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 blaKPC-2 gene was surrounded by ISkp6 and ISkp7.

Keywords: bla, carbapenem resistance, Klebsiella pneumoniae, neurosurgical centre

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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 blaKPC-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 blaKPC-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’Étoile, 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 blaKPC gene was identified by PCR amplification and subsequently sequenced to confirm the variant present in the isolates. The absence of other carbapenemases (blaVIM, blaNDM-1 and blaOXA-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 blaKPC-2 in conjugative plasmids (Table 1).

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

The prevalence of CRE has increased substantially during the last decade. An increased prevalence of \textit{K. pneumoniae} ST258 harbouring KPC was observed Argentina [14]. In addition, a KPC-producing \textit{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 \textit{K. pneumoniae} \textit{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 \textit{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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