C1-125
ICAAC/IDSA 2008
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Polymyxin-Resistant KPC-3-Producing *K. pneumoniae* in the USA and Israel: Challenging Therapies and Molecular Typing Methods

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: KPC-producing isolates are reported in the USA and Israel, limiting the clinical utility of most ß-lactams. Polymyxins are among the last-resort therapies to treat infections caused by these pathogens. We investigated 4 polymyxin resistant KPC-producing *K. pneumoniae* collected in 2007 as part of the SENTRY Antimicrobial Surveillance Program.

Methods: *K. pneumoniae* isolates (1 from USA, 3 from Israel) were tested for bla_{KPC} by PCR. Susceptibility testing used CLSI broth microdilution methods. Amplicons were sequenced on both strands. Clonality was assessed by PFGE. Plasmid extractions and conjugation were also performed. Plasmid digestions were carried out with Dra I. The bla_{KPC} genetic element (Tn*4401*) was amplified, digested with Eag I and sequenced.

Results: All 4 isolates harbored bla_{KPC-3} and were resistant to all agents tested, except tigecycline. PFGE showed isolates were similar. Isolates carried multiple plasmids. Transconjugants harbored plasmids of the same sizes (80 Kb), but different susceptibility profiles (USA isolate yielded aminoglycoside-resistant tranconjugants). Polymyxin resistance was not transferred. All isolates showed the bla_{KPC-3} flanking region was identical to Tn*4401*. USA isolate possessed an insertion of 1.2 Kb downstream of bla_{KPC}, corresponding to the IS*1294*. Dra I digestions of transconjugants plasmids indicated different structures.

Conclusions: We initiated this study to evaluate the possible dissemination of very unusual polymyxin resistant KPC-producing *K. pneumoniae* between two nations. Initially the isolates appeared related, but further molecular tests showed that bla_{KPC-3} genetic backgrounds were distinct. This study demonstrates the importance of thorough molecular investigation. In the USA isolate, Tn*4401* was associated with IS*1294*, an insertion sequence that can mobilize resistance genes similarly to ISCRs increasing the potential of dissemination of bla_{KPC-3}. Israel strains were significantly different.

INTRODUCTION

The gene encoding KPC was initially discovered among *Klebsiella pneumoniae* isolates in hospitals located in the New York City area and North Carolina. Isolates carrying this gene have recently been described in other parts of the United States (USA) and in several other nations, including China, Colombia, France, Argentina and Israel. KPC production that has been considered an endemic problem among *K. pneumoniae* in the USA has also been detected among other Enterobacteriaceae species, emphasizing the risk of interspecies dissemination of resistance genes.

KPC-encoding genes were recently found to be carried on Tn*3*-based transposon, named Tn*4401*, which seems to be involved in acquisition and dissemination of these genes. In addition to the *tnpA* transposase, Tn*4401* possesses the resolvase *tnpR*, the bla_{KPC} gene, and two ISs, IS*Kpn6* and IS*Kpn7*. Tn*4401* was present in all the strains initially tested and portions of this transposon have been identified in every sequence of bla_{KPC}-like genes submitted to the GenBank database. The overall structure of Tn*4401* seemed to be conserved; however a 100-bp deletion was observed upstream of the bla_{KPC} gene in some isolates.

The polymyxins (colistin and polymyxin B) can also be an effective therapeutic alternative for the treatment of KPC-producing *K. pneumoniae*; however, resistance against these compounds is also emerging. In this study, we evaluated four polymyxin B resistant KPC-producing *K. pneumoniae* collected in Israel and New York City that seem to be genetically related, but further molecular evaluation demonstrated different plasmids. Additionally, in one of the strains, a copy of the IS*1294*, a rolling circle transposase, was found within the bla_{KPC}-carrying element that has not been previously related to carbapenemase encoding genes.

MATERIALS AND METHODS

Bacterial isolates. Four polymyxin B-resistant KPC-producing *K. pneumoniae* isolates collected during the SENTRY Antimicrobial Surveillance Program in 2007 were evaluated. The isolates were susceptibility tested against more than 25 antimicrobial agents by the broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI, 2008).

Molecular typing. Isolates were evaluated for clonality by pulsed-field gel electrophoresis (PFGE). Genomic DNA was prepared in agarose blocks and digested with Spe I (New England Biolabs, Beverly, MA). Electrophoresis was performed on the CHEF-DR II (BioRad, Richmond, CA), with the following conditions: 0.5 x TBE, 1% agarose, 13°C , 200V, for 23 h with the switch time ramped from 5 to 60 seconds.

PCR experiments and DNA sequencing. Custom oligonucleotides were used to amplify and sequence the KPC-encoding gene. Three sets of primers targeting the structures of Tn*4401* and bla_{KPC} were used to amplify the surrounding 10-Kb region of KPC-encoding gene. Amplicons obtained were digested with Eag I and compared to the RFLP pattern generated by the Tn*4401* sequence from GenBank (EU176011 and EU176014). Amplicons showing differences from this structure were sequenced on both strands. The nucleotide sequences and deduced amino acid sequences were analyzed using Lasergene software package (DNASTAR, Madison, WI) and compared with sequences available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Plasmid analysis and conjugation. Plasmid extractions were performed using the Plasmid MIDI kit (QIAGEN, Hilden, Germany). Plasmid preparations were resolved in 1% agarose gels and the molecular weights were determined by comparison with plasmids harbored by *E. coli* NCTC 50192 and 50193. Plasmid restriction profiles were determined after digestions of plasmid samples with Dra I followed by gel electrophoresis.

Transference of ß-lactam resistance determinants were assayed by mating experiments in broth by mixing equal volumes of KPC-producing donor and recipient *E. coli* in the exponential phase of growth. The recipients were selected according to the susceptibility profile of the donor clinical strain and *E. coli* J53 derivatives resistant to azide (Az^R) or K12 derivatives resistant to streptomycin (Sm^R) were used in the experiments. Transconjugants were selected in agar plates containing sodium azide or streptomycin (200 µg/ml) and ceftazidime (8 µg/ml). The presence of bla_{KPC} and species identification of the transconjugant strain was confirmed by PCR. Colonies were susceptibility tested with CLSI broth microdilution method.

RESULTS

- Three polymyxin B-resistant KPC-producing *K. pneumoniae* isolates from Israel and one from New York City, USA were detected during the SENTRY Program (2007).
- All four isolates harbored bla_{KPC-3}. The isolate from New York was resistant to almost all antimicrobial agents tested, being susceptible only to tigecycline. The isolates from Israel were susceptible when tested against gentamicin and also against tigecycline (Table 1).
- PFGE patterns for all isolates were similar, varying from 1 to 3 bands. Two of the three isolates from Israel were identical (Figure 1).

Table 1. Antimicrobial susceptibility profiles of polymyxin B resistant KPC-producing *K. pneumoniae* (KPN) isolates and *E. coli* (EC) transconjugant strains.

	MIC (µg/ml)							
Antimicrobial Agent	KPN 15-2771D	KPN 63-6598A	KPN 63-9215A	KPN 63-10848A	ECJ53 p2771D	EC J53 Az^R	ECK12 p10848A	EC K12 Sm^R
Imipenem	>8	>8	>8	>8	4	0.5	4	0.25
Meropenem	>8	>8	>8	>8	4	≤0.12	4	≤0.12
Aztreonam	>16	>16	>16	>16	>16	≤0.12	>16	≤0.12
Cefepime	>16	>16	>16	>16	16	≤0.12	8	≤0.12
Piperacillin/Tazobactam	>64	>64	>64	>64	>64	2	>64	2
Polymyxin B	>4	>4	>4	>4	0.5	0.5	0.5	0.5
Colistin	>4	>4	>4	>4	≤0.5	≤0.5	≤0.5	≤0.5
Ciprofloxacin	>4	>4	>4	>4	≤0.03	≤0.03	≤0.03	≤0.03
Trimethoprim/Sulfamethoxazole	>2	>2	>2	>2	>2	≤0.5	≤0.5	≤0.5
Amikacin	32	32	32	>32	32	2	4	4
Gentamicin	8	≤2	≤2	≤2	>8	≤2	≤2	≤2
Tigecycline	0.5	0.5	0.5	0.5	0.12	0.12	0.12	0.06

Figure 1. PFGE patterns of the four polymyxin B resistant KPC-producing *K. pneumoniae* isolates found in Israel (63-6598A, 63-9215A and 63-10848A) and New York City (15-2771D) during the 2007 SENTRY Program.

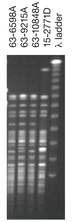
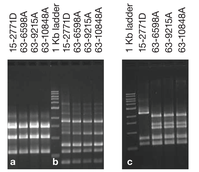
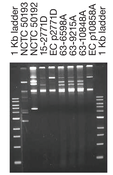


Figure 2. Eag I RFLP patterns of amplicons corresponding to the Tn*4401* transposon carrying bla_{KPC}. Panels (a) and (b) show amplicons of the upstream region of bla_{KPC-3} and panel (c) correspond to the downstream region of this gene. Isolate 15-2771D showed a different restriction pattern and sequencing revealed the presence of IS*1294*.



- The 10-Kb Tn*4401* element was amplified from all four isolates. The bands obtained had the expected sizes for all strains from Israel. The USA isolate possessed an insertion of 1.2-Kb downstream of bla_{KPC-3} (Figure 2) that corresponded to a copy of the IS*1294*.
- The Eag I RFLP showed a genetic arrangement that was consistent with the Tn*4401* structure previously described in KPC-producing strains (Figure 2).
- Isolates displayed multiple plasmids with sizes varying from 19 to 85-Kb (Figure 3).
- Conjugation was performed for one isolate from each country and bla_{KPC-3}-carrying transconjugants were obtained with both strains (Figure 3).
- Plasmids of 80-Kb were detected in both transconjugants. However, the colonies obtained showed distinct susceptibility patterns (Table 1).
- Dra I digestion patterns of transconjugant plasmids were different indicating distinct sequences in the genetic structures.

Figure 3. Plasmid profiles of the polymyxin B resistant KPC-3-producing *K. pneumoniae* clinical isolates and the *E. coli* (EC) transconjugants carrying bla_{KPC-3}.



CONCLUSIONS

- The isolates evaluated in this study were initially considered to be genetically related and possibly representing clonal dissemination within and/or between medical centers. However, a thorough molecular evaluation showed that elements carrying bla_{KPC-3} were distinct indicating different sources of acquisition of this gene.
- IS*1294* is an IS that differs from most elements of this class, in that it replicates by rolling circle and was shown to be able to mobilize surrounding resistance genes. The role of IS*1294* in the mobilization or dissemination of bla_{KPC} is still to be determined.
- KPC-producing strains are an emerging problem worldwide. The association of this resistance mechanism with polymyxin B resistance narrows even more the limited alternatives for the treatment of infections caused by these isolates. Tigecycline was the only agent that was active against these four strains.

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