Identification of plasmid IncQ1 and NTE_{KPC-IId} harboring $bla_{KPC-2}$ in isolates from *Klebsiella pneumoniae* infections in patients from Recife-PE, Brazil

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**Abstract**

**Introduction:** This study investigated the genetic environment of $bla_{KPC-2}$ in *Klebsiella pneumoniae* multi-drug resistant clinical isolates.  

**Methods:** Four carbapenemase gene isolates resistant to carbapenems, collected from infected patients from two hospitals in Brazil, were investigated using polymerase chain reaction and plasmid DNA sequencing.  

**Results:** The $bla_{KPC-2}$ gene was located between IS$_{Kpn6}$ and a resolvase $tnpR$ in the non-Tn$_{4401}$ element (NTE$_{KPC-IId}$). It was detected on a plasmid belonging to the IncQ1 group.  

**Conclusions:** To our knowledge, this is the first report of the presence of the $bla_{KPC-2}$ gene in the NTE$_{KPC-IId}$ element carried by plasmid IncQ1 from infections in Brazil.

**Keywords:** *Klebsiella pneumoniae*. IncQ1. $bla_{KPC-2}$. NTE$_{KPC-IId}$.

*Klebsiella pneumoniae* is one of the pathogens responsible for healthcare-associated infections (HAIs). Infections can, depending on the anatomic site affected and the patient’s immune status, lead to a range of adverse clinical outcomes, including death, as this gram-negative bacterium carries several antibiotic resistance and virulence genes.

The most relevant antibiotic-resistant genes in this bacterial species are those that encode carbapenemases, (KPC). *K. pneumoniae* isolates from different countries, including Brazil, have been found to contain the KPC encoding gene $bla_{KPC-2}$. This gene has been found to be located on plasmids of different sizes and nucleotide sequences and belong to different incompatibility groups (Incs), the most prevalent being IncL/M, IncFII and IncN$_{2,3}$.

Nicoletti et al. (2015) [4] identified the IncQ plasmid in *K. pneumoniae* carrying carbapenemase BKC-1 from São Paulo, Brazil. IncQ1 is a stable and mobilizable plasmid that can be transferred among a wide range of gram negative bacteria through conjugative plasmids present in the same bacterial cell, which facilitates its transmission in a hospital environment. There are few studies in Brazil that characterize the plasmid Incs of *K. pneumoniae*, mainly because some of these are non-typeable plasmids, such as the ones studied by Pereira et al (2013) [5].

These plasmids may also harbor different isoforms of the Tn$_{4401}$ transposon. Nine variants of Tn$_{4401}$ (a–i) have been described, of which variants "a" and "b" are the most common. Non-Tn$_{4401}$ (NTE$_{KPC}$) elements that can carry $bla_{KPC-2}$ have also been described, including those recently detected in two colonization isolates in Brazil [6].

The aim of this study was to investigate the genetic environment of the $bla_{KPC-2}$ gene from clinical isolates of *K. pneumoniae* resistant to carbapenems, thus helping to understand the dissemination of carbapenem resistance. This may help develop new strategies to prevent the spread of these resistance genes in the hospital environment.

Four multi-drug resistant (MDR) clinical isolates of *K. pneumoniae* (K3R2, K4R2, K6R2, and K1E) were selected, following isolation, from infections (peritoneal fluid, blood cultures,
The PCR products were electrophoresed on 1.0% agarose gel in TBE buffer. The IncQ plasmid was also identified using in silico PCR; bioinformatics tools used included sequence manipulation suite (SMS) (http://www.bioinformatics.org/sms2/index.html) and primer-basic local alignment search tool (BLAST), using the primers for determination of all different plasmid incompatibility groups, as defined by Carattoli et al (2005)\textsuperscript{14}.

The isolates of *K. pneumoniae* were MDR, with resistance to β-lactams, and especially to carbapenems (Table 1), and they were suspected of being producers of KPC. Using PCR, the presence of the *bla*\textsubscript{KPC-2} gene in the four *K. pneumoniae* isolates analyzed was confirmed. The ERIC-PCR genotyping test showed that all the isolates presented distinct clonal profiles, with a maximum of 40% similarity, and therefore they did not present a clonal relationship (Table 1). The plasmid DNA was sequenced to a depth of approximately 238 times. The analysis of the plasmid DNA sequences from all isolates using the Resfinder and GenBank databases confirmed the presence of the *bla*\textsubscript{KPC-2} (882 bp) antibiotic-resistance gene. The gene was identified with 100% similarity when compared with a sequence deposited in GenBank (CP023186.1). The *bla*\textsubscript{KPC-2} gene was observed in similar genetic locations of all isolates and was inserted between the ΔISK\textsubscript{pn6} insert sequence and a resolvase *tnpR*; between 402–558 bp upstream of the *bla*\textsubscript{KPC-2} gene, a truncated *bla*\textsubscript{TEM} gene was an evidence that *bla*\textsubscript{KPC-2} was inserted into a non-\textsuperscript{Tn}4401 (variant NTE\textsubscript{KPC-IId}) (Figure 1). We found deletions in *tnpA* and a total deletion of the ISK\textsubscript{pn7} insert sequence (Figure 1).

In the same consensus sequence where the non-\textsuperscript{Tn}4401 was located, plasmid mobility proteins (*mobA*, *mobB*, and *mobC*) and replication proteins (*repA*, *repB*, and *repC*) were found with a 100% similarity to the reference pool of the IncQ1 RSF1010 (M28829.1). The *oriV, oriT*, and *repB* genes were also found inserted into the same consensus sequence of the replication and mobilization genes as the *bla*\textsubscript{KPC-2} gene. Thus, this result suggests that all the isolates have the genes encoding the IncQ1 and the *bla*\textsubscript{KPC-2} gene in the same consensus sequence (Figure 1 and Table 2).

PlasmidFinder confirmed the presence of a plasmid belonging to the variant incompatibility group IncQ1. The in silico PCR with all the isolates, tested positive for the IncQ1 replicons (*oriV*-436bp, *oriT*-191bp, and *repB*-1160bp). Comparative analysis among the four *K. pneumoniae* isolates of this study and reference sequences

| Isolates | Origin |
|----------|--------|
| K3R2     | P1     |
| K4R2     | Blood culture |
| K6R2     | P3     |
| K1E      | Blood culture |

**K**: Klebsiella pneumoniae; **R2**: public hospital; **E**: private hospital; **P**: public hospital; **AMP**: ampicillin; **APS**: ampicillin/sulbactam; **CFL**: cefalotin; **CFZ**: ceftazolin; **CFX**: cefotaxime, **CAZ**: ceftazidine; **CRO**: ceftriaxone; **CXM**: cefuroxime; **CIP**: ciprofloxacin; **ERT**: ertapenem; **GEN**: gentamycin, **IPM**: imipenem, **MER**: meropenem.
FIGURE 1: Comparison among Tn4401, non-Tn4401 (NTE KPC-IId), and IncQ1 carrying the \( \text{bla}_{\text{KPC}} \) gene in \( \text{Klebsiella pneumoniae} \). Tn4401 with \( \text{bla}_{\text{KPC}} \) gene between ISKpn6 and ISKpn7; NTEKPC-IId with deletion of the ISKpn7 and truncated \( \text{bla}_{\text{TEM}} \) gene; K3-R2 isolate with \( \text{bla}_{\text{KPC}} \) gene between truncated \( \text{bla}_{\text{TEM}} \) gene and \( \Delta \text{ISKpn6} \) as well as mobility and replication genes encoding the plasmid IncQ1. Protein-coding sequences are represented by the arrows and labeled with gene name. Vertical lines represent gaps schematizing the termination of one contig and the beginning of another contig in the isolate. Gray represents homologous shared regions. The direct repeat sequence of NTE KPC-IId is represented by a circle.

TABLE 2: Plasmid sequence characteristics of \( \text{K. pneumoniae} \) isolates and comparative analysis with plasmid RSF1010-IncQ (accession number: M28829.1).

| Isolates | K3R2 | K4R2 | K6R2 | K1E | IncQ (M28829.1) |
|----------|------|------|------|-----|----------------|
| Reference | This study | This study | This study | This study | Scholz et al. (1989) |
| Size of the sequenced plasmid DNA | 27,508 bp | 27,325 bp | 59,776 bp | 84,800 bp | 8,684 bp |
| GC | 57.95% | 58.13% | 55.35% | 53.84% | 61% |
| CDS | 31 | 31 | 70 | 94 | 40 |
| Inc | IncQ1 and NTP | IncQ1 and NTP | IncQ1 and NTP | IncQ1 and NTP | IncQ1 and NTP |
| Resistance genes | \( \text{bla}_{\text{KPC}-2} \) | \( \text{bla}_{\text{KPC}-2} \) | \( \text{bla}_{\text{KPC}-2} \) | \( \text{bla}_{\text{KPC}-2} \) | \( \text{str} \; \text{sul} \) |
| Mobility and replication | \( \text{mobA} \) | \( \text{mobA} \) | \( \text{mobA} \) | \( \text{mobA} \) | \( \text{mobA} \) |
| Proteins | \( \text{mobB} \) | \( \text{mobB} \) | \( \text{mobB} \) | \( \text{mobB} \) | \( \text{mobB} \) |
| | \( \text{mobC} \) | \( \text{mobC} \) | \( \text{mobC} \) | \( \text{mobC} \) | \( \text{mobC} \) |
| | \( \text{repA} \) | \( \text{repA} \) | \( \text{repA} \) | \( \text{repA} \) | \( \text{repA} \) |
| | \( \text{repB} \) | \( \text{repB} \) | \( \text{repB} \) | \( \text{repB} \) | \( \text{repB} \) |
| | \( \text{repC} \) | \( \text{repC} \) | \( \text{repC} \) | \( \text{repC} \) | \( \text{repC} \) |
| | \( \text{repF} \) | \( \text{repF} \) | \( \text{repF} \) | \( \text{repF} \) | \( \text{repF} \) |

K: \( \text{Klebsiella pneumoniae} \); R2: public hospital; E: private hospital; GC: guanine and cytosine; bp: base pairs; CDS: coding sequence; Inc: plasmid incompatibility group; KPC: \( \text{Klebsiella pneumoniae} \) carbapenemase; \( \text{str} \): streptomycin; sul: sulfonamide; mob: mobility protein; rep: replication proteins; NTP: not typable.
for the IncQ1 and IncQ-like plasmids deposited in GenBank showed 98% to 100% similarity to the oriV gene (M21475.1), to the oriT gene (X04830.1), and to the repB gene (M28829.1).

The PCR for the IncQ1 replicons also confirmed this result, with the repB, oriV, and oriT genes of the plasmid IncQ1 amplified in all isolates analyzed. In addition to IncQ1, the isolates also presented other plasmids, but these were not typable given the total size of the DNA sequence (Table 2).

Antimicrobial resistance genes are spread among enterobacteria due to the horizontal transfer of mobile genetic elements. The bla

\[ \text{KPC-2} \]

gene is found associated with several different plasmids. However, little was known about the plasmid genetic environment of this gene in clinical isolates of K. pneumoniae in Brazil, and especially in Recife-PE, where the first reports of KPC in Brazil came from.

The bla

\[ \text{KPC-2} \]

gene is often found inserted into transposon Tn4401, which has different isoforms, but it has also been found in a non-Tn4401 mobile element (NTEKPC) in China, Argentina, Brazil, and Russia. NTEKPC has been separated into three groups based on the absence or presence of the bla

\[ \text{TEM} \]

gene, where the second group, NTEKPC-II, includes the variant with a truncated bla

\[ \text{TEM} \]

gene.

The non-Tn4401 variant of the present study resembles the NTEKPC-IIId variant (Figure 1). These findings corroborate the results obtained by our research group with Klebsiella aerogenes from Recife-PE, Brazil, which had 100% similarity with a sequence deposited in GenBank (MG786907, MH000708).

IncQ and IncQ-like plasmids have been found in different bacterial species such as Escherichia coli, Salmonella typhimurium, Salmonella enterica serovar, Pseudomonas aeruginosa, and Enterobacter cloacae from locations in Canada, Italy, the United Kingdom, and Germany.

This report demonstrates the presence of the bla

\[ \text{KPC-2} \]

gene in the non-Tn4401 element (NTEKPC-IId), which is carried by small, mobilizable, and promiscuous plasmids of the type IncQ1, in four clinical MDR isolates of infection by K. pneumoniae in Northeast Brazil. This data indicates that this type of plasmid may have been responsible for spreading the bla

\[ \text{KPC-2} \]

gene among K. pneumoniae in patients from hospitals in Recife, Brazil.

The study by Pereira et al. (2013) used K. pneumoniae isolates from Recife-Pernambuco, Brazil, but it was not possible to type the plasmids. Cerdeira et al. (2019) found the bla

\[ \text{KPC-2} \]

gene in the NTEKPC-IId gene carried by IncQ1 plasmids in two colonization isolates of K. pneumoniae in Brazil (uninformed locality).

Collectively, these results reveal the dynamics of the genetic environment of the bla

\[ \text{KPC-2} \]

gene and emphasize the continuous recombination and evolution of plasmids and transposons. This may make the spread of different resistance genes in K. pneumoniae isolates more likely, introducing additional difficulties to the development of possible measures to control the spread of this form of bacterial resistance.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.
12. Carver T, Berriman M, Tivey A, Patel C, Böhme U, Barrell BG, Parkhill J, Rajandream MA. Artemis and ACT: Viewing, annotating and comparing sequences stored in a relational database. Bioinformatics, 2008;24(23):2672–76.

13. Götz A, Pukall R, Smit E, Tietze E, Prager R, Tschäpe H, et al. Detection and Characterization of Broad-Host-Range Plasmids in Environmental Bacteria by PCR. Appl Environ Microbiol. 1996;62(7):2621-8.

14. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 2005; 63(3):219–28.

15. De Belder D, Lucero C, Rapoport M, Rosato A, Faccone D, Petroni A, Pasteran F, Albornoz E, Corso A, Gomez AS Genetic diversity of KPC -producing Escherichia coli, Klebsiella oxytoca, Serratia marcescens, and Citrobacter freundii isolates from Argentina. Microb Drug Resist. 2018; 24(7):958 -65.

16. Chen L, Mathema B, Chavda KD, DeLeo FR, Bonomo RA, Kreiswirth BN. Carbapenemase -producing Klebsiella pneumoniae: molecular and genetic decoding. Trends Microbiol. 2014;22(12):686-96.

17. Kotsakis SD, Tzouvelekis LS, Lebessi E, Doudoulakis A, Bouli T, Tzelepi E, et al. Characterization of a mobilizable IncQ plasmid encoding cephalosporinase CMY-4 in Escherichia coli. Antimicrob Agents Chemother. 2015;59(5):2964–6.