First Report on Hyper-Epidemic Clone of KPC-3 Producing *Klebsiella pneumoniae* in Israel Genetically Related to a Strain Causing Outbreaks in the United States

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**Short title:** KPC-3 producing *Klebsiella pneumoniae* in Israel genetically related to a clone in the US

**Key words:** epidemics, KPC, genetic similarity, plasmids, carbapenem resistance
A highly epidemic carbapenem-resistant clone of KPC-3-producing *Klebsiella pneumoniae* emerged in Israel in 2006, causing a nationwide outbreak. This clone was genetically related to US outbreak strains isolated in 2000 but differed in KPC-carrying plasmids. The threat of global spread of hyper-epidemic extensively-drug-resistant bacterial strains should be recognized and confronted.
KPC-type enzymes in carbapenem-resistant *Klebsiella pneumoniae* strains were first reported in 2001 in North Carolina (16), and until 2005, the geographical distribution of these enzymes in *K. pneumoniae*, both KPC-2 and KPC-3, was limited to the eastern United States (1, 12, 15). In the New York area, KPC-producing strains have become a frequently-encountered nosocomial pathogen (2, 4). The first case of KPC-producing *K. pneumoniae* outside the US occurred in France where a patient who had been hospitalized in NY carried the strain with him (9). KPC-producing *K. pneumoniae* were reported since then from Israel (6), Colombia (13), China (14), and Greece (8).

Carbapenem resistance in *Klebsiella pneumoniae* carrying *bla*KPC-2 was first observed in Tel Aviv Sourasky Medical Center in late 2005. In February 2006, we noted a sharp increase in *bla*KPC-carrying carbapenem-resistant *K. pneumoniae* strains in our hospital, mainly possessing *bla*KPC-3 (6). These extensively drug resistant (XDR) isolates caused difficult-to-treat infections and had an adverse impact on patients' outcomes (11). Despite infection control efforts that limited the spread of KPC-2 producing clones of *Klebsiella* in our hospital, KPC-3-producing *K. pneumoniae* isolates continued to appear rapidly during 2006, in our hospital and in other hospitals throughout the country (10).

To characterize the extent of the nation-wide occurrence of KPC-3-producing carbapenem resistant *K. pneumoniae* in Israel, 100 single-patient isolates collected during 2006 in eight hospitals and five chronic care centers with wide geographical distribution in the North, center and in the Southern part of the country, were sent to our lab for further study. Six to eight isolates were included from each institution based on their resistance to at least one carbapenem antibiotic (imipenem and or meropenem) using the Vitek2 automated system (bioMerieux, Marcy l'Etoile, France) with an AST-GN09 card. The presence of *bla*KPC in all isolates was verified using PCR reactions.
followed by sequencing to determine the type of KPC gene (6). MICs of imipenem, meropenem, and ertapenem were determined by agar dilution according to the Clinical and Laboratory Standards Institute (3). Susceptibility testing for tigecycline was performed via Etest according to the manufacturer's instructions (AB Biodisk, Solna, Sweden). Interpretive criteria for tigecycline MICs were defined based on the United States Food and Drug Administration breakpoint criteria for *Enterobacteriaceae* (susceptible ≤2 µg/ml, intermediate 4 µg/ml, resistant ≥8 µg/ml). The genetic relatedness of all carbapenem-resistant *K. pneumoniae* strains was determined by pulsed-field gel electrophoresis (PFGE) analysis. DNA was prepared as described previously (6), and chromosomal restriction fragments obtained after SpeI or ApaI cleavage were documented and compared.

Sites of isolation included urine (n = 46), blood (n = 19), respiratory tract (n = 15), wounds (n=15) and other (n=5). Isolates were resistant to nearly all antimicrobial agents including all cephalosporins, beta-lactam/beta-lactamase inhibitor combinations, trimethoprim-sulfamethoxazole and fluoroquinolones; MICs of the carbapenems varied (Table 1). The majority of the isolates (98%) were susceptible only to gentamicin and colistin, while the other 2% were gentamicin resistant and kanamicin susceptible or resistant to both aminoglycosides. Among 20 isolates tested for tigecycline MICs were 1-3 µg/ml. PFGE analysis revealed all isolates belonged to the same genetic clone, indistinguishable from the KPC-3-producing clone described previously in our hospital (6) (figure 1A). This finding suggested nationwide spread of an epidemic carbapenem resistant *K. pneumoniae* strain, designated clone Q.

PCR and sequencing revealed the presence of *bla*KPC-3 in all isolates.

When the Israeli outbreak started, the only country in which KPC-producing *K. pneumoniae* had been reported was the United States. Therefore we compared the
hyper-epidemic strain isolated in Israel to a collection of 26 KPC-producing, carbapenem-resistant *K. pneumoniae* outbreak isolates from the US. These US isolates originated from patients with diverse infections who were hospitalized in five different states between 2000 and 2006 (Table 1).

Testing for genetic relatedness between the Israeli hyper-epidemic XDR clone and the American isolates revealed that nine (35%) of the 26 KPC-3-producing US isolates had PFGE profiles identical or highly similar to each other (Figure 1B). These nine isolates represented outbreaks that occurred in New York (NY) Medical Center in 2000 (15), and in New-Jersey (NJ) and Arizona (AZ) in 2006. Four of these nine isolates were indistinguishable by PFGE from the Israeli epidemic clone and the remaining five isolates were closely related to that (with differences of up to three bands). The other 17 isolates, representing an outbreak during 2004 in a NY Medical Center, and clinical isolates identified in DE, MD, NJ and PA, during 2006 differed from the common PFGE type by more than seven bands.

Plasmid analysis was performed to compare whether the highly genetically related Israeli-American *K. pneumoniae* clone carried the same KPC-3-encoding plasmid. Ten isolates were analyzed; five isolates from Israel (from 2006 to 2008) and five isolates from the US (from 2000 to 2006). Plasmid DNA was purified using NucleoBond PC100 plasmid Midi kit (Macherey-Nagel GmbH, Duren, Germany) and transformation was performed into *E. Coli* GeneHogs (Invitrogen Corp., Dorset, UK). Transformants were selected on Luria-Broth agar plates with ampicillin (100 µg/ml), and selected colonies were subjected to *bla*<sub>KPC</sub> PCR screening to confirm the acquisition of a KPC-encoding plasmid. KPC-encoding plasmids were purified from 10 transformants originating from the 10 genetically-related Israeli and American isolates (Figure 2A). All five Israeli isolates carried the same plasmid with an apparent molecular weight of 100 kb. The
genetically related US isolates carried various plasmids ranging in size from 165 kb to 38 kb, which differed from the typical KPC-3-encoding Israeli plasmid (Figure 2B).

Transfer of the KPC-3-encoding plasmids to the susceptible *E. coli* GeneHogs recipient strain raised the MICs of extended-spectrum cephalosporins and aztreonam by more than 60-fold rendering resistance. MICs of carbapenems, although increased (from 0.012 µg/ml to 0.016-0.5 µg/ml for meropenem and ertapenem, and from 0.094 µg/ml to 0.125-1.0 µg/ml for imipenem) were not in the resistant range. This observation may be due to the presence of additional mechanisms of carbapenem resistance in these strains such as porin alterations (17).

Our finding of the occurrence of closely related *K. pneumoniae* strains carrying *bla*<sub>KPC-3</sub> on different plasmids in two continents is intriguing.

This report on international occurrence of a clone of XDR *bla*<sub>KPC-3</sub>-producing *K. pneumoniae* which has caused outbreaks in the US and Israel, emphasizes the potential for transmission of highly resistant Gram negative pathogens that are difficult and in some cases, impossible, to treat with currently available antimicrobial agents. This situation is exacerbated by the lack of novel agents in the antimicrobial pipeline to treat these pathogens. Our finding of similar strains in the US and Israel raises the possibility of a clonal XDR strain of *K. pneumoniae*, akin to what has been described for the 300.0114 strain of MRSA (5) and the NAP1/027 strain of *C. difficile* (7). This issue will require further investigation, but may help inform future studies directed at characterizing the molecular mechanisms, environmental factors, and selection pressures which promote the spread of this XDR hyper-epidemic strain. In the meantime, currently established infection control measures such as hand hygiene, isolation precautions and judicious antimicrobial use, should be employed throughout the world to limit the emergence and transmission of KPC-producing organisms.
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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.
REFERENCES


Table 1 Carbapenem resistant *K. pneumoniae* isolates included in this bi-national study

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*Outbreak isolates from various states including New York, Delaware, Maryland, Pennsylvania, New Jersey, and Arizona.*
Figure Legends

Figure 1

Pulsed-field gel electrophoresis (Spe-1 restricted) of 14 representative KPC-3-producing carbapenem-resistant *K. pneumoniae* isolates from various Israeli hospitals (Panel A, 1-14) demonstrate a nationwide epidemic clone different from a KPC-2-producing clone reported previously in our hospital (6) (Panel A, lane 15). Genetic relatedness of the predominant Israeli epidemic clone (Panel B, lane 10) with nine KPC-3-producing *K. pneumoniae* isolates involved in outbreaks in the United States (US), from New Jersey (NJ, lanes 1, 2), Arizona (AZ, lanes 3-5), and New York (NY, lanes 6-9). M, Lambda ladder (New England Biolabs).

Figure 2

A comparison of *bla*<sub>KPC-3</sub> encoding plasmids (panel A) and their EcoRI restriction pattern (panel B) from five Israeli *K. pneumoniae* isolates (lanes 1-5 in each panel), and five of the nine genetically related *K. pneumoniae* US isolates (lanes 6-10 in each panel). Analysis was performed on plasmids isolated from *E. coli* GeneHogs transformants carrying the *bla*<sub>KPC-3</sub> encoding plasmids.; lanes 1-5, representative Israeli isolates from 2006-2008; Lanes 6-10, representative US isolates from 2000 and 2006; Lanes 6-7, two isolates from New York Medical Center; Lanes 8-10, one isolate from New Jersey and two isolates from Arizona. panel A, M = BAC-Tracker ™ Supercoiled DNA Ladder, Epicentre Biotechnologies; panel B, M, GeneRuler™ 1 kb DNA Ladder, Fermentas, Life Sciences.
Figure 1

A

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Israel

B

M 1 2 3 4 5 6 7 8 9 10 11

United States

NJ AZ NY

Israel
Figure 2

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