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Complete Sequence of the Multidrug-Resistant IncL/M Plasmid pIMP-HB623 Cocarrying bla_{IMP-34} and *fosC2* in an *Enterobacter cloacae* Strain Associated with Medical Travel to China

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IncL/M plasmids identified recently in clinical and environmental isolates are characterized by insertions of one or more complex multidrug resistance regions (MRRs) (1–3). Here, we analyzed the sequence of plasmid pIMP-HB623, which has a MRR with novel structural features and cocarries the carbapenemase bla_{IMP-34} and the fosfomycin resistance determinant *fosC2*. In 2011, we identified a multidrug-resistant *Enterobacter cloacae* strain, strain CRE623, in an isolate from a rectal swab of a 76-year-old man who was repatriated from Harbin, China, where he had been hospitalized for intracranial hemorrhage (see Table S1 in the supplemental material). PCR and sequencing using previously described primers showed that the strain was positive for bla_{IMP-34}, *fosC2*, and *aacC2* genes (4–6).

In filter mating (2), the bla_{IMP-34}-carrying plasmid could be transferred to the J53 recipient at a frequency of 10^{-1} per donor cell. Plasmid DNA from the transconjugant was sequenced at approximately 150-fold coverage using a 454 GS-FLX system (4). The reads were assembled using a GS *de novo* Assembler (version 2.6) into 24 contigs, and the gaps were closed by additional PCR and Sanger sequencing (see Table S2 in the supplemental material). Plasmid pIMP-HB623 (GenBank accession no. KM877517) was 133,208 bp in size, comprising a 60,166-bp backbone and a 73,042-bp MRR, which was inserted into an integration hot spot between *repA* and *trbC* in IncL/M (Fig. 1). The plasmid backbone of pIMP-HB623 differs from those of pEL1573 (JX101693) and pCTX-M3 (AF550415) by only 3 and 56 nucleotides, respectively (see Table S3). These nucleotide variations were confirmed by Sanger sequencing.

The MRR had probably resulted from the integration of several mobile elements, giving rise to a complex structure with multiple resistance determinants. First, it has a Tn*2* carrying bla_{TEM-1b} (Fig. 1), as identified previously in several other IncL/M plasmids (pCTX-M360, pCTX-M3, pNDM-OM, pNDM-HK, and pEl1573). Second, there is a Tn*21* backbone with copies of IS*5075* inserted into inverted repeats (IRs) IR_{tnp21} and IR_{mer21} (7). The insertion of IS*5075* should have occurred subsequent to Tn*21* inserting into Tn*2*, as the interruption of the IR by IS*5075* prevents further mobilization. A novel class 1 integron carrying *fosC2* and bla_{IMP-34} (designated In1070 by INTEGRALL [http://integrall.bio.ua.pt/]) with a complete set of *tni* modules (*tniR*, *tniQ*, *tniB*, and *tniA*) and the *mer* operon were identified within Tn*5075*. This class 1 integron was bound within two Tn*402* inverted repeats of 25 bp, IRi and IRt (as identified in GQ857074), while the *tniA* gene was split by the insertion of a 47.6-kb sequence with IS*26* termini, flanked by 8-bp direct repeats (GGCGATAG). The 47.6-kb insertion includes multiple copies of IS*26* and other mobile elements, suggesting that it was formed by multiple genetic events. Modular sequences that could be identified within this region include a 5.6-kb fragment carrying *aacC2* (positions 15174 to 20828, with 100% identity to pK245) (DQ449578), a 3.3-kb fragment matching the IncN backbone (positions 22,402 to 25,699, with 100% identity to pHKU1) (KC960485), aminoglycoside resistance genes (*strA* and *strB*), a matching 12.8-kb fragment (positions 29621 to 42455, with 99.4% identity to pKP1780) (JX424614), a partial class 1 integron (In191) carrying *dfrA14*, and a macrolide resistance operon. In conclusion, this is the first report of the cocarriage of bla_{IMP} and a fosfomycin resistance gene. It further highlights the importance of IncL/M plasmids as vectors for emerging resistance genes.

Nucleotide sequence accession number. Sequence data from plasmid pIMP-HB623 have been deposited in GenBank under accession no. KM877517.

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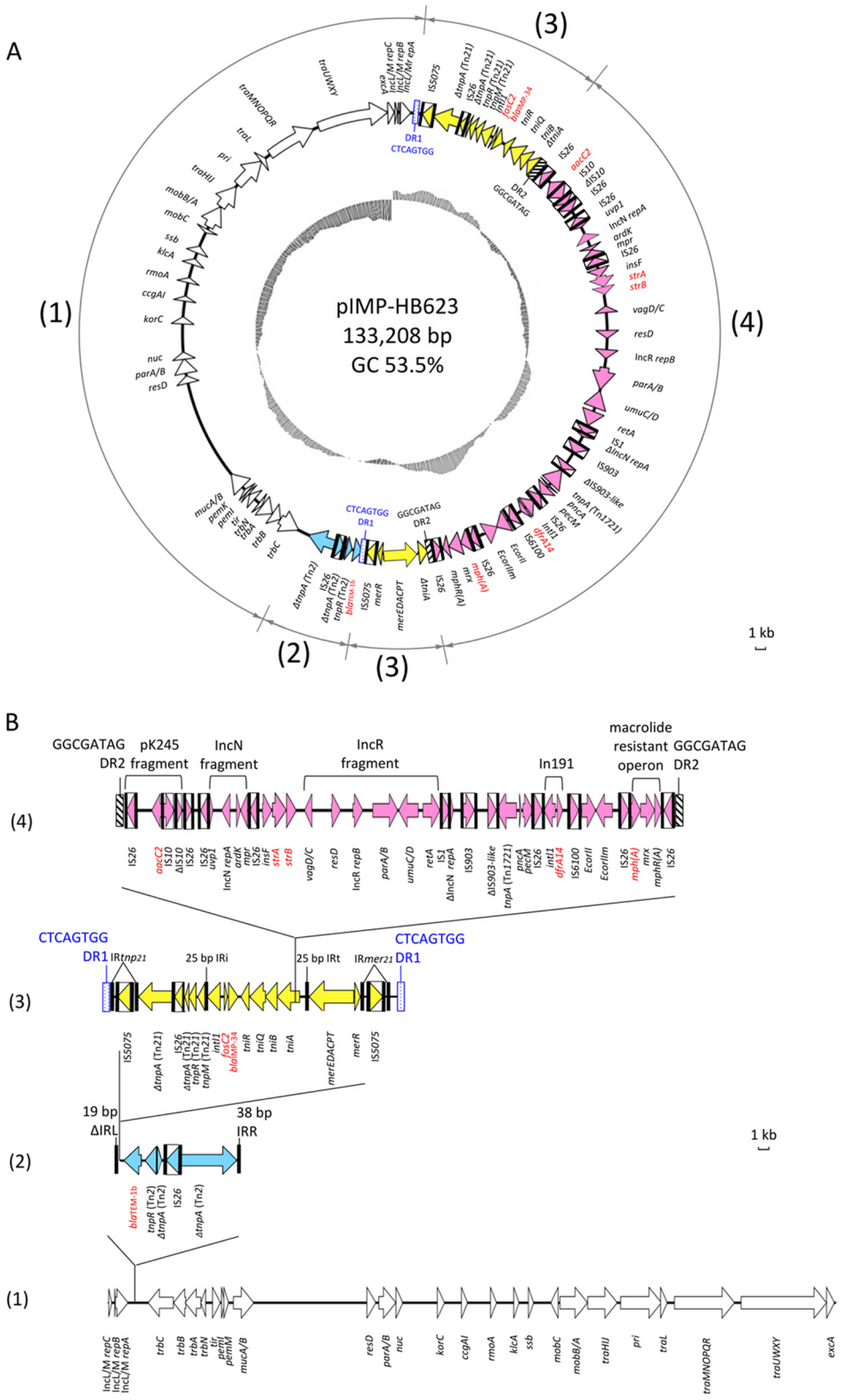

FIG 1 (A) Circular map of pIMP-HB623. Open reading frames (ORFs) are colored coded, with the direction of transcription indicated by arrowheads. ORFs with unknown function are not shown. Insertion sequence (IS) elements are represented by boxes, with the arrows inside the boxes indicating the transposases. Black bars are used to indicate inverted repeats associated with IS elements (IS*1*, IS*3*, IS*10*, IS*26*, IS*903*, IS*5075*, and IS*6100*), Tn*2* (ΔIRL, IRR), Tn*21* family transposons (IR_{tmp21}, IR_{mer21}), and integrons (IRi and IRt). Duplication repeats are represented by rectangular boxes (DR1, blue and dotted boxes; DR2, black and hatched boxes). Resistance genes are indicated in red font. The numbers in the outer circle indicate the following regions: (1) IncL/M plasmid scaffold (white arrows), (2) Tn*2*-based module (blue arrows), (3) Tn*5075* module (yellow arrows), and (4) IS*26* transposon-like structure (pink arrows). The G+C plot is indicated in the inner circle (mean, 53.5%). (B) Linear map of pIMP-HB623 showing the putative insertion events that have contributed to the formation of the multidrug-resistant region. The modules are identified, and other features are represented as described for panel A. Scale bars show the sequence length for 1 kb. For the purpose of illustration, duplication repeats and inverted repeats are not drawn to scale.

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