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Transfer of KPC-2 carbapenemase from *Klebsiella pneumoniae* to *Enterobacter cloacae* in a patient receiving meropenem therapy

Evelin Rodrigues Martins [a], Cássia Fernanda Estofolete [a,b], Andressa Batista Zequini [b], Louise Cerdeira [c], Doroti de Oliveira Garcia [d], Maria Fernanda Campagnari Bueno [d], Gabriela Rodrigues Francisco [d], Leonardo Neves de Andrade [e], Ana Lúcia da Costa Darini [e], Fernanda Modesto Tolentino [g,f], Tiago Casella [a,f], Nilton Lincopan [c,h], Mara Corrêa Lelles Nogueira [a,b,*]

[a] Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, Brazil
[b] Hospital de Base de São José do Rio Preto, São José do Rio Preto, Brazil
[c] Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil
[d] Instituto Adolfo Lutz, São Paulo, Brazil
[e] Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Ribeirão Preto, Brazil
[f] Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto, Brazil
[g] Instituto Adolfo Lutz, São José do Rio Preto, Brazil
[h] Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil

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ABSTRACT

The horizontal transfer of a plasmid bearing the bla_{KPC-2} gene from *K. pneumoniae* to *E. cloacae* infecting the respiratory tract of a patient during meropenem therapy was elucidated. This finding is particularly worrisome, since these drugs are of last resort for multidrug-resistant Gram-negative pathogens.

Carbapenems are the most effective antibiotics for the treatment of infections caused by multidrug-resistant Gram-negative bacteria, mainly extended-spectrum beta-lactamase-producing Enterobacteriaceae. In this regard, the wide dissemination of the *Klebsiella pneumoniae* carbapenemase (KPC) among members of the Enterobacteriaceae family has become a global issue, since KPC can hydrolyze all β-lactam antibiotics (Doi and Paterson 2015). Moreover, most KPC producers often exhibit co-resistance to aminoglycosides and fluoroquinolones, dramatically limiting treatment options (Morrill et al. 2015). Pandemic KPC has occurred primarily by the dissemination of KPC-producing *K. pneumoniae* isolates belonging to international clones. On the other hand, the bla_{KPC} gene, which is frequently located in transposon Tn*4401*, and carried on conjugative plasmids that vary in size and structure, can spread among clinically significant Gram-negative species by horizontal gene transfer (HGT) (Mathers et al. 2015). Evidences for HGT of the bla_{KPC} gene is supported on the isolation of several KPC-producing Enterobacteriaceae species in the same patient (Goren et al. 2010; Kocsis et al. 2014).

On January 16, 2014, a 57-year-old man was transferred from a local medical center to the emergency department of the Hospital de Base de São José do Rio Preto, in São Paulo, Brazil. During admission, the patient presented pallor, cold sweats and dyspnea, and reported epigastric pain of high intensity, irradiating to the back. Previous medical history enclosed diabetes mellitus, hypertension, congestive heart failure by trypanosomiasis and an ischemic stroke. Chest computerized tomography showed a type B aortic aneurysm without rupture with partially thrombosed false lumen and bilateral consolidation in lung parenchyma plus pleural effusion. He was transferred to cardiac intensive care unit (C-ICU), presenting mental confusion, tachypnea and hypertension, evolving with worsening of symptoms and consciousness impairment,

* Corresponding author. Tel.: +55-17-3201-5909; fax: +55-17-3229-1777.
E-mail addresses: evelin.rod.martins@gmail.com (E.R. Martins), cassiafestofolete@gmail.com (C.F. Estofolete), andressa.zequini@yahoo.com.br (A.B. Zequini), lcerdeira@gmail.com (L. Cerdeira), dogarcia@yahoo.com (D. de Oliveira Garcia), mf.campagnari@gmail.com (M.F.C. Bueno), gabis.francisco@gmail.com (G.R. Francisco), leoandrade02es@gmail.com (L.N. de Andrade), aldarini@fcfrp.usp.br (A.L. da Costa Darini), fernandaTollentino@hotmail.com (F.M. Tolentino), tiago_casella@yahoo.com.br (T. Casella), lincopan@usp.br (N. Lincopan), ml.nogueira@famerp.br (M.C.L. Nogueira).

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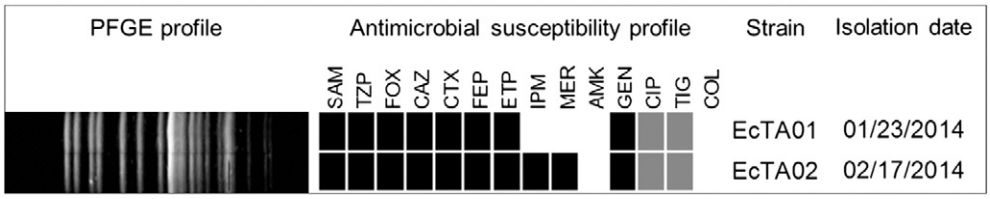

Fig. 1. PFGE profile and antimicrobial susceptibility profiles of *E. cloacae* strains EcTA01 and EcTA02. Black squares (resistant), gray squares (intermediate resistance), white squares (susceptible). SAM (ampicillin/sulbactam), TZP (piperacillin-tazobactam), FOX (cefoxitin), CAZ (ceftazidime), CTX (cefotaxime), FEP (cefepime), ETP (ertapenem), IPM (imipenem), MER (meropenem), AMK (amikacin), GEN (gentamicin), CIP (ciprofloxacin), TIG (tigecicline), COL (colistin). • Fig. 1 is a 2-column fitting image.

and requiring intubation and mechanical ventilation (MV). Amoxicillin/clavulanic acid (500 mg tid) was empirically prescribed, due to the pneumonia hypothesis, and an endotracheal aspirate (EA) sample was collected. Three days after, the treatment was changed to piperacillin-tazobactam (4.5 g qid). Quantitative EA culture showed the presence of *E. cloacae* ($<10^6$ CFU/mL), but the antimicrobial susceptibility test was not performed. Patient's condition worsened, and on January 23, 2014, a new EA sample was collected and an *E. cloacae* ($\geq 10^6$ CFU/mL) susceptible to carbapenems (CS), amikacin and colistin (designated EcTA01) was isolated. Meropenen (500 mg tid) was used for treatment and the patient evolved with clinical improvement. On February 6, 2014, he was submitted to placement of an aortic endoprosthesis. Without MV and invasive devices, he was discharged from C-ICU to hospital ward. On February 16, 2014, due to new septic condition, the patient was readmitted to C-ICU, being submitted to MV and placement of central venous and bladder catheters. Blood cultures were negative, but a carbapenem-resistant (CR) *K. pneumoniae* strain (designated KpU01) was isolated from a urine culture. One day later, another CR *K. pneumoniae* (designated KpTA01) and a CR *E. cloacae* (designated EcTA02) were isolated from EA cultures. Treatment with sodium colistin methanesulfonate (3.000.000UI tid) and ciprofloxacin (500 mg bid) was initiated. However, on the fourth day of this treatment his clinical conditions deteriorated, leading to death by septic shock, after forty-one days of admission. *E. cloacae* strains EcTA01 (CS) and EcTA02 (CR) were isolated in different times from endotracheal aspirate cultures, and *Xba*I-PFGE clonal relatedness (Pereira et al. 2013) elucidated that both strains belong to the same clone (Fig. 1).

The carriage of bla_{KPC} genes was investigated by PCR and sequencing (Doyle et al. 2012). Plasmids from CR strains were extracted and transformed into *Escherichia coli* DH10B. Next, plasmid DNAs were extracted from transformants to construct a Nextera XT DNA library, which was sequenced using the MiSeq platform (Illumina, San Diego, CA), using paired-end reads (300 bp), which produced reads of 3.632,240, 2.814,910 and 148.966 base pair lengths for plasmids pKP4365 (KpU01), pKP4368 (KpTA01) and pEC4365 (EcTA02), respectively. *De novo* assembly was performed using A5-Miseq pipeline (Coil et al. 2015) and this assembly was optimized using Geneious v.R9 (Biomatters Ltd., New Zealand). The three final plasmid sequences were annotated using BLASTp and ISFinder (https://www-is.biotoul.fr/blast.php); the annotation was curated using Artemis software version 16.0.11 (Carver et al. 2012). The DDBJ/ENA/GenBank accession numbers are KX783440 for pKP4365, KX783441 for plasmid pKP4368, and KX783439 for pEC4365.

During meropenem therapy, *E. cloacae* EcTA02 (CR) and *K. pneumoniae* KpTA01 (CR) were isolated from the same endotracheal sample, and curiously, both strains presented an identical ~15.7-kb plasmid harboring bla_{KPC-2} (pEC4365 and pKP4365). Otherwise, the *K. pneumoniae* KpU01 (CR), isolated from the urine sample, carried a ~ 67.1-kb plasmid (pKP4368), which also harbored the bla_{KPC-2} gene. In all CR strains, the presence of the bla_{KPC-2} gene was associated with transposon Tn*4401b* (Fig. 2), whereas none of the studied plasmids presented additional antibiotic resistance genes.

Clinical and experimental studies on antibiotic therapy have showed that the choice of dose and treatment duration can influence the selection of antibiotic-resistant bacteria (Olofsson and Cars 2007). Specifically, to broad-spectrum beta-lactams, some studies have demonstrated that Enterobacteriaceae can acquire carbapenem resistance during antimicrobial therapy through horizontal transfer of an insertion sequence or plasmid (Ding et al. 2016). In this study, we report for the first time (to our knowledge) the transfer of KPC-2 from *K. pneumoniae* to *E. cloacae* in the same patient, during meropenem treatment, since DNA sequences of KPC-2-positive plasmids from the two species were identical (Richter et al. 2011). So, both selection of the KPC-2-producing *E. cloacae* and coexistence of *K. pneumoniae* producing KPC-2, in the respiratory tract of the patient, were triggered by therapy with meropenem. On the other hand, horizontal transfer of identical bla_{KPC-2}- or bla_{NDM-7}-containing plasmids among several different Enterobacteriaceae genera has been previously documented (Cai et al. 2008; Chen et al. 2015), supporting the obtained result.

In conclusion, clonal dissemination and horizontal plasmid transfer can play a significant role in the acquisition of carbapenem resistance *in vivo*, where acquisition of carbapenemases by Enterobacteriaceae can occur during antibiotic therapy. In this regard, treatment of infection caused by KPC-positive bacteria is particularly worrisome, since

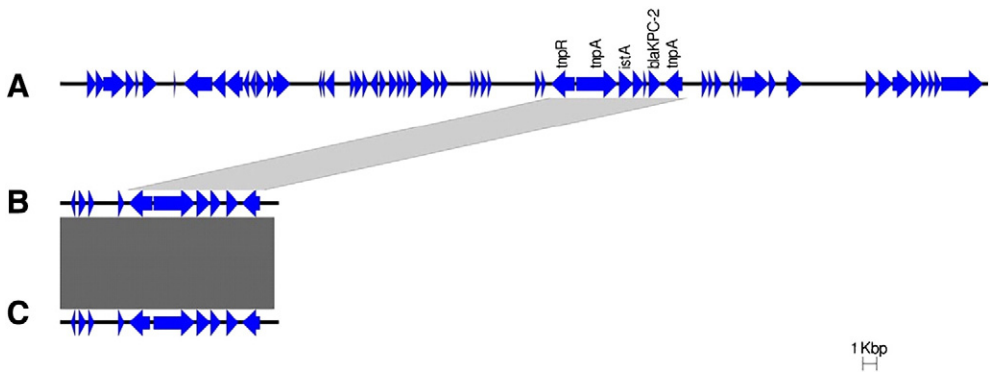

Fig. 2. Comparison of plasmids harboring the bla_{KPC-2} gene from *K. pneumoniae* KPU01 (A: pKP4368, GenBank accession number KX783441), *E. cloacae* ECTA02 (B: pEC4365, GenBank accession number: KX783439) and *K. pneumoniae* KPTA01 (C: pKP4365, GenBank accession number KX783440). • Fig. 2 is a 2-column fitting image.

the carbapenems are often considered of last resort for multidrug-resistant Gram-negative bacterial infections.

Conflicts of interest

None.

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