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Emergence of *Serratia marcescens*, *Klebsiella pneumoniae*, and *Escherichia coli* Isolates Possessing the Plasmid-Mediated Carbapenem-Hydrolyzing β-Lactamase KPC-2 in Intensive Care Units of a Chinese Hospital▽

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Twenty-one *Serratia marcescens*, ten *Klebsiella pneumoniae*, and one *Escherichia coli* isolate with carbapenem resistance or reduced carbapenem susceptibility were recovered from intensive care units (ICUs) in our hospital. Enterobacterial repetitive intergenic consensus-PCR and pulsed-field gel electrophoresis demonstrated that all the *S. marcescens* isolates belonged to a clonal strain and the 10 *K. pneumoniae* isolates were indistinguishable or closely related to each other. The MICs of imipenem, meropenem, and ertapenem for all isolates were 2 to 8 μg/ml, except for *K. pneumoniae* K10 (MICs of 128, 256, and >256 μg/ml). Isoelectric focusing, PCRs, and DNA sequencing indicated that all *S. marcescens* isolates produced KPC-2 and a β-lactamase with a pI of 6.5. All *K. pneumoniae* isolates produced TEM-1, KPC-2, CTX-M-14, and a β-lactamase with a pI of 7.3. The *E. coli* E1 isolate produced KPC-2, CTX-M-15, and a β-lactamase with a pI of 7.3. Conjugation studies with *E. coli* (EC600) resulted in the transfer of reduced carbapenem susceptibility compared to that of the original isolates, and only the bla_{KPC-2} gene was detected in *E. coli* transconjugants. Plasmid restriction analysis showed identical restriction patterns among all *E. coli* transconjugants. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and *ompK35*/*36* gene sequence analysis of outer membrane proteins revealed that *K. pneumoniae* K10 failed to express OmpK36, because of insertional inactivation by an insertion sequence IS*Ecp1*. All these results indicate that KPC-2-producing *S. marcescens*, *K. pneumoniae*, and *E. coli* isolates emerged in ICUs in our hospital. KPC-2 combined with porin deficiency results in high-level carbapenem resistance in *K. pneumoniae*. The same bla_{KPC-2}-encoding plasmid was spread among the three different genera.

Increased emergence of *Enterobacteriaceae* possessing extended-spectrum β-lactamases (ESBLs) has been accompanied by the widespread use of cephalosporins. Carbapenems are considered to be one of the few therapies for serious infections caused by multidrug-resistant, gram-negative bacteria, especially strains producing high levels of AmpC cephalosporinases or ESBLs. Over the past 2 decades, while carbapenem resistance has become a serious problem for the non-lactose-fermenting bacteria, it has remained uncommon in *Enterobacteriaceae*. Recently however, identification of carbapenem-resistant *Enterobacteriaceae* is increasing. Carbapenem resistance in *Enterobacteriaceae* usually requires, in addition to production of β-lactamases that are capable of hydrolyzing carbapenems, other mechanisms, particularly porin loss.

Carbapenem-hydrolyzing KPC β-lactamases are a group of recently identified carbapenemases which belong to Bush group 2f, molecular class A. KPCs are capable of hydrolyzing carbapenems, penicillins, cephalosporins, and aztreonam, and they are inhibited by clavulanic acid and tazobactam. The initial report of one of these β-lactamases, KPC-1, was from a carbapenem-resistant *Klebsiella pneumoniae* strain isolated in North Carolina (27). KPC-2 was then found in isolates of *K. pneumoniae* (17), *Salmonella enterica* (16), *Klebsiella oxytoca* (28), and an *Enterobacter* sp. (11). Soon afterward, KPC-3 was found in *K. pneumoniae* (26) and *Enterobacter cloacae* isolates from New York (2). Recently, KPCs were found in France (18), South America (23), and Israel (19) outside the United States, and KPC-2 was identified for the first time in *Pseudomonas aeruginosa* isolates outside of the family *Enterobacteriaceae* (24). KPC-2 has emerged in China. A *K. pneumoniae* isolate from Hangzhou city producing KPC-2 has been reported (25). Almost simultaneously, we identified KPC-2 in three *Serratia marcescens* isolates from the same city but a different hospital (30). Most KPC enzymes occurred sporadically. However, it is worth noting that KPC-producing *Klebsiella* species are predominant in some facilities in New York (1, 3, 26).

In the present report, we describe the emergence of KPC-2-producing *Enterobacteriaceae* including *S. marcescens*, *K. pneumoniae*, and *Escherichia coli* in intensive care units (ICUs) in our hospital.

MATERIALS AND METHODS

Bacterial strains. Thirty-two isolates of *S. marcescens* (21 isolates, strains S1 to S21), *K. pneumoniae* (10 isolates, strains K1 to K10), and *E. coli* (1 isolate, strain E1) with carbapenem resistance or reduced carbapenem susceptibility were recovered from ICUs in the 2nd Affiliated Hospital of Zhejiang University from April 2006 to February 2007. *K. pneumoniae* K8 and K9 were recovered from the neurology intensive care unit (NICU), and others were recovered from the

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TABLE 1. Antimicrobial susceptibility patterns of *S. marcescens*, *K. pneumoniae*, and *E. coli* isolates and their *E. coli* transconjugants

Antimicrobial agent(s)	MIC (μg/ml)					
	S. marcescens S1 to S21	*K. pneumoniae*		*E. coli* E1	*E. coli* EC600	*E. coli* transconjugants
		K1 to K9	K10			
Imipenem	4–8	2–4	128	2	≤0.125	1–2
Meropenem	2–4	4–8	256	2	≤0.125	2
Ertapenem	4–8	8	>256	8	≤0.125	4
Ampicillin	>256	>256	>256	>256	8	>256
Piperacillin	256	>256	>256	>256	1	256
Piperacillin-tazobactam	128–256	256–>256	>256	256	1	256
Ceftriaxone	32	256	>256	>256	≤0.125	32
Ceftazidime	8–16	64–128	256	64	0.25	16–32
Cefotaxime	16–32	128	256	256	≤0.125	8
Cefepime	8–16	32–64	256	64	≤0.125	8
Cefoperazone-sulbactam	32–64	128–256	>256	128	≤0.125	32
Cefoxitin	16–32	8–16	256	32	8	16
Aztreonam	128–256	256	>256	256	≤0.125	128
Ciprofloxacin	≤0.125	16–>32	>32	>32	≤0.125	≤0.125
Gentamicin	0.5–1	>256	>256	64	0.25	0.25

surgical intensive care unit (SICU). These isolates were recovered from various kinds of specimens, including sputum (all *S. marcescens* isolates, *K. pneumoniae* K1 to K6, and *E. coli* E1), wound swabs (*K. pneumoniae* K7), blood (*K. pneumoniae* K8 and K9), and central vein pipe sample (*K. pneumoniae* K10). Species identification was performed with a Vitek system (bioMérieux, Hazelwood, MO). Most patients were treated with piperacillin-tazobactam (or cefoperazone/sulbactam) and levofloxacin, and about half of the patients received therapy with carbapenems before the organism was isolated. The isolates were obtained 3 to 14 days after the initial administration of the antibiotics.

Antimicrobial susceptibility testing. The MICs of 15 antibiotics were determined using the agar dilution method according to CLSI recommendations (7). Antimicrobial agents were obtained from Sigma (St. Louis, MO) (ampicillin, piperacillin, cefotaxime, and gentamicin) and the pharmaceutical companies Merck & Co., Inc. (imipenem, ertapenem, and cefoxitin), Dainippon Sumitomo Pharma Co., Ltd. (meropenem), Wyeth Holdings Co. (piperacillin-tazobactam), Pfizer (cefoperazone/sulbactam), GlaxoSmithKline (ceftazidime), Roche (ceftriaxone), Bristol-Meyers Squibb (aztreonam, cefepime), and Bayer (ciprofloxacin). *E. coli* ATCC 25922 was used for quality control.

PFGE typing. Pulsed-field gel electrophoresis (PFGE) typing of *K. pneumoniae* isolates was performed according to the procedure described by PulseNet from the website of the Centers for Disease Control and Prevention (http://www.cdc.gov/pulsenet/protocols.htm) in a Rotaphor System 6.0 instrument (Whatman Biometra, Goettingen, Germany). The XbaI restriction patterns of the isolates' genomic DNAs were analyzed and interpreted according to the criteria of Tenover et al. (21).

ERIC-PCR typing. Total DNAs of *S. marcescens* isolates were obtained with an Axyprep bacterial genomic DNA miniprep kit (Axygen Scientific, Union City, CA) and were used as templates in enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) analysis. The sequences of the primers and PCR conditions were as described by Versalovic et al. (22).

Conjugal transfer experiment and analysis of plasmid. The conjugation experiment was carried out in mixed broth cultures. Rifampin-resistant *E. coli* EC600 ($LacZ^-$ Nal^r Rif^r) was used as the recipient strain. Overnight cultures of the donor strain (200 μl) and recipient strain (100 μl) were mixed with 600 μl of fresh Mueller-Hinton broth and were incubated for 24 h at 35°C. Then, the mixture was inoculated on Mueller-Hinton agar plates containing rifampin (Sigma; 700 mg/liter) plus imipenem (0.5 mg/liter) for 24 h at 35°C. The colonies that grew on the selecting medium were picked up and identified by the Vitek system.

Plasmid DNAs were obtained with an Axyprep plasmid miniprep kit (Axygen Scientific) and were digested by various endonucleases, including EcoRI, HindIII, and BcuI (MBI Fermentas, Lithuania). The plasmid DNA and digestion products were separated by electrophoresis at 0.6% agarose gel at a constant voltage of 100 V for 4 h. The PCR-based replicon typing of the bla_{KPC-2}-encoding plasmids was conducted following the method described by Carattoli et al. (5).

IEF of β-lactamases. The crude β-lactamase extracts of original isolates and their *E. coli* transconjugants were prepared by ultrasonic treatment of bacterial cells. Isoelectric focusing (IEF) was performed on PhastGel polyacrylamide gel (pH, 3 to 9; Amersham Biosciences, Uppsala, Sweden) using a PhastSystem instrument (Pharmacia Biotech, Uppsala, Sweden) according to the method of Mathew et al. (15). β-Lactamase activity was visualized by staining the gel with Nitrocefin (Oxoid, Basingstoke, Hampshire, England). The isoelectric points (pIs) were determined after comparison to known β-lactamases TEM-1 (pI of 5.4), TEM-28 (pI of 6.1), SHV-7 (pI of 7.6), and ACT-1 (pI of 9.0).

PCR amplification and DNA sequence analysis of *bla* genes. Plasmid DNAs from original isolates and their *E. coli* transconjugants were used as templates in PCR amplification. The primers used to amplify bla_{KPC}, bla_{TEM}, bla_{SHV}, and bla_{CTX-M} were as described previously (27, 29). The reaction was conducted in a GeneAmp PCR System 9600 thermal cycler (Applied Biosystems, Foster City, CA). The PCR products were cloned into a pGEM-T Easy vector (Promega), and recombinant plasmids were transformed into *E. coli* DH5α as described previously (6). Inserts were sequenced using an ABI3730 sequencer (Applied Biosystems), and the sequences were compared with the reported sequences from GenBank.

Analysis of OMPs. Outer membrane proteins (OMPs) were isolated as described by Hernandez-Alles et al. (9). 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 thick minigel was run at a constant current of 20 mA for 85 min with a Mini Protein 3 slab electrophoresis cell (Bio-Rad, Richmond, CA).

The *ompK35* and *ompK36* genes of *K. pneumoniae* K1 and K10 were amplified by using PCR (13). The products were sequenced, and the sequences were compared with the reported sequences from GenBank.

RESULTS

Antimicrobial susceptibility. Thirty-two clinical isolates of *S. marcescens*, *K. pneumoniae*, and *E. coli* exhibited resistance or reduced susceptibility to imipenem, meropenem, and ertapenem. The MICs of the three drugs for all isolates were 2 to 8 μg/ml, except *K. pneumoniae* K10, for which the MICs were 128, 256, and >256 μg/ml. All *S. marcescens* isolates had similar antimicrobial susceptibility patterns and were resistant or intermediately resistant to penicillins, cephalosporins, cefoxitin, and aztreonam, but they were susceptible to quinolones and aminoglycosides. The *K. pneumoniae* and *E. coli* isolates were highly resistant to all antibiotics listed in Table 1 except cefoxitin.

PFGE and ERIC-PCR typing. PFGE patterns of the XbaI DNA digests of 10 *K. pneumoniae* isolates are shown in Fig. 1. One major PFGE type with three subtypes was identified. *K. pneumoniae* K1 to K7 belonged to a dominant subtype. *K.*

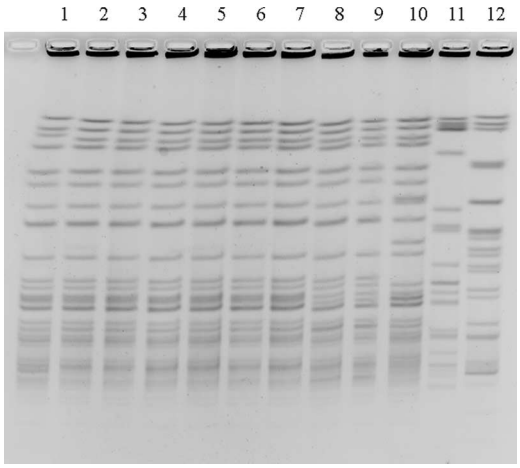

FIG. 1. PFGE patterns of chromosomal DNA restriction fragments from *K. pneumoniae* isolates. Lanes 1 to 10, *K. pneumoniae* K1 to K10; lanes 11 and 12, *K. pneumoniae* control strains that did not produce KPC-2.

pneumoniae K8 and K9, isolated from an SICU rather than an NICU, belonged to the second subtype. High-level carbapenem-resistant *K. pneumoniae* K10 belonged to a single subtype. Two to three band differences were observed among the three subtypes. According to the criteria of Tenover et al. (21), *K. pneumoniae* K1 to K7 were indistinguishable and were considered to represent the same strain, and strains K8, K9, and K10 were considered to be closely related to the predominant strain.

Similar PFGE analyses were also performed for the *S. marcescens* isolates several times but were unsuccessful due to the degradation of the chromosomal DNA. Therefore, we used the ERIC-PCR to analyze their molecular similarity. These isolates which were isolated from the same ward (NICU) were presumed to be clonally related (data not shown).

Transfer of carbapenem resistance and plasmid analysis. Transfer of β-lactam resistance from *S. marcescens*, *K. pneumoniae*, and *E. coli* isolates to *E. coli* EC600 by conjugation was successful. All the *E. coli* transconjugants exhibited significantly reduced carbapenem susceptibility with imipenem and meropenem MICs of ~1 to 2 μg/ml and an ertapenem MIC of 4 μg/ml. They were also resistant to penicillins and aztreonam and were resistant or intermediately resistant to cephalosporins and cefoxitin, but they were susceptible to quinolones and aminoglycosides (Table 1).

K. pneumoniae isolates had identical plasmid profiles, except for that of strain K10. *S. marcescens* isolates and all *E. coli* transconjugants acquired a plasmid with a size of approximately 50 kb similar to the largest plasmid of *E. coli* V517 (data not shown). Plasmid DNA restriction analysis using EcoRI, HindIII, and BcuI showed identical restriction patterns among the representative *E. coli* transconjugants (Fig. 2). PCR-based replicon typing indicated that all bla_{KPC-2}-encoding plasmids were negative for all the replicons tested, while the HI1, A/C, and FIIAs replicons were detected in total plasmid from *K. pneumoniae* K1 (data not shown). These results suggested that the carbapenem resistance-related plasmids existing in clinical isolates of *S. marcescens*, *K. pneumoniae*, and *E. coli* were closely related.

IEF analysis. The IEF results showed that *S. marcescens* isolates produced two β-lactamases with apparent pIs of 6.5 and 6.7. The pI 6.5 β-lactamase was presumed to be either the degradation product or the precursor of the pI 6.7 β-lactamase (30). IEF analysis also revealed four β-lactamases with pIs of 5.4, 6.7, 7.3, and 7.9 in *K. pneumoniae* isolates and three β-lactamases with pIs of 6.7, 7.3, and 9.0 in the *E. coli* isolate. *E. coli* transconjugants produced a single β-lactamase with a pI of 6.7 (Fig. 3).

PCRs and DNA sequence analysis. IEF results suggested the production of β-lactamases of TEM (pI 5.4), KPC (pI 6.7), and other ESBLs. PCRs and DNA sequence analysis were performed to confirm the presence of these β-lactamase genes. bla_{KPC-2} was identified in *S. marcescens* isolates and all *E. coli* transconjugants by comparing their DNA sequences with the known genes existing in GenBank. bla_{KPC-2}, bla_{TEM-1} (pI 5.4), and $bla_{CTX-M-14}$ (pI 7.9) were identified in *K. pneumoniae* isolates. bla_{KPC-2} and $bla_{CTX-M-15}$ (pI 9.0) were identified in *E. coli* E1. The pI 7.3 β-lactamase that was detected by IEF of *K. pneumoniae* and *E. coli* isolates remained unclear.

Analysis of OMPs. The MICs of carbapenems for *K. pneumoniae* K10 were significantly higher than those of other *K.*

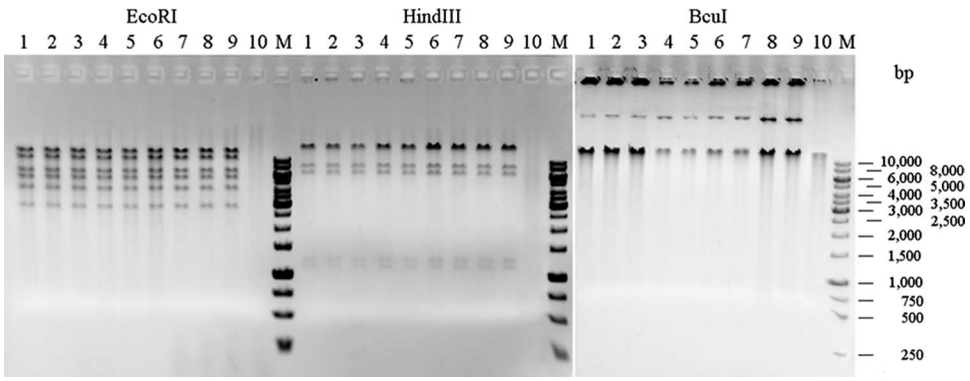

FIG. 2. Restriction patterns of plasmid DNA from *E. coli* transconjugants of *E. coli*, partial *K. pneumoniae* and *S. marcescens* isolates, and partial original *S. marcescens* isolates. Lane 1, *E. coli* transconjugant of *E. coli* E1; lanes 2 to 4, *E. coli* transconjugants of *K. pneumoniae* K1, K8, and K10; lanes 5 to 7, *E. coli* transconjugants of *S. marcescens* S1, S10, and S20; lanes 8 and 9, original *S. marcescens* S1 and S10; lane 10, *E. coli* EC600 as a negative control; M, 1-kb DNA ladder (MBI Fermentas). Plasmid DNA was digested with EcoRI, HindIII, and BcuI endonucleases.

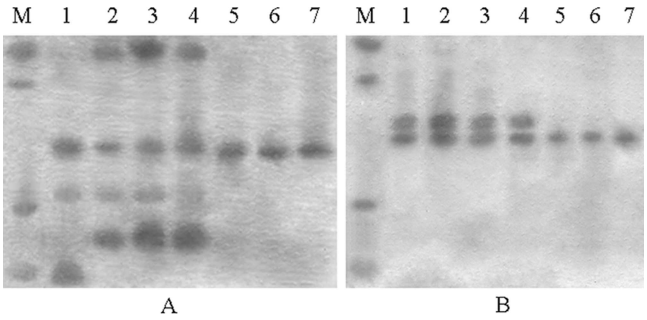

FIG. 3. IEF patterns of crude β-lactamase extracts from partial *S. marcescens*, *K. pneumoniae*, and *E. coli* isolates and their *E. coli* transconjugants. (A) Lane 1, *E. coli* E1; lanes 2 to 4, *K. pneumoniae* K1, K8, and K10; lane 5, *E. coli* transconjugant of *E. coli* E1; lanes 6 and 7, *E. coli* transconjugants of *K. pneumoniae* K1 and K10; M, strains producing TEM-1 (pI of 5.4), TEM-28 (pI of 6.1), SHV-7 (pI of 7.6), and ACT-1 (pI of 9.0). (B) Lanes 1 to 4, *S. marcescens* S1, S5, S10, and S20; lanes 5 to 7, *E. coli* transconjugants of *S. marcescens* S1, S10, and S20; M, strains producing TEM-1 (pI of 5.4), TEM-28 (pI of 6.1), SHV-7 (pI of 7.6), and ACT-1 (pI of 9.0).

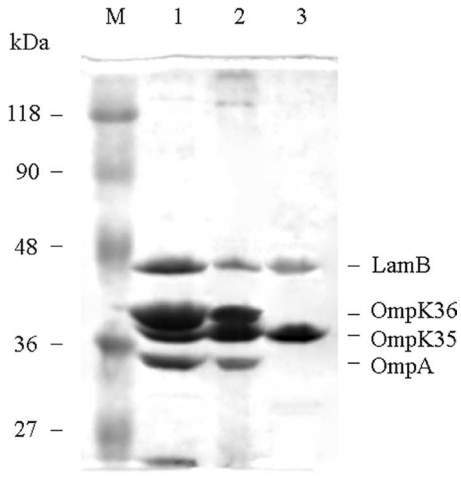

FIG. 4. SDS-PAGE analysis of OMPs extracted from *K. pneumoniae* ATCC 13883 and *K. pneumoniae* K1 and K10. Lane 1, *K. pneumoniae* ATCC 13883; lane 2, *K. pneumoniae* K1; lane 3, *K. pneumoniae* K10; M, protein molecular mass standard (MBI Fermentas).

pneumoniae isolates and *E. coli* transconjugants. This may be due to alterations in outer membrane permeability. Therefore, SDS-PAGE analysis of OMPs was performed, and the result revealed the lack of two OMPs of approximately 39 and 32 kDa in *K. pneumoniae* K10, compared to results for strains K1 and ATCC 13883 (Fig. 4). The 32-kDa OMP corresponds to OmpA, which is a specific porin and does not contribute to antimicrobial resistance phenotypes. It was uncertain whether the 39-kDa OMP was OmpK36 or OmpK35, since in some strains the OmpK36 migrates faster than OmpK35 (9). Amplification and sequencing of the *ompK35* gene indicated that both *K. pneumoniae* K1 and K10 contained identical gene sequences for *ompK35*. For the *ompK36* gene, the size of the amplification product from *K. pneumoniae* K10 was about 1,600 bp larger than that of strain K1. An insertion sequence IS*Ecp1* was identified between nucleotides 4 and 5 upstream of the *ompK36* start codon in strain K10. We inferred that IS*Ecp1* interfered with the upstream regulatory region and resulted in disruption of the *ompK36* gene. These results suggested that OmpK36 was not expressed in *K. pneumoniae* K10.

DISCUSSION

Carbapenems are a class of β-lactam antibiotics with strong activities against many gram-positive, gram-negative, and anaerobic bacteria and are often used as a last resort in infections due to multidrug-resistant, gram-negative bacilli. The emergence and spread of acquired carbapenem resistance brought about problems regarding therapy and control. Therefore, investigation of the molecular mechanisms of carbapenem resistance is critical.

The KPCs are most frequently detected in *K. pneumoniae*, and they are being increasingly detected in *E. coli* (4, 8, 10, 19). In this study, we described the first discovery of KPC-2 in *E. coli* in China. We identified KPC-2 in three *S. marcescens* isolates from the NICU in our hospital in February 2006 (30). From April 2006 to February 2007, 21 KPC-2-producing *S. marcescens* isolates were recovered in the same ward. The similar antimicrobial susceptibility patterns and identical ERIC-PCR patterns, plasmid profiles, restriction patterns, and IEF results suggested that they belonged to a clonal strain. During this period, 10 KPC-2-producing *K. pneumoniae* isolates that were indistinguishable or closely related to each other were recovered from the NICU and SICU. These results indicated that KPC-2-producing organisms were widespread in ICUs, especially in the NICU. To investigate the molecular epidemiology of the KPC-2-encoding plasmids existing in *E. coli*, *S. marcescens*, and *K. pneumoniae* isolates, restriction analysis using various endonucleases was performed. The identical restriction patterns suggested that the same transmissible KPC-2-encoding plasmid had spread among the three different genera and resulted in the spread of carbapenem resistance.

The pI 6.5 and 7.3 β-lactamases detected by IEF were not identified by PCR and DNA sequencing. In a previous study, we had demonstrated that the pI 6.5 β-lactamase was probably either the degradation product or the precursor of KPC-2 (30). Given the uncommon isoelectric point and low activity, we presumed that the pI 7.3 β-lactamase was either the degradation product or the precursor of CTX-M-14 or CTX-M-15.

Ertapenem, a relatively new carbapenem in clinical use, was strongly active against ESBL-producing *Klebsiella* isolates, with an MIC_{90} of 0.06 μg/ml, compared with 0.5 μg/ml for imipenem. Nevertheless, the MICs of ertapenem for ESBL-producing *Klebsiella* isolates were about two- to fourfold above those for nonproducers (14). Paterson et al. (20) reported that 10.9% of ESBL-producing *K. pneumoniae* organisms isolated from intra-abdominal infections worldwide were resistant to ertapenem, while only 4 to 5% were resistant to imipenem and meropenem. Jacoby et al. (12) reported that most *K. pneumoniae* derived from porin-deficient strains which produce plasmid-mediated β-lactamases were resistant to ertapenem but showed only slightly reduced susceptibility to imipenem and meropenem. In the current study, ertapenem showed slightly less activity against KPC-2 producers than did imipenem and meropenem. The MICs of ertapenem for major KPC-2-producing original isolates and all *E. coli* transconju-

gants were about twofold above those of imipenem and meropenem.

The *E. coli* transconjugant that only produced KPC-2 exhibited significantly reduced susceptibility to imipenem and meropenem (MICs of 1 to 2 μg/ml) and ertapenem (MICs of 4 μg/ml), suggesting that reduced carbapenem susceptibility in isolates of *S. marcescens*, *K. pneumoniae*, and *E. coli* was mainly due to production of KPC-2. However, it was not sufficient to explain the high-level carbapenem resistance in *K. pneumoniae* K10 (MICs of 128, 256, and >256 μg/ml), since the *E. coli* transconjugant remained susceptible to carbapenems at CLSI breakpoints (MICs of ≤4 μg/ml). In addition, the plasmid profiles and PFGE patterns of *K. pneumoniae* K10 were slightly different from those of other *K. pneumoniae* isolates. These results suggested that other mechanisms may contribute to carbapenem resistance in strain K10. The loss of a 39-kDa porin was observed in this isolate by SDS-PAGE of OMPs. The lost porin was subsequently proven to be OmpK36 and not the OmpK35 that was described by Yigit et al. (27) and Woodford et al. (26).

KPC-producing organisms are being increasingly detected. The host range of these KPCs is no longer limited to *K. pneumoniae*, and the geographical distribution is no longer limited to the northeastern United States. Sporadic occurrences of KPC-2 were once reported in China. They are now rapidly spreading in ICUs in our hospital. Effective measures for early identification and control should be adopted to prevent the potential continuous dissemination of these carbapenem-resistant pathogens.

REFERENCES

1. **Bradford, P. A., S. Bratu, C. Urban, M. Visalli, N. Mariano, D. Landman, J. J. Rahal, S. Brooks, S. Cebular, and J. Quale.** 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 β-lactamases in New York City. Clin. Infect. Dis. **39:**55–60.
2. **Bratu, S., D. Landman, M. Alam, E. Tolentino, and J. Quale.** 2005. Detection of KPC carbapenem-hydrolyzing enzymes in *Enterobacter* spp. from Brooklyn, New York. Antimicrob. Agents Chemother. **49:**776–778.
3. **Bratu, S., M. Mooty, S. Nichani, D. Landman, C. Gullans, B. Pettinato, U. Karumudi, P. Tolaney, and J. Quale.** 2005. Emergence of KPC-possessing *Klebsiella pneumoniae* in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob. Agents Chemother. **49:**3018–3020.
4. **Bratu, S., S. Brooks, S. Burney, S. Kochar, J. Gupta, D. Landman, and J. Quale.** 2007. Detection and spread of *Escherichia coli* possessing the plasmid-borne carbapenemase KPC-2 in Brooklyn, New York. Clin. Infect. Dis. **44:**972–975.
5. **Carattoli, A., A. Bertini, L. Villa, V. Falbo, K. L. Hopkins, and E. J. Threlfall.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228.
6. **Chen, G. X., R. Zhang, and H. W. Zhou.** 2006. Heterogeneity of metallo-β-lactamases in clinical isolates of *Chryseobacterium meningosepticum* from Hangzhou, China. J. Antimicrob. Chemother. **57:**750–752.
7. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A7, 7th ed. CLSI, Wayne, PA.
8. **Deshpande, L. M., R. N. Jones, T. R. Fritsche, and H. S. Sader.** 2006. Occurrence and characterization of carbapenemase-producing Enterobacteriaceae: report from the SENTRY antimicrobial surveillance program (2000–2004). Microb. Drug Resist. **12:**223–230.
9. **Hernandez-Alles, S., S. Alberti, D. Alvarez, A. Domenech-Sanchez, L. Martinez-Martinez, J. Gil, J. M. Tomas, and V. J. Benedi.** 1999. Porin expression in clinical isolates of *Klebsiella pneumoniae*. Microbiology **145:**673–679.
10. **Hong, T., E. S. Moland, B. Abdalhamid, N. D. Hanson, J. Wang, C. Sloan, D. Fabian, A. Farajallah, J. Levine, and K. S. Thomson.** 2005. *Escherichia coli*: development of carbapenem resistance during therapy. Clin. Infect. Dis. **40:**e84–e86.
11. **Hossain, A., M. J. Ferraro, R. M. Pino, R. B. Dew III, E. S. Moland, T. J. Lockhart, K. S. Thomson, R. V. Goering, and N. D. Hanson.** 2004. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 in an *Enterobacter* sp. Antimicrob. Agents Chemother. **48:**4438–4440.
12. **Jacoby, G. A., D. M. Mills, and N. Chow.** 2004. Role of β-lactamases and porins in resistance to ertapenem and other β-lactams in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **48:**3203–3206.
13. **Kaczmarek, F. M., F. Dib-Hajj, W. Shang, and T. D. Gootz.** 2006. High-level carbapenem resistance in a *Klebsiella pneumoniae* clinical isolate is due to the combination of bla_{ACT-1} β-lactamase production, porin OmpK35/36 insertional inactivation, and down-regulation of the phosphate transport porin PhoE. Antimicrob. Agents Chemother. **50:**3396–3406.
14. **Livermore, D. M., K. J. Oakton, M. W. Carter, and M. Warner.** 2001. Activity of ertapenem (MK-0826) versus *Enterobacteriaceae* with potent β-lactamases. Antimicrob. Agents Chemother. **45:**2831–2837.
15. **Mathew, A., A. M. Harris, M. J. Marshall, and G. W. Ross.** 1975. The use of analytical isoelectric focusing for detection and identification of β-lactamase. J. Gen. Microbiol. **88:**169–178.
16. **Miriagou, V., L. S. Tzouvelekis, S. Rossiter, E. Tzelepi, F. J. Angulo, and J. M. Whichard.** 2003. Imipenem resistance in a *Salmonella* clinical strain due to plasmid-mediated class A carbapenemase KPC-2. Antimicrob. Agents Chemother. **47:**1297–1300.
17. **Moland, E. S., N. D. Hanson, V. L. Herrera, J. A. Black, T. J. Lockhart, A. Hossain, J. A. Johnson, R. V. Goering, and K. S. Thomson.** 2003. Plasmid mediated, carbapenem-hydrolysing β-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. J. Antimicrob. Chemother. **51:**711–714.
18. **Naas, T., P. Nordmann, G. Vedel, and C. Poyart.** 2005. Plasmid-mediated carbapenem-hydrolyzing β-lactamase KPC in a *Klebsiella pneumoniae* isolate from France. Antimicrob. Agents Chemother. **49:**4423–4424.
19. **Navon-Venezia, S., I. Chmelnitsky, A. Leavitt, M. J. Schwaber, D. Schwartz, and Y. Carmeli.** 2006. Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. Antimicrob. Agents Chemother. **50:**3098–3101.
20. **Paterson, D. L., F. Rossi, F. Baquero, P. R. Hsueh, G. L. Woods, V. Satishchandran, T. A. Snyder, C. M. Harvey, H. Teppler, M. J. Dinubile, and J. W. Chow.** 2005. In vitro susceptibilities of aerobic and facultative gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). J. Antimicrob. Chemother. **55:**965–973.
21. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
22. **Versalovic, J., T. Koeuth, and J. R. Lupski.** 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. **19:**6823–6831.
23. **Villegas, M. V., K. Lolans, A. Correa, C. J. Suarez, J. A. Lopez, M. Vallejo, J. P. Quinn, and the Colombian Nosocomial Resistance Study Group.** 2006. First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of *Klebsiella pneumoniae* from South America. Antimicrob. Agents Chemother. **50:**2880–2882.
24. **Villegas, M. V., K. Lolans, A. Correa, J. N. Kattan, J. A. Lopez, J. P. Quinn, and the Colombian Nosocomial Resistance Study Group.** 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing β-lactamase. Antimicrob. Agents Chemother. **51:**1553–1555.
25. **Wei, Z. Q., X. X. Du, Y. S. Yu, P. Shen, Y. G. Chen, and L. J. Li.** 2007. Plasmid-mediated KPC-2 in a *Klebsiella pneumoniae* isolate from China. Antimicrob. Agents Chemother. **51:**763–765.
26. **Woodford, N., P. M. Tierno, Jr., K. Young, L. Tysall, M.-F. I. Palepou, E. Ward, R. E. Painter, D. F. Suber, D. Shungu, L. L. Silver, K. Inglima, J. Kornblum, and D. M. Livermore.** 2004. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A β-lactamase, KPC-3, in a New York medical center. Antimicrob. Agents Chemother. **48:**4793–4799.
27. **Yigit, H., A. M. Queenan, G. J. Anderson, A. Domenech-Sanchez, J. W. Biddle, C. D. Steward, S. Alberti, K. Bush, and F. C. Tenover.** 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:**1151–1161.
28. **Yigit, H., A. M. Queenan, J. K. Rasheed, J. W. Biddle, A. Domenech-Sanchez, S. Alberti, K. Bush, and F. C. Tenover.** 2003. Carbapenem-resistant strain of *Klebsiella oxytoca* harboring carbapenem-hydrolyzing β-lactamase KPC-2. Antimicrob. Agents Chemother. **47:**3881–3889.
29. **Yu, Y. S., S. J. Ji, Y. G. Chen, W. L. Zhou, Z. Q. Wei, L. J. Li, and Y. L. Ma.** 2007. Resistance of strains producing extended-spectrum β-lactamases and genotype distribution in China. J. Infect. **54:**53–57.
30. **Zhang, R., H. W. Zhou, J. C. Cai, and G. X. Chen.** 2007. Plasmid-mediated carbapenem-hydrolysing β-lactamase KPC-2 in carbapenem-resistant *Serratia marcescens* isolates from Hangzhou, China. J. Antimicrob. Chemother. **59:**574–576.