References


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Detection of blaKPC-2 in a carbapenem-resistant Klyuyvera georgiana

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Sir,

Since the redefinition of the enteric group 8 as a new genus of the family Enterobacteriaceae designated Klyuyvera, there have been some reports describing its role as a human pathogen, mostly recovered from respiratory samples. Antimicrobial agents active against Klyuyvera spp. include third-generation cephalosporins, carbapenems, fluoroquinolones and aminoglycosides. Although the Klyuyvera genus is believed to be the source of genes encoding CTX-M-type extended spectrum β-lactamas (ESBLs), reports of multidrug-resistant isolates of this genus are still rare.

Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae have been increasingly reported worldwide and currently represents the major mechanism of carbapenem resistance in this family. Regardless of the fact that these enzymes are more frequently found in K. pneumoniae and Enterobacter spp., they have already been reported in virtually all members of Enterobacteriaceae. In this report, we describe the presence of KPC-2 in Klyuyvera georgiana.

A patient admitted to a hospital in Porto Alegre, Brazil, in 2011 required mechanical ventilation and ventilator-associated pneumonia (VAP) was diagnosed. A carbapenem-resistant isolate, initially identified as Klyuyvera cryocrescens by VITEK2 (bioMérieux, France), and a carbapenem- and cephalosporin-susceptible K. pneumoniae were recovered from a quantitative tracheal aspirate (QTA), both at >10^6 cfu/mL. Only piperacillin/tazobactam had been previously administered to the patient. Meropenem was initiated, but no improvement was observed, and a new QTA was performed 4 days after starting meropenem. A pure growth of K. cryocrescens, at >10^6 cfu/mL, was obtained. The patient was then treated with polymyxin B and recovered from VAP.

The isolate identified as K. cryocrescens was submitted to further analysis by 16S rRNA gene sequencing using primers 27f and 1492r for amplification and primers 27f, 38r, 533f, 1175r, 1194f and 1492r for sequencing. Susceptibility tests were performed by broth microdilution and interpreted according to CLSI criteria. The modified confirmatory test for ESBLs with antibiotic discs containing boronic acid (BA) was performed as described previously. The modified Hodge test (MHT) and combined-disc tests with BA were performed to screen for carbapenemase production. Specific primers were used to detect the presence of blaCTX-M, blaTEM and blaSHV ESBL-coding genes. PCR and sequencing of the blaKPC gene were performed as described previously. PCR products were purified using a GFX kit (GE Healthcare) and sequenced using BigDye Terminator version 3.1 and a 3130xl Genetic Analyzer (Applied Biosystems), according to the manufacturer’s instructions. GenBank was used to access the KPC sequences deposited to date, and the BioEdit program was used to compare the similarity between sequences. Plasmid DNA was obtained from alkaline lysis and it was electro- porated into Escherichia coli Top10 (Invitrogen). Transforms were selected on Luria–Bertani agar containing 1.0 mg/L ceftazidime or 0.05 mg/L meropenem, and an E. coli 398861 was used as a standard for transformant plasmid analysis. Genomic DNA from K. georgiana was submitted to amplification and sequencing using specific primers for evaluation of the genetic environment of the blaKPC-2 gene.
The 16S rRNA gene sequencing revealed that the isolate was in fact a K. georgiana, with 99.85% similarity to the sole available sequence from K. georgiana ATCC 51603 (GenBank accession number AF047186.1), while the similarity index was 98.91% when compared with K. cryocrescens ATCC 33435 (GenBank accession number AF310218.1). The isolate was resistant to all β-lactams (ceftazidime MIC, 16 mg/L; cefepime MIC, 32 mg/L; ampicillin/sulbactam MIC, ≥256 mg/L; imipenem MIC, 256 mg/L; meropenem MIC, 128 mg/L; and ertapenem MIC, 128 mg/L), but susceptible to other antibiotics such as amikacin (MIC, 2.0 mg/L), ciprofloxacin (MIC ≤0.125 mg/mL), tigecycline (MIC, 0.5 mg/L) and polymyxin B (MIC, 0.25 mg/L). ESBL phenotypic and genotypic tests were negative. Both the MHT and BA-based assay for carbapenemase detection were positive. The presence of the \( bla_{\text{KPC}} \) gene was confirmed by PCR and the gene sequencing revealed 100% identity with \( bla_{\text{KPC-2}} \). The plasmid electroporation into E. coli Top10 resulted in transformants that presented positive results using the MHT and the BA-based method, and the presence of the KPC gene was confirmed by PCR. The transformants demonstrated the acquisition of a ~36 kb plasmid and the genetic environment analysis of \( bla_{\text{KPC-2}} \) suggested that the gene was inserted in a Tn801-like transposon. We obtained a partial sequence of 1461 bp of this transposon (130 bp upstream to 459 bp downstream of the KPC gene) demonstrating 99.8% similarity with the sequence of a KPC-2-producing Enterobacter cloacae, in which the KPC gene is inserted in a Tn801-like transposon (GenBank accession number JNO48640.1).

High-level resistance to carbapenem was observed in K. georgiana, suggesting the involvement of additional resistance mechanisms. Since porin expression was not investigated, we cannot exclude the possibility that the K. georgiana phenotype results from the interplay between permeability alteration and KPC-2 production.

KPC-producing isolates have been associated with serious infection and high mortality, probably due to the difficulty of treating these infections. This work presents a description of a KPC-2 enzyme in the rare pathogen K. georgiana, highlighting the ability of KPC to spread to unusual pathogens.

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**Transparency declarations**

None to declare.

**References**


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**Emergence of resistance to fosfomycin used as adjunct therapy in KPC Klebsiella pneumoniae bacteraemia: report of three cases**

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Sir, Fosfomycin has recently been proposed as an adjunct to other active agents for the treatment of KPC-producing Klebsiella pneumoniae (KPC-Kp) infections.1,2 We describe here three