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Presence of Plasmid-Mediated Quinolone Resistance in *Klebsiella pneumoniae* Isolates Possessing bla_{KPC} in the United States▽

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The presence of plasmid-mediated quinolone resistance genes [i.e., *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*, and *qepA*] was evaluated among 42 bla_{KPC}-containing *Klebsiella pneumoniae* isolates collected in the eastern United States. One isolate carried the bla_{KPC-3} and *qnrB19* genes on the same conjugative plasmid, whereas another carried the bla_{KPC-3} and *qnrA1* genes on separate plasmids.

The rapid spread of *Klebsiella pneumoniae* carbapenemases (KPCs) among members of the family *Enterobacteriaceae* represents an escalating global threat (15). Since bla_{KPC} genes confer resistance to all β-lactams, the only therapeutic options for treating infections due to organisms possessing these β-lactamases are quinolones, aminoglycosides, polymyxins, or combinations of agents for which there are few data on efficacy (15). Unfortunately, high-level resistance to ciprofloxacin, gentamicin, and amikacin is also frequently observed among bla_{KPC}-containing *K. pneumoniae* isolates (2, 3).

Quinolone resistance among *Enterobacteriaceae* is usually mediated by chromosomal mutations in the genes encoding DNA gyrase and topoisomerase IV. Plasmid-mediated quinolone resistance (PMQR) can arise from the expression of proteins encoded by the *qnrA*, *-B*, and *-S* genes that are able to protect the DNA gyrase. In addition, an aminoglycoside acetyltransferase encoded by the *aac(6′)-Ib-cr* gene also confers ciprofloxacin resistance (10, 12). The *qnr* and *aac(6′)-Ib-cr* loci are frequently found among *Enterobacteriaceae* producing AmpC enzymes, extended-spectrum β-lactamases, or both (1, 7, 8, 13). A plasmid-mediated quinolone efflux pump (i.e., the *qepA* gene) also has been described in *Escherichia coli* (17).

Recently, Mendes et al. reported the detection of a single *K. pneumoniae* isolate in China possessing bla_{KPC-2} and *qnrB4* on the same conjugative plasmid (6). However, the prevalence of PMQR determinants among *K. pneumoniae* isolates producing KPCs has not yet been examined. Therefore, we studied a set of *K. pneumoniae* isolates collected at five major health care institutions located in the eastern United States to estimate the frequency of PMQR among KPC producers.

Eighty-five nonreplicated *K. pneumoniae* clinical isolates showing reduced susceptibility (i.e., MIC, ≥0.5 mg/liter) to imipenem, meropenem, or ertapenem were studied by using PCR amplification and DNA sequencing to detect the presence of the bla_{KPC} genes and to confirm their identity (forward primer, 5′-ATGTCACTGTATCGCCGTC-3′; reverse primer, 5′-TTACTGCCCGTTGACGCC-3′). The isolates were randomly collected from January 2006 to October 2007 from Mount Sinai Medical Center in New York (MSMC), the University of Pittsburgh Medical Center (UPMC), and three Cleveland institutions, including the University Hospital Case Medical Center (UHCMC), the Cleveland Clinic Foundation (CCF), and the Louis Stokes Cleveland Department of Veterans Affairs Medical Center (LSVAMC).

Overall, 42 *K. pneumoniae* clinical isolates possessed a bla_{KPC} gene (MSMC, $n = 24$; UPMC, $n = 4$; UHCMC, $n = 2$; CCF, $n = 9$; LSVAMC, $n = 3$). In particular, 25 isolates amplified bla_{KPC-2}, and the remaining 17 amplified bla_{KPC-3}. PCR and DNA sequence analysis of the PMQR determinants, the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes, were performed as previously reported (5, 7, 13, 14, 16). Two of the 42 (4.8%) *K. pneumoniae* isolates containing bla_{KPC} also encoded a PMQR determinant. For these two isolates, conjugation experiments were performed using *E. coli* J53 (rifampin resistant) as the

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TABLE 1. Susceptibility test results of the parental *K. pneumoniae* isolates, VA367 and VA375, and the corresponding *E. coli* J53 transconjugants, VA367-J53 and VA375-J53[a]

Antimicrobial drug	VA367 [bla_{KPC-3} *qnrB19* bla_{TEM-1} bla_{SHV-11} bla_{SHV-12} *aac(6′)-Ib*]		VA367-J53 [bla_{KPC-3} *qnrB19* bla_{TEM-1} bla_{SHV-11} *aac(6′)-Ib*]		VA375 (bla_{KPC-3} *qnrA1* bla_{TEM-1} bla_{SHV-11})		VA375-J53 (*qnrA1* bla_{TEM-1} bla_{SHV-11})		*E. coli* J53	
	MIC (μg/ml)	Susceptibility[c]	MIC (μg/ml)	Susceptibility	MIC (μg/ml)	Susceptibility	MIC (μg/ml)	Susceptibility	MIC (μg/ml)	Susceptibility
Levofloxacin	≥8 (≥32)	R	1 (0.75)	S	1 (0.75)	S	1 (1.5)	S	≤0.25 (0.25)	S
Ciprofloxacin	≥4 (≥32)	R	1 (1)	S	1 (0.75)	S	1 (2)	S	≤0.25 (0.25)	S
Gentamicin	4	S	≤1	S	≥16	R	4	S	≤1	S
Amikacin	≥64	R	16	S	≤2	S	≤2	S	≤2	S
Tobramycin	≥16	R	≥16	R	≥16	R	4	S	≤1	S
Trimethoprim-sulfamethoxazole	≥320	R	≤20	S	≥320	R	≥320	R	≤20	S
Nitrofurantoin	≥512	R	≤16	S	64	I	≤16	S	≤16	S
Tigecycline[b]	(3)		(0.5)		(1.5)		(0.5)		(0.5)	
Colistin[b]	(1.5)		(0.38)		(1)		(0.38)		(0.38)	
Piperacillin	≥128	R	≥128	R	≥128	R	≥128	R	≤4	S
Piperacillin-tazobactam	≥128	R	64	I	≥128	R	64	I	≤4	S
Ampicillin-sulbactam	≥32	R	≥32	R	≥32	R	≥32	R	4	S
Cefazolin	≥64	R	≥64	R	≥64	R	≥64	R	≤4	S
Cefuroxime	≥64	R	≥64	R	≥64	R	≥64	R	16	I
Ceftazidime	≥64	R	32	R	≥64	R	≥64	R	≤1	S
Ceftriaxone	≥64	R	8	S	32	I	8	S	≤1	S
Cefepime	≥64	R	2	S	≥64	R	≤1	S	≤1	S
Aztreonam	≥64	R	≥64	R	≥64	R	2	S	≤1	S
Meropenem	2 (8)	S (I)	1 (4)	S (S)	1 (4)	S (S)	≤0.25 (0.38)	S	≤0.25 (0.047)	S
Imipenem	2 (16)	S (R)	4 (4)	S (S)	2 (8)	S (I)	≤1 (0.38)	S	≤1 (0.25)	S
Ertapenem	(16)	(R)	(2)	(S)	(8)	(I)	(0.125)	(S)	(0.023)	(S)

[a] The antimicrobial susceptibility tests were performed with the Vitek 2 system, using AST-GN09 cards (bioMérieux, Durham, NC). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) criteria (4). All MIC and susceptibility data given in parentheses were obtained by the Etest method (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar.

[b] Interpretative criteria for this drug have not yet been released by the CLSI (4).

[c] S, susceptible; I, intermediate; R, resistant.

recipient strain. After overnight incubation, transconjugants were selected by plating the conjugation mixture (donor and recipient strains) onto MacConkey agar supplemented with ceftazidime (10 mg/liter) and rifampin (100 mg/liter) (11). Antimicrobial susceptibility testing results for the two donors and their *E. coli* J53 transconjugants are shown in Table 1. Both *K. pneumoniae* isolates demonstrated increased MICs for carbapenems. In contrast, only one strain (VA367) expressed high-level resistance to quinolones, whereas the other (VA375) showed only a small increase in the MICs.

K. pneumoniae VA367 was isolated in November 2007 from a sputum sample from a 75-year-old man admitted to the surgery service of the LSVAMC with a diagnosis of adenocarcinoma of the esophagus. He had not received antibiotics in the preceding 12 months. On the first hospital day, the patient underwent a transhiatal esophagectomy and received piperacillin-tazobactam (3.375 g every 6 h) perioperatively for 36 h. Since fever and leukocytosis developed on the fifth postoperative day, administration of piperacillin-tazobactam was resumed. On the 11th postoperative day, the patient suffered cardiorespiratory arrest. He was intubated and transferred to the intensive care unit. Fever persisted, and on the 16th postoperative day, a sputum culture grew *Enterobacter aerogenes*, which had intermediate resistance to ceftazidime but was susceptible to piperacillin-tazobactam and carbapenems, and *K. pneumoniae* VA367 as a coisolate (Table 1). On the 22nd postoperative day, therapy was changed to meropenem (500 mg every 8 h). Fever persisted, and on the 27th postoperative day, tigecycline therapy (100 mg followed by 50 mg every 12 h) was initiated. The patient died on the 28th day after hospital admission.

Molecular analysis of *K. pneumoniae* VA367 revealed the following resistance determinants: bla_{KPC-3}, *qnrB19*, bla_{TEM-1}, bla_{SHV-11}, bla_{SHV-12}, and *aac(6′)-Ib*. The *E. coli* transconjugant of strain VA367 contained the bla_{KPC-3}, *qnrB19*, bla_{TEM-1}, bla_{SHV-11}, and *aac(6′)-Ib* genes. Analytical isoelectric focusing (aIEF) was performed as previously described (9). aIEF revealed that the donor and transconjugant strains possessed β-lactamases at pIs of 5.4, 6.7, and 7.6; only the donor had an additional β-lactamase at pI 8.2. Strain VA367 and its transconjugant carried one transferable plasmid of approximately 80 kb.

K. pneumoniae VA375 was recovered on August 2007 from a culture of a blood sample obtained from a 58-year-old man admitted to the UHCMC for a kidney transplant. He received intravenous ciprofloxacin (400 mg/day) and amikacin (500 mg after each hemodialysis) treatment for 14 days, with complete resolution of the infection. Molecular analysis demonstrated that the isolate contained bla_{KPC-3}, *qnrA1*, bla_{TEM-1}, and bla_{SHV-11}. The *qnrA1*, bla_{TEM-1}, and bla_{SHV-11} genes were transferred to *E. coli* J53, whereas bla_{KPC-3} was not. aIEF showed β-lactamases at pIs of 5.4, 5.8, 6.7, 7.0, and 7.6 in the

donor isolate, but the β-lactamases at pIs of 5.4, 5.8, and 7.6 were observed only in the transconjugant strain. VA375 contained at least two plasmids of approximately 80 kb and 130 kb, whereas the transconjugant contained only the larger plasmid.

This is the first molecular epidemiological survey assessing the spread of PMQR genes among bla_{KPC}-containing *K. pneumoniae* isolates. Our limited survey suggests that the prevalence of these isolates in the United States may be approximately 5%. VA367 represents the first *K. pneumoniae* isolate reported to carry both the bla_{KPC-3} and *qnrB* genes on a single conjugative plasmid. Strain VA375 is the first observed *K. pneumoniae* clinical isolate possessing both the bla_{KPC} and *qnrA* genes. In both strains, the *qnr* genes were cotransferred with other important drug-resistant elements [e.g., β-lactamases and aminoglycoside resistance determinants, such as *aac(6′)-1b*]. These findings warn us that novel combinations of transferable resistance determinants continue to emerge and could seriously undermine therapeutic regimens with β-lactams, fluoroquinolones, and aminoglycosides. The possibility of *K. pneumoniae* transferring these resistant plasmids to other *Enterobacteriaceae* and nonfermenting gram-negative bacilli is a serious consideration in the care of hospitalized patients.

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