Carbapenem resistance in Enterobacteriaceae has emerged worldwide mainly by the production of carbapenemases (1). Carbapenemase genes are commonly carried on conjugative plasmids, representing a significant infection control challenge because of the potential horizontal transfer of resistance genes between bacterial isolates, species, and genera. The New Delhi metallo-b-lactamase (NDM), first described in 2008 (2), has been detected in different species and genera without a clear link to dominant plasmids or clones (3). In this study, we characterized three different NDM-producing entero bacterial species isolated from the same patient.

A 65-year-old man presented to the emergency department of a community hospital in Ontario, Canada, with a urinary tract infection, and his stay was complicated by the development of a large sacral decubitus ulcer. He was admitted to the hospital after returning from India with a diagnosis of an infected sacral ulcer. His symptoms improved with the initial treatment (cefazolin and metronidazole), but he had a recurrence of fever. Blood cultures grew Escherichia coli GN568 resistant to quinolones, co-trimoxazole, and tigecycline; and P. stuartii GN576 was also resistant to tetracycline and colistin. IncA/C plasmids were identified in E. coli GN568 (IncFIA and Frep were also detected in this isolate) and P. stuartii GN576 by PCR (5). E. cloacae GN574 was negative for all of the Inc groups tested. In conjugative assays with E. coli J53 as the acceptor (6), blaNDM-1 and biaCMY-4 were cotransferred on the IncA/C plasmid to strains J-568 (derived from E. coli GN568) and J-576 (from P. stuartii GN576) (Table 1). The only b-lactamase-encoding gene transferred to E. coli J-574 (conjugant strain derived from E. cloacae GN574) was blaNDM-1, consistent with its low aminoglycoside MIC.

NDM-1-carrying plasmids were extracted from the conjugant E. coli strains with the QIAGEN Large-Construct kit (Qiagen, Valencia, CA) and sequenced with the Illumina compact MiSeq system. Assembly of the contigs obtained was done with the CLC Genomics Workbench software (CLC bio, Qian, Gaps were filled by PCR amplification and Sanger sequencing. Open reading frames (ORFs) were predicted and annotated by the RAST server (available at rast.nmpdr.org) (7), followed by manual comparative curation and sequence similarity searches directed against the NCBI (www.ncbi.nlm.nih.gov/BLAST).

pNDM-EcoGN568 resulted in a closed circular sequence of 166,750 bp (average GC content of 51.8%). Its annotation revealed 205 predicted ORFs ranging from 117 to 5,487 bp, 98 of which encoded proteins. Bioinformatics analysis suggested both lateral plasmid transfer and independent acquisition of the blaNDM-1 gene in these clinical isolates.

Characterization of Multiple NDM-1-Producing Enterobacteriaceae Isolates from the Same Patient

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A male patient was admitted to a community hospital in Ontario, Canada, with an infected sacral ulcer after returning from India, where he was hospitalized. Carbapenem-resistant Escherichia coli (isolated from blood cultures), Enterobacter cloacae, and Providencia stuartii (from urine samples), all positive for blaNDM-1, were recovered. Comparative NDM-1 plasmid analysis suggests both lateral plasmid transfer and independent acquisition of the blaNDM-1 gene in these clinical isolates.

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which showed homology to proteins with known functions (Fig. 1; see Table S1 in the supplemental material). pNDM-EcoGN568 was almost identical to pNDM10-0505 (plasmid not yet published, GenBank accession no. JF503991), a 166,744-bp IncA/C plasmid detected in an *E. coli* isolate from a female patient hospitalized in Vancouver, British Columbia, Canada (8). Like the patient described here, that patient also received medical attention in India before being transferred to Canada. Moreover, pNDM-EcoGN568 also had ~89% identity with pNDM-KN (GenBank accession no. JN157804), another IncA/C plasmid of 162,746 bp detected in a *K. pneumoniae* isolate from Kenya (9, 10) (Fig. 1A). Differences between these two plasmids were detected mainly immediately upstream of the *bla*<sub>NDM-1</sub> gene (Fig. 1B): the cassette content in a class I integron (four cassettes and *qacEAl* deleted from the 3′-conserved region in pNDM-KN versus only one cassette plus *qacEAl* in pNDM-EcoGN568) and a fragment of ~10 kb flanked by two copies of ISKpn14 between the *rmtC* and *bla*<sub>NDM-1</sub> genes. Besides some transposase genes, this fragment also included two determinants of aminoglycoside resistance. One of these ISKpn14 copies in pNDM-EcoGN568 disrupts ISAba125, part of which was absent from pNDM-KN (Fig. 1). Upstream of this region, a copy of *ISEc23* inserted into the *sugE* gene (close to the *bla*<sub>GXY</sub>-<sub>6</sub> gene) in pNDM-KN was not present in pNDM-EcoGN568.

pNDM-PstGN576 had a circular sequence of 147,886 bp with a GC content of ~52% and 190 predicted ORFs (from 117 to 5,487 bp in size) (Fig. 1; see Table S1 in the supplemental material). Compared to pNDM-EcoGN568, pNDM-PstGN576 showed a deletion of ~19 kb corresponding to a Tn7-like transposon (the missing fragment was flanked by the inverted repeats IR-L and -R, which define the ends of the transposon) (Fig. 1A). The rest of both plasmid sequences showed 99% identity. These similarities suggest possible *in vivo* horizontal transfer between *E. coli* and *P. stuartii* clinical isolates with their subsequent evolution in the colonized patient, although their independent acquisition by each isolate cannot be ruled out.

pNDM-EclGN574 was 110,625 bp in length, with an average GC content of 54.8% and harboring 146 predicted ORFs (114 to 3,045 bp in size) (Fig. 1; see Table S1 in the supplemental material). pNDM-EclGN574 was identical in sequence to pKOX_NDM1 isolated from a *Klebsiella oxytoca* strain recovered in Taiwan from a patient who underwent renal transplantation in China (11, 12). pNDM-EclGN574 showed high identity with pNDM-EcoGN568 and pNDM-PstGN576 only in the region containing the *bla*<sub>NDM-1</sub> gene (Fig. 1B). As described for pKOX_NDM1, this region was flanked by 256-bp direct repeats (Fig. 1B, orange circles) with similarities to miniature inverted-repeat transposable elements (MITEs) suggested to be involved in the acquisition of this *bla*<sub>NDM-1</sub> region (11). The low sequence identity of pNDM-EclGN574 with the other two NDM plasmids recovered from the

### TABLE 1 Antibacterial drug susceptibility profiles, plasmid types, and resistance genes of clinical isolates and their *E. coli* transconjugant

<table>
<thead>
<tr>
<th>Isolate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ampicillin</th>
<th>Cefoxitin</th>
<th>Ceftazidime</th>
<th>Cefotaxime</th>
<th>Cefepime</th>
<th>Aztreonam</th>
<th>Ertapenem</th>
<th>Meropenem</th>
<th>Imipenem</th>
<th>Amikacin</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
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<tbody>
<tr>
<td>Eco GN568</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>256</td>
<td>16</td>
<td>32</td>
<td>6</td>
<td>≥256</td>
<td>≥1,024</td>
<td>≥256</td>
<td></td>
</tr>
<tr>
<td>Ecl GN574</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>128</td>
<td>128</td>
<td>≥32</td>
<td>≥32</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
</tr>
<tr>
<td>Pst GN576</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>16</td>
<td>1.5</td>
<td>0.75</td>
<td>≥32</td>
<td>≥32</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
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<tr>
<td>Eco J5-568</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
<td>16</td>
<td>6</td>
<td>≥32</td>
<td>4</td>
<td>8</td>
<td>≥256</td>
<td>≥1,024</td>
<td>≥256</td>
</tr>
<tr>
<td>Eco J5-574</td>
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<td>4</td>
<td>12</td>
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<td>≥256</td>
</tr>
<tr>
<td>Eco J5-53</td>
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<td>8</td>
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<td>0.094</td>
<td>0.064</td>
<td>0.125</td>
<td>0.008</td>
<td>0.023</td>
<td>0.38</td>
<td>1.5</td>
<td>1.5</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Eco, *E. coli*; Ecl, *E. cloacae*; Pst, *P. stuartii*; Eco J53, recipient *E. coli* J53. *E. coli* J53 transconjugant strains were derived from *E. coli* GN568 (J-568); *P. stuartii* GN576 (J-576), and *E. cloacae* GN574 (J-574).

<sup>b</sup> Susceptibility testing was performed by the Etest (bioMérieux) and agar dilution methods, and results were interpreted according to Clinical and Laboratory Standards Institute guidelines (13), except for colistin and tigecycline, for which the European Committee on Antimicrobial Susceptibility Testing breakpoints were used (http://www.eucast.org /clinical_breakpoints/).

<sup>c</sup> Nontypeable by the PCR-based replicon typing scheme described by Carattoli et al. (5). A new set of primers was designed for detection of this repA allele: FIB F2, 5′-CGTCTATCTCTGCCAGAAGC-3′; FIB R2, 5′-GGCTCAAGCTCATCATGC-3′.

<sup>d</sup> Sequencing of complete genes was performed with samples positive by PCR. PCR included screening for *bla*<sub>TEM</sub>, *bla*<sub>CIP</sub>, *bla*<sub>SHV</sub>-1, *bla*<sub>KPC</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub>, *bla*<sub>TEM-1</sub>, and *bla*<sub>SHV</sub>-1 genes, as well as the *armA*, *rmtA* to -F, and *pmrA* 16S methylase genes (14, 15). A plus sign indicates a positive result, and a minus sign indicates a negative result.

<sup>e</sup> NA, not applicable.
same patient suggests its independent acquisition by *E. cloacae* GN574. We were unable to identify the incompatibility group of plasmids from *E. cloacae* GN574 and its *E. coli* transconjugant by the standard plasmid replicon typing method described by Carattoli et al. (5). However, the repA gene on pNDM-EcGN574 had the highest identity with RepFIB replication protein A. Analysis of the primers used for amplification of this replicon type indicated that one of them (FIB F) was not identical to the repA sequence in this plasmid, and the reverse primer had two mismatches.

In conclusion, plasmid sequencing and comparative analysis suggest both lateral plasmid transfer and independent acquisition of the *blaNDM-1* gene in these three different enterobacterial genera recovered from the same patient. Plasmids with high sequence identity recovered from different bacterial species in different geographic areas support the idea of lateral dissemination as the main mechanism of the spread of this metallo-β-lactamase.

**Nucleotide sequence accession numbers.** The sequences of the plasmids reported here have been deposited in GenBank under accession numbers KJ802404, KJ812998, and KJ802405.

**REFERENCES**


