

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

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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 *ISKpn6* and *ISKpn7*. © 2018 The Author(s). Published by Elsevier Ltd.

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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 Tn3-based transposon, Tn4401, 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

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, fosfomicin; GEN, gentamicin; IMP, imipenem; KPC, *Klebsiella pneumoniae* carbapenemase; MEM, meropenem; TIG, tigecycline; TMS, trimethoprim/sulfamethoxazole.
^aResistance only.

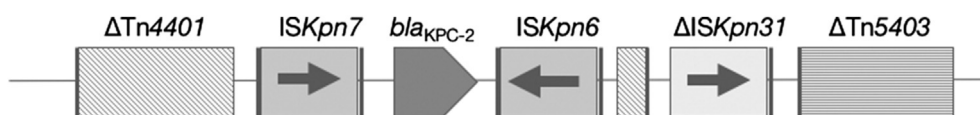


FIG. 1. Genetic environment of *bla*_{KPC-2} gene found in representative strain Kpn8. Transposons Tn4001 and Tn5403 are represented by striped square boxes; inverted repeats are shown as a grey tall line. ISs (*ISKpn7*, *ISKpn6* and *ISKpn31*) are shown with striped square boxes; arrows indicate transcriptional 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 *ISKpn6* and *ISKpn7* flanking this gene (Fig. 1). This structure was disrupting the transposon Tn4401. Moreover, an incomplete copy of *ISKpn31* and a part of Tn5403 were present downstream of the later context (Fig. 1). The association between *ISKpn31* and Tn4401 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.

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Conflict of interest

None declared.

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