LETTER TO THE EDITOR

Molecular characterization of KPC-2–positive *Klebsiella pneumoniae* isolates from a neurosurgical centre in Argentina

S. Montaña[1], M. Hernandez[2], J. S. Fernandez[2], M. Pennini[3], D. Centrón[1], A. Sucari[3], A. Iriarte[4] and M. S. Ramírez[2]

1) Instituto de Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Argentina, 2) Center for Applied Biotechnology Studies, Department of Biological Science, California State University Fullerton, Fullerton, CA, USA, 3) Unidad Microbiología, Stamboulian, Buenos Aires, Argentina and 4) Laboratorio de Biología Computacional, Departamento de Desarrollo Biotecnológico, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Uruguay

Abstract

Carbapenem-resistant *Enterobacteriaceae* is a growing concern worldwide. *Klebsiella pneumoniae* is an important nosocomial pathogen with a high capacity for nosocomial spread. We described the occurrence of plasmid-encoded KPC-2–harbouring *K. pneumoniae* isolates recovered from a neurosurgical centre in Argentina. The bla_{KPC-2} gene was surrounded by IS*kpn6* and IS*kpn7*.

Keywords: *bla*, carbapenem resistance, *Klebsiella pneumoniae*, neurosurgical centre
Original Submission: 15 March 2018; **Revised Submission:** 10 April 2018; **Accepted:** 14 April 2018
Article published online: 21 April 2018

Corresponding author: M.S. Ramírez, Department of Biological Science, California State University Fullerton, 800 N State College Blvd, Fullerton, CA 92831, USA
E-mail: msramirez@fullerton.edu

Klebsiella pneumoniae is an important nosocomial pathogen involved in urinary tract infections, hospital-acquired pneumonia, ventilator-associated pneumonia, surgical-wound infection, bacteraemia and septicaemia [1,2]. It is well known that carbapenem-resistant *Enterobacteriaceae* (CRE) is a growing concern worldwide [3]. In recent years, the ongoing emergence of CRE in Argentina has increased and among them, *K. pneumoniae* harbouring *K. pneumoniae* carbapenemase (KPC) are prevalent [4]. KPCs are the most frequent carbapenemases found in *K. pneumoniae* and in many other members of the *Enterobacteriaceae* family such as *Escherichia coli*, *Enterobacter* spp., *Salmonella enterica*, *Proteus mirabilis* and *Citrobacter freundii* [5].

Because the bla_{KPC-2} gene is mostly plasmid encoded [6–8] and is typically in a Tn*3*-based transposon, Tn*4401*, the capacity of disseminating among *K. pneumoniae* and in Gram-negative genera is a major concern [2,3]. *K. pneumoniae* sequence type (ST) 258 is largely responsible for KPC dissemination throughout North America and other parts of the world. No information describing *K. pneumoniae* KPC-positive isolates or KPC outbreaks in neurosurgical centres can be found in the literature. Only one report describing a KPC-2–producing *Klebsiella pneumoniae* outbreak in patients admitted to a neurosurgery department in a South Korean has been published [9].

The aim of this study was to perform the molecular characterization of the genetic surroundings of the bla_{KPC-2} gene among *K. pneumoniae* (KPC-2 positive) clinical isolates recovered from 70 subjects tested in a neurosurgical centre in Argentina.

During 2014–2016, a total of 22 nonrepeated carbapenem-resistant *K. pneumoniae* KPC-positive isolates were recovered from a variety of samples including blood, urine and respiratory tract. Antibiotic susceptibility was determined using the VITEK 2 System (bioMérieux, Marcy l'Etoile, France) using the panel AST-082 (GNS susceptibility card) and interpreted using the Clinical and Laboratory Standards Institute (CLSI) categories, with the exception of colistin and tigecycline, where the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations was used. The bla_{KPC} gene was identified by PCR amplification and subsequently sequenced to confirm the variant present in the isolates. The absence of other carbapenemases (bla_{VIM}, bla_{NDM-1} and bla_{OXA-48}) was confirmed by PCR.

Conjugation assays were performed to determine the genetic location of the gene [10]. All the strains possessed similar antibiotic susceptibility profiles and harboured bla_{KPC-2} in conjugative plasmids (Table 1).

To further characterize in detail the genetic context of bla_{KPC-2}, one strain was randomly selected (Kpn8). Plasmid extraction was performed using the QIAfilter Midi prep Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Whole-plasmid shotgun sequencing was

https://doi.org/10.1016/j.nmni.2018.04.002

TABLE 1. Study strains

Strain	Material source	Antibiotic profile[a]	KPC PCR
KPC 2	Urine culture	MEM, IMP, ERT, GEN, AKN, TMS, CIP, COL	Positive
KPC 3	Urine culture	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, COL, TIG	Positive
KPC 4	Respiratory secretion	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, TIG	Positive
KPC 5	Blood culture	MEM, IMP, ERT, GEN, AKN, TMS, CIP, TIG	Positive
KPC 6	Bronchoalveolar lavage	MEM, IMP, ERT, TMS	Positive
KPC 8	Cerebrospinal fluid	MEM, IMP, ERT, TMS, CIP, FOS, COL, TIG	Positive
KPC 9	Urine culture	MEM, IMP, ERT, TMS, CIP, FOS, COL, TIG	Positive
KPC 11	Urine culture	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, COL, TIG	Positive
KPC 13	Urine culture	MEM, IMP, ERT, GEN, AKN, TMS, CIP, COL, TIG	Positive
KPC 16	Blood culture	MEM, IMP, ERT, TMS, CIP, FOS, COL, TIG	Positive
KPC 17	Urine culture	MEM, IMP, ERT, TMS, CIP	Positive
KPC 18	Catheter	MEM, IMP, ERT	Positive
KPC 19	Anal swab	MEM, IMP, ERT, TMS, CIP	Positive
KPC 20	Anal swab	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, COL, TIG	Positive
KPC 21	Anal swab	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, COL, TIG	Positive
KPC 22	Anal swab	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, COL, TIG	Positive
KPC 23	Anal swab	MEM, IMP, ERT, TMS, CIP	Positive
KPC 24	Catheter	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, COL, TIG	Positive
KPC 25	Anal swab	MEM, IMP, ERT, GEN, AKN, TMS, CIP, FOS, COL, TIG	Positive
KPC 26	Urine culture	MEM, IMP, ERT, GEN, TMS, CIP, FOS, TIG	Positive
KPC 27	Respiratory secretion	MEM, IMP, ERT, GEN, TMS, CIP, FOS, TIG	Positive

AMK, amikacin; CIP, ciprofloxacin; COL, colistin; ERT, ertapenem; FOS, fosfomycin; GEN, gentamicin; IMP, imipenem; KPC, *Klebsiella pneumoniae* carbapenemase; MEM, meropenem; TIG, tigecycline; TMS, trimethoprim/sulfamethoxazole.
[a]Resistance only.

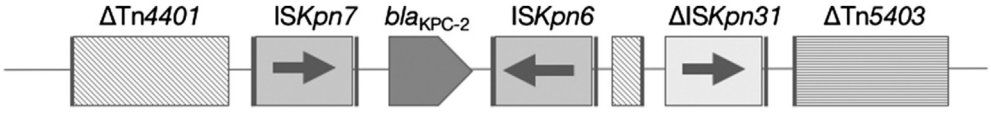

FIG. 1. Genetic environment of bla_{KPC-2} gene found in representative strain Kpn8. Transposons Tn*4001* and Tn*5403* are represented by striped square boxes; inverted repeats are shown as a grey tall line. ISs (IS*Kpn7*, IS*Kpn6* and IS*Kpn31*) are shown with striped square boxes; arrows indicate transcriptional orientation; inverted repeats are shown as a grey tall line. bla_{KPC-2} gene is shown by grey arrow box.

performed using Illumina MiSeq-I, with Nextera XT libraries for sample preparation (Illumina, San Diego, CA, USA). Assemblies were annotated by means of the RAST Server [11] and the SEED source for plasmid annotations [12].

The genetic analysis of the bla_{KPC-2} gene revealed the presence of IS*kpn6* and IS*kpn7* flanking this gene (Fig. 1). This structure was disrupting the transposon Tn*4401*. Moreover, an incomplete copy of IS*kpn31* and a part of Tn*5403* were present downstream of the later context (Fig. 1). The association between IS*kpn31* and Tn*4401* has been previously described [13].

The prevalence of CRE has increased substantially during the last decade. An increased prevalence of *K. pneumoniae* ST258 harbouring KPC was observed Argentina [14]. In addition, a KPC-producing *K. pneumoniae* isolate that belonged to a different ST, ST23, was also reported in the region [15]. The rapid increase and dissemination of the KPC carbapenemases in centres where major surgeries take place is of great concern.

In this study we described the spread of *K. pneumoniae* bla_{KPC-2}-positive strains in a neurosurgical centre. The genetic context and plasmid location of this carbapenemase has been determined. Because in all cases bla_{KPC-2} was plasmid located, we highlight the importance of searching for this gene and installing control measures to stop its dissemination.

Acknowledgements

Supported in part by grants from the 'Secretaría de Ciencia y Técnica de la Universidad de Buenos Aires' (UBACyT) to MSR and PICT 1881 (2014) to DC. SM has a doctoral fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas.

Conflict of interest

None declared.

References

[1] Paczosa MK, Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiol Mol Biol Rev 2016;80:629–61.
[2] Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother 2015;59:5873–84.
[3] Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 2011;17:1791–8.

[4] Pasteran FG, Otaegui L, Guerriero L, Radice G, Maggiora R, Rapoport M, et al. *Klebsiella pneumoniae* carbapenemase-2, Buenos Aires, Argentina. Emerg Infect Dis 2008;14:1178–80.
[5] Shen P, Wei Z, Jiang Y, Du X, Ji S, Yu Y, et al. Novel genetic environment of the carbapenem-hydrolyzing β-lactamase KPC-2 among *Enterobacteriaceae* in China. Antimicrob Agents Chemother 2009;53:4333–8.
[6] Conlan S, Park M, Deming C, Thomas PJ, Young AC, Coleman H, et al. Plasmid dynamics in KPC-positive *Klebsiella pneumoniae* during long-term patient colonization. mBio 2016;7:e00742-16.
[7] Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P. Genetic structures at the origin of acquisition of the beta-lactamase *bla* KPC gene. Antimicrob Agents Chemother 2008;52:1257–63.
[8] Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007;20:440–58.
[9] Hong SK, Yong D, Kim K, Hong SS, Hong SG, Khosbayar T, et al. First outbreak of KPC-2–producing *Klebsiella pneumoniae* sequence type 258 in a hospital in South Korea. J Clin Microbiol 2013;51:3877–9.
[10] Montaña S, Cittadini R, del Castillo M, Uong S, Lazzaro T, Almuzara M, et al. Presence of New Delhi metallo-β-lactamase gene (NDM-1) in a clinical isolate of *Acinetobacter junii* in Argentina. New Microbes New Infect 2016;11:43–4.
[11] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75.
[12] Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 2014;42(Database issue):D206–14.
[13] Partridge SR. Tn*4401* carrying *bla*(KPC) is inserted within another insertion in pKpQIL and related plasmids. J Clin Microbiol 2014;52:4448–9.
[14] Gomez SA, Pasteran FG, Faccone D, Tijet N, Rapoport M, Lucero C, et al. Clonal dissemination of *Klebsiella pneumoniae* ST258 harbouring KPC-2 in Argentina. Clin Microbiol Infect 2011;17:1520–4.
[15] Cejas D, Fernandez Canigia L, Rincon Cruz G, Elena AX, Maldonado I, Gutkind GO, et al. First isolate of KPC-2–producing *Klebsiella pneumonaie* sequence type 23 from the Americas. J Clin Microbiol 2014;52:3483–5.