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Emergence of KPC-Possessing *Klebsiella pneumoniae* in Brooklyn, New York: Epidemiology and Recommendations for Detection

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Among 257 isolates of *Klebsiella pneumoniae* collected in Brooklyn, NY, 24% were found to possess bla_{KPC}. Clinical microbiology laboratories that used automated broth microdilution systems reported 15% of the KPC-possessing isolates as susceptible to imipenem. The imipenem MIC was found to be markedly affected by the inoculum. For accurate detection of KPC-possessing *K. pneumoniae*, particular attention should be paid to proper inoculum preparation for broth-based susceptibility methods. In addition, using ertapenem or meropenem for class reporting of carbapenem susceptibility will improve detection.

Carbapenem resistance among *Klebsiella pneumoniae* isolates had been distinctly unusual. Recently, carbapenem resistance mediated by the KPC β-lactamases has been documented in isolates of *Klebsiella* spp. (1, 6, 7, 9), *Enterobacter* spp. (2, 4), and *Salmonella* (5). Accurate detection of these isolates will be crucial for controlling their spread. In this study, we examined the accuracy of the clinical laboratories and various susceptibility test methods in the detection of KPC-possessing isolates.

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During September and October 2004, isolates of *Escherichia coli*, *Enterobacter* spp., and *K. pneumoniae* were gathered from four hospitals in Brooklyn, NY. Isolates of *E. coli* and *Enterobacter* spp. for which the ceftazidime MIC was ≥1 μg/ml and all isolates of *K. pneumoniae* were screened for the presence of bla_{KPC}. The methods used for PCR screening and bla_{KPC} identification, ribotyping, and isoelectric focusing were as previously described (1, 2). KPC possession was confirmed by a second, independent PCR test; previously characterized clinical isolates (1) were used as positive and negative controls.

In the central research laboratory, the MICs of carbapenems were determined by the broth microdilution method in cation-supplemented Mueller-Hinton broth using inocula of ~5×10^4 and 5×10^5 CFU/ml for all KPC-possessing isolates. Carbapenem susceptibility testing was also performed using the disk diffusion (3) and Etest methodologies with Mueller-Hinton agar. Susceptibility results were defined according to established breakpoints (3).

Carbapenem susceptibility results were correlated with results reported by the clinical microbiology laboratories, all of which used the MicroScan System (Dade Microscan Walk-Away System; Dade International Inc., West Sacramento, CA). All of the clinical laboratories used Prompt Inoculation System-D (3M Company, St. Paul, MN) for inoculum preparation. Selected isolates underwent repeated carbapenem susceptibility testing, using the MicroScan System, with inocula prepared using (i) Prompt Inoculation System-D and (ii) the CLSI (formerly NCCLS)-recommended protocol (3).

A total of 642 isolates of *E. coli* were gathered during the surveillance. None of the 55 isolates for which the ceftazidime MIC was >1 μg/ml possessed bla_{KPC}. Seventy-nine isolates of *Enterobacter* spp. were collected during the 2-month surveillance; none of the 29 ceftazidime-resistant isolates carried bla_{KPC}.

A total of 257 single-patient isolates of *K. pneumoniae* were gathered during the surveillance study; 109 (42%) were considered to possess extended-spectrum β-lactamases. All 257 isolates were screened for the presence of bla_{KPC}, and 62 (24%) were found to carry the gene. Fifty-nine possessed bla_{KPC-2}, and 3 possessed bla_{KPC-3}. All demonstrated a β-lactamase with a pI value of 6.7 by isoelectric focusing; β-lactamases with pIs of 5.4 and 7.6 were also identified in many isolates. Ribotyping revealed that 88% of the 62 isolates belonged to a single ribotype.

The clinical laboratories identified 15% of the 62 KPC-possessing isolates as susceptible to imipenem (Table 1). Of 42 isolates that also had meropenem susceptibility determined, 12% were reported as susceptible to this antibiotic. All were found to be resistant to ceftazidime.

A variety of carbapenem susceptibility testing methods were evaluated in the research laboratory. A pronounced inoculum effect was observed when the broth microdilution method was used for imipenem; using ~10^4 CFU/ml, only 19% were considered resistant (Table 1). Although only 5% were susceptible to meropenem using the lower inocula, 77% were considered intermediate. An inoculum effect was not observed for ertapenem. Virtually all isolates were found resistant to the three carbapenems when agar diffusion (Etest and disk diffusion) techniques were employed.

Ten isolates that were reported susceptible to imipenem by the clinical microbiology laboratories were reexamined. When the inocula were prepared using Prompt Inoculation System-D, 3 of the 10 were found susceptible and 7 intermediate

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TABLE 1. Results of carbapenem susceptibility testing of 62 KPC-possessing *K. pneumoniae* isolates according to various testing procedures

Drug and method	MIC (μg/ml)			% Susceptible	% Intermediate	% Resistant
	50%[a]	90%[b]	Range			
Imipenem						
Clinical laboratory				15	35	50
Broth dilution						
Inoculum = 10^5 CFU/ml	32	>32	≤0.5–>32	5	16	79
Inoculum = 10^4 CFU/ml	8	32	≤0.5–>32	44	37	19
Etest	>32	>32	4–>32	2	2	96
Disk diffusion				2	0	98
Meropenem						
Clinical laboratory (n = 42)				12	5	83
Broth dilution						
Inoculum = 10^5 CFU/ml	32	>32	1–>32	2	0	98
Inoculum = 10^4 CFU/ml	8	32	≤0.5–>32	5	77	18
Etest	>32	>32	2–>32	5	2	93
Disk diffusion				2	2	96
Ertapenem						
Broth dilution						
Inoculum = 10^5 CFU/ml	32	>32	≤0.5–>32	2	0	98
Inoculum = 10^4 CFU/ml	32	>32	≤0.5–>32	2	3	95
Etest	>32	>32	16–>32	0	0	100
Disk diffusion				0	0	100

[a] MIC for 50% of strains tested.
[b] MIC for 90% of strains tested.

to imipenem. Using the same system, two were susceptible and eight resistant to meropenem. When the inocula were prepared according to CLSI procedures, 2 were susceptible and 8 intermediate to imipenem; all 10 were found resistant to meropenem.

All of the 62 KPC-possessing *K. pneumoniae* isolates were resistant to piperacillin-tazobactam and ciprofloxacin (Table 2). Among commonly used antibiotics, gentamicin retained the greatest activity; nearly one-fourth were resistant to polymyxin B.

Numerous reports describing KPC-possessing isolates from the northeastern United States have surfaced (1, 2, 4, 7, 8, 9). Despite heightened awareness and enhanced infection control efforts, medical centers in our region continue to struggle with KPC-possessing *K. pneumoniae*. In this report, a startling 24% of *K. pneumoniae* isolates harbored the KPC carbapenem-hydrolyzing β-lactamase. Accurate detection of KPC-possessing *K. pneumoniae* will be the crucial first step in controlling its spread.

It was anticipated that carbapenem susceptibility results could be used to identify KPC-possessing *K. pneumoniae*. However, the MICs of imipenem were highly dependent on the

TABLE 2. Susceptibility results involving 62 KPC-possessing *K. pneumoniae* isolates

Antibiotic(s)	MIC (μg/ml)			% Susceptible	% Intermediate	% Resistant
	50%[a]	90%[b]	Range			
Piperacillin-tazobactam	>256	>256	>256	0	0	100
Cefotetan	64	>256	16–>256	15	34	51
Ceftazidime	>256	>256	32–>256	0	2	98
Cefepime	>256	>256	16–>256	0	5	95
Gentamicin	4	64	1–>256	65	3	32
Amikacin	64	128	2–>256	6	6	88
Ciprofloxacin	>32	>32	8–>32	0	0	100
Doxycycline	8	16	4–>256	32	50	12
Polymyxin B	2	8	0.5–>16	73		27

[a] MIC for 50% of strains tested.
[b] MIC for 90% of strains tested.

inoculum employed in the susceptibility test procedure. While ~95% of isolates were correctly identified as resistant using agar-based diffusion tests, many isolates were considered susceptible or intermediate to imipenem in broth-based tests, especially when the inocula were lower than recommended. This pronounced inoculum effect likely explains the results obtained from the clinical laboratories, which reported 15% of the KPC-possessing isolates of *K. pneumoniae* as susceptible to imipenem. All of the participating clinical laboratories used Prompt Inoculation System-D for inoculum preparation. As noted by the manufacturer, an adequate sample may not be obtained when the bacterial colonies (such as *K. pneumoniae*) are extremely mucoid, and other methods for inoculum preparation should be considered.

An inoculum effect was also observed with meropenem, although fewer isolates were categorized as susceptible. No inoculum effect was observed with ertapenem, suggesting that susceptibility testing of this antibiotic may be the preferred method for detecting KPC-possessing isolates.

Based on our results, several recommendations can be offered to clinical microbiology laboratories to improve detection of KPC-possessing *K. pneumoniae*. First, a correct inoculum of any mucoid lactose-fermenting gram-negative bacillus undergoing identification and susceptibility testing should be assured. If the proper inoculum cannot be assured using an inoculation wand method, inoculum preparation using the CLSI procedures (3) should be employed. Alternatively, an agar diffusion test could be employed for mucoid isolates. Secondly, *K. pneumoniae* intermediate or resistant to ertapenem or meropenem should be considered resistant to all carbapenems, regardless of the other susceptibility results.

Precisely why some isolates demonstrate an inoculum effect with imipenem while others are highly resistant even with a low inoculum is an area of active investigation. Most of these isolates possess other β-lactamases (1, 9), perhaps contributing to differing levels of resistance. Also, porin defects have been found in some (7, 8) but not all (9) KPC-possessing *Klebsiella* spp. Finally, an inoculum effect with imipenem has also been observed in KPC-possessing *Enterobacter* spp. (2). Since these β-lactamases reside on transmissible plasmids and have been found in other genera (4, 5, 6, 8), an examination of detection methods with other bacteria is warranted.

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