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Prospective investigation of carbapenem-resistant *Klebsiella pneumonia* transmission among the staff, environment and patients in five major intensive care units, Beijing

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SUMMARY

Background: Following the alarming outbreak of carbapenem-resistant *Klebsiella pneumonia* (CRKP) in five intensive care units (ICUs) of a tertiary care hospital in China, a prospective investigation of CRKP colonized/infected patients was conducted.
Aim: To describe the diffusion and transmission of CRKP among epidemiologically linked ICU patients, staff and environment.
Methods: Enhanced CRKP infected/colonized case monitoring was performed by the real-time nosocomial infection surveillance system (RT-NISS). The immediate surroundings of each CRKP patient bed unit and the staff hands/gloves/gowns were sampled and then evaluated for the presence of CRKP. Antimicrobial susceptibility tests, pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (WGS) were used to identify and to characterize these isolates.
Findings: Among 2750 patients monitored, 67 CRKP patients were newly labeled and 11 patients' CRKP isolates were available. A total of 31.34% (21/67) bed units were positive at one or more surrounding surfaces, 7.99% (49/613) environmental samples and 3.57% (4/112) ICU staff samples were CRKP positive. The selected CRKP isolates ($N = 64$) exhibited intermediate to high resistance levels to the antibiotics tested apart from colistin and tigecycline. RT-NISS data combined with MLST and PFGE revealed nine likely transmission clusters. WGS analysis of these CRKP isolates revealed extensive sharing of multiple antimicrobial resistance genes and plasmid replicons among these isolates. Two carbapenemase genes bla_{KPC-2} (62/64) and bla_{OXA-48} (2/64) were identified. These CRKP isolates carried one or more plasmid replicons.

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Conclusions: The contamination of ICU environment and staff's hands, gloves or gowns is frequent with CRKP patients. Our study also supports the hypothesis that an association between environmental contamination and transmission of CRKP bacteria in ICUs.

Introduction

Klebsiella pneumoniae is a major member of the family Enterobacteriaceae. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains are spreading globally, primarily in healthcare settings. Infections with MDR or XDR *K. pneumoniae* strains are often difficult to treat [1–3]. Intensive care unit (ICU) patients are already disproportionally at risk of developing nosocomial carbapenem-resistant *Klebsiella pneumonia* (CRKP) infections, which are associated with high rates of morbidity and mortality [4]. Since the first report of CRKP in Zhejiang Province, China, in 2007 [5], CRKP infection numbers have sharply increased and a series of hospital outbreaks have been described over recent years [6]. Based on the data from the China Carbapenem-resistant Enterobacteriaceae (CRE) Net, CRKP strains account for roughly 70–80% of clinical CRE infections in China [7]. The CRKP outbreak isolates in China have mostly carried bla_{KPC-2} or bla_{NDM-1} that is predominantly plasmid encoded [6–8]. Dissemination of bla_{KPC} involves both horizontal transfer of bla_{KPC} genes and plasmids, and clonal spread [9]. The multiple transmission mechanisms for the bla_{KPC} gene among strains and plasmids in association with transposons, are great challenges for infection prevention and control measures against CRKP in ICU clinical practice [10].

CRKP isolates may contaminate the environment and ICU staff's hands, gloves or gowns, and especially some high-touch surfaces in the CRKP-positive patient zone, and thus increase potential for transmission [11,12]. However, the knowledge relative to the specific environmental and staff sites contaminated by colonized/infected CRKP patients is not well defined, and data concerning dissemination of CRKP bacteria among ICU patients, environment and staff is currently lacking. Understanding these factors may help to lead to interventions to prevent and control transmission of CRKP isolates.

To our knowledge, none of the published studies has assessed the diffusion and transmission of CRKP among epidemiologically linked ICU patients, staff and environment. In this study, a prospective sampling investigation was conducted to evaluate the presence of CRKP from the staff, environment and patients in five major ICUs of a large military hospital in China. The clonal relatedness and molecular data of CRKP isolates recovered through pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (WGS) were further analysed. The sequence types of multilocus sequence typing (MLST) were also analysed, and resistomes and plasmid contents were characterized from the WGS data.

Methods

Sampling strategies

Chinese People's Liberation Army General Hospital (PLAGH), with approximately 2000 licensed beds, is a tertiary care hospital located in Beijing. The PLAGH provides health and medical care to troops stationed in Beijing and is also open to civilian patients. It is also a tertiary centre that provides diagnosis and treatment for critically ill patients transferred from different areas of China. After May 2017, following the alarming and prolonged outbreak due to CRKP isolates, this study was carried out in five different ICUs in which CRKP were endemically spread, including: surgery (SICU), hepatobiliary surgery (HSICU), neurosurgery (NSICU), respiratory (RICU), and neurosciences (NICU). The five ICUs contain 100-bed units, each of which has its own dedicated medical, nursing and environmental cleaning staff. This study prospectively analysed all of the newly identified infected/colonized CRKP cases between 1st June 2017, and 31st December 2017. During the study period, enhanced case monitoring was performed by the real-time nosocomial infection surveillance system (RT-NISS) to find out whether patients admitted to the five ICUs were either infected or colonized with CRKP [13]. The screening algorithms of the RT-NISS are based on microbiological reports, antibiotic usage, serological and molecular testing, imaging reports, and fever history. The system is capable of daily, automatic, and real-time monitoring of all suspicious cases and outbreaks of nosocomial infections. The first clinical culture yielding CRKP was considered the index culture for each patient. If a patient had an index clinical culture for CRKP which was identified by microbiological laboratory, the bed unit of that CRKP infected/colonized patient was distinctly labelled, and the screening of environmental surfaces of patients' immediate surroundings was started. In order to be included in the study, infected/colonized patients had to have occupied their current ICU bed units for at least 24 h prior to the time of environmental sampling. Patients' sputum, urine, and abdominal drainage fluid were collected, if present.

Environmental samples were collected on the same day of each week between 10:00 a.m. and 12:00 p.m. before clothing and sheet replacement. Environmental samples were obtained from up to 20 sites within 2 m of the patient's bed: bed linen around the pillow; bed rails; bedside table; buttons of bed-control panel; call buttons; cardiovascular monitor screen; dedicated stethoscope; door handle; electrical outlet line; enteral feeding pump; floor on either side of bed; gauze pads around endotracheal tube; intravenous pump; mobile nursing cart handrail; nasal catheter; outer surface of bedside drainage bag; oxygen mask; sink; suction machine; and ventilator machine buttons. Not all sites were present in every room.

ICU staff (nurses, physicians and environmental cleaners) who entered the rooms of patients infected/colonized with CRKP were observed. On completion of patient care or bed unit cleaning and before exiting the patient room, the gloves and gowns of the ICU staff were swabbed by investigators. The bare hands of the ICU staff were immediately sampled a second time before hand hygiene. Hand, glove and gown samples were obtained with a standardized process as described by Morgan *et al.* [14].

The samples of environmental surfaces and items related to those infected/colonized patients were collected using a pre-moistened tip wrapped with traditional fiber (CLASSI-QSwabs™) for each site. At each site, an area of about 10 cm^2 was sampled, and the swab was rotated and swiped three times across each surface. The swab was then placed in the physiological saline-containing tube and immediately (within 30 min) transferred to the laboratory for further workup.

Microbiological testing

Swabs were vortexed at maximum rate for approximately 1 min, then 200 μL of the suspension was plated onto MacConkey agar and incubated overnight at 37°C. A VITEK-2 compact system (bioMérieux, Marcy l'Étoile, France) was used to establish the strain identity and antimicrobial susceptibilities of the isolates and these were interpreted in accordance with the guideline document M100-S28 established by Clinical and Laboratory Standards Institute (CLSI) [15]. Susceptibility to colistin and tigecycline was verified with the E-test (bioMérieux, Marcy l'Étoile, France). Because CLSI does not give breakpoints for colistin and tigecycline, those breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/) were applied. The species identity of all isolates was confirmed via matrix-assisted laser desorption/ionization mass spectrometry (Bruker Daltonics, Billerica, MA, USA).

PFGE genotyping

In order to determine the clonal relationships of all environmental, ICU staff's and patients' CRKP isolates, *Xba*I-digested genomic DNA was analysed by PFGE according to the standard protocol for *K. pneumoniae* isolates using a CHEF Mapper system (Bio-Rad), with a running time of 20 h and pulse times ranging from 3 to 40 s [16]. Macrorestriction pattern analysis and figure drawing were carried out using BioNumerics version 6.0 (Applied-Maths, Sint-Martens-Latem, Belgium). The Dice coefficient of similarity was calculated with a position tolerance of 0.8%, and a dendrogram was constructed based on the Unweighted pair-group mean Analysis (UPGMA). The similarity of the PFGE banding patterns was interpreted by the Dice coefficient. The isolates were considered to be genetically related if the Dice coefficient of correlation was 80% or greater [17].

WGS and genomic data analysis

Genomic DNA of the selected isolates ($N = 64$) was extracted using a Qiagen large construct kit (Qiagen, Hilden, Germany) and sequenced from a mate pair library with average insert size of 5000 bp, using a MiSeq sequencer (Illumina, San Diego, CA, USA). Raw reads were trimmed or filtered to remove poor-quality sequences, and then the contigs were assembled using Newbler 3.0 [18]. The web servers of ResFinder (version 3.0), PlasmidFinder (Enterobacteriaceae) (version 2.0), and MLST (version 2.0) at http://www.genomicepidemiology.org/ were used to identify acquired antimicrobial resistance genes, plasmid replicon types, and MLST from the assembled WGS data, respectively [19–21]. For resistance genes, a threshold of 100% identity was used for the genes encoding β-lactamases, and 98% identity was used for all other genes. A lower identity threshold (60%) was initially used to search for putative novel β-lactamase genes.

Results

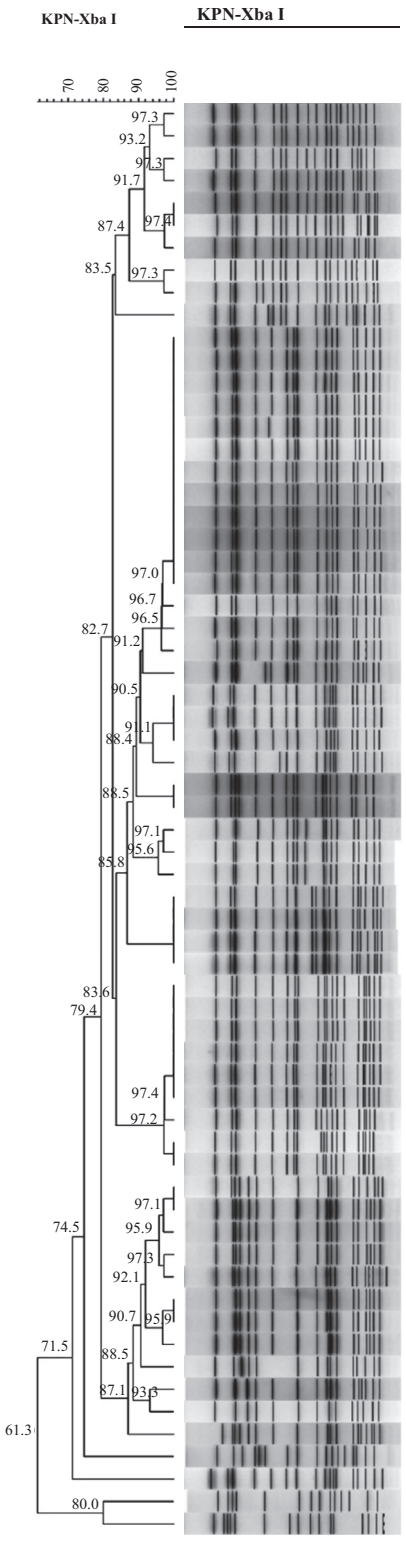
CRKP monitoring and sampling analysis

During the study, 2836 admissions (involving 2750 patients) to five ICUs were recorded, including 95 patients who were already in five ICUs at study initiation, 2652 admitted and discharged, and 98 remaining in five ICUs at study end. Sixty-seven patients (2.44%) were found to be colonized/infected with CRKP in these ICUs and their bed units were labelled and sampled; 31.34% (21/67) were positive at one or more surrounding environmental surfaces of bed units. Six hundred and thirteen surrounding environmental samples were taken from 67 bed units, 49 of the 613 samples (7.99%) were found to be CRKP-positive, the most frequently contaminated sites were gauze pads around endotracheal tubes (34.62%). The frequency of environmental contamination is shown in Table I.

Fifty-two ICU staff (35 nurses 12 physicians, and five environmental cleaners) were selected for participation in the study during routine care activities or bed unit cleaning. One hundred and twelve ICU staff samples (78 from nurses, 24 from physicians and 10 from environmental cleaners) were collected from hands (47), gloves (13), and gowns (52), and 3.57% (4/112) were positive for CRKP isolates. The staff's CRKP isolates were obtained from nurse's gown (1/35), nurse's hand (1/35), nurse's glove (1/8) and ICU environmental cleaner's gown (1/5).

Table I
The frequency of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolation from environmental screening

Site	Samples taken	Samples positive for CRKP	
	N	*N*	%
Bed linen around the pillow	51	7	13.73
Bed rails	81	10	12.35
Bedside table	38	0	0
Buttons of bed-control panel	57	3	5.26
Call buttons	27	1	3.70
Cardiovascular monitor screen	52	2	3.85
Dedicated stethoscope	21	0	0
Door handle	24	0	0
Electrical outlet line	42	0	0
Enteral feeding pump	14	1	7.14
Floor on either side of bed	47	6	12.77
Gauze pads around endotracheal tube	26	9	34.62
Intravenous pump	25	0	0
Mobile nursing cart handrail	40	4	10.00
Nasal catheter	3	1	33.33
Outer surface of bedside drainage bag	22	2	9.09
Oxygen mask	4	1	25.00
Sink	8	0	0
Suction machine	4	1	25.00
Ventilator machine buttons	27	1	3.70
Total	613	49	7.99



ST	Strain ID	ICU Type (Bed No.)	Patient ID	Speciman	PFGE Type	Beta-lactam resistant determinants
11	PCRK093	SICU(12)	P24:Y3053011	ADF	A01	KPC-2, SHV-12. CTX-M-65
11	ECRK095	SICU(12)	P24:Y3053011	BR	A02	KPC-2, CTX-M-14, SHV-12
11	PCRK091	SICU(13)	P22:F819863	UR	A03	KPC-2, SHV-12
11	ECRK094	SICU(12)	P24:Y3053011	FOB	A04	KPC-2, CTX-M-14, SHV-12
11	ECRK086	SICU(13)	P22:F819863	GPT	A05	KPC-2, SHV-12
11	ECRK090	SICU(16)	P23:K0175983	FOB	A05	KPC-2, SHV-11
11	ECRK088	SICU(13)	P22:F819863	BR	A06	KPC-2, SHV-12
11	ECRK012	NSICU(3)	P18:Y2407902	OM	A07	KPC-2, CTX-M-65, SHV-12
11	ECRK013	NSICU		MNH	A08	KPC-2, CTX-M-65, SHV-12
11	PCRK041	SICU(13)	P22:F819863	UR	A09	KPC-2, CTX-M-65, SHV-12
11	PCRK072	NICU(7)	P02:K0166688	SP	A10	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK073	NICU(3)	P01:66781	GPT	A10	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK075	NICU(15)	P03:B393337	GPT	A10	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK076	NICU(6)	P04:K0171343	BLP	A10	KPC-2, CTX-M-65, TEM-1B, SHV-1 1
11	ECRK077	NICU(6)	P04:K0171343	CMS	A10	KPC-2, CTX·M-65, TEM-1B, SHV-11
11	ECRK078	NICU(6)	P04:K0171343	SM	A10	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	PCRK079	NICU(6)	P04:K0171343	SP	A10	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	PCRK081	NICU(16)	P05:K0164282	SP	A10	KPC-2, CTX-M-65, LEN12, TEM-1B
11	PCRK082	NICU(17)	P06:K0158540	SP	A10	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK083	NICU(16)	P05:K0164282	BLP	A10	KPC-2, CTX-M-24, SHV-11
11	ECRKOB7	SICU		MNH	A10	KPC-2. CTX-M-65, TEM-1B. SHV-11
11	ECRK089	SICU(16)	P23:K0175983	BR	A10	KPC-2. CTX-M-65, SHV-11, TEM-1B
11	ECRK046	SICU(19)	P21:K0090906	BR	A11	KPC-2, SHV-11
11	PCRK071	NICU(3)	P01:66781	SP	A12	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK074	NICU(7)	P02:K0166688	VMB	A13	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	SCRK016	RICU		NH	A14	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK043	SICU(19)	P21:K0090906	GPT	A15	KPC-2, SHV-11
11	SCRK044	SICU		NGL	A15	KPC-2, SHV-11
11	ECRK052	SICU(19)	P21:K0090906	CB	A15	KPC-2, SHV-11
11	PCRK042	SICU(19)	P21:K0090906	SP	A16	KPC-2, SHV-11
11	PCRK084	HSICU(12)	P17:K0164357	ADF	A17	KPC-2, CTX-M-65, TEM-1B. SHV-11
11	ECRK085	HSICU(12)	P17:K0164357	OSB	A17	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK009	HSICU(S)	P15:G102247	FOB	A18	KPC-2, CTX-M-65, TEM-1B. SHV-11
11	ECRK024	HSICU(S)	P15:G102247	FOB	A19	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK025	HSICU(S)	P15:G102247	OSB	A20	KPC-2, CTX-M-65, TEM-1B. SHV-11
11	ECRK026	HSICU(13)	P16:G103194	NC	A21	KPC-2, CTX-M-65, TEM-1B, SHV-11
11	ECRK027	HSICU(13)	P16:G103194	EFP	A21	KPC-2, CTX-M-65, TEM-1B. SHV-11
11	ECRK033	HSICU(13)	P16:G103194	BLP	A21	KPC-2, CTX-M·65, TEM-1B, SHV-1 1
11	ECRK034	HSICU(13)	P16:G103194	BR	A21	KPC-2, CTX-M-65, TEM-1B. SHV-11
11	SCRK001	SICU		NGO	A22	KPC-2, CTX-M-15, OXA-1, TEM-1B, SHV-11
11	ECRK002	SICU(14)	P19:607444	CMS	A22	KPC-2, CTX-M-15. OXA-1, OHA-1, TEM-1B, SHV-11
11	ECRK003	SICU		MNH	A22	KPC-2, CTX-M-15, OXA-1, OHA-1, TEM-1B, SHV-11
11	ECRK038	SICU(14)	P19:607444	GPT	A22	KPC-2, CTX·M-15. OXA-1, DHA-1. TEM-1B. SHV-12
11	ECRK039	SICU(14)	P19:607444	BLP	A22	KPC-2, CTX-M-15, OXA-1, DHA-1, TEM-1B, SHV-12
11	ECRK040	SICU(14)	P19:607444	FOB	A22	KPC-2, CTX-M-15. OXA-1, DHA-1, TEM-1B, SHV-11
11	ECRK036	SICU(15)	P20:K0074636	FOB	A23	KPC-2, CTX-M-15, OXA-1, DHA-1, TEM-1B, SHV-11
11	ECRK035	SICU(15)	P20:K0074636	BOP	A24	KPC-2, CTX-M-15. CTX-M-65, OXA-1, OHA-1. TEM-1B
11	ECRK037	SICU(14)	P19:607444	8R	A24	KPC-2, CTX-M-15, OXA-1, DHA-1, TEM-1B, SHV-11
11	SCRK004	RICU		ECG	B01	KPC-2, SHV-11, TEM-1B, CTX·M-3. blaCTX-M-65
11	ECRK006	RICU(15)	P07:62538	GPT	B01	KPC-2, CTX-M-3, CTX-M-65, SHV-11, TEM-1B
11	ECRK008	RICU(15)	P07:62538	BR	B02	KPC-2, CTX-M-65, SHV-11
11	ECRK007	RICU(15)	P07:62538	8LP	B03	KPC-2, CTX-M-3, TEM-1B, SHV-11
11	ECRK049	RICU(15)	P07:62538	GPT	B04	KPC-2, CTX-M-3, CTX-M-65, SHV-11, TEM-1B
11	ECRK017	RICU(12)	P09:A189584	BR	B05	KPC-2, CTX-M-3, CTX-M-65, SHV-11, TEM-1B
11	ECRK020	RICU(15)	P07:62538	GPT	B05	KPC-2, CTX-M-3, CTX·M-65, SHV-11, TEM-1B
11	ECRK019	RICU(13)	P10:A271788	BOP	B06	KPC-2, CTX-M-3, TEM·1B, SHV-11
11	PCRK050	RICU(16)	P12:928093	SP	B07	KPC-2, CTX-M-65, SHV-11
11	ECRK018	RICU(13)	P10:A271788	BR	B08	KPC-2, CTX-M-3, CTX-M-65, SHV-11, TEM-1B
11	ECRK047	RICU		MNH	B09	KPC-2, SHV-11, TEM-1B, CTX-M-3. CTX-M-65
11	ECRK005	RICU(15)	P07:62538	BOP	B10	KPC-2, CTX-M-3, CTX-M-65, SHV-11, TEM-1B
11	ECRK022	RICU(9)	P13:602093	BR	C	KPC-2, CTX-M-65, OXA-1, SHV-12, TEM-1B
11	ECRK045	SICU(19)	P21:K0090906	BLP	D	KPC-2, SHV-11
383	ECRK023	RICU(17)	P14:8715443	GPT	E01	OXA-48, CTX-M-14b, SHV-1, TEM-1B
383	ECRK048	RICU(18)	P11:616342	BLP	E02	OXA-48,SHV-148,SHV-1

Figure 1. Pulsed-field gel electrophoresis (PFGE) dendrogram of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates. Dendrogram analysis, virtual gel image of PFGE patterns and beta-lactam resistant determinants profiles of 64 CRKP strains. Strains were considered to be genetically related if the Dice coefficient of correlation was 80% or greater. ADF, abdominal drainage fluid; BLP, bed linen around the pillow; BOP, buttons of bed-control panel; BR, bed rails; CB, call buttons; CMS, cardiovascular monitor screen; ECG, ICU environmental cleaner gown; EFP, enteral feeding pump; FOB, floor on either side of bed; GPT, gauze pads around endotracheal tube; HSICU, hepatobiliary surgery intensive care unit; MNH, mobile nursing cart handrail; NC, nasal catheter; NGL, nurse glove; NGO, nurse gown; NH, nurse hand; NICU, neurosciences intensive care unit; NSICU, neurosurgery intensive care unit; OM, oxygen mask; OSB, outer surface of bedside drainage bag; RICU, respiratory intensive care unit; SICU, surgery intensive care unit; SM, suction machine; SP, sputum; UR, urine; VMB, ventilator machine buttons.

A total of 64 non-duplicate CRKP isolates were collected from 21 colonized/infected patients during the study, including: 49 isolated from ICU bed unit environment, four isolated from ICU staff, and 11 isolated from ICU patients.

All isolates were MDR, and 17 isolates were XDR and resistant to all of the tested antibiotics apart from colistin and tigecycline. With the exception of colistin and tigecycline, these CRKP isolates exhibited intermediate to high resistance levels to the antibiotics tested. They were resistant to all beta-lactams as well as to quinolones (ciprofloxacin and levofloxacin); twenty-two isolates (34.38%) were resistant to trimethoprim/sulfamethoxazole; 46/64 (71.88%), 47/64 (73.44%), and 47/64 (73.44%) of isolates were resistant to amikacin, gentamicin, and tobramycin, respectively.

Molecular typing and likely transmission events analysis by PFGE

PFGE analysis revealed that the 64 CRKP isolates belonged to three distinct cluster patterns (A, B, and E) and two singletons (C and D) with a similarity of 80% (Figure 1). Sixty-two KPC-2-producing *K. pneumoniae* isolates were categorized into PFGE type A (48/62 = 77.42%), B (12/62 = 19.35%), and unique PFGE patterns (C = 1, and D = 1), and two OXA-48-producing isolates were PFGE type E. Patients' microbiological laboratory data combined with MLST typing and PFGE profiling revealed nine likely transmission clusters (Table II), where patients, staff and environmental isolates had identical undistinguished patterns (100% similarity) and were deemed to be very closely related.

In the A10 PFGE subtype, four ST11/KPC-2 isolates from CRKP-colonized/-infected patients who were in the NICU for an overlapping period, and six further isolates from environmental items were considered to be involved in patient (patient ID P04:K0171343 and P05:K0164282)-to-environment or environment-to-patient (patient ID P06:K0158540) transmission. In the A22 PFGE subtype including six CRKP strains, an ICU staff sample (nurse gown) and five further environmental samples were isolated from SICU, which showed a possible environment-to-staff transmission event. A possible transmission mode was also found between ICU environmental cleaner (ICU

Table II
Nine likely transmission clusters corresponded to the same pulsed-field gel electrophoresis (PFGE) subtypes

Transmission clusters	Strain ID	Sampling data	ICU type (bed no.)	Patient ID	Strain source	Speciman type	PFGE type
1	ECRK090	2017/11/28	SICU (16)	P23:K0175983	Environment	Floor on either side of bed	A05
	ECRK086	2017/11/28	SICU (13)	P22:F819863	Environment	Gauze pads around endotracheal tube	A05
2	PCRK072	2017/11/21	NICU (7)	P02:K0166688	Patient	Sputum	A10
	ECRK073	2017/11/21	NICU (3)	P01:66781	Environment	Gauze pads around endotracheal tube	A10
	ECRK075	2017/11/21	NICU (15)	P03:B393337	Environment	Gauze pads around endotracheal tube	A10
	ECRK076	2017/11/28	NICU (6)	P04:K0171343	Environment	Bed linen around the pillow	A10
	ECRK077	2017/11/28	NICU (6)	P04:K0171343	Environment	Cardiovascular monitor screen	A10
	ECRK078	2017/11/28	NICU (6)	P04:K0171343	Environment	Suction machine	A10
	PCRK079	2017/11/28	NICU (6)	P04:K0171343	Patient	Sputum	A10
	PCRK081	2017/12/12	NICU (16)	P05:K0164282	Patient	Sputum	A10
	ECRK083	2017/12/12	NICU (16)	P05:K0164282	Environment	Bed linen around the pillow	A10
	ECRK089	2017/11/28	SICU (16)	P23:K0175983	Environment	Bed rails	A10
	ECRK087	2017/11/28	SICU		Environment	Mobile nursing cart handrail	A10
	PCRK082	2017/12/12	NICU (17)	P06:K0158540	Patient	Sputum	A10
3	ECRK052	2017/8/14	SICU (19)	P21:K0090906	Environment	Call buttons	A15
	ECRK043	2017/8/1	SICU (19)	P21:K0090906	Environment	Gauze pads around endotracheal tube	A15
	SCRK044	2017/8/1	SICU		ICU staff	Nurse glove	A15
4	PCRK084	2017/11/21	HSICU (12)	P17:K0164357	Patient	Abdominal drainage fluid	A17
	ECRK085	2017/11/21	HSICU (12)	P17:K0164357	Environment	Outer surface of bedside drainage bag	A17
5	ECRK033	2017/7/6	HSICU (13)	P16:G103194	Environment	Bed linen around the pillow	A21
	ECRK034	2017/7/6	HSICU (13)	P16:G103194	Environment	Bed rails	A21
	ECRK027	2017/6/29	HSICU (13)	P16:G103194	Environment	Enteral feeding pump	A21
	ECRK026	2017/6/29	HSICU (13)	P16:G103194	Environment	Nasal catheter	A21
6	ECRK039	2017/7/6	SICU (14)	P19:607444	Environment	Bed linen around the pillow	A22
	ECRK002	2017/6/13	SICU (14)	P19:607444	Environment	Cardiovascular monitor screen	A22
	ECRK040	2017/7/6	SICU (14)	P19:607444	Environment	Floor on either side of bed	A22
	ECRK038	2017/7/6	SICU (14)	P19:607444	Environment	Gauze pads around endotracheal tube	A22
	ECRK003	2017/6/13	SICU		Environment	Mobile nursing cart handrail	A22
	SCRK001	2017/6/13	SICU		ICU staff	Nurse gown	A22
7	ECRK035	2017/7/6	SICU (15)	P20:K0074636	Environment	Buttons of bed-control panel	A24
	ECRK037	2017/7/6	SICU (14)	P19:607444	Environment	Bed rails	A24
8	SCRK004	2017/6/19	RICU		ICU staff	ICU environmental cleaner Gown	B01
	ECRK008	2017/6/19	RICU (15)	P07:62538	Environment	Gauze pads around endotracheal tube	B01
9	ECRK017	2017/6/29	RICU (12)	P09:A189584	Environment	Bed rails	B05
	ECRK020	2017/6/29	RICU (15)	P07:62538	Environment	Gauze pads around endotracheal tube	B05

environmental cleaner gown) and a contaminated environmental reservoir (gauze pads around endotracheal tube) in RICU.

MLST, antimicrobial resistance genes and plasmid profile analysis

The WGS data was used to determine these CRKP isolates MLST types (STs) and antimicrobial resistance genes (AGRs). The data of WGS are shown in Supplementary Table S1. The 64 sequenced CRKP isolates belonged to two different STs. All 62 KPC-2-producing isolates belonged to ST11 (Table I). Two OXA-48-producing isolates were ST383.

Besides the two carbapenemase genes (bla_{KPC-2} and bla_{OXA-48}), a number of additional β-lactamase genes were determined from WGS data using ResFinder. Four variants of the chromosomally encoded SHV β-lactamase genes were identified. SHV-11 was the most frequent subtype found in 75% (48/64) of isolates. Several other SHV allelic variants included SHV-12 (12/64), SHV-1 (2/64), and SHV-148 (1/64). One variant (LEN-12) of chromosomally encoded LEN β-lactamase genes was identified. The majority of CRKP isolates possessed bla_{TEM-1B} (68.75%,44/64), 15.63% (10/64) contained bla_{OXA-1} and 12.5% (8/64) bla_{DHA-1}. Five acquired CTX-M-subtype β-lactamase genes were identified, including 60.94% (39/64) $bla_{CTX-M-65}$, 15.63% (10/64) $bla_{CTX-M-3}$, 14.06% (9/64) $bla_{CTX-M-15}$, 4.69% (3/64) $bla_{CTX-M-14}$ and 1.56% (1/64) $bla_{CTX-M-24}$. Plasmid-mediated AmpC β-lactamase gene bla_{DHA-1} was detected in 12.5% (8/64) of isolates.

A total of 58 (90.63%) CRKP isolates were positive for fluoroquinolone-resistance gene *oqxAB*. Among 58 *oqxAB* -positive CRKP isolates, 25.86% (15/58) co-harboured *aac(6′)Ib-cr*.

WGS indicated that between two and six aminoglycoside resistance genes were identified among 59 CRKP isolates and in various combinations. The major frequent aminoglycoside resistance genes were *aadA2* and *rmtB* (82.81% and 68.75%, respectively).

All 64 CRKP isolates were positive for fosfomycin resistance gene *fosA* and 23.44% (15/64) co-harboured *fosA3* (plasmid-mediated fosfomycin resistance gene).

WGS also revealed the presence of two sulfonamide resistance genes (*sul1* (53) and *sul2* (13)), six phenicol resistance genes (*catA2* (29), *catB4* (9), *floR* (9), *cmlA1* (3), *catA1* (1) and *catB3* (1)), two tetracycline resistance genes (*tetA* (29) and *tetB* (1)), and five trimethoprim resistance genes (*dfrA1* (10), *dfrA12* (10), *dfrA14* (2), *dfrA17* (2) and *dfrA27* (1)) among one or more CRKP isolates.

Using the Plasmid Finder webserver, a total of 14 plasmid replicon types were detected (Table III). The similarity in alignment between the best matching plasmid in the database and the corresponding sequence in the input genome ranged from 97.97% to 100%. All of sixty-four CRKP isolates carried one or more plasmid replicons. *ColRNAI*, *IncFII(pHN7A8)*, and *IncR* were predominant, with 96.88% (62/64), 93.75% (60/64) and 64.06% (41/64) positivity, respectively. Among 21 (33.87%, 21/62) KPC-2-producing isolates, 11 possessed *IncFII(K)* and another 10 contained *IncFIB(K)*. All two OXA-48-producing *K. pneumoniae* isolates contained an *IncL/M(pOXA-48)* plasmid.

Table III
Distribution of plasmid replicon types

Plasmid replicon types	CRKP isolates ($N = 64$) *n* (%)
ColRNAI	62 (96.88)
IncFII(pHN7A8)	60 (93.75)
IncR	41 (64.06)
IncFIB(pKPHS1)	12 (18.75)
IncFII(K)	11 (17.19)
IncHI1B	11 (17.19)
IncQ1	11 (17.19)
IncFIB(K)	10 (15.63)
IncFIB(Mar)	5 (7.81)
IncFII	3 (4.69)
Col156	2 (3.13)
IncFIB(AP001918)	2 (3.13)
IncFII(29)	2 (3.13)
IncL/M(pOXA-48)	2 (3.13)

CRKP, carbapenem-resistant Klebsiella pneumoniae.

Discussion

CRKP infection is an imminent threat to ICU patients because of its ability to contaminate environmental surfaces surrounding patients and then further spread among patient, ICU staff and environment. The degree of environmental contamination by CRKP strains during a patient's stay in five ICUs was investigated. The sites most commonly contaminated in our study, such as gauze pads around endotracheal tubes, nasal catheters, oxygen masks, suction machines, bed linen around pillows, floor on either side of beds, bed rails, mobile nursing cart handrails, and outer surfaces of bedside drainage bags are environmental reservoirs of CRKP related to transmission in ICU settings, and these sites are commonly touched by healthcare workers during routine patient care. These results are consistent with studies of other important nosocomial pathogens [22–25], and suggest that more emphasis should be placed on the cleaning and disinfection of these surfaces. One study showed the existence of CRE contamination in the patients' bed area in different wards [11]. These findings highlight the importance of standard cleaning regimens for surfaces and items in patients' immediate surroundings and awareness of their roles in CRE dissemination and transmission to other patients and staff.

Many studies have shown that different activities were associated with a greater likelihood of ICU staff hands, gloves or gown contamination, including contact with wound dressings, artificial airways, side rails, lines, infusion pumps, catheters or drains, and direct patient contact including performing a physical examination or spending a longer duration in a room [26,27]. Our study found that during routine clinical care of CRKP-infected/-colonized patients, healthcare workers' gowns and/or gloves are contaminated. The source of contamination of ICU staff hands or gloves may have been prior patient contact or contact with the contaminated environment. The ICU environment can become extensively contaminated with CRKP that is not eliminated by standard cleaning methods. After our study period, in addition to standard environmental cleaning regimens, twice-daily cleaning with chlorine dioxide solution (100 ppm) was performed in the room environment of the CRKP carriers by microfibre cloths and mops. Further work is needed to determine the importance of environmental contamination with CRKP and the effect of effective decontamination on hospital infection rates.

To monitor the transmission events of identical CRKP strains among epidemiologically linked patients, staff and

environment, the CRKP strains were analysed through PFGE and WGS. The PFGE results showed that most of the CRKP isolates belonged to three major clusters of genetically related groups. The genome-wide sequence analysis provided strong evidence that these isolates were in fact genetically linked. However, it is important to note that the mechanisms underlying the spread of CRKP are complex and that epidemics may be due to the horizontal transmission of plasmids rather than clones [28,29]. WGS analysis demonstrated possible transmission of KPC-2 or OXA-48 producers in our ICU settings during a 7-month period at the strain and plasmid level. Consequently, our analysis hypothesized possible patient-/staff-to-environment, patient-/environment-to-staff, or staff-/environment-to-patient transmission events, mostly on the basis of the identical PFGE subtypes, the same MLST types (STs) and bla_{KPC-2} or bla_{OXA-48} gene correlations between the isolates of patients, staff and environment which were in the same ICU for an overlapping period. These results showed that contamination of the ICU environments and staff with clinical CRKP strains plays an important role in some instances of patient-to-patient transmission in the studied ICUs. ICU staff might contribute to nosocomial transmission of CRKP as a reservoir and/or as vectors.

Our isolates co-harboured various resistance determinants confirmed by WGS analysis, these genes conferring resistance to β-lactam antibiotics, aminoglycosides, fluoroquinolones, phenicol, sulfonamide, tetracyclines and trimethoprim. Our data showed that most of the isolates in this study possessed KPC-2 and coharboured three or more classes of resistance genes. The prevalence rate of KPC-2 among these isolates was higher than other investigations across China [7—9,29]. The majority of CRKP isolates belonged to clone ST11/KPC-2, and only two isolates were ST383/OXA-48.

The PlasmidFinder analysis showed that all of the tested strains had multiple replicon types, which have been classified into different incompatibility (Inc) groups or families on the basis of the inability of closely related plasmids to stably propagate within the same bacterial strain [9]. Together with the resistome of antimicrobial resistance genes analysed by ResFinder, the specific characteristics of plasmid replicon content were associated with multidrug resistance. Various combinations of several replicon types were probably responsible for wide dissemination of multiple resistance genes in CRKP bacteria. IncF plasmids have been widely distributed in our 64 CRKP strains, and they harbour a number of resistance determinants, including extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpCs, as well as quinolone and aminoglycoside resistance genes. The frequent identification of KPC-encoding IncF plasmids suggests that IncF plasmids may be the primary vehicle for the dissemination of KPC. Controlling the dissemination of bla_{KPC} is problematic, as the KPC-encoding gene is located on Tn4401 or Tn4401-like transposons and carried on variable transferable plasmids, thereby facilitating the inter- and intraspecies dissemination of resistance [30]. The IncL/M(pOXA-48) plasmid type was observed in our two OXA-48-producing *K. pneumoniae* isolates, suggesting that IncL/M associated with OXA-48 producers [31].

Although a significant isolation rate of CRKP was still found, our sampling technique may have been insensitive compared with others. And further, string tests are needed in order to identify the hypermucoviscous phenotype of the isolated strains. In addition, corresponding researches of the genetic environment surrounding bla_{KPC-2} gene and the predominant bla_{KPC} plasmid type are deficiencies in the present study; further studies and explorations are still warranted.

In conclusion, CRKP isolates can transfer between patients, ICU staff and environment. Environmental contamination and CRKP-positive patients were the most sources of transmission to ICU staff's hands, gloves or gowns. Compliance with contact precautions and more aggressive environmental cleaning and disinfection may decrease transmission of CRKP isolates. Although the mechanism of and interventions for environmental contamination and transmission of MDR bacteria are not well defined, our study supports the idea that contamination of a CRKP colonized/infected patient's bed unit increases the likelihood of transmission.

Conflict of interest statement
None declared.

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Authors' contributions

Y.L. and Z.Y. designed and supervised the study. M.D., B.L. and M.G. carried out data collection, environmental sampling, isolation and identification of CRKP isolates. Y.B. and H.S. performed PFGE genotyping by macrorestriction analysis. Z.Y., Y.Z. and Y.T. selected CRKP isolates for WGS, and performed the WGS data analysis. Z.Y. and Y.Z. wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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Appendix A: Supplementary data

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