Isolation of KPC 3-producing *Enterobacter aerogenes* in a patient colonized by MDR *Klebsiella pneumoniae*

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**SUMMARY**

We describe the interspecies transmission of the plasmid-mediated *bla*KPC-3 gene, which confers carbapenem resistance, between clinically relevant gram-negative bacteria in a single patient. A KPC-3 producing *Enterobacter aerogenes* was isolated from a hospitalized patient previously colonized and then infected by a *Klebsiella pneumoniae* ST101 carrying the *bla*KPC-3 gene. The strains showed identical plasmids. Since intense horizontal exchanges among bacteria can occur in the gut, clinicians should be aware that patients colonized by carbapenem-resistant *K. pneumoniae* could become carriers of other carbapenem-resistant Enterobacteriaceae.

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**Key words:** *Magnusomyces capitatus*, Pleural infection, Galactomannan antigen, MALDI-TOF identification, Posaconazole.

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were observed, and the patient was finally transferred to a rehabilitation unit.

All K. pneumoniae isolates were highly resistant to all beta-lactams tested, including carbapenems and penicillin/inhibitor combinations, colistin, and ciprofloxacin. Two isolates were also resistant to aminoglycosides (Table 1). The E. aerogenes strain was resistant to carbapenems and aminoglycosides, but susceptible to colistin and ciprofloxacin. Identification of genes encoding carbapenemases (blaoXA-48, blaoXA-60, blaTEM, blaoX-01, blaoX-42, blaoX-04 genes), extended-spectrum β-lactamases (ESBLs) (such as blaoX-24, blaoX-48) and plasmid-mediated ampC beta-lactamases was performed by PCR as previously described (Mabilat et al., 1990; Pérez et al., 2002; Woodford et al., 2006; Poirel et al., 2011). Sequences were analyzed using the BioEdit software and BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST). As reported in Table 2, PCR screening and sequencing results showed that K. pneumoniae and E. aerogenes isolates carried the blaoX-48 and blaoX-01 genes; the six K. pneumoniae isolates were positive for the intrinsic, chromosomally located blaoX-48 gene, as expected. Multilocus sequence typing (MLST) was carried out according to Diancourt et al., (2005) and sequence types were analyzed using the Institut Pasteur database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). Analyses revealed that all K. pneumoniae isolates belonged to the epidemic clone sequence type 101 (ST101). Further analyses by PCR-based replicon typing (PBRT) (DIATHEVA, Italy) (Carattoli et al., 2005) showed that plasmids carried by the K. pneumoniae and E. aerogenes isolates were positive for the IncFIIK replicon, and two strains of K. pneumoniae (2105Kp and 128) also harboured the IncR-type replicon.

One of the six K. pneumoniae isolates (2105Kp) and the only E. aerogenes 163E were chosen as prototypes to compare plasmids carrying the blaoX-01 gene. Plasmid DNAs were purified with the PureLinkTM HiPure Plasmid Filter Midiprep Kit (Invitrogen, USA) and transformed into competent E. coli DH5α cells (Invitrogen, USA). PCR analysis (Villa et al., 2010) on transformants (TF) 2105Kp and 163E revealed that the blaoX-01 genes were located on the IncFIIK-type plasmid. In particular, sequence analysis showed that IncFIIK replicon sequences were homologous (100% identity) to those identified in the pKpQIL IncFIIK plasmid (GUS959196) (15). The identification of the pKpQIL-like plasmid, carrying the blaoX-01 gene, was an interesting finding. This plasmid is the most frequently identified carrier of blaoX-01 genotype in the most commonly spread K. pneumoniae clone ST258. Plasmids purified from TF2105Kp and TF163E transformants were then compared by EcoRI and PstI restriction fragment length polymorphism (RFLP) analysis. The plasmids isolated by transformation from the ST101 K. pneumoniae

**Table 1 - Summary of clinical data and susceptibility pattern of clinical isolates.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Date of isolation</th>
<th>Clinical specimen</th>
<th>Clinical ward</th>
<th>Identification</th>
<th>Antibiotic MIC values (mg/L) ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>06/10/13</td>
<td>Rectal swab</td>
<td>ICU</td>
<td>K. pneumoniae</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
<tr>
<td>134</td>
<td>17/10/13</td>
<td>Rectal swab</td>
<td>ICU</td>
<td>K. pneumoniae</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
<tr>
<td>2060</td>
<td>17/10/13</td>
<td>Blood</td>
<td>ICU</td>
<td>K. pneumoniae</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
<tr>
<td>2105Kp</td>
<td>07/11/13</td>
<td>Blood</td>
<td>ICU</td>
<td>K. pneumoniae</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
<tr>
<td>708</td>
<td>06/12/13</td>
<td>Blood</td>
<td>ICU</td>
<td>K. pneumoniae</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
<tr>
<td>708B</td>
<td>13/12/13</td>
<td>Abcess culture</td>
<td>ICU</td>
<td>K. pneumoniae</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
<tr>
<td>210</td>
<td>16/01/14</td>
<td>Abcess culture</td>
<td>ICU</td>
<td>E. aerogenes</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
<tr>
<td>163E</td>
<td>06/02/14</td>
<td>Rectal swab</td>
<td>ICU</td>
<td>E. aerogenes</td>
<td>AMK (ND), AMC (ND), FEP (ND), CTX (ND), CAZ (ND), CIP (ND), ERT (ND), FOE (ND), GEN (ND), IMP (ND), MEM (ND), TZP (ND), TGC (ND), SXT (ND)</td>
</tr>
</tbody>
</table>

¹MICs were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. Susceptibility MICs are highlighted in gray shading. Abbreviations: AMK, amikacin; AMC, amoxicillin-clavulanic; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; ERT, ertapenem; FOE, fosfomycin; GEN, gentamicin; IMP, imipenem; MEM, meropenem; TZP, piperacillin-tazobactam; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole. ICU: Intensive Care Unit.

**Table 2 - Summary of genetic features of clinical isolates and transformants.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Identification</th>
<th>Resistance determinants</th>
<th>PCR-based replicon typing</th>
<th>Sequence Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>K. pneumoniae</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>R, FIIk</td>
<td>101</td>
</tr>
<tr>
<td>134</td>
<td>K. pneumoniae</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>FIIk</td>
<td>101</td>
</tr>
<tr>
<td>2060</td>
<td>K. pneumoniae</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>FIIk</td>
<td>101</td>
</tr>
<tr>
<td>708</td>
<td>K. pneumoniae</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>FIIk</td>
<td>101</td>
</tr>
<tr>
<td>708B</td>
<td>K. pneumoniae</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>FIIk</td>
<td>101</td>
</tr>
<tr>
<td>2105Kp</td>
<td>K. pneumoniae</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>R, FIIk</td>
<td>101</td>
</tr>
<tr>
<td>163E</td>
<td>E. aerogenes</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>FIIk</td>
<td>ND</td>
</tr>
<tr>
<td>TF2105Kp</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>FIIk</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>TF163E</td>
<td>blaoX-01, blaoX-01, blaoX-01</td>
<td>FIIk</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

ND: not determined.
and E. aerogenes showed indistinguishable restriction profiles (Fig. 1).

Regarding the acquisition of the MDR E. aerogenes, it must be pointed out that no carbapenem-resistant microorganisms other than K. pneumoniae had been detected in our surveillance system up to that time. Considering that a carbapenem-susceptible E. aerogenes strain was previously isolated from a subcutaneous abscess (Table 1), we hypothesize that the IncFII blaKPC-3-carrying plasmid was transferred in vivo between the two species. Coexistence of multiple multidrug-resistant Enterobacteriaceae in one patient and transmission of the blaKPC gene between different strains was reported previously (Tsakris et al., 2010; Kocsis et al., 2014; Ding et al., 2015). So far, the transmission of the plasmid carrying the blaKPC gene among K. pneumoniae isolates has been shown to occur independently of the sequence types (Corbellini et al., 2014, Manageiro et al., 2015), suggesting that its dissemination is due to lateral gene transfer rather than clonal spread. To our knowledge, this is the first observation of blaKPC in ST101 and its possible transmission between K. pneumoniae and E. aerogenes. The ST101 is one of three clones prevalent in our hospital, together with ST258 and ST512 (data not shown), and has already been reported in Italy: an outbreak of carbapenem-resistant K. pneumoniae in Palermo (Mammina et al., 2012), Valle D’Aosta region (Del Franco et al., 2015) and an extremely drug-resistant K. pneumoniae in Padua (Frasson et al., 2012). In all cases the blaKPC was identified as the mechanism of carbapenem resistance in the K. pneumoniae isolates. The ST101 strain gene is likely to be endemic in Italy also in the community, as several strains, most carrying the blaCTX-M-15 gene, have been identified in a collection of ESBL producers isolated from animal samples between 2006 and 2012 (Donati et al., 2014). Moreover, international studies have described the dissemination of blakPC2, blactMX-M or blacTX-M-1 and blaxa-M genes in K. pneumoniae ST101 (Oteo et al., 2013; Potron et al., 2013; Mshana et al., 2015).

Horizontal transmission of the carbapenem-resistant plasmid pKpQIL across strains, species, and genera of bacteria has been widely observed (Goren et al., 2010; Mathers et al., 2011), and KPC-producing Enterobacteriaceae such as K. pneumoniae, Klebsiella oxytoca, Escherichia coli, Serratia marcescens, Enterobacter cloacae, E. aerogenes, Citrobacter freundii have been detected worldwide (Li et al., 2011, Piazza et al., 2016). In the last countrywide survey in Italy, KPC-type enzymes were the most common carbapenemases and the most affected species was K. pneumoniae, while only a minority of cases were Enterobacter spp (Giani et al., 2013). However, blakPC3-carrying E. aerogenes have been isolated in Portugal (Manageiro et al., 2015) and Italy (Kocsis et al., 2014). These observations indicate that KPC-dependent resistance can occur in Enterobacteriaceae other than K. pneumoniae, possibly leading to the establishment of further reservoirs of carbapenem-resistance genes. Our first finding of a highly resistant E. aerogenes strain occurred during a routine surveillance programme aimed at detecting all CRE. Our experience, together with other similar reports (Manageiro et al., 2015; Pesesky et al., 2015), suggests that attention should be given to all relevant KPC-carrying bacteria, including less pathogenic species, as part of an effective surveillance to reduce the number of CRE infections (Chen et al., 2012). Further investigations should focus on the mechanisms that can facilitate the horizontal transfer of plasmids carrying genes conferring antibiotic resistance.

References


Figure 1 - Restriction fragment length polymorphisms (RFLPs) of transformants. Restriction pattern of plasmid DNA of transformants TF2105Kp and TF163E obtained by digestion with EcoRI (lanes 2 and 3 respectively) and PstI (lanes 5 and 6), showing identical profiles. A 1Kb plus ladder size standard was run in lanes 1 and 4 (Invitrogen, USA).


