Detection of GES-5-producing Klebsiella pneumoniae in Brazil

Renata C. Picão*, Anderson F. Santos, Adriana G. Nicoletti, Guilherme H. Furtado and Ana C. Gales

Laboratório ALERTA, Universidade Federal de São Paulo, São Paulo, Brazil

*Corresponding author. Tel: +55-11-50846538; Fax: +55-11-50812965; E-mail: renata.picaoo@lemc.com.br

Keywords: β-lactam resistance, β-lactamases, ESBLs, carbapenemases, porin loss

Sir,
The production of carbapenemases or extended-spectrum β-lactamases (ESBLs) and/or AmpC β-lactamases coupled with porin loss are the most common mechanisms of carbapenem resistance in Klebsiella spp.1,2 Amino acid substitutions in the active site of GES-type ESBLs may enhance their spectrum of activity against carbapenems. The variants GES-2, -4, -5, -6 and -11 are able to hydrolyse imipenem; GES-5 hydrolyses this agent most efficiently.1,3,4 To date, GES-5-producing Klebsiella pneumoniae has only been identified in Korea.5 In this report we investigated the mechanisms responsible for carbapenem resistance in a K. pneumoniae subsp. ozaenae from Brazil.

KOZ-Ban2 was isolated from a rectal surveillance swab of an elderly patient admitted to a private hospital in São Paulo, Brazil, in 2008. The patient was admitted with the diagnosis of community-acquired pneumonia and was successfully treated with piperacillin/tazobactam and moxifloxacin. Two weeks later, the patient presented a new episode of pneumonia that was empirically treated with meropenem and teicoplanin, with good clinical outcome. After 5 weeks of hospitalization, the patient was admitted to the intensive care unit with septic shock and respiratory failure. Candida sp. was isolated from the bloodstream at the same time that KOZ-Ban2 was recovered from the rectal swab. The patient died 24 h later.

KOZ-Ban2 identification was performed by the BD Phoenix™ Automated Microbiology System. Susceptibility testing was performed by CLSI agar dilution,6 and showed that KOZ-Ban2 was resistant to all β-lactams including carbapenems, amikacin, gentamicin, nalidixic acid and ciprofloxacin (Table 1).

PCR and amplicon sequencing, using previously reported conditions and primers specific for KPC-, MBL- and ESBL-encoding genes and the 5‘ and 3‘ conserved sequences of integrons,7 identified the blaGES-5 gene. This gene was harboured in the first position of a class 1 integron, followed by the aacA4, blaOXA-17, dfrA21 and catB3 cassettes, encoding an aminoglycoside acetyltransferase, an extended-spectrum oxacillinase capable of hydrolysing cefotaxime and ceﬁpime better than ceftazidime,8 a dihydrofolate reductase and a chloramphenicol acetyltransferase, respectively. This new integron, named In91, had a weak P2 version with the sequence −35 TGGACA and −10 TAAGCT and an inactive P2 (GQ139471). The search for plasmid-mediated quinolone resistance determinants yielded negative results.

PCR and sequencing of ompK35 and ompK36 genes revealed that the size of the ompK36 amplicon was ~650 bp lower than expected. Sequencing showed that 674 bp was missing in KOZ-Ban2 ompK36 (GQ139473). In addition, although the ompK35 amplicon was the expected size, its sequencing revealed one change at position 689, where the codon TGG changed to the stop codon TAG, probably resulting in a deficient porin that lacked the last 130 amino acids (GQ139472). SDS–PAGE of outer membrane protein (OMP) profiles was performed as previously reported,9 using a carbapenem-susceptible K. pneumoniae for comparison. The carbapenem-susceptible isolate showed three bands near the 36 kDa band of the molecular weight marker, whereas KOZ-Ban2 had a single band, probably corresponding to OmpA (data not shown). These findings suggest that neither OmpK35 nor OmpK36 was expressed in KOZ-Ban2.

Mating-out assays were performed using streptomycin-resistant Escherichia coli K12 as the recipient strain, and selection was performed using ampicillin, clavulanic acid, ticarcillin and ticarcillin plus clavulanic acid (Table 1).

Table 1. Antimicrobial susceptibility profile of the KOZ-Ban2 clinical isolate, the recipient strain E. coli K12 and the transconjugant E. coli K12 carrying blaGES-5

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>KOZ-Ban2</th>
<th>Transconjugant E. coli K12 carrying blaGES-5</th>
<th>E. coli K12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticarcillin</td>
<td>256</td>
<td>&gt;256</td>
<td>4</td>
</tr>
<tr>
<td>Ticarcillin +2 mg/L clavulanic acid</td>
<td>64</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;256</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>32</td>
<td>4</td>
<td>≤2</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>64</td>
<td>4</td>
<td>≤2</td>
</tr>
<tr>
<td>Cefepime</td>
<td>64</td>
<td>128</td>
<td>≤2</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>128</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Ertopenem</td>
<td>32</td>
<td>≤0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Meropenem</td>
<td>16</td>
<td>≤0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;32</td>
<td>0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Imipenem +2 mg/L clavulanic acid</td>
<td>16</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>&gt;512</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>32</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Amikacin</td>
<td>16</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>32</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

MICs are expressed as mg/L.
performed on Mueller–Hinton agar plates containing 500 mg/L streptomycin and 50 mg/L ticarcillin. The presence of blaGES-5 in transconjugants was confirmed by PCR. Conjugation experiments revealed that blaGES-5 was carried by a self-conjugative plasmid of ~37 kb. Although the transconjugant carrying blaGES-5 had low carbapenem MICs (Table 1), a spectrophotometric assay, performed as previously described, revealed that its crude extract had hydrolytic activity against imipenem (data not shown).

Our study constitutes the first report of GES-5-producing Klebsiella spp. on the American continent. The presence of antimicrobial resistance determinants coupled with OmpK35 and OmpK36 loss have contributed to the multidrug resistance phenotype observed. Clinical data analysis suggests that patient colonization by KOZ-Ban2 was probably favoured by previous β-lactam therapy.

Acknowledgements
We thank Dr Laurent Poirel and Dr Minghua Wang for kindly providing positive controls used in this study.

Funding
The study was carried out as part of our routine work. A. C. G. is a researcher from the ‘National Council for Science and Technological Development (CNPq)’, Ministry of Science and Technology, Brazil (grant number 307714/2006-3).

Transparency declarations
None to declare.

References


Low prevalence of 16S methylases among extended-spectrum-β-lactamase-producing Enterobacteriaceae from a Turkish hospital

Béatrice Berçot1,2, Laurent Poirel1, Melda Özdamar3, Elif Hakko4, Salih Türkoglu3 and Patrice Nordmann5*

1INSERM U914 ‘Emerging Resistance to Antibiotics’, K.-Bicêtre, France; 2Service de Bactériologie–Virologie, Hôpital Lariboisière, Assistance Publique/Hôpitaux de Paris, Université Paris Diderot, Faculté de Médecine, Paris VII, France; 3Department of Microbiology, Anadolu Medical Center, Kocaeli, Turkey; 4Department of Infectious Diseases, Anadolu Medical Center, Kocaeli, Turkey; 5Service de Bactériologie–Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K.-Bicêtre, France

*Corresponding author. Tel: +33-1-45-21-36-32; Fax: +33-1-45-21-63-40; E-mail: nordmann.patrice@bct.aphp.fr

Keywords: aminoglycosides, RmtB, AAC(6’)-Ib-cr, Turkey, ESBLS

Sir,

Enzymatic inactivation mediated by aminoglycoside phosphotransferases (APhs), aminoglycoside nucleotidyltransferases (ANTs) or aminoglycoside acetyltransferases (AACs) is the most common mechanism of resistance to aminoglycosides identified among Gram-negative organisms. Among these resistance determinants, two acetyltransferases, AAC(6’)-Ib (which acetylates kanamycin, amikacin, tobramycin and netilmicin) and AAC(3)-II (which acts on kanamycin, tobramycin, netilmicin and gentamicin), and one adenylyltransferase, ANT(2’)-I (which modifies kanamycin, tobramycin and gentamicin), are the most prevalent.1 Since 2003, plasmid-mediated 16S rRNA methylases conferring high levels of resistance to all aminoglycosides that are used systemically (kanamycin, amikacin, tobramycin, netilmicin, gentamicin, and isepamicin) have been described. Five of these enzymes methylate the G1405 of the A site of 16S ribosomal RNA (ArmA, RmtA, RmtB, RmtC and RmtD), whereas one (NpmA) methylates the A1408 of the A site of the 16S rRNA site.2,3 In this work, we have conducted an epidemiological survey to evaluate the prevalence of 16S rRNA methylases among extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae collected from a Turkish hospital.

A collection of 139 non-repetitive and consecutive ESBL-producing enterobacterial clinical isolates was recovered from...