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Emergence of Klebsiella pneumoniae Isolates Possessing KPC ß-lactamase in Israel, Puerto Rico, Colombia and Greece

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Revised Abstract

Background: *Klebsiella pneumoniae* carbapenemases (KPCs) are carbapenem-hydrolyzing ß-lactamases belonging to Bush group2f, molecular class A. KPC enzymes confer resistance to all ß-lactam agents including penicillins, monobactams, and cephalosporins, as well as carbapenems. KPC ß-lactamases have great potential to spread due to their location on plasmids. While initially limited to the eastern United States, KPC enzymes have recently been reported in France, Colombia, China and Israel. In this report, we describe the detection of KPC enzymes in *K. pneumoniae* (Kpn) isolates collected from 2005 to 2008 in the Tigecycline Evaluation Surveillance Trial (T.E.S.T.) from Israel, Puerto Rico, Colombia, and Greece and additionally describe data from S. Africa, S. Korea, Taiwan, Italy, Argentina and Brazil. **Methods**: Isolates with meropenem (MER) or imipenem (IMI) MIC values of $\geq$4, or ertapenem (ERT) MIC values of $\geq$2 were screened by PCR for the presence of bla_{KPC}, the gene responsible for the KPC enzyme. MICs were determined using manual broth microdilution following CLSI guidelines. **Results**:

Country	Number Tested for KPC	Number positive for KPC (KPC+)	Number of sites with KPC	MIC range for KPC-positive isolates (mg/L)		
				ERT	IMI	MER
Greece	11	1	1	>16	16	32
Israel	14	13	4	>16	8 - >32	8 - >32
Puerto Rico	8	8	1	0.5 - >16	2 - 16	1 - 32
South Africa	5	0	0	-	-	-
South Korea	2	0	0	-	-	-
Taiwan	1	0	0	-	-	-
Italy	1	0	0	-	-	-
Argentina	3	0	0	-	-	-
Brazil	1	0	0	-	-	-
Colombia	4	4	2	4 - 16	2 - 32	1 - >32

Conclusions: Monitoring the world-wide dissemination of KPC outside of the US via the T.E.S.T. global surveillance program revealed isolates in four countries, including the first cases reported from Puerto Rico and Greece. Since therapy of infections caused by these multi-drug resistant organisms is difficult, it is essential to monitor their spread into new regions of the world.

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 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). The occurrence of bla_{KPC} carrying isolates appears to be increasing (2) and not restricted to the United States, whereby reports of bla_{KPC} genes in isolates in Israel (5) and in South America (6) have also been documented. Recently, six KPC-producing *K. pneumoniae* have been isolated in Norway and Sweden that demonstrated clonal relationships to strains in Greece, Israel and Hungry (7).

In the present study, we have evaluated 86 isolates of *K. pneumoniae* from various countries outside of the United States 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 and carbapenems.

Materials & Methods

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, body fluids, 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 21 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 in line with CLSI guidelines (8).
- The following oligonucleotide primers were used for amplification (9):
  - KPC-1-3-F 5' ATGTCACTGTATCGCCGTCT
  - KPC-1-3-R 5' TTTTCAGAGCCTTACTGCCC
  - Segment size: 850 bp
- 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 µl 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 (10µl) 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 0.5mg/L, 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 (-).

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Results

Results from this study are shown in the following tables:

Table 1. Yearly origin of Isolates of *K. pneumoniae.*

Country	2005	2006	2007	2008	Total
Argentina			3		3
Brazil			1		1
Colombia			1	3	4
Greece		4	8		12
Israel	2	4	20		26
Italy			1		1
Korea		1	1		2
Puerto Rico		14			14
South Africa		2	20		22
Taiwan			1		1
Grand Total	2	25	56	3	86

Table 2. Geographical origin of isolates and occurrence of KPC-positive and KPC-negative isolates.

Country	No. of Isolates	PCR KPC-Negative	PCR KPC-Positive
Argentina	3	3	0
Brazil	1	1	0
Colombia	4	0	4
Greece	12	11	1
Israel	26	13	13
Italy	1	1	0
Korea	2	2	0
Puerto Rico	14	6	8
South Africa	22	22	0
Taiwan	1	1	0
Total N	86	60	26

Table 3. Susceptibility of KPC-positive and KPC-negative isolates to tigecycline and carbapenems.

PCR	Drug	MIC (mg/L) MIC_{50}	MIC_{90}	Min	Max	%Sus*
KPC Negative (n=60)	Tigecycline	0.5	2	0.25	4	96.6
	Ertapenem	0.12	>16	$\leq$0.008	>16	63.4
	Imipenem	0.5	16	0.025	>32	80.1
	Meropenem	0.06	16	0.03	>32	75
KPC Positive (N=26)	Tigecycline	1	2	0.25	2	100
	Ertapenem	>16	>16	0.5	>16	3.8
	Imipenem	16	32	2	>32	19.2
	Meropenem	>32	>32	0.25	>32	19.2

*Percent of isolates susceptible to each agent. Susceptibility based on FDA breakpoints for tigecycline. For other agents CLSI (7) breakpoints were used.

Table 4. Frequency distribution and cumulative percent inhibited activity of tigecycline and carbapenems against 86 *K. pneumoniae* classified by KPC.

PCR	Drug	MIC (mg/L) $\leq$0.03	0.05	0.06	0.12	0.25	0.5	1	2	4	8	16	$\geq$32
KPC Negative (N=60)	Tigecycline					3	29	14	12	2			
						5	53.3	76.6	96.6	100			
	Ertapenem	21		7	4	2	2	1	1	1	3	2	16
		35	35	46.7	53.4	56.7	60	61.7	63.4	65.1	70.1	73.4	100
	Imipenem	1	1		1	18	14	4	8	1	3	3	6
		1.7	3.4	3.4	5.1	35.1	58.4	65.1	78.4	80.1	85.1	90.1	100
	Meropenem	9		21	4	5		1		5	4	5	6
		15	15	50	56.7	65	65	66.7	66.7	75	81.7	90	100
KPC Positive (n=26)	Tigecycline					1	7	10	8				
						3.8	30.7	69.2	100	100	100	100	100
	Ertapenem						1			4	2		19
							3.8	3.8	3.8	19.2	26.9	26.9	100
	Imipenem								2	3	3	5	13
									7.7	19.2	30.7	50	100
	Meropenem					1		2	2		3		18
						3.8	3.8	11.5	19.2	19.2	30.7	30.7	100

Conclusions

- Monitoring the world-wide dissemination of KPC outside of the US via the T.E.S.T. global surveillance program revealed isolates in four countries, including the first cases reported from Puerto Rico and Greece and new cases from Colombia and Israel.
- The majority of KPC-negative isolates were susceptible to Tigecycline, ertapenem, imipenem and meropenem with % susceptibilities of 96.6, 63.4, 80.1 and 75 %, respectively.
- All KPC-positive isolates were susceptible to Tigecycline only.
- Further monitoring of KPC occurrence in various countries is warranted.