Worldwide Diversity of \textit{Klebsiella pneumoniae} That Produces β-Lactamase \textit{bla}_{KPC-2} Gene\textsuperscript{1}

Gaëlle Cuzon, Thierry Naas, HaVy Truong, Maria-Virginia Villegas, Karin T. Wiseli, Yehuda Carmeli, Ana. C. Gales, Shiri Navon-Venezia, John P. Quinn, and Patrice Nordmann

\textit{Klebsiella pneumoniae} isolates that produce carbapenemases (KPCs) are rapidly disseminating worldwide. To determine their genetic background, we investigated 16 \textit{bla}_{KPC-2}-harboring \textit{K. pneumoniae} isolates from 5 countries. The isolates were multidrug resistant, possessed the \textit{bla}_{KPC-2} gene, and differed by additional β-lactamase content. They harbored a naturally chromosome-encoded \textit{bla} gene (\textit{bla}_{SHV-1} [12.5%], \textit{bla}_{SHV-11} [68.7%], or \textit{bla}_{OXA-48} [18.8%]) and several acquired and plasmid-encoded genes (\textit{bla}_{TEM-1} [81.3%], \textit{bla}_{GTX-M-2} [31.3%], \textit{bla}_{CTX-M-12} [12.5%], \textit{bla}_{CTX-M-15} [18.7%], and \textit{bla}_{OXA-2} [37.5%]). The \textit{bla}_{KPC-2} gene was always associated with 1 of the \textit{Tn4401} isoforms (a, b, or c). \textit{Tn4401} was inserted on different-sized plasmids that belonged to different incompatibility groups. Several \textit{bla}_{KPC}-containing \textit{K. pneumoniae} clones were found: 9 different pulsotypes with 1 major (sequence type 258) and 7 minor distinct allelic profiles. Different clones harboring different plasmids but having identical genetic structure, \textit{Tn4401}, could be at the origin of the worldwide spread of this emerging resistance gene.

Resistance of \textit{Klebsiella pneumoniae} to carbapenems is mainly associated with acquired carbapenem-hydrolyzing β-lactamases (1). These β-lactamases can be metallo-β-lactamases (IMP, VIM), expanded-spectrum oxacillinases (OXA-48), or Ambler class A enzymes (NMCA, IMI, SME, GES, and KPC) (1–4). The most common class A carbapenemases in \textit{K. pneumoniae} are the \textit{K. pneumoniae} carbapenemases (KPCs) (4). KPCs in carbapenem-resistant \textit{K. pneumoniae} strains were first reported in 2001 in North Carolina (5), and until 2005, the geographic distribution of these enzymes in \textit{Enterobacteriaceae}, including \textit{K. pneumoniae}, was limited to the eastern part of the United States (5,6). Now, KPC-producing \textit{K. pneumoniae} isolates are frequently identified among nosocomial pathogens (7). Recently, dissemination of a single major clone of KPC-producing \textit{K. pneumoniae} (sequence type [ST] 258) in the eastern United States has been suggested (8). KPCs have been observed more rarely among other gram-negative rods such as \textit{Pseudomonas} spp. (9,10).

Outside the United States, KPC-producing \textit{K. pneumoniae} are also being reported more often. The first case of KPC-producing \textit{K. pneumoniae} infection was reported in 2005 in France and had a US origin (11). The first outbreak of KPC-producing \textit{K. pneumoniae} outside the United States was in Israel (12). In South America, dissemination of KPC-producing \textit{K. pneumoniae} was initially reported in 2006 in Colombia (13) and then in Brazil and Argentina (14,15). KPC enzymes have also been identified in \textit{P. aeruginosa} isolates from Colombia (16). In the People’s Republic of China, KPC enzymes in several enterobacterial species are being increasingly reported (17). Finally, in Europe a few cases of KPC-producing \textit{K. pneumoniae} infection have been described, but in Greece, outbreaks have occurred (18). In Europe, different variants of KPCs (KPC-2 and KPC-3) have been described; some patients

\textsuperscript{1}This study was presented in part at the Interscience Conference on Antimicrobial Agents and Chemotherapy; 2009 Sep 12–15; San Francisco, CA, USA.

DOI: 10.3201/eid1609.091389
carrying KPC-positive isolates had been transferred from the United States, Israel, or Greece (19–21).

Reports of this β-lactamase being found in novel locations are increasing worldwide, probably signaling active spread. The genetic element carrying the \( \text{bla}_{\text{KPC}-2} \) gene, Tn4401, was recently elucidated (22). Three isoforms of this Tn3-like transposon (a, b, and c) are known. Several other genetic environments of \( \text{bla}_{\text{KPC}} \) gene have been described; other insertion sequences have been found upstream of the \( \text{bla}_{\text{KPC}} \) gene (23,24). Nevertheless, the downstream sequences of the \( \text{bla}_{\text{KPC}} \) gene matched perfectly with Tn4401, which suggests that these insertion sequences have been inserted into Tn4401.

Insertion sequences may play major roles in the evolution of Tn4401, but little information is available about the bacterial strains and the plasmids that may explain this rapid spread. Our goal, therefore, was to characterize the genetic background of several \( \text{bla}_{\text{KPC}-2} \)-harboring \( \text{K. pneumoniae} \) isolates from various geographic origins.

**Materials and Methods**

**Bacterial Strains**

\( \text{K. pneumoniae} \) isolates used in this study and their origin are listed in Table 1 (11,13,16,21,25). Electrocompetent \( \text{Escherichia coli} \) DH10B (Invitrogen, Eragny, France) was used as a recipient in electroporation experiments. \( \text{E. coli} \) J53AzR, which is resistant to sodium azide, was used for conjugation experiments. \( \text{E. coli} \) 50192 was used as a reference strain for plasmid extraction (22).

**Antibiograms and MIC Determinations**

Antibiograms were created by using the disk-diffusion method on Mueller-Hinton agar (Bio-Rad Laboratories, Marnes-La-Coquette, France), and susceptibility break points were determined as previously described and interpreted as recommended by the Clinical and Laboratory Standards Institute (22,26). All plates were incubated at 37°C for 18 h. MICs of β-lactams were determined by using the Etest technique (bioMérieux, Marcy l’Etoile, France).

**Electroporation and Plasmid Extraction**

Direct transfer of resistance into azide-resistant \( \text{E. coli} \) J53 was attempted as reported (22). Plasmids were introduced by electroporation into \( \text{E. coli} \) DH10B (22) by using a Gene Pulser II (Bio-Rad Laboratories).

Plasmid DNA was extracted by using a QIAGEN Plasmid Maxi Kit (QIAGEN, Courtaboeuf, France) and analyzed by agarose gel electrophoresis (Invitrogen, Paris, France). Natural plasmids were extracted by using the Kieser extraction method (27) and subsequently analyzed by electrophoresis on a 0.7% agarose gel.

**Hybridization**

DNA–DNA hybridization was performed as described by Sambrook et al. (28) with Southern transfer of an agarose gel containing Kieser method–extracted total DNA. The probe consisted of a 796-bp PCR-generated fragment from recombinant plasmid pRYC-1 (22) and was internal to the \( \text{bla}_{\text{KPC}-2} \) gene. Labeling of the probe and detection of signal were conducted by using an ECL nonradioactive labeling and detection kit according to the

---

**Table 1. Geographic origin and structure of Tn4401 and other β-lactamases of \( \text{Klebsiella pneumoniae} \) isolates**

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Isolate type</th>
<th>Origin</th>
<th>KPC-2</th>
<th>TnpA</th>
<th>ISKP7</th>
<th>ISKP6</th>
<th>Deletion, bp</th>
<th>Other β-lactamases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>YC</td>
<td>USA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–100</td>
<td>SHV-11 TEM-1 OXA-9</td>
</tr>
<tr>
<td>2</td>
<td>GR</td>
<td>Greece</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–100</td>
<td>SHV-11 TEM-1 OXA-9</td>
</tr>
<tr>
<td>3</td>
<td>K271</td>
<td>Sweden</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td>SHV-11 TEM-1 OXA-9</td>
</tr>
<tr>
<td>4</td>
<td>KN2303</td>
<td>Colombia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>KN633</td>
<td>Colombia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td>OKP-A TEM-1 CTX-M-12</td>
</tr>
<tr>
<td>6</td>
<td>INC H1521-6</td>
<td>Colombia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td>SHV-1 TEM-1 CTX-M-15</td>
</tr>
<tr>
<td>7</td>
<td>INC H1516-6</td>
<td>Colombia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td>SHV-1 TEM-1 CTX-M-15</td>
</tr>
<tr>
<td>8</td>
<td>HPTU 27635</td>
<td>Colombia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td>OKP-B TEM-1 CTX-M-15</td>
</tr>
<tr>
<td>9</td>
<td>HPTU 2020532</td>
<td>Colombia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td>OKP-A TEM-1 CTX-M-12</td>
</tr>
<tr>
<td>10</td>
<td>A28006</td>
<td>Brazil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>SHV-11 TEM-1 CTX-M-2</td>
</tr>
<tr>
<td>11</td>
<td>A28008</td>
<td>Brazil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>SHV-11 TEM-1 CTX-M-2</td>
</tr>
<tr>
<td>12</td>
<td>A28009</td>
<td>Brazil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>SHV-11 TEM-1 CTX-M-2</td>
</tr>
<tr>
<td>13</td>
<td>A28011</td>
<td>Brazil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>SHV-11 TEM-1 CTX-M-2</td>
</tr>
<tr>
<td>14</td>
<td>A33504</td>
<td>Brazil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
<td>SHV-11 TEM-1 CTX-M-2</td>
</tr>
<tr>
<td>15</td>
<td>475</td>
<td>Israel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–200</td>
<td>SHV-11 TEM-1 OXA-9</td>
</tr>
<tr>
<td>16</td>
<td>588</td>
<td>Israel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–200</td>
<td>SHV-11 TEM-1 OXA-9</td>
</tr>
</tbody>
</table>

*KPC, \( \text{K. pneumoniae} \) carbapenemase.
PCR Amplification and Sequencing

The bla<sub>CTX-M</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub>, and bla<sub>OXA-1/9</sub>-like genes were searched for and characterized as described (21). PCR experiments were performed on an ABI 2700 thermocycler (Applied Biosystems, Les Ulis, France) by using laboratory-designed primers (Table 2). PCR products were then analyzed on agarose gel and sequenced.

Both strands of the PCR products were sequenced by using laboratory-designed primers with an automated sequencer (ABI PRISM 3100; Applied Biosystems). The nucleotide and the deduced protein sequences were analyzed using software from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

Isoelectrofocusing

Crude β-lactamase extracts, obtained as described (21) from 10-mL cultures of clinical isolates and their E. coli transconjugants or electroporants were subjected to analytical isoelectrofocusing on an ampholine-containing polyacrylamide gel, pH 3.5–9.5 (Ampholine PAG plate; GE Healthcare, Orsay, France) for 90 min at 1,500 volts, 50 milliamps, and 30 watts. The focused β-lactamase extracts, obtained as described (21), were analyzed by using the recommendation of Tenover et al. (30).

Multilocus Sequence Typing

Multilocus sequence typing (MLST) with 7 housekeeping genes (rpoB, gapA, mdh, pgi, phoE, infB, and tonB) was performed according to Diancourt et al. (31). Allele sequences and STs were verified at http://pubmlst.org/kpneumoniae. A different allele number was given to each distinct sequence within a locus, and a distinct ST number was attributed to each distinct combination of alleles.

Replicon and Transposon Typing

PCR-based replicon typing of the main plasmid incompatibility groups reported for Enterobacteriaceae was performed as described (32). Genetic structures surrounding the bla<sub>KPC-2</sub> gene were determined according to the Tn4401 PCR-mapping scheme as described (22).

Results

Pulsotypes

Molecular typing by PFGE identified 9 major pulsotypes among the isolates (Table 3). The first pulsotype (pulsotype A) corresponded to the strains from the United States and Greece. We found 4 different pulsotypes (B–E) among strains from Colombia, which suggested polyclonal diffusion inside this country. We also identified 2 different clones among strains from Brazil (pulsotypes F and G) and from Israel (pulsotypes H and I). These results indicate much heterogeneity among KPC-producing isolates from various geographic regions.

MLST of the 16 isolates resulted in 8 distinct allelic profiles: ST 258 (allelic profile 3–3–1–1–1–1–79) corresponding to isolates K. pneumoniae YC (United States), K. pneumoniae GR (Greece), and K. pneumoniae K271 (Greece); ST 14 (allelic profile 1–6–1–1–1–1) corresponding to isolates K. pneumoniae INC H1521–6 and K. pneumoniae INC H1516–6 (Colombia); ST 11 (allelic profile 3–3–1–1–1–1–4) corresponding to isolates from Brazil; ST 277 (allelic profile 3–1–1–1–1–1–43) corresponding to isolate K. pneumoniae 475 (Israel); novel ST 337 (allelic profile 2–1–11–1–1–1–13) corresponding to isolate K. pneumoniae KN2303 (Colombia); ST 338 (allelic profile 17–19–22–39–34–21–52) corresponding to isolates K. pneumoniae KN633 and K. pneumoniae HPTU 2020532 (Colombia); ST 339 (allelic profile 18–15–24–11–13–51) corresponding to isolate K. pneumoniae HPTU 27635 (Colombia); and ST 340 (allelic profile 3–3–1–1–1–1–18) corresponding to isolate K. pneumoniae 588 (Israel).

Table 2. Primers used for PCR of Klebsiella pneumoniae isolates producing β-lactamase bla<sub>KPC-2</sub> gene

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer no.†</th>
<th>Sequence, 5′ → 3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>KpcA</td>
<td>1</td>
<td>CGTCTGGTCGTGCATGGCC</td>
</tr>
<tr>
<td>KpcB</td>
<td>2</td>
<td>CGTTCGCTGTGCTCATCC</td>
</tr>
<tr>
<td>4281</td>
<td>3</td>
<td>GCACGCGCAATGACTA</td>
</tr>
<tr>
<td>4714</td>
<td>4</td>
<td>GGATAGTCGAAGTCTGAGTC</td>
</tr>
<tr>
<td>EccRout</td>
<td>5</td>
<td>CACCCGACCTGACAGAACACTA</td>
</tr>
<tr>
<td>3′YEnd</td>
<td>6</td>
<td>GACTACAAAGGAAGCANAAG</td>
</tr>
<tr>
<td>3781L</td>
<td>7</td>
<td>CACAGGCGACAGAACAGAAAGGC</td>
</tr>
<tr>
<td>3098U</td>
<td>8</td>
<td>TGACCTGGAGCGCGAGAG</td>
</tr>
<tr>
<td>905L</td>
<td>9</td>
<td>GGGACGGGTCAAGTCTGCTT</td>
</tr>
<tr>
<td>816U</td>
<td>10</td>
<td>CACCTACACCCGACGACGACC</td>
</tr>
<tr>
<td>141IR-6</td>
<td>11</td>
<td>TCACCGGCGCCCTCACCCTTTCGG</td>
</tr>
<tr>
<td>5′endYC</td>
<td>12</td>
<td>CTTGCCGAAATTGAGTCGAAGC</td>
</tr>
<tr>
<td>Pre-SHV-5U</td>
<td></td>
<td>GGTACGACGAGGAAGACCTC</td>
</tr>
<tr>
<td>Pre-SHV-5L</td>
<td></td>
<td>AATTACGCGATCGCCATC</td>
</tr>
<tr>
<td>Pre-TEM 1</td>
<td></td>
<td>GTACCCGCTGATAGACAATA</td>
</tr>
<tr>
<td>Pre-TEM 2</td>
<td></td>
<td>TGTAAAGGTTATGAGTGAAGAAGT</td>
</tr>
<tr>
<td>OXA-9 A</td>
<td></td>
<td>CCCTTGTCGCGGCATCCCTC</td>
</tr>
<tr>
<td>OXA-9 B</td>
<td></td>
<td>TGCAGGATAATCGTCGTCG</td>
</tr>
<tr>
<td>Ctx-M A</td>
<td></td>
<td>GGCTTTGCGGTAGTCGAC</td>
</tr>
<tr>
<td>Ctx-M B</td>
<td></td>
<td>ATTCGCGATACTGCTTGAT</td>
</tr>
</tbody>
</table>

†Primers from (21).

Numbers correspond to those in Figure 1, panel A. –, primer not shown in Figure 1, panel A.
analysis of STs by eBURST (http://pubmlst.org) showed that ST 11 and ST 340 are single-locus variants of ST 258 and that ST 277 is a double-locus variant of ST 258. These results matched perfectly with PFGE results. One isolate from Brazil (K. pneumoniae A33504) showed a different pattern by PFGE but the same ST (ST 11) as other isolates from the same origin, which suggests a strong genetic relatedness.

**Antimicrobial Drug Susceptibility**

All isolates were resistant to penicillins and cephalosporins but showed varying levels of susceptibility to carbapenems (Table 4). Resistance to other drug classes varied among the isolates. For aminoglycosides, 2 clones (A and I) were susceptible to gentamicin only, 1 clone (H) was susceptible to amikacin only, and 3 clones (C, D, and G) were resistant to all tested aminoglycosides. Six clones (A, C, D, F, G, and I) showed resistance to fluoroquinolones. Percentages of nonsusceptible isolates to the non-β-lactam drugs were as follows: gentamicin, 75%; amikacin, 81.3%; ciprofl oxacin, 81.5%; trimethoprim/sulfamethoxazole, 81.5%; and tetracycline, 87.5%. Two isolates were also resistant to colistin (K. pneumoniae GR and K. pneumoniae K271); each was from Greece, where this drug is often used (33).

**β-Lactamase Genes**

Positive results of CTX-M-, TEM-, SHV-, and OXA-specific PCRs are indicated in Table 1. All isolates possessed the bla_{KPC-2} gene and a naturally chromosome-encoded bla gene: bla_{SHV-1} (12.5%), bla_{SHV-11} (68.7%), or bla_{OKP-A/B} (18.8%). The bla_{OKP} genes are 1 of the 3 families of the chromosomal β-lactamase genes found in K. pneumoniae (34) with bla_{SHV} and bla_{KPC-2} and share 88% similarity with bla_{SHV-1}. K. pneumoniae isolates also harbored several acquired and plasmid-encoded genes: bla_{TEM-1} (81.3%), bla_{CTX-M-2} (31.3%), bla_{CTX-M-12} (12.5%), bla_{CTX-M-15} (18.7%), and bla_{OXA-48} (37.5%).

**Characterization Results for Tn4401**

Primer couples specific for the different genes found on Tn4401 (Table 2; Figure 1, panel A) obtained similar-sized fragments for all strains, which suggests that the strains have a similar genetic organization. For only 1 primer pair, hybridizing in IS_{Kpn7} and bla_{KPC} gene (primers 7 and 8 in Figure 1, panel A), located upstream of the bla_{KPC} gene,
an ≈100-bp (K. pneumoniae YC, K. pneumoniae GR, and K. pneumoniae 271) or 200-bp (K. pneumoniae 475 and K. pneumoniae 588) shorter fragment was observed, compared with the Tn4401b structure, thus indicating that the 3 isoforms of Tn4401 were present in this collection of isolates (Figure 1, panel B).

To investigate the flanking sequences of Tn4401, we used PCR primers located in the Tn4401 structure and in the flanking sequences derived from K. pneumoniae YC (22). PCR products of expected size were obtained for K. pneumoniae GR and K. pneumoniae K271 isolates only. For all other strains, no PCR product could be obtained, suggesting that the Tn4401 insertion site might differ from that found in K. pneumoniae YC.

Genetic Support for \( \text{bla}_{\text{KPC}} \) in the Isolates

The carbapenem-resistant K. pneumoniae isolates contained several plasmids of different sizes, ranging from <5 kb to >170 kb (Figure 2, left panel). At least 1 plasmid hybridized with an internal probe for \( \text{bla}_{\text{KPC}} -2 \) gene in each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3).

Mating-out assays showed that the ≈75–80-kb plasmids harboring \( \text{bla}_{\text{KPC}} -2 \) from K. pneumoniae YC, GR, K271, and KN2303 were self-transferable to E. coli. The smaller plasmid from K. pneumoniae KN633 was not transferred to E. coli.

Origin of Replication

PCR-based replicon typing of the major plasmid incompatibility groups showed that the \( \text{bla}_{\text{KPC}} -2 \)-positive plasmids belonged to at least 3 incompatibility groups (IncFIIAS, IncN, and IncL/M) (Table 3). The plasmids of K. pneumoniae KN633, HPTU-2020532 from Colombia and K. pneumoniae A33504 from Brazil gave negative results with the Inc primers tested and could not be classified into a major plasmid incompatibility group.

Discussion

Rapid spread of KPC-producing K. pneumoniae is a major clinical and public health concern. These broad-spectrum \( \beta \)-lactamases are increasing in new locations worldwide, indicating an ongoing process. Recently, a novel Tn3-based transposon, Tn4401, was identified in nonclonally related KPC-producing K. pneumoniae and P. aeruginosa isolates (22). This transposon is in most recently described isolates (20,35,36), although a recently characterized novel variant from China had another insertion sequence inserted upstream of \( \text{bla}_{\text{KPC}} -2 \) gene (24). Identification of Tn4401 inserted at different loci, on different plasmids, and flanked by different 5-bp target site duplications indicates a frequent and dynamic process of transposition.
It has been suggested that this novel transposon is at the origin of \( \text{bla}_{\text{KPC}} \)-like gene acquisition and dissemination (22). Sixteen \( \text{K. pneumoniae} \) isolates that express the \( \text{bla}_{\text{KPC}} \) gene from 5 countries were characterized here.

PFGE and MLST showed that several clones are currently spreading in different geographic locations. In Colombia, 3 pulsotypes could be identified. Overall, among the 16 isolates, 1 major ST (258) and its derivative ST 11 seemed to predominate. In a recent study that gathered isolates from 10 US states, ST 258 accounted for 70% of isolates, according to a database of KPC-producing \( \text{K. pneumoniae} \) PFGE results maintained by the Centers for Disease Control and Prevention (8). This ST has also been identified for KPC-producing \( \text{K. pneumoniae} \) in Sweden (in isolates imported from Greece and Israel) and more recently in Poland (36,37). These findings suggest possible international dissemination of KPC-producing ST 258. Apparently, the \( \text{K. pneumoniae} \) clone that contains the extended-spectrum \( \beta \)-lactamase (ESBL) determinant CTX-M-15 belongs to ST 11 (38).

KPC-producing \( \text{K. pneumoniae} \) contained diverse \( \beta \)-lactamases. All except 2 isolates harbored at least another \( \beta \)-lactamase; \( \text{bla}_{\text{TEM-1}} \) and a \( \text{bla}_{\text{CTX-M}} \)-type ESBLs were expressed by >80% and 62.5% of isolates, respectively. KPC producers have already been associated with other \( \beta \)-lactamase genes, such as the widespread ESBL gene \( \text{bla}_{\text{CTX-M-15}} \) (17). SHV ESBLs have been found among isolates, as has been described for strains from the United States (39) and Norway (36). These additional \( \beta \)-lactamases are likely to complicate phenotype-based identification of KPC producers. Three isolates harbored the chromosome-encoded \( \text{bla}_{\text{OKP-A/B}} \) genes and belonged to phylogenetic group KpII, which accounts for <10% of \( \text{K. pneumoniae} \) strains (34). Coexpression of OKP enzymes and ESBLs has rarely been reported.

Isolates also demonstrated diversity in their molecular features. In this study, the KPC-2 genes were encoded on a broad variety of plasmids, as shown by previous studies (22,35). These plasmids differed in size and incompatibility groups. Similar plasmids were observed among isolates with the same ST, whereas different plasmids were also associated with similar STs. Therefore, epidemiologic investigation of KPC producers should be performed at different molecular levels.

\( \text{Tn4401} \) was present in all tested strains. The overall structure of \( \text{Tn4401} \) seemed to be conserved, except for the 100-bp to 200-bp deletion. Of the 16 isolates, 11 encoded the full-length \( \text{Tn4401b} \) isoform, 3 encoded the \( \text{Tn4401a} \) isoform containing a 100-bp deletion (ST 258), and 2 encoded the \( \text{Tn4401c} \) isoform containing a 200-bp deletion upstream of the \( \text{bla}_{\text{KPC}} \) gene. These types of transposons tend to evolve by capturing various insertion sequences, as illustrated for the \( \text{vanA} \)-containing \( \text{Tn}1546 \) transposon (40). For \( \text{Tn4401} \), three descriptions have been published in which different insertion sequences were present upstream of \( \text{bla}_{\text{KPC-2}} \) (22–24). None of these atypical structures were found in our strains. Observation of \( \text{Tn4401} \) on different
plasmids further supports the hypothesis that this transposon contributes to the mobilization and dissemination of the bla_KPC genes.

Our analysis of several *K. pneumoniae* isolates from 5 geographic origins indicates the spread of different clones that were harboring different plasmids but with an identical genetic structure, Tn4401, that sustained a bla_KPC gene acquisition, which could likely be at the origin of the world-wide spread of this emerging resistance gene. Finally, taken together, our findings and those of recent studies report a major KPC-producing clone with ST 258, even if novel ST types could also be evidenced, especially from Colombia. Our data suggest that KPC genes benefit all molecular ingredients (transposon location, self-transferable plasmids, efficient STs) by facilitating their rapid spread to *K. pneumoniae* and other bacterial species.

**Acknowledgment**

We thank Platform Genotyping of Pathogens and Public Health (Institut Pasteur) for coding MLST alleles and providing the data.

Dr Cuzon is an assistant professor of microbiology at the University of Paris South Medical School. Her primary research interest is the genetic basis of antimicrobial drug resistance gene acquisition.

**References**


Address for correspondence: Thierry Naas, Service de Bactériologie–Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre CEDEX, France; email: thierry.naas@bct.aphp.fr