Spread of a *Klebsiella pneumoniae* Strain Producing a Plasmid-Mediated ACC-1 AmpC β-Lactamase in a Teaching Hospital Admitting Disabled Patients

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We describe a large outbreak involving a *Klebsiella pneumoniae* strain producing a plasmid-encoded ACC-1 type AmpC β-lactamase in a hospital caring for patients with motor impairment. The epidemic strain was isolated from 57 patients in six wards between September 1999 and May 2003 and caused clinical infections in 19 patients.

Large nosocomial epidemics involving AmpC-producing *Klebsiella pneumoniae* strains have never been reported to date. The first reported nosocomial outbreak due to AmpC-producing *K. pneumoniae* strains occurred at the Miriam Hospital (Rhodes, Greece) in 1988 and involved 11 patients (8). A number of other outbreaks have since been described, but they rarely involved more than 10 individuals (7). We report the largest outbreak involving a plasmid-encoded AmpC-producing *K. pneumoniae* strain and the first such case in a department of physical medicine and rehabilitation (PMR). The Raymond Poincaré hospital (Garches, France) is a 440-bed teaching hospital that includes a 150-bed PMR department caring for patients with motor impairment. In November 1999, a 26-year-old patient with tetraplegia transferred from Tunisia to the PMR department was found to have urine samples positive for an ACC-1-producing *K. pneumoniae* strain. After the identification of three other cases from January to March 2000 in the PMR department, the screening policy used for the detection of extended-spectrum β-lactamase-producing organisms by rectal swabbing and inoculation of Dri-galski agar plates containing 0.5 ml/liter of cefotaxime (1) was extended to ACC-1-producing *K. pneumoniae* strains. Oxidase-negative colonies detected on cefotaxime selection plates were tested with the disk diffusion method (3) for susceptibility to ticarcillin-clavulanate, ceftazidime, cefotaxime, aztreonam, cefepime, cefoxitin, and cefotetan. Epidemic ACC-1-producing *K. pneumoniae* was suspected in the presence of the susceptibility pattern shown in Fig. 1.

The epidemic involved a total of 57 cases (at least one clinical sample and/or rectal swab positive for ACC-1-producing *K. pneumoniae*) from September 1999 to May 2003, mainly in the PMR department (46 cases) but also in other hospital units (surgical intensive care unit, 7 cases; medical intensive care unit, 3 cases; and septic orthopedic surgery, 1 case). The epidemic was controlled by strictly isolating carriers of ACC-1-producing *K. pneumoniae*, a measure implemented only at a late stage because it is in contradiction with our reeducation strategy. ACC-1-producing *K. pneumoniae* isolates were detected in diagnostic cultures (12 cases), screening cultures (24 cases), or both (21 cases). Nineteen patients met the criteria for nosocomial infection (4) (urinary tract infection, 16 cases; screening cultures (24 cases), or both (21 cases). Nineteen patients met the criteria for nosocomial infection (4) (urinary tract infection, 16 cases; screening cultures (24 cases), or both (21 cases). Nineteen patients met the criteria for nosocomial infection (4) (urinary tract infection, 16 cases; screening cultures (24 cases), or both (21 cases). Nineteen patients met the criteria for nosocomial infection (4). Nineteen patients met the criteria for nosocomial infection (4). Nineteen patients met the criteria for nosocomial infection (4). Nineteen patients met the criteria for nosocomial infection (4).

All ACC-1-producing *K. pneumoniae* isolates collected during the outbreak showed the same pattern of resistance to β-lactams with the disk diffusion method and were resistant to gentamicin, tobramycin, netilmicin, trimethoprim-sulfamethoxazole, and rifampin; most isolates were resistant to tetracycline and/or intermediate or resistant to ciprofloxacin. MICs of ceftazidime, cefotaxime, cefoxitin, and cefotetan were determined by the agar dilution method on the first ACC-1-producing *K. pneumoniae* isolates recovered from cases 1 to 12. The results confirmed the results of previous disk diffusion susceptibility tests (Table 1) and suggested the presence of an AmpC-type enzyme, most likely an ACC-1 enzyme because of the susceptibility to cefoxitin (2).

The first isolates recovered from cases 1, 2, and 3 (referred to as isolates 1, 2, and 3, respectively) were each tested for the ability to transfer ceftazidime resistance to *Escherichia coli* 

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K12C600 (Str<sup>b</sup>) by conjugation and to *E. coli* NM554 (Str<sup>b</sup>) by transformation. Transconjugants and transformants were selected on Drigalski agar plates containing streptomycin (100 mg/liter) and ceftazidime (10 mg/liter). Transconjugants (obtained only with isolate 2) and transformants displayed the same pattern of resistance to β-lactam agents as the donor *K. pneumoniae* isolates. Other resistance markers were cotransferred: (i) gentamicin, tobramycin, and netilmicin (isolates 1, 2, and 3); (ii) rifampin (isolates 1, 2, and 3); (iii) sulfamethoxazole (isolates 1 and 2); (iv) trimethoprim (isolate 3); and (v) tetracycline (isolate 3). Each of the transconjugants and transformants contained a single additional plasmid of approximately 100 kb (data not shown), suggesting that all of the transferred resistance genes were carried by this genetic element.

PCR was carried out on the epidemic *K. pneumoniae* isolates 1 to 3 and their transconjugants and transformants in *E. coli* using the sets of primers ACC3-ACC2, SHV01-SHV02, and TEMA1-TEMB1 (Table 2). The amplification conditions were 45 cycles of 30 s at 94°C, 30 s at the annealing temperature, and 30 s at 72°C and a final extension step at 72°C for 10 min. The PCR products were sequenced with the Big Dye terminator sequencing kit (Perkin-Elmer/Applied Biosystems, Courtabeuf, France) using the primers listed in Table 2 and an ABI Prism sequencer (Perkin-Elmer/Applied Biosystems). The three

Ampicillin 512–512 64–256 16 2
Cefalotin >512–512 >512–512 2 2
Cefoxitin 4–4 2–4 0.06 0.06
Cefotetan 2–2 2–2 ≤0.03 ≤0.03
Ceftazidime 32–64 32–64 ≤0.03 ≤0.03
Aztreonam 32–64 32–64 ≤0.03 ≤0.03
Cefepime 0.25–0.5 0.25–0.25 ≤0.03 ≤0.03
Imipenem 0.06–0.125 ND 0.125 ND

**TABLE 1. β-Lactam susceptibility of epidemic ACC-1-producing *K. pneumoniae***

<table>
<thead>
<tr>
<th>β-Lactam</th>
<th>ACC-1-producing <em>K. pneumoniae</em></th>
<th>Control&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-Lactam alone&lt;sup&gt;a&lt;/sup&gt;</td>
<td>β-Lactam + Cla&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>512–512</td>
<td>64–256</td>
</tr>
<tr>
<td>Cefalotin</td>
<td>&gt;512–512</td>
<td>&gt;512–512</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4–4</td>
<td>2–4</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>2–2</td>
<td>2–2</td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>8–8</td>
<td>4–8</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>1–2</td>
<td>1–2</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.25–0.5</td>
<td>0.25–0.25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.06–0.125</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Extreme MICs obtained with 12 epidemic isolates (first isolate from cases 1 to 12). Abbreviations: Cla, clavulanate; ND, not determined.

<sup>b</sup> β-Lactam MICs in the presence of a fixed concentration of clavulanate (2 mg/liter).

<sup>c</sup> *K. pneumoniae* B0004479-001 (wild-type β-lactam susceptibility pattern).

**FIG. 1. β-Lactam susceptibility pattern of the epidemic ACC-1-producing *K. pneumoniae* strain (disk diffusion method on Mueller-Hinton agar).** Note the following: (i) the resistance to cefotaxime, ceftazidime, aztreonam, and cefotetan; (ii) the susceptibility to cefepime and cefoxitin; (iii) the lack of synergy between ceftazidime, cefotaxime, aztreonam, cefepime, and clavulanate; and (iv) the inhibition zone of cefoxitin was greater than that of cefotetan (diameters of 19 to 25 mm versus 16 to 20 mm, respectively) and antagonism between cefotaxime and clavulanate. Abbreviations: TCC, ticarcillin-clavulanate; CAZ, ceftazidime; FOX, cefoxitin; PEP, cefepime; ATM, aztreonam; CTX, cefotaxime; and CTT, cefotetan.

*K. pneumoniae* isolates and their transconjugants and transformants yielded an amplicon of 1,276 bp with the primer set ACC3-ACC2, which was identical to the first ACC-1 *K. pneumoniae* gene described (2). PCR studies also revealed the presence of a *bla<sub>SHV</sub>* gene in epidemic *K. pneumoniae* isolates but not in the transconjugants and transformants and of a *bla<sub>TEM</sub>* gene, identified as a *bla<sub>TEM</sub>* gene by sequencing, both in the epidemic isolates and in their transconjugants and transformants.

ACC-1-producing *K. pneumoniae* isolates were characterized by random amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) analysis of XbaI-digested DNA. RAPD was carried out as previously described using the primers HLWL74 (5'-ACGTATCTGC-3') and R108

**TABLE 2. Primers used for PCR and sequencing**

<table>
<thead>
<tr>
<th>β-Lactam resistance gene</th>
<th>Primer name and sequence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>T&lt;sub&gt;23&lt;/sub&gt;, (°C) Fragment size (bp) Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bla&lt;sub&gt;SHV&lt;/sub&gt;</em></td>
<td>SHV-F: 5'-CACTCAAGGATGTATTGTG-3' SHV-R: 5'-TACGGTGCTGGAAT-3'</td>
<td>49 822 10</td>
</tr>
<tr>
<td><em>bla&lt;sub&gt;TEM&lt;/sub&gt;</em></td>
<td>A1: 5'-ATGAAATTTGAGAAGGGACTC-3' B1: 5'-TTACGGTTAATC-3' V167*: 5'-ATCCTTTGAGGTTTGC-3' V267*: 5'-GCTTTTCTGACTTGG-3'</td>
<td>42 1,075 12</td>
</tr>
<tr>
<td><em>bla&lt;sub&gt;ACC-1&lt;/sub&gt;</em></td>
<td>ACC3: 5'-AGGAGGATGGAATGTAGAG-3' ACC2: 5'-GTGAAAGCAGTGGGATT-3' ACC7*: 5'-GGGTTCCTCAATC-3'</td>
<td>55 1,276</td>
</tr>
</tbody>
</table>

<sup>a</sup> An asterisk indicates that the primer was used only for sequencing.
(5′-GTATTCGCCCT-3′) (5). PFGE was carried out using the GenePath system with a CHEF DRII apparatus (Bio-Rad Laboratories, Marna-la-Coquette, France), as previously described (13). All of the epidemic ACC-1-producing \textit{K. pneumoniae} isolates analyzed shared the same RAPD profile and were of the same PFGE pulotype according to Tenover et al. (15) (data not shown).

This is the first large, nosocomial outbreak involving a unique strain of \textit{K. pneumoniae} producing a plasmid-mediated AmpC \(\beta\)-lactamase. The epidemic strain harbored a plasmid-borne \textit{bla}_{ACC-1} gene, known to be derived from the chromosomal \textit{bla} gene of \textit{Hafnia alvei} (9). This strain was imported from a region of Tunisia that constitutes a persistent focus of \textit{Hafnia alvei} (13) (data not shown). PFGE was carried out using the \textit{bla}_{ACC-1} GTATTGCCCT-3′ (5).

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REFERENCES


