Emergence of *Escherichia coli* Sequence Type 131 Isolates Producing KPC-2 Carbapenemase in China

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Twenty-two KPC-2-producing *Escherichia coli* isolates were obtained from three hospitals in Hangzhou, China, from 2007 to 2011. One isolate, with OmpC porin deficiency, exhibited high-level carbapenem resistance. Pulsed-field gel electrophoresis showed that few isolates were indistinguishable or closely related. Multilocus sequence typing indicated that sequence type 131 (ST131) was the predominant type (9 isolates, 40.9%), followed by ST648 (5 isolates), ST405 (2 isolates), ST38 (2 isolates), and 4 single STs, ST69, ST2003, ST2179, and ST744. Phylogenetic analysis indicated that 9 group B2 isolates belonged to ST131, and 5 of 11 group D isolates belonged to ST648. Only one group B1 isolate and one group A isolate were identified. A representative plasmid (pE1) was partially sequenced, and a 7,788-bp DNA fragment encoding Tn3 transposase, Tn3 resolvase, ISKpn8 transposase, KPC-2, and ISKpn6-like transposase was obtained. The *bla*_{KPC-2}-surrounding sequence was amplified by a series of primers. The PCR results showed that 13 isolates were consistent with the genetic environment in pE1. It is the first report of rapid emergence of KPC-2-producing *E. coli* ST131 in China. The *bla*_{KPC-2} gene of most isolates was located on a similar genetic structure.

Carbapenems are considered to be the most efficient antibiotics for treatment of serious infections caused by multidrug-resistant Gram-negative bacilli producing extended-spectrum β -lactamases (ESBLs) or AmpC. However, the rise in the number of carbapenem-resistant *Enterobacteriaceae* with the extensive use of these agents is of great concern. The resistance rates increased significantly, from <2% to ~5% in 2010 and 2011 according to the surveillance data of CHINET (1–3). Carbapenem resistance in the *Enterobacteriaceae* is attributed mainly to production of carbapenemases, the most common one of which is KPC. This enzyme has been detected in many genera of the *Enterobacteriaceae* and nonfermenting bacteria and has spread globally, in particular, in the eastern United States, Israel, Colombia, Greece, and Puerto Rico within a few years (4, 5). The initial report of KPC in China was for a *Klebsiella pneumoniae* isolate of the 1st Affiliated Hospital of Zhejiang University in Hangzhou in 2007 (6). We identified KPC-2 in *Serratia marcescens* (7), *Citrobacter freundii* (8), *K. pneumoniae*, *Escherichia coli* (9), *Enterobacter cloacae* (10), and *Proteus mirabilis* (11) isolates from our hospital (2nd Affiliated Hospital of Zhejiang University) in the same city from 2007.

KPC enzymes have been detected most frequently in *K. pneumoniae* and much less in *E. coli* and other enterobacterial species (4, 5). The majority of KPC-producing *K. pneumoniae* isolates worldwide belonged to sequence type 258 (ST258) (12), while the dominant clone in the Chinese mainland and Taiwan was ST11, which was closely related to ST258 (13, 14). The molecular epidemiology investigation of KPC-producing *E. coli* was limited. Recently, KPC was detected in *E. coli* ST131, an international epidemic and multidrug-resistant clone (15), 1 isolate of which was from France (16), 1 from Ireland (17), and 7 from the United States (18). Fourteen *E. coli* ST410 isolates and one *E. coli* ST2281 isolate with KPC-2 were identified in Greece (19). During the period from 2007 to 2011, we collected 22 *bla*_{KPC}-positive carbapenem-nonsusceptible *E. coli* isolates in 3 hospitals in Hangzhou, China. For the current study, we describe the mechanisms of carbapenem resistance and the molecular epidemiology analysis of the 22 *E. coli* isolates, including pulsed-field gel electrophoresis

(PFGE), multilocus sequence typing (MLST), and phylogenetic typing, as well as the genetic structures surrounding the *bla*_{KPC} gene.

MATERIALS AND METHODS

Bacterial strains. Twenty-two clinical isolates of *E. coli* with carbapenem resistance or reduced carbapenem susceptibility were collected from 3 hospitals in Hangzhou from 2007 to 2011. Twelve were from the 2nd Affiliated Hospital of Zhejiang University, 9 were from Hangzhou Traditional Chinese Medicine Hospital, and 1 was from Zhejiang Provincial People's Hospital. Seventeen patients were hospitalized in intensive care units (ICUs). Four isolates were recovered in 2007, 2 in 2009, 4 in 2010, and 12 in 2011. The *E. coli* E1 isolate has been described previously (9). Most *E. coli* isolates were obtained from elderly patients with cardiovascular and cerebrovascular disease or respiratory disease in intensive care units. Twelve of the 22 isolates were recovered from sputum, and others were from body fluid or secretion. Half of the patients had been exposed to carbapenems before the organism was isolated, and half received therapy with anti-Gram-positive-bacterium antibiotics, including vancomycin, teicoplanin, and linezolid. Species identification was performed with the Vitek 2 compact system (bioMérieux, North Carolina).

Antimicrobial susceptibility testing. MICs of ciprofloxacin and amikacin were determined by Etest (AB Biodisk, Sweden), and those of other 12 antibiotics were determined using the agar dilution method according to Clinical and Laboratory Standards Institute recommendations (20). *E. coli* ATCC 25922 was used as a quality control strain.

Conjugation and transformation experiment. Conjugation experiment was carried out in mixed broth cultures as described previously (9). Rifampin-resistant *E. coli* EC600 (LacZ⁻; Nal^r Rif^r) was used as the recip-

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ient strain. Plasmids DNAs which were unable to be transferred to *E. coli* EC600 by conjugation were transformed into competent *E. coli* EC600 using the heat shock method. *E. coli* transconjugants and transformants were selected on Mueller-Hinton agar containing meropenem (0.25 µg/ml) and rifampin (500 µg/ml). Plasmid DNAs of *E. coli* transconjugants and transformants were obtained using the AxyPrep Plasmid Miniprep kit (Axygen Scientific, California) and were separated by electrophoresis.

PCR amplification of drug resistance genes. Plasmid DNA from *E. coli* isolates and *E. coli* transconjugants and transformants were used as templates in PCR amplification. The most common carbapenemase genes in the *Enterobacteriaceae*, *bla*_{KPC} (21) and *bla*_{IMP} (22), the broad-spectrum and extended-spectrum β-lactamase genes *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} (23), and plasmid-mediated AmpC enzymes genes (24) were amplified using previously described conditions. The reaction was conducted in a Tpersonal thermal cycler (Whatman Biometra, Germany). PCR amplification products were purified and directly sequenced using an ABI3730 sequencer (Applied Biosystems, California). DNA sequences were compared with the reported nucleotide sequences from the GenBank database using the BLASTN software program (<http://www.ncbi.nlm.nih.gov>).

Analysis of OMPs. Outer membrane proteins (OMPs) of *E. coli* were extracted as described by Hernandez-Alles et al. (25). Strains were grown in Mueller-Hinton broth overnight at 37°C with shaking. Electrophoretic analysis of OMPs by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 11.6% acrylamide–0.4% bisacrylamide–0.1% SDS gels. The 0.75-mm-thickness minigel was run at a constant current of 20 mA with a Mini Protein 3 slab electrophoresis cell (Bio-Rad, California). The gel was visualized by staining with Coomassie brilliant blue. PCR amplification and DNA analysis of the *ompF* and *ompC* genes were performed (26).

PFGE analysis. Twenty-two carbapenem-nonsusceptible *E. coli* were genotyped by pulsed-field gel electrophoresis (PFGE) following the PulseNet protocol from the website of the U.S. Centers for Disease Control and Prevention (<http://www.cdc.gov/pulsenet/pathogens/index.html>). The bacterial cells were digested with the XbaI restriction enzyme and were separated in a Rotaphor System 6.0 instrument (Whatman Biometra). The strain of *Salmonella enterica* serotype Braenderup (H9812) restricted with XbaI was used as a molecular weight standard. The restriction patterns of the genomic DNA from the isolates were analyzed and interpreted according to the criteria of Tenover et al. (27). PFGE band patterns of 22 isolates were analyzed using the UVIBand software program (UVItect Ltd., Cambridge, United Kingdom), and homology was calculated (unweighted pair group method using average linkages [UP-GMA], 0.5% Master+Lane).

MLST and phylogenetic typing. Multilocus sequence typing (MLST) of 22 carbapenem-nonsusceptible *E. coli* isolates was performed according to the protocol of Wirth et al. (28). Allelic profiling and sequence type (ST) determination were performed using the *E. coli* MLST scheme from the website of the University College Cork (<http://mlst.ucc.ie/mlst/dbs/Ecoli>). Phylogenetic groups were determined by a triplex PCR described by Clermont et al. (29).

Sequence analysis of genetic structures surrounding the *bla*_{KPC-2} gene. Previous study demonstrated that the restriction patterns of the *bla*_{KPC-2}-encoding plasmid pE1 from *E. coli* E1 was identical to those of *Serratia marcescens* and *Klebsiella pneumoniae* isolates in our hospital (9). This representative plasmid pE1 was selected for sequencing by primer walking. The putative open reading frames (ORFs) were identified using the FramePlot 4.0beta program (<http://nocardia.nih.go.jp/fp4/>). DNA sequences and putative amino acid sequences were compared with the reported data in the GenBank database online using the BLAST program available at <http://www.ncbi.nlm.nih.gov/BLAST/>. Five pairs of primers were designed (see Table 3) based on the known *bla*_{KPC}-surrounding sequences of plasmid pE1 to amplify the upstream and downstream regions of the *bla*_{KPC} gene, respectively.

RESULTS

Antimicrobial susceptibility. Twenty-two *E. coli* isolates exhibited various levels of carbapenem resistance. All isolates were resistant to ertapenem, with a MIC range of 2 to 64 µg/ml, and were resistant or intermediately resistant to imipenem and meropenem, with a MIC range of 1 to 16 µg/ml. Only 3 isolates were susceptible to ciprofloxacin, and 4 were resistant to amikacin (Table 1). *E. coli* E6 and E21 showed high-level carbapenem resistance compared with the others.

Transfer of carbapenem resistance and production of β-lactamases. Resistance of carbapenems was transferred from *E. coli* isolates to the *E. coli* EC600 standard recipient by conjugation except for strains E4, E6, and E8. Plasmid DNA extracted from these 3 isolates was transformed into competent *E. coli* EC600 by heat shock. All the *E. coli* transconjugants and transformants showed a significant increase in carbapenem MICs, and their β-lactam susceptibility profiles were similar to those of the donor strains. Six *E. coli* transconjugants and transformants remained resistant or intermediately resistant to ciprofloxacin, with MICs of 3 or 4 µg/ml, and 3 remained resistant to amikacin at a high level, suggesting the presence of plasmid-mediated quinolone and aminoglycoside resistance determinants. All *E. coli* isolates contained a plasmid with a size of approximately 60 kb, and most isolates had several extra, small plasmids. Isolates E1 and E3 and isolates E15 and E17 showed identical plasmid profiles, respectively. All *E. coli* transconjugants and transformants acquired the 60-kb plasmid (Fig. 1).

All *E. coli* isolates produced KPC-2, and most of them coproduced a variety of β-lactamases. Twenty-two CTX-M-type ESBLs were found in 20 isolates. The most prevalent CTX-M genotype was CTX-M-14, followed by CTX-M-15. Coproduction of SHV-type ESBL and plasmid-mediated AmpC enzymes was rather uncommon, with one SHV-12, two DHA-1, and one CMY-2 enzyme. In addition, 14 isolates produced TEM-1. All *E. coli* transconjugants and transformants produced KPC-2, and some produced additional ESBLs and/or AmpC enzymes (Table 2). No *bla*_{IMP} metallo-β-lactamase gene was detected.

Analysis of OMPs. *E. coli* E6 and E21 had MICs of carbapenems significantly higher than those of their *E. coli* transconjugants and other *E. coli* isolates. Since reduced permeability, which confers carbapenem resistance in the *Enterobacteriaceae*, may be involved, OMPs were examined. *E. coli* ATCC 25922, E1, E13, and E6 expressed two major porins, with molecular masses of approximately 38 kDa and 40 kDa, whereas E21 lacked the 40-kDa porin (Fig. 2). PCR amplification and DNA sequences of both porin genes were normal except that the *ompC* gene was not amplified in E21, which may be due to the presence of an insertion sequence in the gene. These results suggested that *E. coli* E21 did not express the OmpC porin.

Molecular typing of KPC-2-producing *E. coli*. PFGE indicated different restriction fragment length polymorphism (RFLP) patterns among the 22 *E. coli* isolates instead of monoclonality, except for 5 pairs of strains (E1 and E3, E7 and E9, E11 and E12, E13 and E18, and E15 and E17) (Fig. 3A), which showed high genetic homology (>70%) (Fig. 3B). This was confirmed by MLST as well as phylogenetic analysis. MLST indicated that ST131 was the dominant strain (9/22; 40.9%), followed by ST648 (5/22; 22.7%), ST405 (2 isolates), and ST38 (2 isolates). Four single STs, ST69, ST2003, ST2179, and ST744, were identified. Phylogenetic analysis indi-

TABLE 1 Susceptibility results for *E. coli* isolates and *E. coli* transconjugants and transformants

Isolate(s) ^a	MIC(s) (μg/ml) of antimicrobial agent for <i>E. coli</i> isolate and <i>E. coli</i> transconjugant or transformant ^b									
	IPM	MEM	ETP	CAZ	CTX	FEP	CFS	FOX	CIP	AK
E1/TE1	2/2	2/2	8/4	64/16	256/8	64/8	64/32	32/16	>32/0.19	4/1
E2/TE2	2/2	4/1	16/2	32/16	256/8	64/4	128/16	64/16	>32/0.125	12/0.75
E3/TE3	2/2	2/2	8/4	64/16	256/8	64/8	64/32	32/16	>32/0.125	4/1
E4/TE4	4/4	4/8	8/16	32/64	32/64	32/64	64/128	64/64	0.25/0.19	1.5/0.75
E5/TE5	4/8	8/8	16/32	32/128	128/64	64/64	128/128	64/64	>32/0.19	1.5/1.0
E6/TE6	16/8	16/16	64/32	128/64	>256/64	128/32	>256/128	>256/64	>32/4	>256/0.75
E7/TE7	2/2	2/2	8/8	64/64	>256/>256	128/64	64/64	32/32	16/4	12/8
E8/TE8	2/4	4/4	16/8	256/128	>256/32	256/16	64/32	>256/128	>32/0.25	1.5/0.75
E9/TE9	4/4	4/4	4/8	32/32	128/128	128/64	64/64	16/32	8/4	12/6
E10/TE10	2/2	2/2	4/4	128/128	>256/>256	128/64	64/64	32/32	>32/0.19	1.5/0.75
E11/TE11	2/1	2/1	4/2	8/32	128/64	32/16	32/32	256/64	>32/0.75	>256/>256
E12/TE12	2/1	2/1	2/2	8/32	128/64	32/16	32/32	256/64	>32/0.75	>256/>256
E13/TE13	4/8	8/8	16/16	128/64	>256/64	256/32	256/256	32/64	>32/0.19	4/0.75
E14/TE14	2/4	2/4	2/8	64/32	>256/32	128/32	128/64	32/32	32/4	2/1.0
E15/TE15	2/2	2/2	8/4	16/16	64/16	32/8	64/32	16/16	>32/0.19	3/0.75
E16/TE16	4/4	4/4	4/16	16/64	128/32	32/32	64/64	16/32	8/3	3/0.75
E17/TE17	2/2	2/2	8/8	16/16	64/16	32/8	64/32	16/16	>32/0.25	2/0.75
E18/TE18	2/4	1/4	2/8	16/64	128/32	8/32	32/64	16/32	>32/3	6/1.0
E19/TE19	2/4	2/4	4/8	64/64	64/32	16/32	64/64	16/32	2/0.25	2/0.75
E20/TE20	2/2	2/2	2/4	16/16	>256/32	16/16	64/32	16/16	>32/0.19	>256/>256
E21/TE21	16/2	16/2	32/2	32/64	32/32	64/32	128/64	128/32	1.0/0.19	2/1.0
E22/TE22	2/2	4/2	16/4	256/256	>256/32	128/16	128/64	128/16	>32/0.19	1.5/1.5
EC600	≤0.125	≤0.125	≤0.125	0.25	≤0.125	≤0.125	≤0.125	4	0.19	1

^a E1 to E22, 22 isolates of KPC-2-producing *E. coli*; TE1 to TE22, 22 *E. coli* transconjugants or transformants of KPC-2-producing *E. coli*; EC600, *E. coli* EC600.
^b IPM, imipenem; MEM, meropenem; ETP, ertapenem; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; CFS, cefoperazone/sulbactam; FOX, ceftiofur; CIP, ciprofloxacin; AK, amikacin. MICs of ampicillin and piperacillin for all *E. coli* isolates and the *E. coli* transconjugants and transformants were ≥256 μg/ml. MICs of piperacillin-tazobactam and aztreonam were ≥128 μg/ml, except for strains of E11, TE11, E12, and TE12 (MICs of 64 to 128 μg/ml).

cated that 9 group B2 isolates belonged to ST131 and that 5 of 11 group D isolates were ST648. Only one isolate each was identified as group B1 and group A, respectively (Table 2). A Dendrogram of PFGE patterns showed that 3 of 4 *E. coli* ST131-B2 isolates from Hangzhou Traditional Chinese Medicine Hospital were clustered

together on one branch, and 4 of 5 *E. coli* ST131-B2 isolates from 2nd Affiliated Hospital of Zhejiang University and one from Hangzhou Traditional Chinese Medicine Hospital clustered on another branch (Fig. 3B).
Genetic structures surrounding the *bla*_{KPC-2} gene. A 7,788-bp

FIG 1 Plasmid profiles of *E. coli* isolates and *E. coli* transconjugants. E1 to E22, 22 *E. coli* isolates; TE1 to TE22, transconjugants of *E. coli* isolates; *E. coli* V517 was used as molecular size reference.

TABLE 2 β -Lactamases production and molecular typing of *E. coli*

Isolate	β -Lactamase(s) produced		Molecular typing result			PCR amplification of <i>bla</i> _{KPC-2} ⁺ surrounding sequences				
	Clinical isolate	<i>E. coli</i> transconjugant or transformant	PFGE	MLST	Phylogenetic group	P1	P2	P3	P4	P5
E1	KPC-2, CTX-M-2212;15	KPC-2	A	ST648	D	+	+	+	+	+
E2	KPC-2, TEM-1, CTX-M-14	KPC-2	B	ST405	D	+	+	+	+	+
E3	KPC-2, CTX-M-15	KPC-2	A	ST648	D	+	+	+	+	+
E4	KPC-2, TEM-1	KPC-2	C	ST69	D	+	+	+	+	+
E5	KPC-2, CTX-M-14	KPC-2	D	ST648	D	+	+	+	+	+
E6	KPC-2, CTX-M-55	KPC-2	E	ST2003	D	+	+	+	+	+
E7	KPC-2, TEM-1, CTX-M-3	KPC-2, TEM-1, CTX-M-3	F1	ST131	B2	–	+	+	+	–
E8	KPC-2, CTX-M-55, CMY-2	KPC-2, CMY-2	G	ST405	D	–	+	+	+	+
E9	KPC-2, TEM-1, CTX-M-3, CTX-M-14	KPC-2, TEM-1, CTX-M-3	F2	ST131	B2	–	+	+	–	–
E10	KPC-2, TEM-1, CTX-M-15	KPC-2, CTX-M-15	H	ST131	B2	–	–	+	+	+
E11	KPC-2, TEM-1, CTX-M-14, DHA-1	KPC-2, CTX-M-14, DHA-1	I1	ST131	B2	–	+	+	+	+
E12	KPC-2, TEM-1, CTX-M-14, DHA-1	KPC-2, CTX-M-14, DHA-1	I2	ST131	B2	–	–	+	+	+
E13	KPC-2, CTX-M-15	KPC-2	J1	ST648	D	–	+	+	+	+
E14	KPC-2, TEM-1, CTX-M-3, CTX-M-14	KPC-2	K	ST38	D	+	+	+	+	+
E15	KPC-2, TEM-1, CTX-M-14	KPC-2	L1	ST131	B2	+	+	+	+	+
E16	KPC-2, TEM-1, CTX-M-14	KPC-2, TEM-1	M	ST131	B2	+	+	+	+	+
E17	KPC-2, TEM-1, CTX-M-14	KPC-2	L2	ST131	B2	+	+	+	+	+
E18	KPC-2, CTX-M-15	KPC-2	J2	ST648	D	+	+	+	+	+
E19	KPC-2, TEM-1, CTX-M-65	KPC-2, TEM-1	N	ST2179	B1	–	+	+	+	+
E20	KPC-2, TEM-1, CTX-M-14	KPC-2, TEM-1, CTX-M-14	O	ST131	B2	–	+	+	+	+
E21	KPC-2, TEM-1	KPC-2	P	ST744	A	+	+	+	+	+
E22	KPC-2, SHV-12, CTX-M-14	KPC-2, SHV-12	Q	ST38	D	+	+	+	+	+

DNA fragment containing the *bla*_{KPC-2} gene of plasmid pE1 was analyzed. This DNA fragment consisted of 7 genes, those encoding a Tn3 transposase, a hypothetical protein with 110 amino acids, a Tn3 resolvase, and an ISKpn8 transposase, the *bla*_{KPC-2} gene, and genes encoding an ISKpn6-like transposase and a transcriptional repressor protein, KorC (Fig. 4 and Table 3). The sequence of the 7,788-bp DNA fragment was identical to the partial sequence of plasmid pKP048 (20,518 bp; GenBank accession number FJ628167), which contained an integration structure of a Tn3-based transposon and a partial Tn4401 segment (30). The plasmid pKP048 was extracted from KPC-2-producing *K. pneumoniae* KP048 isolated from another hospital in Hangzhou in 2006. Series PCR amplification showed that fragments containing the ISKpn8 transposase, *bla*_{KPC-2}, and the ISKpn6-like transposase were de-

tected in 18 of 22 *E. coli* isolates, and 13 isolates were consistent with the genetic environment in pE1 (Table 2).

DISCUSSION

E. coli was the most common pathogen among clinical isolates of the *Enterobacteriaceae* (>25%) in China. According to CHINET data published in Chinese from 2010 to 2012, a high prevalence of *E. coli* (>50%) consisted of ESBL-producing strains; however, resistance to carbapenems was rare (1–3). During a 5-year period, 22 KPC-2-producing *E. coli* strains were isolated in Hangzhou, where KPC had been identified for multiple pathogens. More than half of them (12/22) were isolated in 2011, suggesting an increasing trend of carbapenem-resistant *E. coli* in our region. As in Hangzhou, KPC-producing *E. coli* have emerged in other regions, such as New York (31, 32), Israel (33, 34), and Greece (19), where the prevalence of KPC-positive *K. pneumoniae* is high. Although KPC-producing *K. pneumoniae* in these regions has been shown to be clonal, few KPC-producing *E. coli* isolates were genetically related by PFGE, excepting the 14 *E. coli* ST410 isolates from Larissa (19). ESBLs were frequently identified in KPC-producing *E. coli*, especially in isolates of our study (91% were CTX-M-type producers). A review of clinical characteristics showed that older age of patient, ICU admission, and prior exposure to carbapenems may be important risks for infection or colonization with KPC-2-producing *E. coli*. The high-level carbapenem resistance in *E. coli* E21 can be explained by the lack of the OmpC porin. The MICs of carbapenems for *E. coli* transconjugant TE6 were much higher than those for most other *E. coli* transconjugants and similar to that for E6. It is presumed that the high-level carbapenem resistance may be related to high-level expression of the *bla*_{KPC} gene in the plasmid from E6 or other unknown mechanisms.

FIG 2 SDS-PAGE analysis of outer membrane proteins extracted from *E. coli* ATCC 25922, E1, E6, E13, and E21.

FIG 3 (A) PFGE patterns of chromosomal DNA restriction fragments from 22 *E. coli* isolates. *Salmonella* serotype *Braenderup* H9812 restricted with *Xba*I was used as molecular size standard. (B) Dendrogram of PFGE patterns. ZJUH, 2nd Affiliated Hospital of Zhejiang University; HZTCMH, Hangzhou Traditional Chinese Medicine Hospital; ZJPPH, Zhejiang Provincial People's Hospital.

FIG 4 Schematic representation of the genetic structure surrounding the *bla*_{KPC-2} gene on plasmid pE1 from *E. coli* E1. Genes and their corresponding transcription orientations are indicated by arrows with various types of shading. Triangles represent the inverted repeats of the respective mobile elements, and target site duplications are presented as nucleotide letters. Short arrowheads show the primers (listed in Table 3) used for PCR amplification of the *bla*_{KPC-2}-surrounding sequences from other KPC-2-producing *E. coli* strains.

ST131, detected in 40.9% KPC-2-producing *E. coli* isolates in our study, was the international epidemic clone which caused community-associated infections (15). *E. coli* ST131 produced a variety of β -lactamases, most frequently the CTX-M-type ESBLs and less frequently the TEM, SHV, and AmpC enzymes (15, 35, 36) and recently KPC (16–18). Our study found for the first time the emergence of KPC-2-producing *E. coli* ST131 in China, including both clonal spread and independent isolates. ST131 isolates possessing KPC have been identified in France (16), Ireland (17), the United States (18), and China, suggesting that this ST may contribute to the spread of the *bla*_{KPC} gene. On the other hand, KPC probably enhanced the spreading potential of the ST131 clone. Besides *E. coli* ST131-B2, ST648-D was another common clone (5 isolates). Detection of a CTX-M-type ESBL and NDM- and KPC-type carbapenemases in *E. coli* ST648-D has been reported (18, 37, 38).

The *bla*_{KPC-2} gene was located on a Tn3-based transposon in pE1, a plasmid disseminating among different enterobacterial species in our hospital (9). The presence of the same or similar genetic structure in most carbapenem-resistant *E. coli* isolates suggested that the *bla*_{KPC-2} gene was carried by a transposon-associated element and is widely spread among many enterobacterial species in eastern China.

At least half of KPC-2-producing *E. coli* isolates were intermediately resistant to imipenem and meropenem, with a MIC of 2 μ g/ml. This means that such strains might be easily neglected by standard antimicrobial susceptibility testing in a clinical microbiology laboratory, and the plasmids might be spread widely before attention is paid and preventative actions are taken. Among 9 KPC-2-producing *E. coli* ST131-B2 strains, one was isolated in 2010 and 8 were isolated in 2011. Acquisition of the KPC-encoding plasmid by ST131 and the rapid emergence of this clone in

China are worrisome and should be carefully monitored and controlled.

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TABLE 3 Primers used for PCR amplification of *bla*_{KPC-2}-surrounding sequences

Primer	Sequence (5'–3')	Position (bp)	Product length (bp)
P1F	TCCAGAAGCCTTCCAGCAC	800–820	2,683
P1R	AACCGAACCAGCAGACCCCTGA	3462–3482	
P2F	ACAAGGCATCCGGCAGTTCAA	3314–3334	1,410
P2R	CGCAGCGTCGATTCCAGTTC	4704–4723	
P3F	GTGGAGCTGCGCAAGACGTT	4101–4120	1,793
P3R	CCAGACGACGCATAGTCAT	5874–5893	
P4F	TGTAAGTTACCGCGCTGAGG	5311–5330	1,337
P4R	AGCAGTTCAGGCCAACACCG	6628–6647	
P5F	ACTCGGCTTGACCTTGTTCCA	6325–6345	1,206
P5R	GTTCGCTGAAGATTGGCAGGC	7510–7530	

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