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ARTICLE

An outbreak of colistin-resistant *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* in the Netherlands (July to December 2013), with inter-institutional spread

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Abstract We describe an outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* (KPC-KP) ST258 that occurred in two institutions (a hospital and a nursing home) in the Netherlands between July and December 2013. In total, six patients were found to be positive for KPC-KP. All isolates were resistant to colistin and exhibited reduced susceptibility to gentamicin and tigecycline. In all settings, extensive environmental contamination was found. Whole genome sequencing revealed the presence of $bla_{\text{KPC-2}}$ and $bla_{\text{SHV-12}}$ genes, as well as the close relatedness of patient and environmental isolates. In the hospital setting, one transmission was detected, despite contact precautions. After upgrading to strict isolation, no further spread was found. After the transfer of the index patient to a nursing home in the same region, four further transmissions occurred. The outbreak in the nursing home was controlled by transferring all KPC-KP-positive residents to a separate location outside the nursing home, where a dedicated nursing team cared for patients. This outbreak illustrates that the spread of pan-resistant Enterobacteriaceae can be controlled, but may be difficult, particularly in long-term care facilities. It, therefore, poses a major threat to patient safety. Clear guidelines to control reservoirs in and outside the hospitals are urgently needed.

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Introduction

Up to now, hospital outbreaks with carbapenemase-producing Enterobacteriaceae (CPE) occur sporadically in Northern and Western Europe, and most reports concern isolates that are introduced by patients from high prevalence areas. However, the frequency of such outbreaks is increasing, and regional spread has been reported [1]. Information about the modes of transmission and potential reservoirs are urgently needed in order to design strategies to control the spread of these bacteria.

Hospital infections caused by CPE, and, in particular, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumonia* (KPC-KP), have been associated with increased cost and length of hospital stay, frequent treatment failures, and death [2]. Few antimicrobial agents exist to treat infections with these bacteria, and colistin is one of the last treatment options. Although resistance to colistin is still rare, there have been several reports of colistin-resistant KPC-KP in the past few years [3–5].

Although the KPC gene has been identified in several other Gram-negative bacteria, it is still most commonly found in *Klebsiella* species. One specific sequence type (ST), ST258, is particularly successful and represents the vast majority of KPC-KP isolates found globally. ST258 isolates have the ability to rapidly disseminate in healthcare settings and are frequently associated with outbreaks in hospitals and long-term care facilities [3, 6–8]. Analysis of the ST258 genome may provide further insight into factors related to the epidemiological success of this clone [9–11].

In this paper, we report an outbreak of KPC-KP ST258 that occurred in the Netherlands between July and December 2013 and involved two institutions (a hospital and a nursing home). We describe the control measures taken to prevent further spread, the results of the microbiological and molecular investigations, and the results of the environmental screening cultures taken during the outbreak.

Methods

Detection of the outbreak

The outbreak was recognized on July 13, 2013, when a KPC-KP isolate was detected in the pleural fluid of a 65-year-old man (patient 2) admitted to the pulmonary ward of a 1,370-bed teaching hospital in Breda, the Netherlands. This event followed the earlier repatriation of a 69-year-old woman (the index patient) from an intensive care unit in a Greek hospital on June 25, 2013, who was found to be colonized with KPC-KP two days after admission to the pulmonary ward. The index patient had been nursed using contact precautions since admission to the hospital. Because carbapenem-resistant Enterobacteriaceae have been practically absent in this hospital, the finding was considered indicative of nosocomial transmission, and an outbreak management team was formed.

Twelve weeks after the transfer of the index patient to a nursing home in the same region (on August 5, 2013), another KPC-KP isolate was found in a rectal screening culture of one of the nursing home residents (patient 3) during a short admission to the previously mentioned hospital (November 5, 2013). This initiated an investigation to assess the extent of transmission in the nursing home.

Setting

The hospital has approximately 40,000 admissions/year at three separate locations. Carbapenems are rarely used (<2 defined daily doses/100 admissions), and, although patients colonized with CPE had been detected previously, no cases of nosocomial transmission had occurred. The infection control department consists of six infection control practitioners (ICPs), headed by a clinical microbiologist, and is part of the microbiology laboratory. The pulmonary ward has five single, three double, and two four-patient bedrooms. The mean length of stay is 7.3 days.

The nursing home consists of four departments (a total of 150 beds), each with specific patient populations, including a 34-bed rehabilitation ward for patients with neurological diseases (mostly cerebrovascular accidents), and patients who underwent orthopedic or surgical operations . The rehabilitation ward has four single, five double, and five four-patient bedrooms, and a shared area for social activities. Patients reside in this ward for periods of up to 6 months. Before the outbreak, diagnostic cultures were sent to a commercial laboratory, and there was no dedicated ICP. On-site or telephone consultations by a self-employed ICP were requested by the nursing home ad hoc.

Infection control measures

Hospital

Because the index patient was transferred from a country where multidrug-resistant organisms (MDROs) are highly endemic [12], isolation measures were implemented while awaiting the results of screening cultures, in accordance with national guidelines [13]: the patient was placed in a single room, and healthcare workers (HCWs) donned gloves and gowns before entering the patient room. Following the detection of KPC-KP carriage of the index patient, an ICP visited the pulmonary ward on a daily basis to educate staff and clarify practical infection control issues.

When, despite these measures, a second patient colonized with a KPC-KP was found, isolation measures were increased: both patients were transferred to a single room with anteroom and negative air pressure, and HCWs donned gloves, gowns, face masks, and caps before entering the patient room. In addition, a dedicated nursing team was appointed (cohorting) for the two patients. Both rooms were cleaned daily at the end of the cleaning shift. On August 5, 2013, the index patient was discharged to the rehabilitation ward of a nursing home and patient 2 was discharged home. The patient rooms were disinfected with a quaternary ammonium compound. To guarantee direct isolation measures upon future readmission, KPC-colonized patients were flagged in the electronic patient record system. At the time of transfer to the nursing home, information regarding the carriage of a pan-resistant KPC-KP and the fact that transmission had occurred despite standard isolation measures was provided to a nurse at the rehabilitation ward by telephone.

Nursing home

Upon transfer to the rehabilitation ward of the nursing home, the index patient was admitted to a single room with private sanitary facilities. HCWs wore gloves and gowns during care moments. There were no restrictions for social activities and the patient had unrestricted access to communal areas in the facility.

On November 5, 2013, when transmission on the rehabilitation ward was detected, isolation measures were intensified: the KPC-positive patients were cared for in single rooms with the door closed, and were no longer allowed to leave their rooms. HCWs donned gloves, gowns, and face masks before entering the patient room, and were instructed to remove

personal protective equipment and rub their hands with 70 % alcohol before leaving the patient room. Frequent audits of hand hygiene compliance were performed by an ICP and direct feedback was given to the HCWs and cleaning staff. Both rooms were cleaned daily at the end of the cleaning shift.

Contact screening

In the hospital, contact screening for the carriage of KPC-producing Gram-negative bacteria was performed for all patients who had been hospitalized for at least 48 h on the pulmonary ward since June 25, 2013, including those already discharged. Prospective screening was performed twice weekly on all hospitalized patients of the pulmonary ward, until 2 weeks after the discharge of both KPC-positive patients.

In the nursing home, contact screening for KPC carriage was initiated after the third KPC-positive patient was identified on November 5, 2013, among all patients discharged from the rehabilitation ward since August 5, 2013. Prospective screening of all patients on the rehabilitation ward was performed once a week from November 8. All nursing home residents (including those on other wards) were screened for KPC carriage twice at a 6-week interval on November 27, 2013 and January 7, 2014. HCWs in the hospital and nursing home were not tested for KPC-producing Gram-negative bacteria carriage.

Case definition

A case was defined as any patient infected or colonized with KPC-producing Gram-negative bacteria. Swabs from the rectum, sputum (in case of cough), insertion sites, sites of infection, and urine (in case of an indwelling catheter) were collected by nursing staff using the eSwab medium (Copan, Murrieta, CA, USA).

Sedimentation cultures and environmental sampling

Air sedimentation cultures were taken by placing solid agar plates (n=9) in the hall, anteroom, next to the bed, on the window sill, and on the bathroom sink. The agar plates were opened for 1.5 h, during the time that the index patient received physiotherapy and tracheostomy care, which included suction of secretions and rinsing of the inner cannula of the trachea cannula in the bathroom.

In the rehabilitation ward of the nursing home, a range of high-touch surfaces (e.g., night cabin, door knob) and equipment (e.g., glucose meter, patient lift) were sampled using 10-cm×10-cm sterile gauzes moistened with sterile saline.

Microbiological methods

Patient swabs were inoculated on the Colorex KPC plate (CHROmagar, bioTRADING, the Netherlands) and EbSA plates (Cepheid, Ledeberg, Belgium). The remaining eSwab fluid was transferred in 5 mL of tryptic soy broth with vancomycin (8 mg/L) and cefotaxime (0.25 mg/L) (TSB-VC; Cepheid), which was inoculated on the same media overnight. Gauzes used for environmental sampling were immediately placed in 10 mL of selective broth (TSB-VC) and grown overnight at 35–37 °C. Ten microliters of the broth was transferred to a Colorex KPC plate and an EbSA plate and grown overnight at 35–37 °C.

For all oxidase-negative Gram-negative rods growing on the selective media, species identification and susceptibility testing was performed using automated systems (Vitek MS and Vitek 2; bioMérieux, Marcy-l'Étoile, France) and the Etest (bioMérieux, Marcy-l'Étoile, France).

Molecular methods

Resistance genes were detected using a commercial microarray (Check-MDR CT103, Check-Points, Wageningen, the Netherlands) that can detect the β-lactamase genes of TEM, SHV, and CTX-M, pAmpC (CMY-2, DHA, FOX, ACC-1, ACT/MIR, and CMY-1/MOX), and carbapenemase genes (KPC, OXA-48-like, VIM, IMP, and NDM).

Typing was performed for every first unique KPC-producing isolate in each patient, and for four isolates found in the environment of the rehabilitation ward. *K. pneumoniae* isolates were typed using both amplified fragment length polymorphism (AFLP) as described by Mohammadi et al. [14] and multilocus sequence typing (MLST), whereas other species were typed using AFLP only. Whole-genome sequencing (WGS) and subsequent MLST+ typing and resistome analysis was performed on the same isolates, as well as one additional isolate of the index patient (isolated on December 9, 2013). Genomic DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. The DNA concentration was measured using the Qubit dsDNA HS and BR Assay Kit (Life Technologies, Carlsbad, CA, USA). The DNA library was prepared using the Nextera XT v2 kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions, and then run on the MiSeq system (Illumina) for generating paired-end 250-bp reads.

De novo assembly was performed by the CLC Genomics Workbench v7.0.4 (Qiagen, Hilden, Germany) after quality trimming (Qs≥20) with optimal word sizes. The assembled genomes were uploaded to a webtool called ResFinder v2.1 (http://cge.cbs.dtu.dk/services/ResFinder/) for identifying the acquired resistance genes with default settings. Assembled genomes were typed by MLST+ using SeqSphere v1.0

(Ridom GmbH, Münster, Germany). Briefly, an in-house defined MLST+ scheme including 3,102 *K. pneumoniae* genes was used as a reference for extracting open reading frames (ORFs) from the genome of each isolate by SeqSphere. Only the ORFs shared by all samples analyzed here without premature stop codons and ambiguous nucleotides were kept for MLST+ typing and, further, for generating the minimum spanning tree. This Whole 201 Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under accession numbers JTFU00000000, JTFV00000000, JTFW00000000, JTFX00000000, JTFY00000000, JTFZ00000000, and JTGA00000000.

Results

Outbreak investigation and control

From June through November 2013, a total of six patients were found to be colonized by colistin-resistant KPC-KP. The characteristics of the patients with KPC-producing Enterobacteriaceae are shown in Table 1.

The sequence of events (including time points of contact screening) is indicated in Fig. 1. In the hospital, contact screening (n=114) up to 2 weeks after the discharge of both KPC carriers revealed no further cases. In the nursing home, transmission occurred from the index patient to four additional patients. The second patient in the nursing home (patient 3) suffered from severe diarrhea upon admission to the rehabilitation ward in September, and, therefore, staff had worn gloves and gowns during care moments since admission. Despite these measures, she was found to carry the outbreak strain on November 5, 2013, and isolation measures were upgraded as described above. Following this event, contact screening (n=29) of discharged patients and the first of three prospective contact screening rounds (November 8, 2013, n=23) in the rehabilitation ward revealed no secondary cases. However, the second (November 18, 2013, n=22) and third (November 22, 2013, n=19) contact screenings revealed two and one additional KPC-KP carriers, respectively (patients 4, 5, and 6). After the detection of patients 4 and 5, cohorting of all KPC-positive patients to one side of the ward with dedicated nursing staff was implemented on November 20, 2013.

Despite these stringent measures, a sixth patient was found to be colonized by the outbreak strain. Subsequently, all KPC-positive patients were transferred to a separate, empty location outside the main building of the nursing home, with dedicated nursing staff, on November 27, 2013. After this transfer, the rehabilitation ward was disinfected with hydrogen peroxide vapor. Surveillance cultures taken on November 27, 2013 and January 7, 2014 in all nursing home patients (n=270) and the environment revealed no further transmission or environmental contamination.

Table 1 Characteristics of patients with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* (KPC-KP)

Patient no.	Sex	Age (years)	Comorbidities	Antibiotic use in the last 30 days	Colonization/infection	Medical devices	Duration of carriage in days
1	F	69	Cerebrovascular accident	Meropenem, Mycamine	Colonization	Trachea stoma, urinary catheter, percutaneous endoscopic gastrostomy catheter	226
2	M	65	Chronic obstructive pulmonary disease, Crohn's disease	Clindamycin, cefuroxime, ceftazidime, meropenem, metronidazole, penicillin, tobramycin, piperacillin/tazobactam	Colonization (pleura drain fluid)	Pleural drain	320
3	F	74	Recurrent *Clostridium difficile* infections	Vancomycin	Infection (urine)	Urinary catheter	97
4	M	70	Cerebrovascular accident	None	Colonization	Urinary catheter, percutaneous endoscopic gastrostomy catheter	26 (died, death unrelated to KPC)
5	M	78	Diabetes mellitus (leading to an amputated leg)	None	Infection (urine)	Urinary catheter	34 (died, death associated with KPC)
6	M	49	Postanoxic encephalopathy after cardiopulmonary resuscitation, comateus	None	Colonization	Urinary catheter, percutaneous endoscopic gastrostomy catheter	3

Several recognized risk factors for KPC colonization and infection were present in all KPC-positive patients, such as previous use of carbapenems, chronic obstructive pulmonary disease, and poor functional status

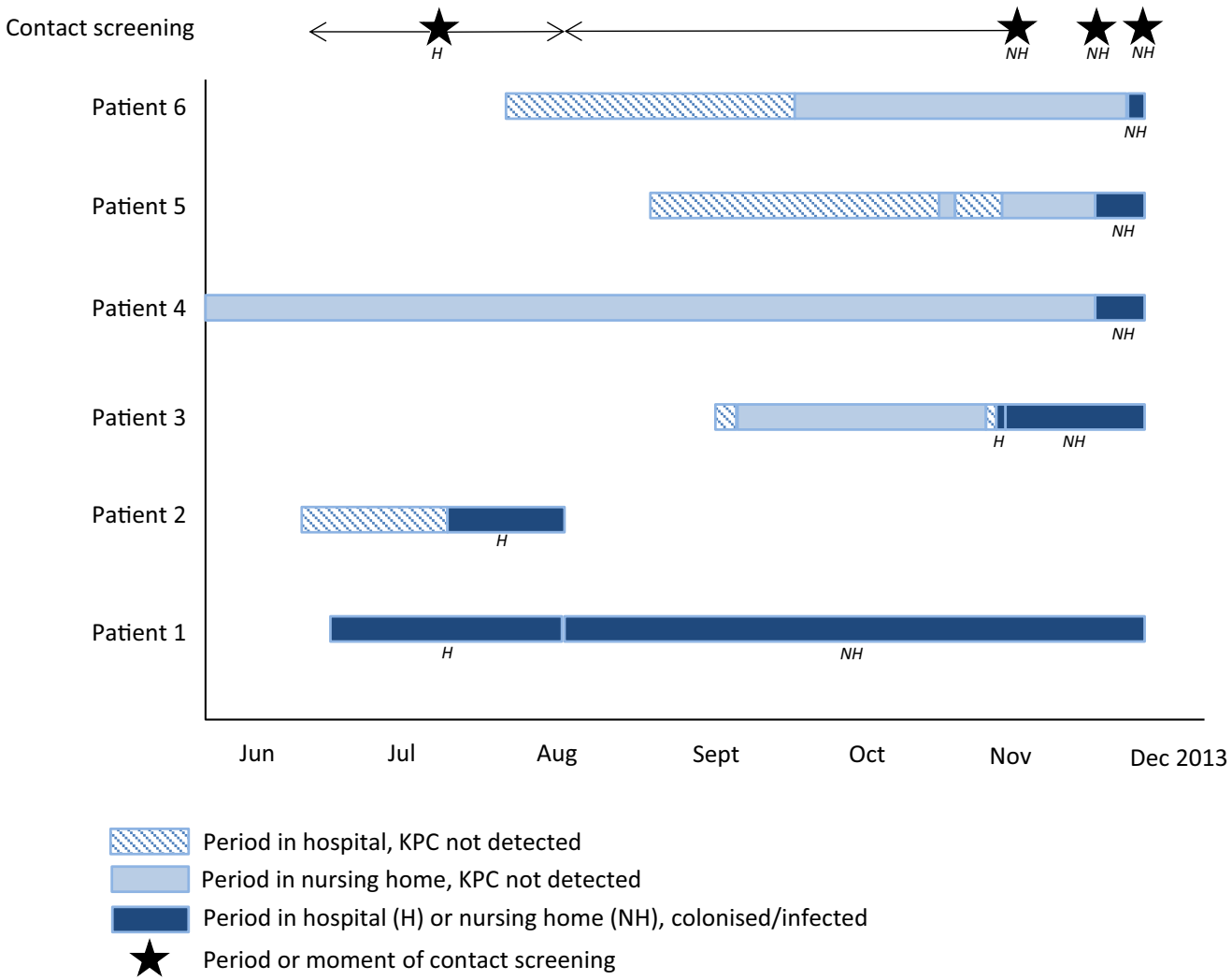


Fig. 1 Sequence of patient detection in the hospital and nursing home

Adherence to infection control

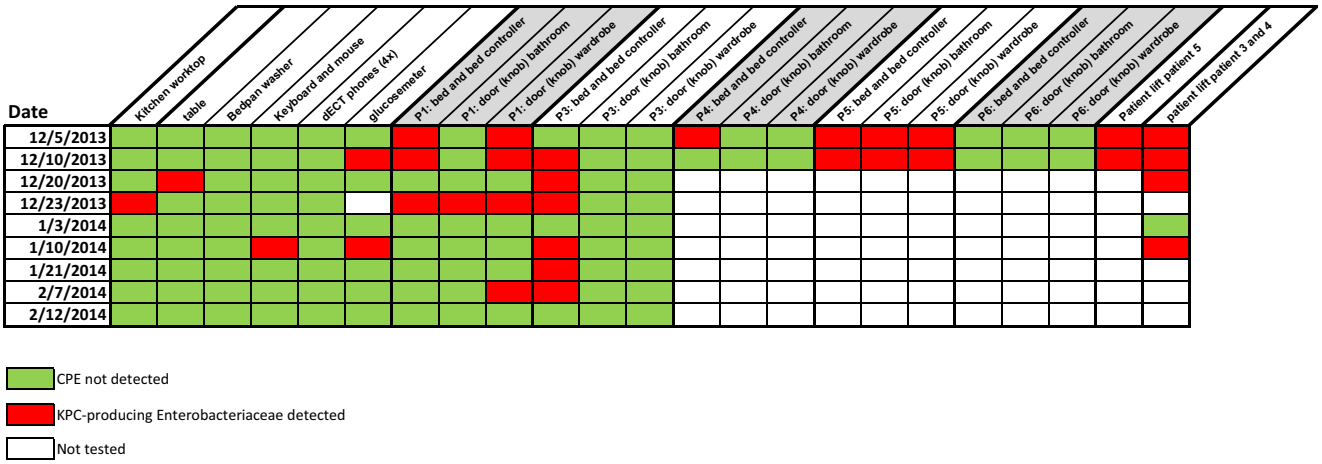
Audits in the nursing home revealed several shortcomings in the infection control measures during the outbreak period. Among these were unnecessary and inappropriate glove use and poor hand hygiene compliance by HCWs and cleaning staff, whereby personal protection was mentioned as the mean reason for wearing gloves. During several days in September, a shared storage of gowns was installed in the room of the index patient. Gowns were retrieved from the index patient's room for use for patient 3, who resided in a nearby room. A single glucose meter was available on the rehabilitation ward that was used for the index patient and for patients 5 and 6. In addition, patients were frequently transferred to different rooms within the ward: patient 4 was relocated four times in 3 weeks, once to a room that had previously been occupied by the index patient and cleaned afterwards.

In the separate facility, all five KPC-positive patients had a single room with private sanitary facilities. They had unrestricted access to communal areas (where standard precautions were employed), but only after adequate hand hygiene before leaving the patient room. HCWs only donned gloves and gowns before entering the patient room and removed them before leaving the room. Staff working on this separate facility was not screened for KPC carriage. In March 2014, the separate location was closed because patients were found to be KPC-negative (n=3) or died (n=2, of which one associated to KPC-KP infection).

Environmental contamination

One day after the KPC-positive patients were cohorted to one side of the ward in the nursing home, KPC-KP was identified in 4/24 samples (taken from night cabins, door knob, and the glucose meter). No KPC-producing isolates were found in the environmental cultures (n=24) from the non-cohorting side of the ward.

Table 2 Environmental cultures of the separate location



Environmental cultures of the separate location from three items per patient room [bed including bed controller, door (knobs), bathroom and door (knob), wardrobe] and six items in the common areas [kitchen worktop, table, handle and keypad of bedpan washer, computer keyboard and mouse in nursing office, dECT telephones (4x), and the glucose meter]. On five occasions, the two patient lifts were also cultured. Patient behavior appeared to be associated with the degree of environmental contamination. For patient 3, who was immobile and bed-bound, KPC-KP was detected on the bed/bed controller, but not on other sites in the patient room. By contrast, for patient 5 (who exhibited unhygienic behavior with urine and feces), all items in the patient room were positive for KPC-KP

In the separate facility, environmental contamination was monitored by taking weekly environmental cultures from predefined items (Table 2). A high level of environmental contamination of KPC-producing Gram-negative bacteria

Table 3 Susceptibility and molecular profile of every first unique carbapenemase-producing isolate

No.	Isolate	Date first isolated (2013)	Samples	Institute	MIC (mg/L)				*bla* genes	ST	AFLP
					IMP	MER	TIG	COL			
P1 (index)	*K. pneumoniae*	June 24	Rectal, urine, decubitus wound	H	3	6	2	6	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	E. aerogenes	November 8	Rectal	NH	24	>32	ND	ND	KPC-2	ND	B
P2	*K. pneumoniae*	July 5	Pleural fluid, rectal	H	32	32	1.5	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
P3	*K. pneumoniae*	October 31	Rectal, urine (catheter)	H[a]	4	6	1	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	E. aerogenes	October 31	Rectal, urine (catheter)	H[a]	12	4	1.5	ND	KPC-2, TEM *wildtype*	ND	B
	E. coli	December 30	Rectal	SL	1	0.38	ND	ND	KPC-2, TEM *wildtype*	ND	ND
P4	*K. pneumoniae*	November 18	Rectal	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	E. aerogenes	December 9	Rectal	SL	6	>32	ND	ND	KPC-2	ND	B
P5	*K. pneumoniae*	November 18	Rectal, urine (catheter), foot wound	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	E. aerogenes	December 9	Rectal	SL	12	1.5	ND	ND	KPC-2, TEM *wildtype*	ND	C
P6	*K. pneumoniae*	November 22	Rectal	NH	8	4	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E1	*K. pneumoniae*	November 21	Night cabin (room of patient 1)	NH	3	3	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E2	*K. pneumoniae*	November 21	Door knob (room of patient 1)	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E3	*K. pneumoniae*	November 21	Night cabin (room of patients 4/5)	NH	32	6	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E4	*K. pneumoniae*	November 21	Glucose meter	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A

H hospital, *NH* nursing home, *SL* separate location

[a] Isolate detected in hospital, while acquisition occurred during admission at the nursing home

was found in the patient rooms and on the two patient lifts, and less frequently in communal/kitchen areas. In addition to KPC-KP, KPC-producing *Enterobacter aerogenes* (room of index patient and patient 5, kitchen worktop) and KPC-producing *Acinetobacter baumannii* complex (glucose meter) were detected in the environment. In contrast to the *E. aerogenes* strains, the KPC-producing *A. baumannii* complex strain was not detected in cultures of the KPC-positive patients.

Sedimentation cultures

In the hospital, KPC-KP was detected in the room of the index patient on 3 out of 9 sedimentation agar plates placed on the left and right sides of the footboard of the bed, and on the sink. Similarly, KPC-KP was detected on 2 of 20 solid agar plates that were placed in the direct patient environment during routine care of the patients admitted to the separate location (on the right side of the headboard of the night cabin of patient 5).

Microbiology and molecular typing

All KPC-KP isolates were multidrug-resistant and exhibited intermediate levels of resistance to gentamicin (4 mg/L) and tigecycline (Table 3). The microarray revealed the presence of KPC, SHV, and (non-ESBL) TEM.

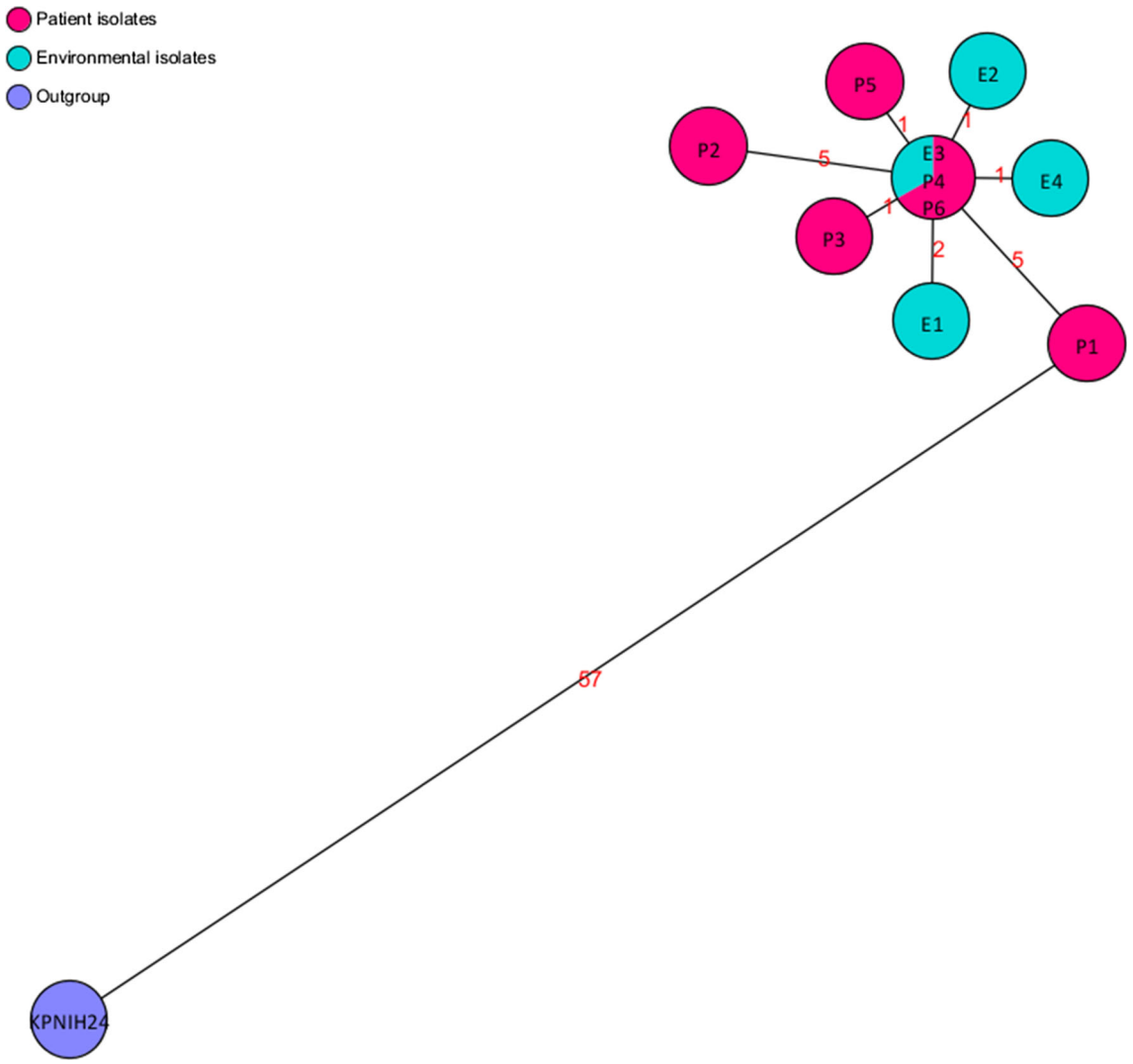


Fig. 2 Core genome typing (MLST+) of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* (KPC-KP) outbreak strains. This minimum spanning tree was generated by SeqSphere, which was based on comparing 3,042 alleles that were defined as the core genome of the strains analyzed in this study. The values indicate the number of different alleles. Different colors represent different epidemic groups, as shown in the legend. An ST258 strain *K. pneumoniae* KPNI H24 (GenBank accession number CP008797) was included in the analysis as the outgroup

MLST indicated that the isolates belonged to the pandemic sequence type ST258 and were clustered together by AFLP. In addition to *K. pneumoniae*, KPC-producing *E. coli* and KPC-producing *E. aerogenes* were detected in the index patient, patient 3, patient 4, and patient 5. The KPC-producing *E. aerogenes* strains of the index patient, patient 3, and patient 4 belonged to the same AFLP cluster, and the strain of patient 5 belonged to a different genotype.

The MLST+ result was concordant with that of AFLP. Of the 3,102 target genes included in the scheme, 3,042 were present in all isolates and were subsequently used in the MLST+ typing. The minimum spanning tree shows that all patient and environmental isolates were tightly clustered, exhibiting only up to seven different alleles (a seven-allele difference was found between P1 and E1, not indicated in Fig. 2). Isolates of patients 4 and 6 (P4 and P6) were identical to that of environmental isolate E3, which had 1–5 different alleles compared with the other isolates (Fig. 2). This clearly suggests that all isolates were related to the outbreak and that transmissions occurred between patients and the environment. The gene-by-gene approach was confirmed by a single nucleotide polymorphism (SNP) analysis. In total, 26 SNPs were detected among the 11 isolates (Table 4). Remarkably, the two strains that were epidemiologically most related (P1 and P2) had the largest number of SNP differences ($n=10$).

In accordance with the microarray results, the resistome analysis of all KPC-KP isolates showed the presence of $bla_{\text{OXA-9}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, and $bla_{\text{KPC-2}}$. Moreover, the aminoglycoside resistance gene *aac*(6′)*Ib*, fluoroquinolone resistance genes *oqxA* and *oqxB*, and fosfomycin resistance gene *fosA* were found in all isolates. All isolates, except the isolate of patient 2, also harbored resistance genes *aadA*2 and *aph*(3′)-*Ia* for aminoglycoside, *mph(A)* for macrolide, *catA*1 for phenicol, *sul*1 for sulfonamide, and *dfrA*12 for trimethoprim.

Discussion

In the outbreak described here, nosocomial transmission of KPC-KP occurred despite the implementation of contact precautions following the National Guideline on the Control of Multidrug-Resistant Microorganisms for the index patient. Although the outbreak could be controlled relatively easily in the hospital setting by transferring patients to an isolation room with anteroom and negative air pressure and appointing a dedicated nursing team, inter-institutional spread occurred after the index patient was transferred to a nursing home in the same region. This was detected only after one of the nursing home residents was admitted to the hospital, and further transmission in the nursing home was difficult to control.

During this outbreak, we found extensive environmental contamination in all settings. The last transmission occurred after separating all positive patients and appointing a dedicated nursing team, with the most probable route of transmission being a shared device (glucose meter). Our findings are consistent with recent studies showing that *Klebsiella* spp. are found in the environment more frequently than other coliforms [15, 16], and add to growing evidence that the environment can act as an important reservoir for transmission during KPC-KP outbreaks. The results of the air sedimentation cultures suggest that HCWs and the patient's environment may not only be contaminated by direct contact, but also by droplets or other (airborne) particles. To what extent this contributes to transmission is, as yet, unclear, and it should be noted that bacteria may survive longer on the sedimentation agars than on other inanimate surfaces. It is, however, certainly an aspect that is not taken into consideration in many infection control guidelines and questions the effectiveness of contact precautions on multiple bed wards. Taken together, the ability to contaminate and persist in the environment may explain the high transmission potential of this clone, and must be taken into account in environmental cleaning procedures. Other factors explaining the success of this clone, such as colonization potential and duration of carriage, need to be investigated.

Adherence to infection control measures is generally more difficult in long-term care facilities than in hospitals, due to differences in population characteristics, length of stay, staff education level, setup, and the level of social interaction between patients/residents. In addition, the diagnostic sampling frequency is generally low. As a result, detecting and preventing the transmission of highly resistant microorganisms in these facilities is particularly challenging, as illustrated by this report. Once a

Table 4 Single nucleotide polymorphisms (SNPs)

No.	Isolation date	Single Nucleotide Polymorphism																													
P1 (Index)	24-Jun-13	C	G	C	G	A	C	C	T	T	G	T	T	A	G	C	A	G	A	C	C	A	G	A	C	A	T	G	C	T	C	C
P1-dec (Index)	9-Dec-13	T	A	T	T	G	T	T	T	T	G	T	T	A	G	A	A	G	A	C	C	A	G	A	C	A	T	G	C	T	C	C
P2	5-Jul-13	C	G	C	T	G	T	T	A	A	A	A	G	C	G	C	A	G	A	C	C	A	G	A	C	A	T	G	C	T	C	C
P3	31-oct-14	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	T	T	G	C	C	A	G	A	C	A	T	G	C	T	C	C
P4	18-Nov-13	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	A	G	A	C	C	A	G	A	C	A	T	G	C	T	C	T
P5	18-Nov-13	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	A	G	A	A	C	A	G	A	C	A	T	G	C	T	C	C
P6	22-Nov-14	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	A	G	A	C	C	A	G	A	C	A	T	G	C	T	C	C
E1	21-Nov-13	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	A	G	A	C	A	G	T	A	C	A	T	G	C	T	C	C
E2	21-Nov-13	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	A	G	A	C	C	A	G	C	T	A	C	G	C	T	C	C
E3	21-Nov-13	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	A	G	A	C	C	A	G	A	C	G	C	G	C	T	C	C
E4	21-Nov-13	T	A	T	T	G	T	T	T	T	G	T	T	A	G	C	A	G	A	C	C	A	G	A	C	A	T	G	C	T	C	T

SNPs were detected by the basic unit of the CLC Genomics Workbench. The first KPC-KP isolate from the index patient was selected as the reference for detecting SNPs. To acquire high quality of SNPs, SNPs were filtered out if: (1) they were located within regions relating to mobile genetic elements, such as phages, transposases, integrases, or plasmids; (2) they were located within repetitive regions; (3) the Qs of SNPs was lower than 30; (4) the 10-bp window surrounding the putative SNP contained base calls with Qs lower than 20; (5) they were within two positions of a second putative SNP

reservoir has been established, this will result in frequent (and potentially unnoticed) introductions into surrounding hospitals. The monitoring of patient movements and direct communication between hospitals and long-term care facilities is of utmost importance to prevent inter-institutional spread, and future efforts should focus on optimizing these processes.

This report also illustrates the possible applications of WGS during outbreak investigations. WGS has the potential to become an important molecular epidemiological tool by obtaining almost all available DNA information in one single method. It could, thus, replace multiple conventional (molecular) methods used in current routine laboratories. Its high typing resolution combined with detailed epidemiological data will allow the identification of transmission chains during outbreaks.

Lastly, this outbreak raises the important question of how the spread of KPC (and other MDROs) in nursing homes and other long-term care facilities can be controlled. Possible solutions include the design of an effective decolonization strategy (for example, selective gut decontamination followed by a fecal transplant) or cohorting of KPC-positive patients in dedicated (regional) locations with highly trained personnel. Information about the effectiveness of decolonization strategies is urgently needed.

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Conflict of interest None.

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