Outbreak of KPC-producing *Klebsiella pneumoniae* ST15 strains in a Chinese tertiary hospital: resistance and virulence analyses

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Research

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Abstract

Background

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a major cause of clinical infection. However, KPC-producing *K. pneumoniae* ST15 strains are occasionally identified and have never been reported to cause hospital outbreaks in China.

Methods

In this study, pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (WGS) were used to characterize 32 KPC-producing *K. pneumoniae* ST15 strains, which were collected at a Chinese tertiary hospital. Susceptibilities, resistance genes, virulence factors and clinical performances of the ST15 strains were comparatively analysed with corresponding ST11 strains.

Results

The ST15 strains were distributed in 7 wards, mainly in the intensive care unit (ICU, 24/32, 75%). PFGE results from 24 strains covering all the wards presented a similarity of more than 92%, indicating clonal spread. In 84% of the ST15 strains, the imipenem (IPM) and meropenem (MEM) minimum inhibitory concentrations (MICs) were ≤ 8 and ≤ 16 $\mu\text{g/mL}$, respectively, both of which were 4-fold lower than those of the ST11 strains. The infection rates of the ST15 and ST11 strains were 28.1% and 43.9%, respectively ($P = 0.018$). PCR evaluation and WGS indicated that all the CRKP strains shared a 100% identical *bla*_{KPC-2} genetic structure, though a synonymous mutation and a Gly115-Asp116 (GD) insertion in the OmpK36 were identified in the ST15 and ST11 strains, respectively. None of the ST15 strains were positive for hypervirulence factors; however, the positive rate was 92.7% (38/41) in the ST11 isolates.

Conclusion

To the best of our knowledge, this is the first description of nosocomial outbreaks caused by KPC-producing *K. pneumoniae* ST15 strains in China. The ST15 strains had low carbapenem resistance and virulence, emphasizing the importance of careful evaluation of phenotypic and genetic characteristics for anti-infection therapy.

Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is an urgent public health problem throughout the world, and *K. pneumoniae* carbapenemase (KPC) is one of the most powerful enzymes that can hydrolyse nearly all β -lactams [1, 2]. Recently, Zhang and colleagues performed a nationwide survey covering a total of 1105 carbapenem-resistant *Enterobacteriaceae* (CRE) strains collected from 25 geographical locales and showed that the *bla*_{KPC} genes were detected in 58% of the CRE strains in China [3]. Another study from the China CRE network demonstrated that 73.9% of CRE cases were caused by *K. pneumoniae* isolates [4]. To date, dozens of *K. pneumoniae* strain types have been reported to be carriers of *bla*_{KPC} genes [5]. Unlike other countries, where the *K. pneumoniae* ST258 strain is the major strain that produces KPC enzymes [5, 6], ST11 is the dominant strain in China, accounting for nearly 60% of the carbapenemase-producing *K. pneumoniae* strains, followed by the ST23 (4.1%), ST15 (2.6%), ST37 (2.0%) and ST147 (1.8%) strains [3, 7]. However, little is known about the phenotypic and genetic characteristics of KPC-producing *K. pneumoniae* ST15 strains.

In a previous study, we systematically analysed the molecular epidemiology of KPC-producing *K. pneumoniae* from 2010 to 2016 and found that 92.8% (103/111) of the *K. pneumoniae* strains belonged to the ST11 type with sporadic cases of the ST1, ST35, ST437, ST494, ST592 and ST1883 strains in our hospital [8]. However, a nosocomial outbreak caused by the clonal spread of KPC-producing *K. pneumoniae* ST15 strains was characterized in 2018. In this study, we comparatively analysed the phenotypic characteristics and genetic backgrounds of the *K. pneumoniae* ST15 strains.

Materials And Methods

Bacterial strains and clinical data collection

In total, 102 non-repetitive CRE strains were isolated using the VITEK2 Compact System (bioMérieux VITEK, USA) in Lishui Hospital of Zhejiang University (Zhejiang, China) in 2018. Species were further confirmed by a VITEK mass spectrometry microbial identification system (bioMérieux VITEK). Ward distribution and sample source of the isolates were collected. Clinical presentations (colonization or infection) caused by *K. pneumoniae* were evaluated in a double-blind manner by a senior doctor with anti-infection experience.

Clonal characterization

Based on the 7 housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB* and *tonB*), multilocus sequence typing (MLST) was performed on all the *K. pneumoniae* strains as described previously [8, 9]. To further analyse the genetic relatedness of the *K. pneumoniae* ST15 strains, 24 isolates covering all the wards were randomly selected, and pulsed-field gel electrophoresis (PFGE) was performed using XbaI digestion and analysed by the software BioNumerics (Applied Math, Belgium) [10]. Strains that exhibited a similarity cut-off $\geq 85\%$ were considered the same pulsotype [11].

Antimicrobial susceptibility testing and phenotypic assays

Susceptibilities of all the isolates were initially tested by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Then, for all the ST15 strains and 20 randomly selected ST11 isolates, the minimum inhibitory concentrations (MICs) of imipenem (IPM), meropenem (MPM), piperacillin/tazobactam (TZP), ceftazidime (CAZ), cefepime (FEP), aztreonam (ATM), levofloxacin (LVX), gentamicin (GN), amikacin (AMK), polymyxin (POL) and tigecycline (TGC) were further detected by the broth microdilution method [12]. Carbapenemase activity was evaluated using the modified carbapenem inactivation method (mCIM) as previously described [12, 13]. The *E. coli* strain ATCC 25922 was used as a quality control.

Detection of resistance and virulence genes

A total of 13 carbapenemase-encoding genes, *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{SME}, *bla*_{GES}, *bla*_{IMI}, *bla*_{SIM}, *bla*_{SPM}, *bla*_{GIM}, *bla*_{OXA-23}, *bla*_{OXA-24} and *bla*_{OXA-58}, from all the *K. pneumoniae* strains were detected as previously described [3, 4, 14, 15]. The *bla*_{KPC} genetic structures were identified by PCR as described in our previous study [8]. The *ompK35* and *ompK36* genes were amplified and sequenced with specific primers as follows: 35F: 5'-TGCCCTTCAGACACCAAACCTC-3', 35R: 5'-CGAATTGCCAACCGTTTGCG3', 36F: 5'-TAGCCGACTGATTAGAAGGG-3', and 36R: 5'-GACAAGAGTATACCAGCGAGGT-3'. Virulence factors, such as the K serotype-specific alleles for K1, K2, K5, K20, K54 and K57; the exopolysaccharide synthesis regulators rpmA1, rpmA2 and cRmp; the iron uptake system; and aerobactin, were determined by PCR as described in previous studies [16, 17].

Whole-genome sequencing

The genome of a representative ST15 strain, KP18069, was extracted, and whole-genome sequencing (WGS) was performed using next-generation sequencing (NGS, Illumina HiSeq, USA) combined with single-molecule real-time (SMRT, PacBio, USS) sequencing as previously reported [18]. Then, the genome was de novo assembled with MUMer (v3), Pilon (v1.22) and BLASTN software and annotated with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

Results

Bacterial strains

During 2018, a total of 84 CRKP, 8 *Escherichia coli*, 5 *Klebsiella aerogenes*, 3 *Citrobacter freundii* and 2 other bacterial strains were isolated in our hospital. Among the CRKP strains, 43 (51.2%) were ST11 strains; 32 (38.1%) were ST15 strains; and 9 (10.7%) were other strains, such as ST23, ST147, ST340, ST395, ST897 and ST1883. The 32 ST15 strains were mainly isolated from the ICU ward (24/32, 75%) and sporadically distributed in another 6 wards: Cardiovasology (2/32), Traumatology (2/32), Neurosurgery (1/32), Gastroenterology (1/32), Respiratory Medicine (1/32) and Infectious Diseases (1/32) Wards. Specimens were sputum (26/32, 81.3%), blood (4/32, 12.5%), ascitic fluid (1/32, 3.1%) and urine (1/32, 3.1%).

Clonal relatedness of the *K. pneumoniae* ST15 strains

PFGE experiments were successfully performed on the 24 *K. pneumoniae* ST15 strains. The 6 strains isolated from the Neurosurgery (KP18091), Traumatology (KP18077), Infectious Disease (KP18029), Respiratory Medicine (KP18038), Cardiovasology (KP18035) and Gastroenterology (KP18062) Wards presented a close relationship to the strains from ICU wards with a similarity of more than 92% (Fig. 1). As expected, the ST11 strains KP148 and KP25 showed distinct PFGE patterns with similarities of 71.2% and 54.3%, respectively, to the ST15 strains.

Antimicrobial susceptibility and carbapenemase testing

All the *K. pneumoniae* strains were resistant to IPM, MPM, TZP, CAZ, FEP, ATM and LVX. However, 84.4% (27/32) of the *K. pneumoniae* ST15 strains exhibited IPM and MEM MICs of ≤ 8 and ≤ 16 $\mu\text{g/mL}$, respectively (Table 1). Only 1 of the ST15 strains (KP18069) exhibited both IPM and MEM MICs of ≥ 64 $\mu\text{g/mL}$. In contrast, except for 3 isolates that showed IPM MICs of 16 $\mu\text{g/mL}$, all the ST11 strains exhibited IPM and MEM MICs of ≥ 32 and ≥ 64 $\mu\text{g/mL}$, respectively. In addition, 3.1% (1/32) and 96.9% (31/32) of the ST15 strains versus 74.4% (32/43) and 81.4% (35/43) of the ST11 isolates had susceptibilities to GN and AMK, respectively. The POL and TGC MICs of all the strains were 0.5 to 2 $\mu\text{g/mL}$. All the selected *K. pneumoniae* strains were positive for carbapenemase activity using the mCIM.

Table 1
Clinical distribution and MICs of the *Klebsiella pneumoniae* strains tested in this study

Strain (KP)	ST type	Wards	Specimens	CAZ	FEP	ATM	TZP	IPM	MEM	GEN	AMK	POL	TGC	LVX	SXT	Infection Rates
18012	15	ICU	S	≥ 256	≥ 256	≥ 256	≥ 1024/4	8	32	32	≤ 4	0.5	0.5	16	≥ 64/1216	28.1% (9/32)
18013	15	ICU	S	128	≥ 256	≥ 256	≥ 1024/4	8	16	64	≤ 4	1	0.5	8	≥ 64/1216	
18028	15	Cardio	S	128	128	≥ 256	512/4	16	16	32	≤ 4	0.5	0.5	16	≥ 64/1216	
18029	15	Infect	S	≥ 256	≥ 256	≥ 256	≥ 1024/4	8	16	32	≤ 4	0.5	0.5	16	≥ 64/1216	
18030	15	ICU	S	128	≥ 256	≥ 256	512/4	8	8	32	≤ 4	0.5	0.5	16	≥ 64/1216	
18031	15	ICU	S	128	128	≥ 256	512/4	4	16	32	≤ 4	1	0.5	8	≥ 64/1216	
18032	15	ICU	S	≥ 256	128	≥ 256	256/4	8	16	32	≤ 4	0.5	0.5	8	≥ 64/1216	
18035	15	Cardio	S	≥ 256	≥ 256	≥ 256	≥ 1024/4	8	16	32	≤ 4	0.5	0.5	16	≥ 64/1216	
18036	15	ICU	S	128	128	≥ 256	≥ 1024/4	8	16	16	≤ 4	0.5	0.5	16	≥ 64/1216	
18038	15	Respir	S	≥ 256	128	≥ 256	512/4	8	16	64	≤ 4	0.5	0.5	16	≥ 64/1216	
18039	15	ICU	B	128	128	≥ 256	512/4	8	16	16	≤ 4	0.5	1	32	≥ 64/1216	
18047	15	ICU	S	≥ 256	128	≥ 256	≥ 1024/4	16	16	32	≤ 4	1	0.5	8	≥ 64/1216	
18051	15	ICU	S	128	128	≥ 256	512/4	8	8	16	≤ 4	0.5	0.5	16	≥ 64/1216	
18054	15	ICU	S	128	128	≥ 256	512/4	8	8	32	≤ 4	0.5	0.5	16	≥ 64/1216	
18055	15	ICU	S	128	128	≥ 256	512/4	16	16	≥ 512	≤ 4	0.5	0.5	16	≥ 64/1216	
18058	15	ICU	S	≥ 256	≥ 256	≥ 256	512/4	8	16	16	≤ 4	0.5	0.5	16	≥ 64/1216	
18059	15	ICU	S	64	64	64	≥ 1024/4	16	32	16	≤ 4	0.5	1	8	≥ 64/1216	
18060	15	ICU	S	128	≥ 256	≥ 256	≥ 1024/4	8	32	128	≤ 4	0.5	0.5	16	≥ 64/1216	
18062	15	Gastro	B	≥ 256	≥ 256	≥ 256	≥ 1024/4	8	16	32	≤ 4	0.5	0.5	16	≥ 64/1216	
18066	15	ICU	S	128	128	≥ 256	≥ 1024/4	8	16	32	≤ 4	0.5	0.5	16	≥ 64/1216	
18065	15	ICU	S	128	128	≥ 256	256/4	4	8	16	≤ 4	0.5	0.5	8	≥ 64/1216	
18068	15	ICU	S	≥ 256	128	≥ 256	256/4	4	8	16	≤ 4	0.5	0.5	16	≥ 64/1216	
18069	15	ICU	AF	≥ 256	≥ 256	≥ 256	512/4	≥ 64	≥ 64	≥ 512	≤ 4	0.5	0.5	16	≥ 64/1216	
18071	15	ICU	S	64	64	≥ 256	256/4	4	8	16	≤ 4	0.5	0.5	16	≥ 64/1216	
18076	15	ICU	S	128	128	≥ 256	512/4	8	16	≥ 512	≥ 1024	1	0.5	16	≥ 64/1216	

Cardio, Cardiovasology; Gastro, Gastroenterology; Infect, Infectious Disease; Neuro, Neurosurgery; Respir, Respiratory Medicine; Traum, Traumatology; Urol, Urology; AF, ascitic fluid; B, blood; S, sputum; U, urine; IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin/tazobactam; ATM, aztreonam; GEN, gentamicin; AMK, amikacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; POL, polymyxin; TGC, tigecycline.

Strain (KP)	ST type	Wards	Specimens	CAZ	FEP	ATM	TZP	IPM	MEM	GEN	AMK	POL	TGC	LVX	SXT	Infection Rates
18077	15	Traum	S	128	128	≥ 256	256/4	8	16	32	≤ 4	1	0.5	16	$\geq 64/1216$	
18078	15	ICU	B	128	64	≥ 256	256/4	8	8	32	≤ 4	0.5	0.5	8	$\geq 64/1216$	
18079	15	Traum	U	128	≥ 256	≥ 256	$\geq 1024/4$	8	16	≥ 64	≤ 4	0.5	1	16	$\geq 64/1216$	
18081	15	ICU	S	≥ 256	≥ 256	≥ 256	512/4	16	32	32	≤ 4	1	0.5	16	$\geq 64/1216$	
18091	15	Neuro	S	≥ 256	128	≥ 256	512/4	8	8	32	≤ 4	0.5	0.5	16	$\geq 64/1216$	
18096	15	ICU	B	≥ 256	≥ 256	≥ 256	512/4	8	16	32	≤ 4	1	0.5	16	$\geq 64/1216$	
18103	15	ICU	S	32	16	128	256/4	8	16	32	≤ 4	0.5	0.5	16	$\leq 0.5/9.5$	
18001	11	Neuro	B	64	128	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	32	$\geq 64/1216$	43.9% (18/41)
18003	11	ICU	S	128	128	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	32	$\geq 64/1216$	
18004	11	Neuro	S	128	128	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	16	$\geq 64/1216$	
18007	11	ICU	U	≥ 256	≥ 256	≥ 256	$\geq 1024/4$	≥ 64	≥ 64	8	≤ 4	0.5	0.5	16	$\geq 64/1216$	
18014	11	Infect	S	≥ 256	≥ 256	≥ 256	$\geq 1024/4$	16	≥ 64	256	≥ 1024	0.5	1	64	$\geq 64/1216$	
18015	11	ICU	S	128	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	32	$\geq 64/1216$	
18016	11	Respir	S	64	128	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	32	$\geq 64/1216$	
18017	11	Infect	B	≥ 256	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	16	$\geq 64/1216$	
18019	11	Urol	S	≥ 256	≥ 256	≥ 256	$\geq 1024/4$	≥ 64	≥ 64	≥ 512	≥ 1024	0.5	0.5	64	$\leq 0.5/9.5$	
18021	11	Respir	S	64	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	1	64	$\geq 64/1216$	
18023	11	ICU	S	128	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≥ 512	≤ 4	2	1	64	$\geq 64/1216$	
18024	11	ICU	S	128	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	64	$\geq 64/1216$	
18025	11	ICU	S	64	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	64	$\geq 64/1216$	
18026	11	Respir	S	≥ 256	≥ 256	≥ 256	$\geq 1024/4$	16	≥ 64	≤ 2	≤ 4	0.5	0.5	64	$\geq 64/1216$	
18052	11	ICU	S	128	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	64	$\geq 64/1216$	
18086	11	ICU	B	64	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≤ 2	≤ 4	0.5	0.5	32	$\geq 64/1216$	
18089	11	Gastro	AF	64	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≥ 512	≥ 1024	0.5	0.5	64	$\geq 64/1216$	
18112	11	ICU	B	≥ 256	≥ 256	≥ 256	$\geq 1024/4$	32	≥ 64	≥ 512	≥ 1024	0.5	0.5	32	$\geq 64/1216$	
18114	11	Infect	U	≥ 256	≥ 256	≥ 256	$\geq 1024/4$	≥ 64	≥ 64	≥ 512	≥ 1024	0.5	0.5	32	32/608	

Cardio, Cardiovasology; Gastro, Gastroenterology; Infect, Infectious Disease; Neuro, Neurosurgery; Respir, Respiratory Medicine; Traum, Traumatology; Urol, Urology; AF, ascitic fluid; B, blood; S, sputum; U, urine; IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin/tazobactam; ATM, aztreonam; GEN, gentamicin; AMK, amikacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; POL, polymyxin; TGC, tigecycline.

Strain (KP)	ST type	Wards	Specimens	CAZ	FEP	ATM	TZP	IPM	MEM	GEN	AMK	POL	TGC	LVX	SXT	Infection Rates
18115	11	ICU	S	128	≥ 256	≥ 256	≥ 1024/4	32	≥ 64	≤ 2	≤ 4	0.5	0.5	32	≥ 64/1216	
25	11	ICU	S	32	8	≥ 256	≥ 1024/4	8	16	8	≤ 4	0.5	0.5	16	≥ 64/1216	
148	11	ICU	S	128	≥ 256	≥ 256	512/4	32	≥ 64	≥ 512	≥ 1024	0.5	0.5	16	≥ 64/1216	
Cardio, Cardiology; Gastro, Gastroenterology; Infect, Infectious Disease; Neuro, Neurosurgery; Respir, Respiratory Medicine; Trauma, Traumatology; Urol, Urology; AF, ascitic fluid; B, blood; S, sputum; U, urine; IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin/tazobactam; ATM, aztreonam; GEN, gentamicin; AMK, amikacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; POL, polymyxin; TGC, tigecycline.																

Detection of resistance genes

The *bla*_{KPC-2} gene was detected in 94% (79/84) of *K. pneumoniae* strains, including all 32 ST15 isolates. Five of the ST11 strains were positive for *bla*_{NDM} genes. None of the other carbapenem genes were detected in this study. Further analysis revealed that all the *bla*_{KPC-2} genes were embedded in the Tn3-Tn4401 chimera, isoform B (CTB). Compared with the low carbapenem resistance *K. pneumoniae* strain KP25 (CP040391) that was characterized in our previous study [18], synonymous mutations of the *ompK35* gene, and 100% identical sequences of the *ompK36* gene were detected in 100% and 96.9% (31/32), of the *K. pneumoniae* ST15 strains, respectively. The strain that contained a nonsense mutation at amino acid position 248 (K248X) of OmpK36 was the highly carbapenem-resistant strain KP18069. However, a nonsense mutation of OmpK35 at amino acid position 63 (L63X) and the Gly115-Asp116 (GD) insertion of OmpK36 [19], which were identical to those of the highly carbapenem-resistant strain KP148 (CP040122) [18], were characterized in 100% (20/20) of the ST11 strains in this study.

Virulence factors and clinical presentations

Evaluation of the virulence factors revealed that all 32 ST15 strains were negative for the virulence genes tested in this work. However, virulence genes were detected in 92.7% (38/41) of the *K. pneumoniae* ST11 strains, and 30 of them were positive for at least 4 virulence genes (*rmpA1*, *rmpA2*, *iroN* and *iucA*). *K* serotype-specific genes were identified in 1 ST11 isolate. According to clinical presentations, 9 *K. pneumoniae* ST15 and 18 *K. pneumoniae* ST11 strains were identified to cause infections in patients. The other strains were considered to be colonization, except for 2 ST11 *K. pneumoniae* strains; colonization in the 2 strains was not determined. The infection rates of the ST15 strains were significantly lower than those of the ST11 isolates (28.1% vs 43.9%, *P* = 0.018, Table 2).

Table 2
Molecular characteristics of the KPC-producing *Klebsiella pneumoniae* strains KP18069, LSH-KPN25, LSH-KPN148, KP17-16 and their plasmids

Strain	KP18069	KP25	KP148	KP17-16
Accession Number *	CP059889-C CP059892	CP040391, CP040180 and CP040181	CP040122- CP040125	CP034077, MK191023, MK192097, MK191024 and MK181634
ST type	15	11	11	15
Plasmids	pKP18069-KPC pKP18069-CTX pKP18069-3	pLSH-KPN25-1 pLSH-KPN25-2	pLSH-KPN148-1 pLSH-KPN148-2 pLSH-KPN148-3	p17-16-KPC p17-16-CTX p17-16-vir p17-16-ColRNAI
Drug resistance genes	<i>bla</i> _{KPC-2} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{SHV-161} , <i>sul1</i> , <i>dfrA12</i> , <i>aadA2</i> , <i>norB</i> , <i>mph(A)</i>	<i>bla</i> _{KPC-2} , <i>aadA2</i> , <i>sul1</i> , <i>sul2</i> , <i>fosA5</i> , <i>dfrA12</i> , <i>norB</i>	<i>bla</i> _{KPC-2} , <i>bla</i> _{CTX-M-65} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-11} , <i>bla</i> _{SHV-66} , <i>aadA3</i> , <i>sul2</i> , <i>fosA5</i> , <i>norB</i>	<i>bla</i> _{KPC-2} , <i>bla</i> _{DHA-1} , <i>bla</i> _{SHV-1} , <i>bla</i> _{OXA-1} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1B} , <i>bla</i> _{SHV-11} , <i>armA</i> , <i>aadA2</i> , <i>aac(6')-Ib-cr</i> , <i>qnrB4</i> , <i>fosA</i> , <i>sul1</i> , <i>tet(A)</i> , <i>dfrA12</i> , <i>mph(A)</i>
<i>bla</i> _{KPC} genetic structure	CTB	CTA	CTB	CTB
<i>ompK36</i>	Synonymous mutation	Reference Sequence	Mutation, eg.: GD insertion	Synonymous mutation
Virulence genes*	<i>irp1</i> , <i>irp2</i> , <i>fimABCDEFGHI</i>	<i>iutA</i> , <i>irp1</i> , <i>irp2</i> , <i>fimABCDEFGHI</i>	<i>iutA</i> , <i>irp1</i> , <i>irp2</i> , <i>fimABCDEFGHI</i>	<i>iucABCD-iutA</i> , <i>rmpA2</i> , <i>irp1</i> , <i>irp2</i> , <i>iroE</i> , <i>entB</i> , <i>fimABCDEFGHIK</i>
*, Chromosome sequences and hypervirulent genes are highlighted in bold font.				

WGS and comparative analysis

The multidrug-resistant (MDR) *K. pneumoniae* ST15 strain KP18069 has a chromosome of 5,379,538 bp and three plasmids, pKP18069-CTX, pKP18069-KPC and pKP18069-3 of 189,307 bp, 95,050 bp and 10,060 bp, respectively. The genetic environment (CTB) of the *bla*_{KPC} and nonsense mutation of the

ompK36 (K248X) genes was further confirmed. Compared with the chromosome sequences of the 2 representative *K. pneumoniae* ST11 strains KP25 (CP040391) and KP148 (CP040122) [18], which have low and high carbapenem resistance, respectively, the chromosome sequence of the ST15 strain KP18069 shared a query coverage of 91% but had identities of 99.36% and 99.34%, respectively. The *bla*_{KPC-2} plasmid pKP18069-KPC shared query coverage of 80% and 76% to pLSH-KPN25-1 (CP040180) and pLSH-KPN148-1 (CP040123), respectively, but had an identity of 99.69% with both (Fig. 2). The *bla*_{CTX-M} plasmid pKP18069-CTX shared query coverage and identity of only 7% and 98.98% with pLSH-KPN148-2 (CP040124), respectively.

The genome sequence of KP18069 was highly homologous to the *K. pneumoniae* ST15 strain KP17-16, which was characterized in Henan Province of China [20]. They shared chromosome query coverage and identity of 99% and 100%, respectively. Particularly, the *bla*_{KPC-2} plasmid pKP18069-KPC shared 98% query coverage and 98.7% identity to the corresponding p17-16-KPC (MK191023). The main difference was that pKP18069-KPC lacks the plasmid SOS inhibition (psi)A/B system that is present in p17-16-KPC (Fig. 2). The *bla*_{CTX-M} plasmid pKP18069-CTX that shared 7% query coverage with pLSH-KPN148-2 (CP040124) had 84% query coverage and 98.7% identity to p17-16-CTX (MK192097). However, the virulence plasmid p17-16-vir (MK191024) that is present in the strain KP17-16 was absent in KP18069.

In agreement with the MDR phenotype, the resistance genes of β -lactams (*bla*_{KPC-2}, *bla*_{CTX-M-15} and *bla*_{SHV-161}), trimethoprim-sulfamethoxazole (*sul1* and *dhfrA12*), aminoglycosides (*aadA2*), quinolone (*norB*) and macrolide (*mph(A)*) were identified in KP18069 by WGS, and these genes, except *bla*_{SHV-161} and *norB*, were plasmid-borne. The plasmid pKP18069-KPC, which belongs to the IncFII group, carries only 1 resistance gene: *bla*_{KPC-2}. Although the *bla*_{KPC-2} gene was present in all strains, it was embedded in the CTB chimera in KP18069 and KP148, while it was harboured in isoform A (CTA) in KP25. Additional resistance genes of *bla*_{CTX-M-65}, *bla*_{TEM-1}, *bla*_{SHV-11}, *bla*_{SHV-66}, *aadA3* and *fosA5* were detected in KP148, and *bla*_{SHV-11}, *dhfrA12*, *sul2*, *aadA2* and *fosA5* were detected in KPN5 (Table 1). Consistent with the PCR results, WGS confirmed the absence of hypervirulence gene in KP18069; however, the aerobactin-associated factor *iutA* was detected in the chromosomes of KP25 and KP148 (Table 1).

Discussion

KPC-type carbapenemases are initially and most frequently identified in *K. pneumoniae* strains [1, 3, 5]. Clonal spread of the *K. pneumoniae* ST11 strain is one of the major causes mediating *bla*_{KPC} dissemination in China [3, 4]. Although the *K. pneumoniae* ST15 strain is occasionally isolated, nosocomial spread of OXA-232-producing ST15 strains has been reported in a teaching hospital in China [21]. However, little is known about the KPC-producing *K. pneumoniae* ST15 strain in China, and it has never been identified before 2018 in our hospital [3, 8].

During 2018, 32 KPC-producing *K. pneumoniae* ST15 strains that accounted for 38.1% of the total CRKP strains were isolated in our hospital. The ratio is much higher than that of the average ratio (2.6%) reported in China [3]. In addition, 75% of these isolates were collected from the ICU ward, and there were strains distributed in 7 different wards that showed PFGE similarity greater than 92%, greatly indicating a clonal spread of the KPC-producing *K. pneumoniae* ST15 strains and suggesting an outbreak in our hospital.

Similarly, as reported by Li and colleagues [20], the KPC-producing *K. pneumoniae* ST15 strains that were characterized in our hospital were multidrug resistant, though the IPM and MEM MICs of most strains were 4-8-fold lower than those of the corresponding ST11 strains. In particular, the IPM MICs of 81.3% (26/32) of strains were 8 μ g/mL or even less, which is considered to be the threshold of the high-dose continuous infusion of carbapenem (HDCI) strategy for anti-infection therapy [22]. The reason that KP18069 showed IPM and MEM MICs of both $\geq$ 64 μ g/mL may be caused by the nonsense mutation (K248X) of *OmpK36*, which has been reported to be responsible for carbapenem resistance [19]. Thus, as the last choice to achieve complete killing of the KPC-producing *K. pneumoniae* ST15 strains, an HDCI strategy is suggested.

In addition to carbapenem resistance, hypervirulence is another factor that greatly impacts the anti-infection procedure. A fatal outbreak of carbapenem-resistant hypervirulent *K. pneumoniae* ST11 strains has been reported in a Chinese hospital [17]. The convergence of *bla*_{KPC} and hypervirulence genes within the same *K. pneumoniae* ST15 strain has also been identified in China and Vietnam [20, 23]. Fortunately, none of the ST15 strains detected in this study were positive for hypervirulence genes, and the infection rates of the KPC-producing *K. pneumoniae* ST15 strains were lower ($P=0.018$) than those of the corresponding ST11 strains in our hospital. These results indicate that the KPC-producing *K. pneumoniae* ST15 strains are less virulent than the ST11 strains.

In agreement with phenotypic analysis, genomic analysis revealed significant discrepancies in chromosomes and plasmids between the *K. pneumoniae* ST15 strain KP18069 and ST11 strains KP25 and KP148. Briefly, the *bla*_{KPC-2} gene was embedded in the CTB chimera in KP18069; however, it was embedded in CTA and CTB in KP25 and KP148, respectively. Compared with KP25, a nonsense mutation and a GD insertion of *OmpK36* were identified in KP18069 and KP148, respectively [18]. In addition, unlike the ST11 strains, no hypervirulence genes were detected in KP18069. Although the genome sequence was highly homologous to that of the *K. pneumoniae* ST15 strain KP17-16, KP18069 lacks the virulence plasmid p17-16-vir (MK191024), which is greatly associated with severe infections [20].

Conclusions

In summary, we characterized a clonal spread and outbreak of KPC-producing *K. pneumoniae* ST15 strains in our hospital. Phenotypic and genetic analyses indicated that the ST15 strains were less resistant to carbapenem and less virulent than the corresponding ST11 isolates. Hence, careful evaluation of carbapenem MICs and virulence factors may be helpful for precision anti-infection therapy. To the best of our knowledge, this is the first description of a nosocomial outbreak of KPC-producing *K. pneumoniae* ST15 strains in China.

Abbreviations

CRE
Carbapenem-resistant *Enterobacteriaceae*;
CRKP
Carbapenem-resistant *Klebsiella pneumoniae*;
KPC
Klebsiella pneumoniae carbapenemase
MIC
Minimum inhibitory concentration;
MDR
Multidrug resistant;
MLST
Multilocus sequence typing;
PFGE
Pulsed-field gel electrophoresis;
WGS
Whole genome sequencing.

Declarations

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Availability of data and materials

Complete nucleotide sequences of the *K. pneumoniae* KP18069 have been deposited in NCBI under the accession numbers CP059889-CP059892.

Authors' contributions

JH, XC, RW and ZZ designed the study. JH and XC wrote the manuscript. YZ, YS and HD participated in the laboratory experiment. JX, XZ, RW and ZZ participated in the data collection and analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Research Ethics Board at Lishui Hospital of Zhejiang University. Strains analyzed in this study were collected from clinical routine. No written informed consent was acquired due to the retrospective nature of the study and the information was de-linked.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

PFGE results of the *K. pneumoniae* strains. Experiments were performed on the 24 ST15 strains from all 7 of the different wards, and the 2 ST11 strains (KP25 and KP148) that were completely sequenced were adopted as outliers. RS, reference sequence (CP040391); S, synonymous mutation; Ins, insertion mutation; K248X, nonsense mutation at amino acid position 248.

Figure 2

Comparative analysis of the KPC-carrying plasmids pLSH-KPN148-1 (CP040123), pLSH-KPN25-1 (CP040180), pKP18069-KPC (CP059889) and p17-16-KPC (MK191023). Grey shading denotes shared regions of homology in each adjacent plasmid. Open reading frames (ORFs) are depicted by boxes and are

coloured based on predicted gene function. Resistance genes, mobile elements, the repA gene, backbone genes, and conjugal transfer system are indicated with deep blue, orange, red, pink and green, respectively.