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Outbreak of KPC-2-producing *Klebsiella pneumoniae* Isolates in Two Texas Hospitals: Report from the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Background: KPC-producing Enterobacteriaceae have been described in various USA regions. However, the occurrence of these strains is somehow limited in the Southwest. We noted a recent increase in the prevalence of carbapenem-resistant Enterobacteriaceae in Texas hospitals participating in the SENTRY Antimicrobial Surveillance Program and this study describes the molecular characterization of these isolates.

Methods: A total of 23 carbapenem-resistant Enterobacteriaceae strains (imipenem or meropenem MIC values of ≥2 μg/mL) from Texas hospitals were evaluated. Isolates were screened using Modified Hodge Test (MHT) and PCR targeting carbapenemase-encoding genes. Amplicons were sequenced. *K. pneumoniae* KPC-producers were typed by PFGE. S1 nuclease and ICeuI preparations were resolved in agarose gels, blotted and hybridized with a *bla*_{KPC} probe. The *bla*_{KPC}-carrying element (Tn*4401*) was amplified with various primer pairs, digested with Eag I and different profiles were sequenced.

Results: Among 879 Enterobacteriaceae strains from three Texas hospitals submitted to the SENTRY Program, 23 (2.6%) isolates were carbapenemresistant. These strains belonged to four bacterial species (K. pneumoniae [20 strains], S. marcescens [one strain], E. aerogenes [one strain], C. freundii [one strain]) and 18 (2.0%) isolates were *bla*_{KPC-2} positive by PCR and MHT: 18 K. pneumoniae and one C. freundii. No other carbapenemases were detected. KPC-2producers were collected in 2008 (3/150 strains; 2.0%) and 2010 (15/289 strains; 5.1%). Three clusters were detected among KPN: PFGE type A (eight strains; three subtypes, Hospital B, 2008 and 2010), type B (five strains, four subtypes, Hospitals A and B, 2010) and type E (two strains; Hospital B, 2010). The remaining two strains were genetically unique. All strains carried *bla*_{KPC} on plasmids, one harbored multiple plasmids carrying this gene. 14 strains carried *bla*_{KPC-2} on a copy of Tn*4401*a and one on Tn*4401*b. Three strains had differences in the genetic structure carrying the carbapenemase gene, two carried a Tn3 transposase upstream of *bla*_{KPC-2} and the other had two adjacent copies of *bla*_{KPC}.

Conclusions: Surveillance in prior years show sporadic detection of KPC-producers in Texas, however, a recent increase in carbapenem-resistant Enterobacteriaceae in this state was due to the spread of two KPC-producing *K. pneumoniae* clusters. Further surveillance and implementation of infection control measures seem prudent.

INTRODUCTION

Enterobacteriaceae have lately been responsible for a significant number of infections and deaths in the United States and worldwide, and have created a significant healthcare threat in both acute and long-term care facilities. In addition to the high prevalence rate of Enterobacteriaceae causing nosocomial infections, the antimicrobial resistance rates among these pathogens continue to rise. Initially caused by the dissemination of extended-spectrum β -lactamase-encoding genes during the 80 and 90's (more recently $bla_{\text{CTX-M}}$), bla_{KPC} has been an additional reason for the increased resistance rates, not only to penicillins and cephalosporins, but also to carbapenems.

*bla*_{KPC} was first detected in a *Klebsiella pneumoniae* clinical isolate collected in 1996 from a patient hospitalized in North Carolina, and the encoded enzyme remains the most clinically significant β-lactamase among Class A derivatives. Several outbreaks of KPC-producing Enterobacteriaceae have been reported and these strains have become endemic in the Northeast region of the USA. However, this gene has also been detected in other USA regions, and has now emerged and disseminated worldwide, affecting Israel, Asia, Europe, Canada, as well as Central and South America, South Africa and Australia.

Overall, the dissemination of $bla_{\rm KPC}$ has been associated with clonally-related strains. Moreover, there is mounting evidence that $bla_{\rm KPC}$ genes are also consistently associated with a specific genetic element (i.e. transposon ${\rm Tn}4401$). ${\rm Tn}4401$ is a ${\rm Tn}3$ -like transposon that has been identified in distinct plasmids carried by KPC-producing Enterobacteriaceae and Pseudomonas aeruginosa isolates from different geographic areas. In addition, the majority of $bla_{\rm KPC-2}$ -carrying K. pneumoniae belong to multilocus sequence type (MLST) 258 and double-locus variants, which may have contributed to the worldwide dissemination of KPC-producing strains. In this study, we report a recent increase in the prevalence of carbapenem-resistant Enterobacteriaceae in Texas hospitals participating in the SENTRY Antimicrobial Surveillance Program and provide the molecular epidemiology characteristics of these isolates.

MATERIALS AND METHODS

Bacterial isolates. A total of 879 Enterobacteriaceae clinical isolates were submitted from three hospitals in Texas (372, 177 and 330 from Hospital A [Houston], B [Houston] and C [Galveston], respectively) as part of the SENTRY Program (2007-2010). Strains exhibiting MIC results for imipenem or meropenem of ≥2 μg/mL were selected for further analysis. Identification was performed by the submitting medical institution using local methodologies and confirmed by the monitoring central laboratory (JMI Laboratories, North Liberty, lowa) by standard algorithms and the automated Vitek 2 system (bioMérieux, Hazelwood, Missouri), when necessary.

Antimicrobial susceptibility testing. Isolates were tested for susceptibility by the broth microdilution method using 96-well panels with cation-adjusted Mueller-Hinton broth according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. Validation of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S22) quality control (QC) strains: Escherichica coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. In addition, the inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event.

Genotypic characterization of β-lactamases. Screening for carbapenemase-encoding genes (*bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM-1}, *bla*_{KPC}, *bla*_{SME}, *bla*_{IMI}, *bla*_{NMC-A}, *bla*_{GES} and *bla*_{OXA-48}) was performed by standard PCR. Amplicons were purified and both strands were subjected to sequencing. Nucleotides and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Molecular typing. KPC-producing *K. pneumoniae* isolates were selected for epidemiology typing. Clonality was evaluated by pulsed-field gel electrophoresis (PFGE). Genomic DNA was prepared in agarose blocks and digested with Spe I (New England, Beverly, Massachusetts). Electrophoresis was performed on the CHEF-DR II (BioRad, Richmond, California) as described elsewhere. One representative strain of each PFGE type was selected for multilocus sequence typing (MLST) according to the instructions on the website

http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html.

Gene location and transposon analysis. Total cellular DNA embedded in 1% agarose plugs was subjected to partial digestion with S1 nuclease. Plasmids were resolved by electrophoresis performed on the CHEF-DR II (BioRad), with the following conditions: 0.5 x TBE, 1% agarose, 13°C, 200V, for 6 hours with switch time ramping from 5 to 25 seconds and 14 hours with the switch time from 30 - 45 seconds. ICeul digested genomic DNA was also resolved on PFGE. DNA gels were transferred to nylon membranes by southern blotting and hybridized with a digoxigenin labeled *bla*_{KPC}-specific probe (Roche Diagnostics GmbH, Mannheim, Germany). The blakeccarrying element (Tn4401) was amplified with several primers and PCR products digested with Eag I. Those amplicons exhibiting an unusual digestion profile were sequenced.

RESULTS

- Among strains received from three medical institutions in Texas from 2007 through 2010, 23 (2.6% of total) isolates collected from Hospitals A and B (both in Houston) met the screening criteria for carbapenemase production (Table 1). These strains belonged to four bacterial species, as follows: *K. pneumoniae* (20 strains), *Serratia marcescens* (one strain), *Enterobacter aerogenes* (one strain), *Citrobacter freundii* (one strain).
- All 23 selected strains were recovered from blood cultures and displayed elevated MIC results for the majority of antimicrobial agents tested, except for tigecycline (0.12 2 μg/mL). Seven of 20 (35.0%) *K. pneumoniae* were also resistant to colistin (Table 1).
- bla_{KPC-2} was detected in 18 (2.0% of total) isolates (17 *K. pneumoniae* and one *C. freundii*) and these strains were collected in 2008 (3/150 strains; 2.0%) and 2010 (15/289 strains; 5.1%; Table 2).
- Isolates clustering within PFGE A were collected from Hospital B in 2008 (two strains) and 2010 (six strains). Five KPC-2-producers grouped within PFGE type B and were collected during 2010 from both Hospitals A and B. Two other genetically-related strains (PFGE type E) were collected from Hospital B (2010) and one remaining strain each from Hospital A and B had an unique PFGE band patterns (C and D, 2010).
- All KPC-2 producers carried at least two plasmids and strains showing PFGE patterns A1-3, B1, C and E possessed very similar *bla*_{KPC-2}-harboring plasmids in size (ca. 25-kb; Figure 1A and 1B). Similarly, strains displaying PFGE profiles B3 and B2 had a *bla*_{KPC-2}-carrying plasmid of ca. 80-kb, while *K. pneumoniae* with PFGE patterns B4 and D showed smaller *bla*_{KPC-2} plasmid bands (ca. 50-kb).
- The *bla*_{KPC-2}-carrying *C. freundii* appeared to possess three large plasmid bands of ca. 200-, 290- and 400-kb (Figure 1). Hybridization signals were observed in the 200- and 400-kb bands (Figure 1A and 1B).
- One and 14 strains carried bla_{KPC-2} on copies of Tn4401b and Tn4401a, respectively. Three strains had differences in the genetic structure carrying the carbapenemase gene, two carried a Tn3 transposase upstream of bla_{KPC-2} and the other had two adjacent copies of bla_{KPC}.
- One representative K. pneumoniae strain from each PFGE type was selected for further MLST analysis. Strains displaying PFGE type A and E were associated to ST258, while those showing PFGE types B, C and D were ST307, ST901 and ST494, respectively (Table 2).

Table 1. Enterobacteriaceae clinical isolates collected in two medical institutions in Texas (2007-2010) that met the screening criteria and were selected for the study.

laalata			Haa						MI	C (µg/r	nL)ª								A -l : :	Oultons	
Isolate No.	Organism	Year	Hos pital	P/T	AZT	CAZ	CRO	СРМ	IPM	MER	AMK	CIP	COL	GEN	TET	TGC	Age	Sex	Admission Date	Culture Date	ICUb
13731	K. pneumoniae	2007	В	>64	>16	>32	>32	>16	1	2	32	>4	≤0.5	>8	≤1	0.12	57	F	16-Nov-07	05-Dec-07	No
3646	C. freundii	2008	В	>64	>16	16	32	8	4	4	0.5	4	≤0.5	>8	4	1	81	M	31-Mar-08	18-May-08	No
14034	K. pneumoniae	2008	В	>64	>16	>32	>32	>16	1	2	32	>4	≤0.5	>8	4	0.5	58	F	27-Nov-08	05-Dec-08	No
13998	K. pneumoniae	2008	В	>64	>16	>32	>32	16	>8	8	32	>4	>4	≤1	4	1	68	F	09-Oct-08	25-Oct-08	No
13989	K. pneumoniae	2008	В	>64	>16	>32	>32	>16	>8	8	32	>4	>4	≤1	8	1	68	F	04-Jul-08	05-Oct-08	No
9508	K. pneumoniae	2009	В	>64	>16	>32	>32	>16	2	4	1	>4	>4	>8	>8	0.5	53	F	01-Jul-09	15-Sep-09	Yes
48293	E. aerogenes	2010	В	>64	>16	>32	>32	>16	4	8	2	>4	≤0.5	>8	>8	0.25	67	M	03-Dec-10	04-Dec-10	No
17098	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	0.5	>4	≤0.5	8	>8	0.5	58	M	16-Jul-10	17-Jul-10	Yes
48277	K. pneumoniae	2010	В	>64	>16	>32	>32	16	8	8	32	>4	≤0.5	≤1	4	0.5	61	F	24-Nov-10	03-Dec-10	No
38892	K. pneumoniae	2010	В	>64	>16	8	>32	4	8	8	1	≤0.03	>4	≤1	2	0.25	63	F	09-Nov-10	10-Nov-10	No
17070	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	8	>8	32	>4	>4	2	4	0.5	72	M	07-Jun-10	08-Jun-10	Yes
78	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	8	>8	32	>4	≤0.5	≤1	4	0.5	57	M	22-Dec-09	07-Jan-10	No
15322	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	32	>4	≤0.5	2	4	0.5	57	F	24-Mar-10	18-May-10	No
15329	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	16	>4	≤0.5	≤1	4	0.5	57	F	10-May-10	22-May-10	No
15334	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	32	>4	>4	2	4	1	72	M	28-May-10	29-May-10	Yes
12028	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	32	>4	>4	≤1	4	0.5	51	M	16-Mar-10	17-Mar-10	No
48279	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	32	>4	≤0.5	2	4	1	83	F	03-Dec-10	04-Dec-10	Yes
48281	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	>32	>4	≤0.5	2	4	1	58	F	18-Nov-10	03-Dec-10	Yes
34813	K. pneumoniae	2010	В	>64	>16	>32	>32	>16	>8	>8	32	>4	≤0.5	>8	>8	0.25	59	F	11-Oct-10	12-Oct-10	Yes
14091	S. marcescens	2007	Α	2	0.5	≤1	2	1	2	2	4	0.12	>4	≤1	>8	0.5	NA^c	NA	NA	NA	NA
37523	K. pneumoniae	2010	Α	>64	>16	32	>32	16	8	>8	1	>4	≤0.5	≤1	>8	1	NA	NA	NA	NA	NA
37516	K. pneumoniae	2010	Α	>64	>16	>32	>32	>16	>8	>8	1	>4	≤0.5	>8	8	1	50	M	NA	19-Aug-10	NA
34494	K. pneumoniae	2010	Α	>64	>16	>32	>32	>16	>8	>8	0.5	>4	1	>8	>8	2	83	F	NA	06-Jun-10	NA

Figure 1A. S1 partial-digested genomic DNA of *bla*_{KPC-2}-carrying *K. pneumoniae* and *C. freundii* (no. 3646) strains collected from Hospitals A and B. PFGE typing results obtained for *bla*_{KPC-2}-carrying *K. pneumoniae* are also shown (NA, reads not applicable [*C. freundii*]). λ represents Lambda ladder PFGE marker also used as negative control (New England Biolabs, Ipswich, Massachusetts). **1B**. Hybridization signal (horizontal arrow) obtained with a *bla*_{KPC-2}-specific probe.

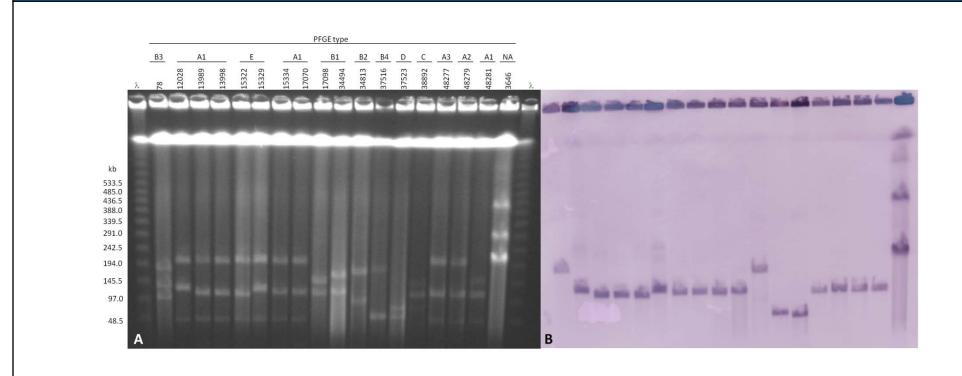


Table 2. Molecular epidemiology results of *bla*_{KPC-2}-carrying *K. pneumoniae* strains collected from Hospitals A and B in Texas.

		_	Epidemiol	ogy typing			_	Epidemiology typing		
Isolate No.	Year	Hospital	PFGE	MLST	Isolate No.	Year	Hospital	PFGE	MLST	
13989	2008	В	A1		34494	2010	Α	B1		
13998	2008	В	A1		34813	2010	В	B2		
12028	2010	В	A1		37516	2010	Α	B4		
15334	2010	В	A1	258	78	2010	В	В3		
17070	2010	В	A1		38892	2010	В	С	901	
48281	2010	В	A1		37523	2010	Α	D	494	
48279	2010	В	A2		15322	2010	В	Е	258	
48277	2010	В	A3		15329	2010	В	Е		
17098	2010	В	B1	307						

CONCLUSIONS

- Among the clinical isolates from three medical institutions in Texas submitted as part of the SENTRY Program, three (2.0%) bla_{KPC-2}-carrying strains were detected in the 2008 sampling year, followed by an increase in the number of KPC-producing isolates (15/289; 5.1%) in 2010 (P=0.1098; OR=0.37 [0.08-1.40]).
- The increase in number of KPC-2 producers was mostly due to the spread of two clonal clusters (PFGE type A and B) associated with different lineages of *K. pneumoniae* (ST258 and ST307). Plasmid dissemination also seemed to have played a role regarding the spread of bla_{KPC-2}, since similar bla_{KPC-2}-harboring plasmids were detected in strains from distinct PFGE and MLST types.
- The prevalence of KPC-2 producers in Texas remains low. However, this investigation shows a trend towards increased rates over the study interval (2007-2010), warranting continued surveillance and implementation of infection control measures to minimize the dissemination of such strains.

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