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Susceptibility Profiles, Molecular Epidemiology, and Detection of KPC-Producing *Escherichia coli* Isolates from the New York City Vicinity▽

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The detection of *Enterobacteriaceae* carrying the carbapenemase KPC has been problematic. Thirty isolates of KPC-possessing *Escherichia coli* were gathered from hospitals in New York City and Connecticut. The imipenem, meropenem, doripenem, and ertapenem MIC_{50} values were 4, 2, 1, and 4 μg/ml, respectively. Over half of the isolates belonged to a single ribotype. Using an ertapenem breakpoint of 0.25 μg/ml would efficiently detect these isolates.

Klebsiella pneumoniae isolates possessing the carbapenemase KPC are endemic in the northeastern United States (3, 11, 21) and have been recovered in Europe, South America, and Asia (15). Isolates of KPC-possessing *K. pneumoniae* may have MICs just below the breakpoint for resistance for imipenem and meropenem, although most of these isolates have been resistant to ertapenem (1, 4, 17). Difficulty in detecting these isolates, based on carbapenem susceptibility testing, has undoubtedly contributed to their dissemination. Current guidelines recommend testing all cephalosporin-resistant, carbapenem-susceptible isolates with relatively high carbapenem MICs for evidence of carbapenemase activity (6).

Epidemiological studies have emphasized the geographic spread of KPC-possessing *K. pneumoniae* isolates with a common lineage (3, 4, 10). However, bla_{KPC} usually resides on a transmissible element (Tn*4401*) (13) and has been recovered in isolates of other *Enterobacteriaceae*, *Acinetobacter* spp., and *Pseudomonas* spp. (16, 20). The spread of bla_{KPC} to *Escherichia coli* is particularly worrisome, and reports of these isolates from the United States and Israel have surfaced (2, 12, 14, 19). A propensity for these pathogens to be recovered from residents of long-term-care facilities has been noted (2, 12, 14, 19). In this report, the susceptibility patterns and molecular epidemiology of isolates of KPC-possessing *E. coli* from the New York City area are documented.

Bacterial isolates. Isolates from Brooklyn, NY, were collected during borough-wide surveillance studies performed in 2006 and 2009, as previously described (11). For the 2009 surveillance, unique patient isolates were gathered from March to May from 16 hospitals in the boroughs of Brooklyn and Staten Island. Isolates were identified as *E. coli* in the clinical microbiology laboratories by standard techniques. All cephalosporin-resistant surveillance isolates were screened for the presence of bla_{KPC} by using previously reported primers and PCR conditions (3, 4). PCR amplicons of bla_{KPC} were identified by bidirectional DNA sequencing, as previously described (3, 4). Isolates of *E. coli* from Queens were screened with meropenem disk diffusion testing; nonsusceptible isolates were also tested for the presence of bla_{KPC}. Some of the isolates from Brooklyn and Queens have been included in prior reports (2, 19). Isolates from Connecticut were referred to the Research Laboratory in Brooklyn because of their relatively high MICs to carbapenems.

Microbiological methods. All surveillance isolates had susceptibility testing performed by using the agar or broth dilution techniques (7). A new investigational polymyxin B analogue, CB-182,804 (Cubist Pharmaceuticals, Lexington, MA), was also included for susceptibility testing of the surveillance isolates. For the KPC-possessing isolates, MICs were determined using Etest methodology (AB Biodisk, Solna, Sweden) as well. MICs of imipenem, meropenem, doripenem, and ertapenem were also determined by the broth microdilution method by using established techniques (7). MICs were analyzed according to breakpoints established at the time of the study (8). For the KPC-possessing isolates, carbapenem susceptibility rates were also defined according to the proposed 2010 CLSI breakpoints (9). Isolates were considered susceptible to tigecycline, colistin, and polymyxin B if the MIC value was ≤2 μg/ml. Modified Hodge tests were performed as recommended (5), using disks containing 10 μg of imipenem, meropenem, or ertapenem. Disk diffusion assays were performed using meropenem disks with and without the addition of 400 μg of phenylboronic acid (Sigma-Aldrich, St. Louis, MO) as previously described (18); an increase of ≥5 mm of the zone diameter around the disk with boronic acid was considered indicative of the presence of a KPC-possessing isolate. Isolates underwent ribotyping using the RiboPrinter microbial characterization system (Qualicon, Wilmington, DE) according to the manufacturer's recommendations.

A total of 30 isolates were confirmed to possess bla_{KPC}. Nine isolates were collected from Brooklyn (from five different hospitals), 15 isolates from Queens, and six isolates from Connect-

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TABLE 1. Susceptibility of 30 KPC-producing *E. coli* isolates to β-lactams

Isolate	Ribotype	MIC (μg/ml)[a]									
		Broth microdilution method				Etest method					
		IPM	MER	DOR	ETP	IPM	MER	DOR	ETP	CAZ	TZB
Brooklyn isolates											
DM133	A	4	1	1	1	6	0.75	1	1.5	8	>256
KB86	A	4	0.5	1	1	4	0.5	1.5	0.75	24	>256
KB801	A	4	4	2	8	4	2	2	8	12	192
ME57	B	4	1	1	4	6	1	3	1.5	8	96
ME236	A	4	0.5	1	2	3	0.5	2	0.5	24	256
LU60	C	2	4	2	16	8	2	2	6	48	>256
MA82	A	8	16	4	32	12	6	12	24	>256	>256
MA46	D	8	2	2	4	12	3	3	6	256	>256
ME366	C	2	2	0.5	4	1	0.75	2	1.5	32	24
Queens isolates											
E1	A	4	1	1	2	4	0.75	4	1	12	>256
E2	E	8	16	8	32	12	12	>32	>32	>256	>256
E3	F	4	2	2	4	8	3	4	3	>256	>256
E4	G	1	8	4	8	3	4	8	12	128	96
E5	H	4	2	4	2	12	3	6	3	12	>256
E6	A	4	2	1	8	6	3	2	8	48	>256
E7	I	8	8	2	16	8	8	4	>32	32	64
E10	A	4	1	1	2	4	1	4	1.5	12	>256
E11	A	2	8	2	8	8	8	3	6	8	>256
E12	I	32	32	8	>32	>32	32	8	>32	>256	>256
E13	J	4	4	1	8	8	3	3	4	24	256
E14	K	8	2	2	2	12	4	6	2	192	>256
E16	I	4	4	4	16	8	4	8	8	>256	>256
E18	A	4	4	0.5	8	3	4	8	6	24	>256
E19	A	8	8	8	16	16	6	16	16	128	>256
Connecticut isolates											
F5483	A	2	0.25	0.5	1	2	0.38	0.5	0.5	8	>256
H1788	A	2	0.5	0.5	0.5	2	0.38	0.5	0.5	4	24
J0597	A	2	0.5	0.5	1	2	0.5	0.38	0.75	6	48
N3201	A	4	1	2	1	3	0.38	0.38	1.5	4	48
T9322	A	4	1	1	2	3	0.75	0.5	2	12	96
W3490	A	2	0.25	1	0.5	2	0.75	0.75	0.5	4	32

[a] IPM, imipenem; MER, meropenem; DOR, doripenem; ETP, ertapenem; CAZ, ceftazidime; TZB, piperacillin-tazobactam.

icut. Using the broth microdilution technique, imipenem, meropenem, doripenem, and ertapenem MIC_{50} values were 4, 2, 1, and 4 μg/ml, respectively (Table 1). Using the Etest method, results were not appreciably different, with imipenem, meropenem, doripenem, and ertapenem MIC_{50} values of 6, 2, 3, and 3 μg/ml, respectively. The presence of scattered colonies within the Etest zone of inhibition made reading the endpoint difficult. If the 2009 breakpoints for carbapenems were used, many of the isolates would have been considered susceptible to these agents (Table 2). Using the proposed 2010 CLSI breakpoints, many of the isolates would have still been considered susceptible to meropenem and doripenem but few to imipenem and none to ertapenem (Table 2).

TABLE 2. Susceptibility rates of 30 bla_{KPC}-possessing *E. coli* isolates according to the 2009 and 2010 CLSI breakpoints

Breakpoint yr	% susceptible[a]							
	Broth microdilution method				Etest method			
	IPM	MER	DOR	ETP	IPM	MER	DOR	ETP
2009	77	77	17[b]	43	47	80	17[b]	47
2010	3	40	50	0	3	43	23	0

[a] IPM, imipenem; MER, meropenem; DOR, doripenem; ETP, ertapenem.
[b] Values calculated based on the 2009 FDA-recommended breakpoint for doripenem.

All bla_{KPC}-possessing isolates had ceftazidime MIC values of >1 μg/ml (extended-spectrum β-lactamase [ESBL] screen positive), and none were susceptible to piperacillin-tazobactam (Table 1). For the non-β-lactam agents, 3% of isolates were susceptible to ciprofloxacin, 47% to gentamicin, 77% to amikacin, 97% to tigecycline, and 100% to colistin.

All isolates were positive for carbapenemase activity by the modified Hodge test regardless of the carbapenem disk used. Most isolates had reduced zone diameters with meropenem disk diffusion testing. Fifteen isolates were screened by this method for inclusion into the study; for the remaining 15 isolates, one was susceptible, two were intermediate, and 12 were resistant to meropenem by disk testing. For all isolates, the addition of boronic acid increased the meropenem disk zone diameter by >5 mm (from 8.9 ± 2.9 mm to 22.4 ± 1.9 mm [mean ± standard deviation]). In contrast, there was no change in meropenem zone diameters with the addition of boronic acid for 11 randomly selected ESBL-possessing *E. coli* isolates (24.5 ± 1.0 mm to 24.0 ± 1.0 mm)

TABLE 3. Susceptibility results for 3,050 isolates of *E. coli* collected from hospitals in Brooklyn during a 3-month surveillance study conducted in 2009

Antimicrobial agent	MIC			% of isolates		
	50%	90%	Range	Susceptible	Intermediate	Resistant
Imipenem	0.25	0.25	≤0.12–8	99.97	0.03	0
Meropenem	≤0.12	≤0.12	≤0.12–8	99.97	0.03	0%
Ertapenem	≤0.12	≤0.12	≤0.12–8	99.7	0.1	0.2
Doripenem	≤0.06	≤0.06	≤0.06–4	99.8		
Ceftriaxone	≤0.25	0.5	≤0.25–>64	93.7	1.5	4.8
Ceftazidime	≤0.25	2	≤0.25–>32	93.1	1.5	5.4
Cefepime	≤0.25	≤0.25	≤0.25–>32	96.5	1.9	1.6
Piperacillin-tazobactam	2	4	≤0.5–>128	98	1.0	1.0
Ciprofloxacin	≤0.12	>4	≤0.12–>4	67.2	0.4	32.4
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5–>4	67.6		32.4
Polymyxin B	1	1	≤0.12–>4	99.8		0.2
CB-182,804	2	4	≤0.12–>4			
Tigecycline	0.25	0.25	0.03–2	100		

or seven cephalosporin-susceptible isolates (24.3 ± 1.1 mm to 25.3 ± 0.8 mm).

Seventeen of the 30 isolates, recovered in all three regions, belonged to a single ribotype, suggesting clonal spread of this resistant pathogen. Two ribotypes consisted of three isolates each and were localized to a single region. The remaining seven isolates belonged to unique ribotypes. Twenty-four isolates carried KPC-2, while the remaining six isolates had KPC-3.

During the 3-month surveillance study conducted in 2009 in Brooklyn, 3,050 unique patient isolates were collected. Susceptibility results are noted in Table 3; 10.1% had MICs of ceftazidime of >1 μg/ml (ESBL screen positive), and 0.1% (four isolates) were bla_{KPC} positive. Nearly all were susceptible to polymyxin B, 95.5% were inhibited by ≤4 μg/ml of CB-182,804, and all were susceptible to tigecycline. If carbapenem-susceptible, ESBL-positive isolates were selected for modified Hodge testing, 299 isolates would have been examined to detect the four bla_{KPC}-possessing isolates. If carbapenemase testing was limited to carbapenem-susceptible, piperacillin-tazobactam-nonsusceptible isolates, 50 isolates would have required examination. If an ertapenem breakpoint of 0.25 μg/ml was used, only 22 isolates would have required additional testing.

In the New York City region, KPC-possessing *K. pneumoniae* isolates have become established in many medical centers; approximately one-third of all isolates carry this carbapenemase (11). In contrast, KPC-possessing *E. coli* isolates have not become commonplace, accounting for <1% of isolates in Brooklyn, NY. However, the epidemiology of KPC-possessing *E. coli* in our region does parallel that of KPC-possessing *K. pneumoniae* in that most of the isolates belong to a single ribotype (3, 4). Although bla_{KPC} resides on a transmissible element (13) and has been recovered in other genera of bacteria (15), it is remarkable that the great majority of KPC-producing isolates of *K. pneumoniae* and *E. coli* are clonally related. Further investigation will be needed to determine if these strains possess other features that provide a survival advantage in the hospital environment.

Detection of KPC-producing *Enterobacteriaceae* in the clinical laboratory has been difficult (1, 4, 17). The MICs of imipenem and meropenem remain in the susceptible range for a significant minority of isolates of KPC-possessing *K. pneumoniae* (1, 4, 17). However, virtually all KPC-possessing *K. pneumoniae* isolates are frankly resistant to ertapenem, and screening with this agent has been recommended (4). Identification of KPC-producing isolates of *E. coli* in the clinical laboratory is also problematic. Using the 2009 CLSI (and FDA, for doripenem) breakpoints, many isolates would have been considered susceptible to carbapenems. Using the revised 2010 CLSI breakpoint recommendations, many isolates would still be considered susceptible to meropenem and doripenem. However, only one of our 30 isolates would be identified as susceptible to imipenem, and none would have been susceptible to ertapenem (using a susceptibility breakpoint of ≤0.25 μg/ml).

Based on our results, use of ertapenem as the class agent provides the most efficient and sensitive method for clinical laboratories to screen for KPC-producing *E. coli*. Using the revised breakpoint of 0.25 μg/ml, 22 isolates in our surveillance study would have been selected for additional testing in order to detect the four bla_{KPC}-possessing strains. The presence of a carbapenemase could be confirmed using the modified Hodge test as suggested or by using a disk diffusion test with and without boronic acid.

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