KPC-4 Is Encoded within a Truncated Tn4401 in an IncL/M Plasmid, pNE1280, Isolated from Enterobacter cloacae and Serratia marcescens

Kendall A. Bryant,¹ Trevor C. Van Schooneveld,² Ishwor Thapa,³ Dhundy Bastola,³ Laurin O. Williams,⁴ Thomas J. Safranek,⁵ Steven H. Hinrichs,⁵ Mark E. Rupp,⁵ Paul D. Fey,⁵b

Departments of Pathology and Microbiology⁴ and Internal Medicine;⁴ University of Nebraska Medical Center, Omaha, Nebraska, USA; College of Information Science and Technology, University of Nebraska at Omaha, Omaha, Nebraska, USA;⑤ Division of Laboratory Science and Standards, Centers for Disease Control and Prevention, Atlanta, Georgia, USA;⑥ Nebraska Department of Health and Human Services, Lincoln, Nebraska, USA

We describe the transfer of bla₄KPC-4 from Enterobacter cloacae to Serratia marcescens in a single patient. DNA sequencing revealed that KPC-4 was encoded on an IncL/M plasmid, pNE1280, closely related to pCTX-M360. Further analysis found that KPC-4 was encoded within a novel Tn4401 element (Tn4401f) containing a truncated tnpA and lacking tnpR, ISKpn7 left, and Tn4401 IRL-1, which are conserved in other Tn4401 transposons and movement to multiple plasmid backbones that results in acquisition by multiple species of Gram-negative bacilli.

Klebsiella pneumoniae carbapenemase (encoded by blaKPC), a functional group 2f, molecular class A serine β-lactamase conferring resistance to all β-lactam antibiotics except cephamycins, is the most common carbapenemase isolated in the United States (1). There are at least 12 known blaKPC types published in the literature or deposited in GenBank; however, the biological significance of these types is unknown. Although blaKPC is commonly isolated from K. pneumoniae sequence type 258 (ST258) worldwide (2, 3), it is also found in other Gram-negative species, including Serratia marcescens and Enterobacter cloacae (4–6). In addition, KPC is encoded on a highly conserved transposon, Tn4401, found on several different transferable plasmid replications in multiple species (4, 6–10). Tn4401 has at least 6 known isoforms (a through e, with two isoforms called Tn4401id) that are distinct due to deletions found just upstream of blaKPC (99-bp deletion [isoform a], no deletion [isoform b], 215-bp deletion [isoform c], 68-bp deletion [isoform d], and 255-bp deletion [isoform e]) (2, 3, 11–14). A separate Tn4401d that contains a partial deletion in blaKPC has been reported (11). We report the isolation of blaKPC-4 from E. cloacae and its subsequent in vivo transfer to S. marcescens in a single patient at an academic medical center. blaKPC-4 was isolated within a unique Tn4401 isoform (Tn4401f) on an IncL/M plasmid, pNE1280, from both E. cloacae and S. marcescens. Bioinformatic analysis of pNE1280 demonstrated high nucleotide identity to the previously sequenced IncL/M plasmid pCTX-M360, carrying blaCTX-M (15).

MATERIALS AND METHODS

Susceptibility testing and molecular methods. Antibiotic susceptibility testing of all isolates was performed using either Etest (bioMérieux, Marcy l’Etoile, France) or disk diffusion (16) and interpreted using standards from the Clinical and Laboratory Standards Institute (CLSI) (17). The modified Hodge test, used to phenotypically detect the presence of a carbapenemase, was performed according to the CLSI guidelines (17). K. pneumoniae BAA1705 and BAA1706 were used as positive and negative controls, respectively (17). Extended-spectrum β-lactamases (ESBLs) were phenotypically detected by testing the MICs of cefotaxime and ceftriaxone alone and in combination with clavulanate. A ≥3, twofold concentration decrease in an MIC for either ceftriaxone or cefotaxime tested in combination with clavulanate in comparison to the MIC when tested alone was considered positive for an ESBL (17). The primers used to detect the presence of blaKPC and IncL/M repA were as follows: KPC forward (ATGTCACGTCTAGGCCGTCT) and KPC reverse (TCTTCAGAGCTCTACTGCCC), and IncL/M forward (GGATGAAAACATACGCACTGAG) and IncL/M reverse (CTGAGGGGCGATTCTTTAGG) (18–20). Isoelectric focusing, performed as described by Mathew et al. (21), was used to detect the presence and pIs of specific β-lactamases. Pulsed-field gel electrophoresis (PFGE) was performed according to the standard PulseNet protocol for Escherichia coli, as described by Ribot et al. (22).

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Address correspondence to Paul D. Fey, pfey@unmc.edu. Copyright © 2013, American Society for Microbiology. All Rights Reserved. doi:10.1128/AAC.01062-12
from the plasmid sequences EU938349 and EU176011. The output of this pipeline was used to create a TBL file, which contains all sequence features in a tabular format. These data, together with published information, were converted with a TBL2ASN program (http://ncbi.nlm.nih.gov/genbank/tbl2asn2/) to generate an SQN file that could be submitted to GenBank.

RESULTS AND DISCUSSION

While performing a statewide public health surveillance study assessing the prevalence of expanded-spectrum cephalosporin and carbapenem resistance in the Enterobacteriaceae, we identified a 60-year-old female with a medical history of mitral and aortic valve stenosis, pulmonary hypertension, restrictive lung disease, and diabetes in January 2010. She was admitted for progressive dyspnea and renal failure and underwent aortic valve replacement 10 days later. Her postoperative course was complicated by renal failure requiring hemodialysis, pneumonia due to Pseudomonas aeruginosa, which was treated with 2 weeks of cefepime followed by 4 weeks of ciprofloxacin, ending in mid-July. A blood culture from late July was positive for S. marcescens isolate 1637, which had an antibiotic susceptibility profile identical to that of S. marcescens 1609. However, a sputum culture from early August detected an S. marcescens isolate, 1638, susceptible only to amikacin, gentamicin, and tobramycin; resistant to multiple β-lactam antibiotics, including ertapenem and imipenem; and exhibiting intermediate susceptibility to meropenem (Table 1). Subsequent PCR experiments with S. marcescens 1638 using primers KPC forward and reverse demonstrated that it also encoded a KPC carbapenemase. Neither K. pneumoniae nor P. aeruginosa was positive for blaKPC by PCR. Due to a poor prognosis, the patient was placed on palliative care, and she died in August 2010.

DNA sequencing of the blaKPC PCR products from both E. cloacae 1623 and S. marcescens 1638 confirmed that these isolates encoded a KPC-4 β-lactamase (19, 28). Furthermore, the PFGE patterns of S. marcescens 1609, 1637, and 1638 were indistinguishable (Fig. 1A), suggesting the possible transfer of a plasmid carrying blaKPC-4 from E. cloacae 1623 into a colonizing isolate of S. marcescens. To address this hypothesis, Southern blot analysis was performed on PFGE-separated DNA using a blaKPC-4 DNA probe. These analyses demonstrated that the blaKPC-4 DNA probe hybridized to a band of similar size in both E. cloacae 1623 and S. marcescens 1638, but not S. marcescens 1609 or 1637 (Fig. 1A and B).

To determine whether the blaKPC-4 gene was transferred between the two genera on a common plasmid, total plasmid DNA was extracted from E. cloacae 1623 and S. marcescens 1638 and used to transform E. coli ElectroMax Stbl4. Potential E. coli Stbl4 strains harboring plasmids carrying blaKPC-4 from E. cloacae 1623 and S. marcescens 1638 were selected by plating on Luria-Bertani (LB) agar (Becton Dickinson, Sparks, MD) containing 1 µg/ml of imipenem (Sigma, St. Louis, MO), producing E. coli Stbl4 1623 and 1638, respectively. The transformation frequency of DNA isolated from both E. cloacae 1623 and S. marcescens 1638 that conferred reduced susceptibility to imipenem was measured at approximately 10⁻⁷ per µg plasmid DNA. To facilitate analysis, total plasmid DNA was isolated from imipenem-resistant E. coli Stbl4

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**TABLE 1 Antibiotic susceptibilities (MICs) of E. cloacae, S. marcescens, and E. coli DH5α 1623 and 1638**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. marcescens</th>
<th>E. cloacae 1623</th>
<th>E. coli DH5α</th>
<th>E. coli DH5α 1623</th>
<th>E. coli DH5α 1638</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1609</td>
<td>1637</td>
<td>1638</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMC</td>
<td>16 (I)</td>
<td>16 (I)</td>
<td>128 (R)</td>
<td>16 (I)</td>
<td>&lt;0.016 (S)</td>
</tr>
<tr>
<td>TZP</td>
<td>2 (S)</td>
<td>2 (S)</td>
<td>128 (R)</td>
<td>32 (R)</td>
<td>0.5 (S)</td>
</tr>
<tr>
<td>AMP</td>
<td>8 (S)</td>
<td>8 (S)</td>
<td>&gt;256 (R)</td>
<td>&gt;256 (R)</td>
<td>1 (S)</td>
</tr>
<tr>
<td>FOX</td>
<td>64 (R)</td>
<td>64 (R)</td>
<td>128 (R)</td>
<td>64 (R)</td>
<td>2 (S)</td>
</tr>
<tr>
<td>CAZ</td>
<td>0.5 (S)</td>
<td>0.5 (S)</td>
<td>&gt;256 (R)</td>
<td>&gt;256 (R)</td>
<td>0.125 (S)</td>
</tr>
<tr>
<td>CTX</td>
<td>0.5 (S)</td>
<td>0.5 (S)</td>
<td>&gt;256 (R)</td>
<td>8 (R)</td>
<td>0.032 (S)</td>
</tr>
<tr>
<td>CRO</td>
<td>0.5 (S)</td>
<td>0.5 (S)</td>
<td>&gt;256 (R)</td>
<td>8 (R)</td>
<td>&lt;0.016 (S)</td>
</tr>
<tr>
<td>FEP</td>
<td>0.5 (S)</td>
<td>0.5 (S)</td>
<td>16 (I)</td>
<td>2 (S)</td>
<td>0.032 (S)</td>
</tr>
<tr>
<td>ETP</td>
<td>0.032 (S)</td>
<td>0.032 (S)</td>
<td>8 (R)</td>
<td>4 (R)</td>
<td>0.008 (S)</td>
</tr>
<tr>
<td>MEM</td>
<td>0.032 (S)</td>
<td>0.032 (S)</td>
<td>2 (I)</td>
<td>4 (R)</td>
<td>0.032 (S)</td>
</tr>
<tr>
<td>IPM</td>
<td>0.5 (S)</td>
<td>0.5 (S)</td>
<td>16 (R)</td>
<td>8 (R)</td>
<td>0.25 (S)</td>
</tr>
</tbody>
</table>

* AMC, amoxicillin/clavulanate; TZP, piperacillin-tazobactam; AMP, ampicillin; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; ETP, ertapenem; MEM, meropenem; IPM, imipenem.

a MIC (µg/ml) and interpretation (susceptible [S], intermediate [I], or resistant [R]) as defined by the Clinical and Laboratory Standards Institute (17).
KPC-4 Encoded in an IncL/M Plasmid, pNF1280

DNA sequencing revealed that pNE1280 was 66,532 bp (GenBank JQ837276). Open reading frame (ORF) analysis detected 72 ORFs within pNE1280, with 42 showing significant amino acid similarity to proteins with known functions. Sequence alignment comparisons against the GenBank database determined high nucleotide identity between the pNE1280 backbone and pCTX-M360 (NCBI reference sequence NC_011641.1), a previously described IncI1 plasmid carrying blaCTX-M isolated in China (15). Primers described by Carattoli et al., specific for IncI1 plasmids, confirmed that pNE1280 is an IncI1 plasmid backbone (18). pNE1280 contained one major insertion of approximately 13 kb containing the Tn3 family transposon Tn440I harboring blaKPC-4, here called Tn440If (31) (Fig. 3). The blaKPC-4 gene was flanked by ISKpn71 IR right and ISKpn6 IR left, similar to other previously described Tn4401-containing plasmids, and did not contain the 100-bp deletion found in pNYC and other Tn4401 transposons (Fig. 3) (31). However, pNE1280 contained a truncated tnpA gene and lacks both ISKpn71 IR left and the tnpR gene. In addition, Tn440If does not contain the flanking upstream Tn4401 IR-L sequence in comparison to other known Tn4401 sequences (Fig. 3) (14). Therefore, these data suggest that Tn4401 and ISKpn7 are not functional within pNE1280, although a putative Tn4401 IR-L was located just upstream of istB (Fig. 3).

In conclusion, we found a high likelihood that blaKPC-4 was transferred from E. cloacae 1623 to S. marcescens 1638 within a single patient. This was the first case of blaKPC-mediated carbapenem resistance at our institution and, to our knowledge, in Nebraska. DNA sequencing revealed that KPC-4 was carried on an IncI1 plasmid, pNE1280; IncI1 plasmids have rarely been reported to carry blaKPC (2, 32). Although KPC-4 was encoded within the highly conserved Tn4401 element, several structural features were found, most notably the loss of tnpR and IRL-1, which suggests that the Tn440If transposon described in this report is not functional. These observations further highlight the continued recombination and evolution of Tn4401 elements en-
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The conclusions of this study are the sole responsibility of the authors and do not necessarily represent the official views of NCRR or NIH. Further, the findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES


