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Detection of KPC Enzymes in Klebsiella pneumoniae Isolates from NY/NJ Sites in the TEST Program

M. Renteria[1], J. Johnson[1], R. Badal[1], S. Hawser[2], M. Hackel[1], S. Bouchillon[1], B. Johnson[1], D. Hoban[1], M. Dowzicky[3]

[1]International Health Management Associates, Inc., Schaumburg, IL, USA
[2]International Health Management Associates Europe Sàrl, Epalinges, Switzerland
[3]Wyeth Pharmaceuticals, Collegeville, PA, USA

IHMA, Inc.
2122 Palmer Dr.
Schaumburg, IL
60173
Tel: 847.303.5003
Fax: 847.303.5601

Revised Abstract

Background: Carbapenem resistance in *Klebsiella pneumoniae* (KPN) is mainly due to the presence of an acquired carbapenem-hydrolyzing ß-lactamase known as *Klebsiella pneumoniae* carbapenemase (KPC). The KPC enzyme confers resistance to all ß-lactam agents including penicillins, monobactams, and cephalosporins, as well as carbapenems. Since the initial discovery in North Carolina in 2001, KPC enzymes have been reported in several outbreaks in the eastern United States, as well as sporadic cases in France, Colombia, China and Israel. We evaluated 168 ESBL+ KPN isolates from New York and New Jersey (NY/NJ) collected in the 2004 to 2008 Tigecycline Evaluation Surveillance Trial (TEST) for the presence of the KPC gene via PCR. **Methods**: Imipenem (IMI), meropenem (MER) and ertapenem (ERT) MICs were determined for 168 ESBL+ KPN from 22 sites in New York and New Jersey. A subset of isolates was screened for the presence of bla_{KPC} by PCR. All MIC testing was done by manual broth microdilution following CLSI protocols. **Results**: 86 of 168 (51.2%) ESBL+ KPN had carbapenem MICs of $\geq$2. Of these, 83 (96.6%) were positive for the KPC gene. 20 isolates with carbapenem MICs of $\leq$1 were also screened and all were negative for KPC.

N (%) of KPC positive ESBL+ KPN

MIC (mg/L)	ERT	MER	IMI
16	66/66(100)	31/31(100)	18/18(100)
8	12/13(92.3)	9/9(100)	53/54(98.1)
4	4/6(66.7)	11/12(91.6)	12/13(92.3)
2	1/1(100)	2/2(100)	0/1(0)
1	0/4(0)	0/0(0)	0/5(0)

Conclusions: The emergence and rapid spread of KPC is of concern, as therapeutic options against these multi-resistant organisms are limited. *K. pneumoniae* with bla_{KPC} have been present in multiple sites in the NY/NJ area since at least 2004. The prevalence of KPC may be underreported due to the widespread use of commercial automated testing systems. The TEST study isolates were identified through manual microdilution testing, and may give a more accurate depiction of the prevalence of these organisms.

Introduction

Carbapenems, such as ertapenem, imipenem and meropenem, are widely used to treat infections caused by *Enterobacteriaceae* that produce extended-spectrum β-lactamases (ESBL) (1). Although carbapenem resistance in Enterobacteriaceae is relatively rare, the emergence of β-lactamases with activity against such antimicrobials is becoming more frequent. *K. pneumoniae* carbapenemases (KPC) are not solely restricted to *K. pneumoniae* as they have also been detected in other Enterobacteriaceae and in *Pseudomonas aeruginosa*. The majority of such KPC producers have been collected in Northeastern parts of the United States of America with sporadic reports from elsewhere (2).

Several outbreaks of carbapenem-resistant *K. pneumoniae* related to KPC producers have been reported in the United States (3,4). Recently the occurrence of bla_{KPC} carrying isolates appears to be increasing (2) and not restricted to the United States of America, whereby reports of bla_{KPC} genes in isolates in Israel (5) and in South America (6) have also been documented

In the present study, we have evaluated 168 ESBL producing isolates of *K. pneumoniae* from New York/New Jersey collected during the 2004 to 2008 Tigecycline Evaluation Surveillance Trial (TEST) for the presence of the KPC gene via PCR and describe the susceptibility of these isolates to tigecycline, carbapenems and other agents.

Materials & Methods

- All isolates were derived from skin & skin structure, blood, respiratory tract, genitor-urinary, catheter/drain, gastrointestinal and other defined sources. Only one isolate per patient was accepted into the study. Clinical isolates were collected and tested between 2004 and 2008 from 22 study centers. Isolates were identified to the species level and tested at each site by the participating laboratory.
- Organism collection, transport, confirmation of organism identification, and development and management of a centralized database, were coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, IL, USA.
- All organisms were deemed clinically significant by local participant criteria. Isolate inclusion was independent of medical history, antimicrobial use, age, or gender. All sites identified each study isolate utilizing local laboratory criteria.
- Susceptibility testing was performed via manual microdilution testing using CLSI guidelines (7).
- The following oligonucleotide primers were used for amplification [8]:
  - KPC-1-3-F 5' ATGTCACTGTATCGCCGTCT
  - KPC-1-3-R 5' TTTTCAGAGCCTTACTGCCC
- Primers were commercially obtained from Operon Biotechnologies, Inc., Huntsville, AL. iQ Supermix was obtained from Bio-Rad Laboratories, Hercules, CA. DNA was isolated from overnight cultures using the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). PCR for detecting the KPC genetic determinant was performed in 25 µL reaction volumes with the following constituents: 12.5 ul 2X iQ Supermix, 5µL DNA template, 200 nM of primers KPC-1-3-F and KPC1-3-R, and 6.5 ul water. All reactions were performed in duplicate. GeneAmp PCR System 9600 (Perkin Elmer Applied Biosystems) DNA thermal cycler was used for the amplifications. The cycle program was predenaturation for 10 min at 95°C, 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, and 2 cycles of postextension for 7 min at 72°C. Amplicons (10ul) were resolved in a 1.5% agarose (Mo-Bio Laboratories, Inc, Carlsbad, CA) gel containing in 1X Tris-Acetate-EDTA buffer (Fisher Scientific, Itasca, IL) at 100V for 1 hr. Gels were stained for 20 minutes in ethidium bromide in TAE buffer at a concentration of 0.5ug/mL, then destained in distilled water for 20 minutes. All PCR reactions were performed in duplicate. Each experiment included a positive control and two negative controls (ATCC 29213 and boiled filtered water with no added DNA). Isolates resulting in a band of approximately 850 bp were called positive (+). Isolates producing no band were called negative (-).

References

1. Paterson, D.L., *Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs).* Clin Microbiol Infect, 2000. *6:* p. 460-3
2. Rasmussen, J.W. & N, Hoiby, *Class A carbapenemases*. J Antimicrob Chemother, 2007. 60: p. 470-82.
3. Woodford, N, Tierno, P.M.,Jr., Young, K. et al, *Outbreak of Klebsiella pneumoniae producing a new carbapenem-hydrolyzing class A β-lactamase, KPC-3, in a New York medical center.* Antimicrob Agents Chemother, 2004. 48: p. 4793-9.
4. Bratu, S., Landman, D., Haag, R., et al, *Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New Yor City: a new threat to our antibiotic armamentarium*. Arch Intern Med, 2005. 165: p. 1430-5.
5. Navon-Venezia, S., Chmelnitsky, I., Leavitt, A., et al, *Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant clonres in Israel.* Antimicrob Agents Chemother, 2006. 50: p. 3098-101.
6. Villegas, M.V., Lolans, K., Correa, A., et al, *First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of Klebsiella pneumoniae from South America.* Antimicrob Agents Chemother, 2006. 51: p. 2880-2.
7. Clinical Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement*. CLSI document M100-S19. Wayne, PA, 2009.
8. Bradford, P. A., S. Bratu, C. Urban, et al, 2004. *Emergence of carbapenem resistant Klebsiella spp. possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 _-lactamases in New York City*. Clin. Infect. Dis. 39: p55–60.

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Results

Results from this study are shown in the following tables:

Table 1. Infection source and frequency of KPC-positive isolates from a sub-set of *Klebsiella pneumoniae* isolates.

Infection source	No. KPC-Positive / Total	
	New Jersey (n = 54, 10 sites)	New York (n = 53, 12 sites)
Skin & skin structure	5/5	2/6
Blood	6/7	10/12
Catheter / Drain	1/1	2/2
Gastrointestinal	1/1	1/1
Genito / Urinary	14/17	6/14
Other	1/1	1/1
Respiratory	19/22	14/17
Total	47/54 (87%)	36/53 (68%)

Table 3. Activity of tigecycline and carbapenems KPC-positive and KPC-negative isolates of *Klebsiella pneumoniae.*

PCR	Drug/n/Cum%	MIC (mg/L)										
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	$\geq$16
KPC-Negative (n=24)	Tigecycline						3	12	7	1	1	
							12.5	62.5	91.7	95.9	100	
	Ertapenem	4	3	3	4	2	1	4		2	1	
		16.7	29.2	41.7	58.4	66.7	70.9	87.6	87.6	95.9	100	
	Imipenem			1	4	9	2	5	1	1	1	
				4.2	20.9	58.4	66.7	87.5	91.7	95.9	100	
	Meropenem	3	6	7	4	2			1	1		
		12.5	37.5	66.7	83.4	91.7	91.7	91.7	95.9	100		
KPC-Positive (n=83)	Tigecycline					2	27	37	15	2		
						2.4	34.9	79.5	97.6	100	100	100
	Ertapenem								1	4	12	66
									1.2	6	20.5	100
	Imipenem									12	53	18
										14.5	78.4	100
	Meropenem								1	11	9	62
									1.2	14.5	25.3	100

Table 2. Frequency distribution (n) and cumulative percent inhibited of tigecycline and carbapenem at each MICs for 107 KPC-positive and KPC-negative determinations.

PCR	Drug	MIC (mg/L)				%Sus
		MIC_{50}	MIC_{90}	Min	Max	
KPC-Negative (n=24)	Tigecycline	1	2	0.5	8	91.7
	Ertapenem	0.12	4	0.015	8	87.6
	Imipenem	0.25	2	0.06	8	95.9
	Meropenem	0.06	0.25	0.015	4	100
KPC-Positive (n=83)	Tigecycline	1	2	0.25	4	97.6
	Ertapenem	16	$\geq$16	2	$\geq$16	1.2
	Imipenem	8	16	4	16	14.5
	Meropenem	16	$\geq$16	2	$\geq$16	14.5

Conclusions

- Isolates from this study originated from 10 and 12 sites in New Jersey and New York States, respectively. Analysis of the sub-set of 107 isolates confirmed 85 % and 68 % of isolates from New Jersey and New York States, respectively to be KPC-positive.
- KPC-positive isolates were seen only in *K. pneumoniae* that had a carbapenem MIC of 2 mg/L or higher in this study. None were identified with a carbapenem MIC of 1 mg/L of below. KPC-positive isolates were present in all sites irrespective of infection source.
- While carbapenems and tigecycline were active against the majority of KPC-negative isolates, only tigecycline was active against KPC-positive isolates.
- The prevalence of KPC may be underreported due to the widespread use of commercial automated testing systems: TEST study isolates were identified through manual microdilution testing, and may give a more accurate depiction of the prevalence of these organisms.