Characterization of KPC-type carbapenemase-producing Klebsiella pneumoniae strains isolated in the Arabian Peninsula

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Sir, Since its first description in 1996, variants of KPC have spread throughout the world mostly in the ST258 clone of Klebsiella pneumoniae within isosforms of the Tn4401 transposon located on various plasmids.1 While the Middle East is considered as a reservoir of various carbapenemase-producing bacteria, to date the only Enterobacteriaceae carrying the blaKPC gene reported from the Arabian Peninsula has been an Escherichia coli ST131 strain isolated in Kuwait.2 In a collection of 34 carbapenem-non-susceptible Enterobacteriaceae strains, comprising all clinically relevant, non-repeat isolates recovered in Rashid Hospital, Dubai, United Arab Emirates (UAE) between March 2012 and February 2014, we identified by PCR3: 3 blaNDM and 1 blaOXA-48-like E. coli; and 3 blaNDM, 6 blaOXA-48-like, 17 blaNDM + blaOXA-48-like and 2 blaKPC K. pneumoniae. Since blaKPC-positive K. pneumoniae has not been described from this region to date, we subjected the two blaKPC-positive K. pneumoniae isolates (ABC220 and ABC224) to detailed investigations.

K. pneumoniae ABC220 was isolated in October 2012 from a necrotizing wound of a previously healthy, 40-year-old Filipino male, 25 days after he had been admitted for perforated appendix and sepsis. K. pneumoniae ABC224 was recovered in May 2013 from the sputum of an 80-year-old UAE national with multiple chronic illnesses, continuously hospitalized for >1 year in a different ward for left leg amputation and sepsis. Prior to the isolation of these strains, various samples from both patients had been repeatedly negative for carbapenem-resistant Enterobacteriaceae.

As detected by Etest (bioMérieux, Marcy-l’Étoile, France) or microdilution, ABC220 and ABC224 exhibited susceptibility to tigecycline (MIC = 0.75 mg/L) and amikacin (MIC = 12 mg/L) only. The MICs of all antimicrobials tested are listed in Table S1 (available as Supplementary data at JAC Online). The XbaI-digested macrorestriction patterns3 of strains exhibited >95% similarity. Both ABC220 and ABC224 belonged to ST14 by MLST.4

Strains were subjected to PCR assays specific for ESBL (blaCTX-M, blaSHV, blaTEM and blaOXA) and AmpC β-lactamase genes, plasmid-mediated quinolone and aminoglycoside resistance genes and virulence genes (rmpA, wabG, wcaG, fimH, mrkD, lutA, fyuA, iroN, ireA, kfuBC, traT and clpK).5 6 Sequencing of the amplicons revealed that they both possessed blaTEM-1, blaSHV-1, blaCTX-M-15 and oac-6'-1b-cr genes and also virulence-related genes fyuA, kfuBC, fimH, mrkD, traT, clpK, ugeA and wabG. Both strains carried sequences suggestive of a K2 capsule type.6

Outer membrane protein analysis by SDS–PAGE revealed the lack of a band corresponding to OmpK35 from both isolates as compared with the patterns of control strains CSUB10S (Omp36K) and CSUB10S/pSH166 (OmpK35 and OmpK36).7

Plasmid transfer and analysis by electrophoresis, Southern blotting, hybridization, replica typing and RFLP was performed as described previously.8 Although blaKPC-carrying plasmids from both strains were conjugally transferred into E. coli J53R2, they were not stably maintained in this recipient. Therefore, plasmids were transferred into E. coli GM2163 by transformation. Both ABC220 and ABC224 harboured a 45 kb IncX3 plasmid carrying blaKPC as identified by hybridization. The plasmids exhibited the same RFLP profile when digested with EcoRI and HindIII (data not shown). Sequencing of blaKPC and its flanking regions by PCR mapping and direct sequencing identified the blaKPC-2 allele in both strains located within the Tn4401b transposon inserted into the umuD gene of the IncX3 plasmid (Figure 1) (GenBank accession no. KM983022).

At the time of submission of our manuscript, no KPC-producing K. pneumoniae had been reported from the Arabian Peninsula; although, not without causing considerable confusion, the terminology ‘KPC’ was used earlier referring to a strain exhibiting putative carbapenemase activity by modified Hodge test, without any analysis of the bla gene.9 Here, we describe two K. pneumoniae ST14 strains, identical in all features tested, carrying blaKPC-2 on IncX3 plasmids. blaKPC-2-bearing IncX3 plasmids described earlier in ST258 K. pneumoniae in France and Hong Kong10 originated from Greece and the USA, respectively, i.e. from KPC-endemic regions. Compared with these strains, the genetic context of blaKPC-2 is different in the UAE isolates: they map within the Tn4401b isoform inserted in the opposite direction into the
genetic load region of the IncX3 plasmid disrupting the umuD gene (Figure 1).

It is noteworthy that neither of our patients had a history of travel or recent hospitalization prior to their current admission, suggesting that both of them acquired the strains while being treated in different wards. Based on the data available, we cannot exclude or confirm the possibility that the clone was concealed in the hospital environment (including personnel) during the considerable time that elapsed between the isolation of the two strains. At present, it is not possible to predict whether these cases indicate the beginning of a local spread of KPC-positive K. pneumoniae or whether their incidence will remain low. To answer these questions, which may have implications in understanding the global spread of the blaKPC gene and the clones carrying it, systemic surveillance of these isolates in the region is needed.

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Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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