Persistence of NDM-1-producing enterobacteria over a 6-month period in a French community patient.

ARPIN C.1, NOURY P.2, M’ZALI F.1, ANDRE C.1, COULANGE L.1, C QUENTIN1.

1 UMR CNRS 5234, Université de Bordeaux 2, Bordeaux, France - 2 Laboratoire EXALAB, Villeneuve d’Ornon, France.

Introduction

On 09/01/2011, Kp5196, a colistin susceptible (CS) and nitrofurantoin resistant (NF) K. pneumoniae isolated for a urinary tract infection (UTI) in a French community patient without history of foreign travel (1). Two months later, a second UTI episode was this time due to the CS-NF Kp5196 producing K. pneumoniae, Kp5289. Thus, nitrofurantoin therapy was implemented. The aim of this study was to look for the persistence of NDM-1-producing enterobacteria in the urine and the stools of this patient over a 6-month period. The strains were analyzed for their epidemiological relationship, antibiotic resistance phenotype and plasmidic content.

Case report

Time distribution of NDM-1-producing enterobacteria

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample</th>
<th>Isolation date (day/month/2011)</th>
<th>Date of first isolation (day/month/2011)</th>
<th>Date of last isolation (day/month/2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kp5196</td>
<td>Urine</td>
<td>09/01/2011</td>
<td>09/01/2011</td>
<td>06/06/2012</td>
</tr>
<tr>
<td>Kp5241</td>
<td>Urine</td>
<td>11/05/2011</td>
<td>11/05/2011</td>
<td>01/06/2012</td>
</tr>
<tr>
<td>Kp5289</td>
<td>Urine</td>
<td>03/01/2012</td>
<td>03/01/2012</td>
<td>29/04/2012</td>
</tr>
<tr>
<td>Ex5301/Ea5315</td>
<td>stool</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
</tr>
<tr>
<td>Ex5319</td>
<td>Stool</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
</tr>
</tbody>
</table>

Material and Methods

- Screening of enterapen-resistant enterobacteria in the fecal samples: CHROMagar KPC medium (CHROMagar).
- Strain identification: API20E system (bioMérieux) and urea indole medium (bioMérieux).
- Strain typing: PFGE using XbaI and the CHEF DR3 apparatus (BioRad).
- Resistance gene analysis: PCR amplification and sequencing.
- Plasmid analysis:
  - Conjugal transfer to Escherichia coli C600 (AdeR resistant).
  - Plasmid size determination by S1 nuclease-PFGE analysis (2).
- Analysis of resistance gene loss after repeated subcultures without selective pressure.

Antibiotic resistance phenotypes and associated genes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample</th>
<th>Isolation date</th>
<th>Date of first isolation</th>
<th>Date of last isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kp5196</td>
<td>Urine</td>
<td>09/01/2011</td>
<td>09/01/2011</td>
<td>06/06/2012</td>
</tr>
<tr>
<td>Kp5241</td>
<td>Urine</td>
<td>11/05/2011</td>
<td>11/05/2011</td>
<td>01/06/2012</td>
</tr>
<tr>
<td>Kp5289</td>
<td>Urine</td>
<td>03/01/2012</td>
<td>03/01/2012</td>
<td>29/04/2012</td>
</tr>
<tr>
<td>Ex5301/Ea5315</td>
<td>stool</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
</tr>
<tr>
<td>Ex5319</td>
<td>Stool</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
<td>20/02/2011</td>
</tr>
</tbody>
</table>

In vitro loss of resistance genes and concomitant evolution of plasmid size

- Antibiotic resistance genes (a)
  - blaCTX-M-15
  - blaOXA-23
  - blaDHA-16
  - blaNDM-1
- Genes or open reading frames (ORFs) are indicated by boxes and their transcriptional orientations are indicated by arrows. The same label is used to represent homologous genes. The antibiotic resistance genes are indicated in italic for blaNDM-1
- Genes are not present in all strains.

Schematic representation of the pNDM-FRA plasmid

<table>
<thead>
<tr>
<th>pNDM-FRA DNA sequences surrounding the blaNDM-1 (A) and armA (B) genes and comparison with the sequences of pNDM-MAR (A) and pCTX-M3 (B)</th>
</tr>
</thead>
</table>

Material and Methods

- Analysis of strain persistence
- Identification of NDM-1-producing K. pneumoniae
- Antibiotic susceptibility testing
- Conjugative transfer experiments
- Plasmid analysis
- Analysis of resistance gene loss

Results

- Antibiotic resistance phenotypes and associated genes
- Schematic representation of the pNDM-FRA plasmid

Conclusions

- Analysis of in vitro plasmid evolution
- Persistence of NDM-1-producing enterobacteria over a 6-month period
- Antibiotic resistance genes
- Conjugative plasmid

References