First description of KPC-2-producing Klebsiella oxytoca in Brazil

Running Title: KPC-2-producing Klebsiella oxytoca in Brazil

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The present work reports the detection of first case of nosocomial *K. oxytoca* producing Class A carbapenemase KPC-2 in Brazil. The isolate KPN106 carried a 65 kb IncW-type plasmid that harbors *bla*KPC gene and Tn4401b. Moreover, we detected the presence of a class 1 integron containing a new allele *arr*-8 followed by 5′-truncated *dhfr*IIIc gene. In view of the recent results, we emphasize the high variability of the bacterial and genetic hosts of this resistance determinant.

Despite the KPC enzymes are frequently associated with several members of Enterobacteriaceae family, few reports have described its presence in *Klebsiella oxytoca* isolates (1). This work reports the first case of KPC-2-producing *K. oxytoca* isolate in Brazil and describes its clinical data, susceptibility profile and molecular analysis.

A 79-years-old female patient with chronic obstructive pulmonary disease and chronic renal failure was readmitted in the ICU at the University Hospital Oswaldo Cruz, Recife, Brazil, in December 2008. Due to her prior hospitalization (forty-one days), the patient received upon admission imipenem (250 mg every 6h, for two days) and polymyxin B (500 000 U every 12h, for eight days). Blood cultures revealed the presence of a carbapenem-resistant *K. oxytoca* isolate (KPN106). The treatment with polymyxin B was maintained in combination with ciprofloxacin (400 mg every 12h) during seven days. The blood cultures remained positive after antimicrobial therapy. At 12th day of stay the patient died with a diagnosis of renal and respiratory failure and sepsis. Here we describe the microbiological and molecular analysis of this isolate.

Broth microdilution method showed that the KPN106 isolate was highly resistant to most of the antimicrobial agents tested (Table 1) according to CLSI (2). The isolate was tested for the presence of class 1 integron, ESBL and class A and B carbapenemases.
Molecular analysis was carried out as described (3). Beyond to \textit{bla}_{KPC-2}, KPN106 presented class 1 integron and additional \textit{bla} genes (Table 1). Moreover it carried three plasmids (c.a 65 kb, 15 kb, 12 kb) that were used in the transference experiments. The sequencing of the variable region of class 1 integron revealed a new allele of rifampin ADP-ribosylating transferase gene named \textit{arr}-8 (GenBank accession number KC199968), involved in resistance to rifampin, with 75\% protein similarity with the enzymes coded by \textit{arr}-2 and \textit{arr}-3 alleles. Furthermore, we found a 5'-truncated form of \textit{dhhfH} gene that encodes for dihydrofolate dehydrogenase, followed by a partial putative insertion sequence. The \textit{E. coli} DH5\(\alpha\) cells (TF106) selected on LB agar plates containing 100\(\mu\)g/ml ampicillin acquired the 65 kb plasmid together with \textit{bla}_{KPC-2} gene and class 1 integron as attested by PCR. The plasmid incompatibility groups were determined as described previously (4) and demonstrated these plasmids belong to the IncW in both donor and transformant cells. This acquisition increased MICs of \textit{E. coli} DH5\(\alpha\) cells for extended spectrum cephalosporins, carbapenens and rifampin (Table 1). Analysis of the genetic environment (5) of \textit{bla}_{KPC} revelead the presence of the transposon Tn4401b isoform, as observed in a KPC-producing \textit{K. oxytoca} isolate (1) and in other \textit{Enterobacteriaceae} members (5). Recently, it was described the presence at the same hospital of 65 kb IncW-type plasmid carrying \textit{bla}_{KPC} in a Tn4401c among \textit{Enterobacteriaceae} species (6). In the present study we report a 65 kb IncW-type plasmid carrying \textit{bla}_{KPC} in a Tn4401b in a \textit{K. oxytoca} isolate, highlighting to diversity of mobile genetic elements related to \textit{bla}_{KPC} gene in this institution and the high variability of the genetic hosts of this gene.

Acknowledgments
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References


2. Clinical and Laboratories Standards Institute. 2012. CLSI. M100-S22-U.


Table 1. Phenotypic and genetic characteristics of the bacterial strains used in this work.

<table>
<thead>
<tr>
<th>Strain</th>
<th>betalactamases/class 1 integron cassette array</th>
<th>MIC (µg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>AMK</td>
<td>GEN</td>
</tr>
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<td>KPN106</td>
<td>bla_KPC-2, blaCTX-M-2, blaSHV-11, bla_TEM-1, arr-8, dhfrIIIc</td>
<td>16</td>
</tr>
<tr>
<td>TF106</td>
<td>bla_KPC-2, arr-8, dhfrIIIc</td>
<td>16</td>
</tr>
<tr>
<td>DH5α</td>
<td>None</td>
<td>0,12</td>
</tr>
</tbody>
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* KPN106, K. oxytoca isolate; TF106, E. coli transformant KPN106; DH5α, E. coli recipient strain.

AMK, amikacin; GEN, gentamicin; CEF, cefalothin; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; ETP, ertapenem; CIP, ciprofloxacin; TZP, piperacillin-tazobactam; AMC, amoxicillin/clavulanate; RIF, rifampin; PMB, polymyxin B; TGC, tigecycline.
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