

## Detection of *bla*<sub>KPC-2</sub> in *Proteus mirabilis* in Brazil

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### ABSTRACT

**Introduction:** Infections caused by *Klebsiella pneumoniae* carbapenemase (KPC)-producing isolates pose a major worldwide public health problem today. **Methods:** A carbapenem-resistant *Proteus mirabilis* clinical isolate was investigated for plasmid profiles and the occurrence of  $\beta$ -lactamase genes. **Results:** The isolate exhibited resistance to ertapenem and imipenem and was susceptible to meropenem, polymyxin, and tigecycline. Five plasmids were identified in this isolate. DNA sequencing analysis revealed the presence of *bla*<sub>KPC-2</sub> and *bla*<sub>TEM-1</sub> genes. An additional PCR using plasmid DNA confirmed that *bla*<sub>KPC-2</sub> was present in one of these plasmids. **Conclusions:** We report the detection of *bla*<sub>KPC-2</sub> in *P. mirabilis* in Brazil for the first time. This finding highlights the continuous transfer of *bla*<sub>KPC</sub> between bacterial genera, which presents a serious challenge to the prevention of infection by multidrug-resistant bacteria.

**Keywords:** *Proteus mirabilis*. *bla*<sub>KPC-2</sub>. Plasmid DNA.

*Klebsiella pneumoniae* carbapenemase (KPC)-type enzymes were first reported in carbapenem-resistant *K. pneumoniae* strains in North Carolina, United States<sup>(1)</sup>. Until 2005, the geographical distribution of these enzymes in members of the family *Enterobacteriaceae* - *K. pneumoniae*, in particular - was limited to the eastern part of the United States. In Brazil, KPC has been described since 2006, and it was first reported in *K. pneumoniae* isolates from Recife<sup>(2)</sup>, followed by reports<sup>(3)</sup> in other species from the *Enterobacteriaceae* and non-fermenting bacilli<sup>(4) (5) (6)</sup>. Carbapenem resistance in *Proteus mirabilis* caused by KPC-2 was also first reported in the United States<sup>(7)</sup> in 2008, and currently, there are a few reports of *P. mirabilis* KPC producers worldwide<sup>(8) (9)</sup>. The present report describes the detection of *bla*<sub>KPC-2</sub> in *P. mirabilis* strains in Brazil for the first time.

In May 2012, an isolate of *Proteus mirabilis* was recovered from the blood culture of a patient admitted to the intensive care unit of a tertiary hospital located in Recife, Pernambuco, Brazil. Biochemical identification and determination of minimum inhibitory concentrations (MICs) of the isolate were performed using the BD Phoenix<sup>TM</sup> Automated Microbiology System, Franklin Lakes, New Jersey, USA and the susceptibility profile was determined according to the guidelines of the Clinical and Laboratory Standards Institute (2013). The isolate was characterized as multidrug resistant, showing resistance

to amikacin (MIC>32 $\mu$ g/mL), amoxicillin/clavulanate (MIC>16 $\mu$ g/mL), ampicillin (MIC>16 $\mu$ g/mL), cefazolin (MIC>16 $\mu$ g/mL), cefepime (MIC>16 $\mu$ g/mL), cefotaxime (MIC>32 $\mu$ g/mL), cefoxitin (MIC=16 $\mu$ g/mL), ceftriaxone (MIC>32 $\mu$ g/mL), ciprofloxacin (MIC>2 $\mu$ g/mL), ertapenem (MIC>4 $\mu$ g/mL), imipenem (MIC>8 $\mu$ g/mL), gentamicin (MIC>8 $\mu$ g/mL), levofloxacin (MIC>4 $\mu$ g/mL), piperacillin/tazobactam (MIC>64 $\mu$ g/mL), and tobramycin (MIC>8 $\mu$ g/mL). It was susceptible only to meropenem, polymyxin, and tigecycline. Plasmid DNA was extracted by the UltraClean Endotoxin-Free Mini Plasmid Prep Kit (Mo Bio Lab, Carlsbad, CA, USA) and was visualized and analyzed by electrophoresis on a 0.7% agarose gel. The molecular weight of each plasmid was determined by comparison with plasmids of known molecular weight, e.g., with that of *K. pneumoniae* K16-P strain<sup>(3)</sup>. Five plasmids with estimated molecular sizes of >150kb, 150kb, 120kb, 90kb, and 70kb were identified in the isolate. Genes *bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>SPM</sub>, *bla*<sub>GES</sub>, *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>CTX-M</sub> were analyzed by PCR using genomic DNA and specific primers<sup>(3)</sup>. *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>SPM</sub>, *bla*<sub>GES</sub>, *bla*<sub>CTM-M</sub>, and *bla*<sub>SHV</sub> were not present, while *bla*<sub>KPC</sub> and *bla*<sub>TEM</sub> were detected, amplifying fragments of approximately 1,000bp. An additional PCR using plasmid DNA for *bla*<sub>KPC</sub> detection was performed to confirm the plasmid origin of this gene. The coding regions of *bla*<sub>KPC</sub> and *bla*<sub>TEM</sub> were sequenced, and the analysis of the nucleotide sequences and deduced protein sequences with BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and EXPASY (<http://web.expasy.org/tools/translate>) showed that the isolate harbored *bla*<sub>KPC-2</sub> and *bla*<sub>TEM-1</sub> (GenBank accession numbers KC736925 and KF811201). *bla*<sub>TEM</sub> has been described in *P. mirabilis* from Brazil by Dropa et al.<sup>(10)</sup> and Abreu et al.<sup>(11)</sup>, but they did not sequence the gene. The analysis of antimicrobial agents revealed susceptibility to meropenem, polymyxin, and tigecycline. Tibbetts et al.<sup>(7)</sup> identified KPC-producing *Proteus mirabilis* isolates

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that were susceptible only to piperacillin/tazobactam and ciprofloxacin in USA, and Sheng et al.<sup>(8)</sup> identified a panresistant KPC-producing *P. mirabilis* isolate in China. According to Castanheira et al.<sup>(12)</sup>, meropenem, ceftazidime-avibactam, and tigecycline were the most active antimicrobials against the KPC-producing *Escherichia coli*, *K. pneumoniae*, *Klebsiella oxytoca*, and *P. mirabilis* isolates collected in 2012 from the all United States Census Bureau-designated regions. The identification of *bla*<sub>KPC-2</sub> as the underlying basis of carbapenem resistance in *P. mirabilis* isolates is worrisome, although this finding is not totally unexpected, given the recent documented spread of *bla*<sub>KPC</sub> carbapenemase to a number of gram-negative bacteria<sup>(4)(5)(13)(14)</sup>, including in *K. pneumoniae* and *Pseudomonas aeruginosa* in the same hospital in Recife, Brazil<sup>(3)(6)</sup>. These data indicate the intra-bacterial transference of *bla*<sub>KPC-2</sub> at this hospital. According to the literature, *bla*<sub>KPC</sub> is encoded by transposon Tn4401, found in a variety of transferable plasmids<sup>(2)(15)</sup>. In this study, PCR experiments using plasmid DNA confirmed that *bla*<sub>KPC-2</sub> was present in the plasmids. Considering that transposons are mobile genetic elements, *bla*<sub>KPC-2</sub> could be present in any of the 5 plasmids detected (>150 kb, 150kb, 120kb, 90kb, and 70kb). The data presented herein confirm that *P. mirabilis* strains harboring *bla*<sub>KPC-2</sub> are emerging in Brazil, suggesting the continuous transfer of *bla*<sub>KPC</sub> between bacterial genera. This poses a serious challenge to prevent infections caused by multidrug-resistant bacteria.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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