As for the STs identified here, both ST167 as well as ST617 *E. coli* emerged from the ST10 clonal complex and are known for causing outbreaks due to unique virulence and surface antigen features (34), which were also detected in our isolates. They are also carriers of NDM-type carbapenemases and are often isolated in healthcare settings (35–37). In our case, transmission of $bla_{\text{NDM-5}}$ between the two different *E. coli* sequence types via plasmid exchange may be assumed, as the long-read sequencing data demonstrate a highly similar genetic

Fig. 2 Hot water high pressure cleaning of the drainpipes after complete removal of the toilet and view of the drainpipe biofilm before cleaning

context of $bla_{\text{NDM-5}}$ in all four analyzed isolates despite the differences between the detected plasmids. The NDM gene is commonly located within recombination-prone and transposon-rich genomic regions (38) which is consistent with our findings. Indeed, recombination between multiple plasmid types carrying $bla_{\text{NDM-5}}$ has been well documented in various bacterial species, even in a single patient (39, 40). Additionally, plasmid DNA has been shown to remain viable for several days in aqueous environments with favorable conditions for biofilm formation (41), and long-distance dissemination of antimicrobial resistance genes via outer membrane vesicles has recently been described (42, 43). These factors suggest that horizontal transfer of the NMD-5 gene between the two *E. coli* STs could have occurred within the hospital's wastewater systems. Nevertheless, the overall differences observed between the plasmids in the two sequence types raise the possibility of a second independent introduction of NDM-5-EC during this outbreak, although this remains epidemiologically unlikely regarding the rarity of such strains in Austria (44).

Taken together, all these factors contributed to the difficulties in terminating this outbreak. Only a combination of measures covering installations (complete exchange of parts, high-pressure cleaning, chlorination), infection control (single room patient care with PPE, screening) and patient behavior (education, use of commodes for colonized patients) was successful.

Conclusions

Detection of wastewater-associated NDM-5-EC in a low-incidence setting should prompt aggressive outbreak management, especially in high-risk wards such as hemato-oncology. The study demonstrated that traditional disinfection protocols were insufficient to eliminate persistent contamination in wastewater pipes, necessitating mechanical interventions such as

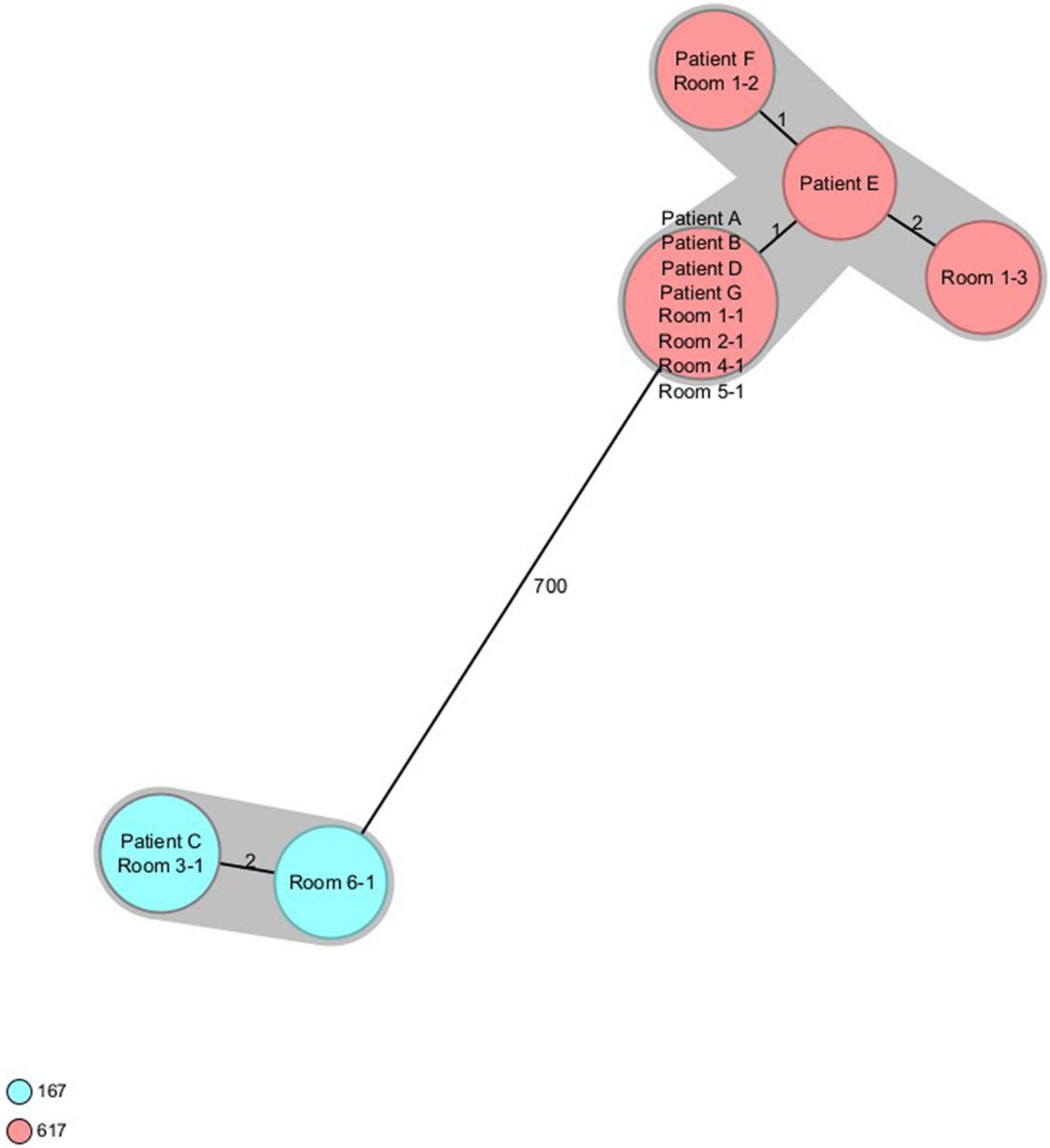

Fig. 3 Minimum spanning tree showing the two clusters detected in the outbreak. ST167 isolates are colored blue, ST617 isolates are colored red

disassembly and high-pressure hot water cleaning, followed by infrastructure replacements. Further research including long-term genomic surveillance is essential to explore the mechanisms of plasmid exchange and resistance gene dissemination in hospital environments. Additionally, optimized products and protocols to prevent contamination of sanitary installations are needed to prevent the spread of antibiotic-resistant pathogens in healthcare facilities.

Table 3 Whole genome sequencing data for all isolates. Data is sorted by sampling date, isolates showing the cgMLST 2791 complex type are colored light grey.

Sample ID	Sampling Date	MLST (Warwick) ST	cgMLST CT	Beta-Lactam Resistance Genes				Other Resistance Genes and Point Mutations													
				blaNDM-5	blaTEM-1	blaCTX-M-15	blaOXA-1	aac(6')-Ib-cr5	aac(3)-IId	mph(A)	sul1	sul2	dfrA12	dfrA17	ftsI_ I336IKYRI	ftsI_ N337NYRIN	glpT_ E448K	gyrA_ D87N	gyrA_ S83L	parC_ S80I	parE_ S458A
Patient A	2018-05-15	617	12607	x	x	x	x	x		x	x	x	x	x	x		x	x	x	x	x
Patient B	2018-05-20	617	12607	x	x	x	x	x		x	x	x	x	x	x		x	x	x	x	x
Patient C	2018-08-24	167	2791	x	x	x	x	x	x	x	x		x	x		x		x	x	x	x
Patient D	2018-09-19	617	12607	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x
Room 2-1	2018-09-25	617	12607	x	x	x	x	x		x	x	x	x	x	x		x	x	x	x	x
Room 1-1	2018-09-25	617	12607	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x
Room 3-1	2018-10-01	167	2791	x	x	x	x	x	x	x	x		x	x		x		x	x	x	x
Patient E	2018-11-27	617	12607	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x
Patient F	2019-07-03	617	12607	x		x	x	x		x	x		x	x	x		x	x	x	x	x
Room 1-2	2019-07-08	617	12607	x		x	x	x		x	x		x	x	x		x	x	x	x	x
Room 1-3	2019-09-09	617	12607	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x
Patient G	2019-11-12	617	12607	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x
Room 4-1	2019-11-14	617	12607	x	x	x	x	x		x	x	x	x	x	x		x	x	x	x	x
Room 5-1	2019-11-14	617	12607	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x
Room 6-1	2019-11-18	167	2791	x	x	x	x	x	x	x	x		x	x		x		x	x	x	x

Sample ID	Virulence Genes																					Genes encoding biofilm/acid-resistance regulators or tolerance to disinfectants and/or heavy metals			
	AslA	aamR:FN 554766	anr	capU	csgA	fdeC	fyuA	hha	hlyE	hra	irp2	iss	iucC	iutA	nlpI	sitA	terC	traJ	traT	yehA	yehB	yehC	yehD	ymgB	qacEdelta1	terD	terZ
Patient A	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x		x	x	x	x	x	x		
Patient B	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x		x	x	x	x	x	x		
Patient C	x	x		x	x	x	x	x	x	x	x	x			x		x		x		x	x	x	x	x		
Patient D	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Room 2-1	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x		x	x	x	x	x	x		
Room 1-1	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Room 3-1	x	x		x	x	x	x	x	x	x	x	x			x		x		x		x	x	x	x	x		
Patient E	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Patient F	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Room 1-2	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Room 1-3	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Patient G	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Room 4-1	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x		x	x	x	x	x	x		
Room 5-1	x		x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Room 6-1	x	x		x	x	x	x	x	x	x	x	x			x		x		x		x	x	x	x	x		

Fig. 4 Comparison of common identical nucleotide blocks between the plasmids of four isolates. The image was created using DNA Features Viewer. A minimum length of 1000 bp was required for a common block. Location of resistance conveying genes was added. Patient C and Room 6–1 (NDM-5-EC ST167) plasmids share large common blocks (block 2 and 3), while Patient F and room 2–1 (NDM-5-EC ST617) plasmids share a common plasmid backbone (block 1 and 4). Some resistance gene cassettes, including $bla_{\text{NDM-5}}$, are common to all four plasmids (block 5 and 6)

Abbreviations

AD	Allelic Differences
bp	Base Pairs
cgMLST	Core genome Multilocus Sequence Typing
CT	Complex type
DNA	Deoxyribonucleic Acid
E. coli (EC)	*Escherichia coli*
GvHD	Graft versus host disease
MLST	Multilocus Sequence Typing
MRSA	Methicillin resistant *Staphylococcus aureus*
MST	Minimum Spanning Tree
NDM	New Delhi Metallo-beta-lactamase
ONT	Oxford Nanopore Technologies
PCR	Polymerase chain reaction
SCT	Stem Cell Transplant
ST	Sequence type
VRE	Vancomycin resistant *Enterococci*
WGS	Whole Genome Sequencing

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Author contributions
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Availability of data and materials
This Whole Genome Shotgun project has been deposited at the Sequence Read Archive (SRA) under the accession no. PRJNA1165667 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1165667). The version described in this paper is the first version.

Declarations

Ethics approval and consent to participate
The study was approved by the ethics committee of the medical faculty of Johannes Kepler University Linz (vote EK1077/2021). Consent to participate was not obtained since the study was done retrospectively and without additional interventions for patients.

Competing interests
The authors declare no competing interests.

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