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Letter to the Editor

Plasmidome of a multiresistant *Salmonella enterica* serovar Typhimurium isolate from Uruguay

Sir,

We recently sequenced the genome of multiresistant *Salmonella enterica* serovar Typhimurium strain STM3224 isolated from a human immunodeficiency virus (HIV)-positive patient in Montevideo, Uruguay [1]. Here we describe the plasmidome of strain STM3224 and compare it with similar enterobacterial plasmids.

Plasmids were annotated using Rapid Annotation using Subsystem Technology (RASTtk) and were manually curated using Artemis 17.0.1 (https://www.sanger.ac.uk/science/tools/artemis). Plasmid multilocus sequence typing (pMLST) was performed using pMLST 2.0 software (https://cge.cbs.dtu.dk/services/pMLST/), incompatibility (Inc) groups were determined using PlasmidFinder 2.0 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) and non-coding RNAs (ncRNAs) were sought using the Rfam database (https://rfam.xfam.org/). Antimicrobial resistance genes were sought using the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/). Plasmid transfer was assessed by conjugation assay.

Strain STM3224 carried two plasmids (pUY_STM62 and pUY_STM96). Their complete nucleotide sequences were deposited in GenBank under accession nos. **MN241904** and **MN241905**, respectively.

Plasmid pUY_STM62 belonged to IncN plasmid group, plasmid sequence type 5 (pST5) and was 62 060 bp in size with an average G + C content of 51.2%. It harboured 77 predicted coding sequences (CDS) and a single ncRNA sequence. The backbone of pUY_STM62 included replication, maintenance and conjugative transfer genes. Conversely, the variable region included three antimicrobial resistance gene modules and various mobile genetic elements inserted in 'integration hotspots' (Fig. 1A).

The first module, located between *repA* and *uvp1*, consisted of the β-lactamase genes bla_{TEM-1} and $bla_{CTX-M-14}$, flanked by insertion sequence IS*26* on both sides in facing orientation. A truncated copy of *tnpR* was located upstream of bla_{TEM-1}, suggesting that this β-lactamase was recruited from a Tn*3* transposon. Downstream of bla_{TEM-1}, genes encoding a hypothetical protein and a putative methyl-accepting chemotaxis protein were detected. In contrast, $bla_{CTX-M-14}$ was flanked upstream by ΔIS*Ecp1* (truncated by insertion of IS*10*) and downstream by IS*903*. This antimicrobial resistance region is highly similar to Tn*6652* in pUR-ECO7 (**MH674341.1**), an IncI plasmid obtained from an *Escherichia coli* clinical isolate from Uruguay [2]. Although IncN plasmids have been associated with the dissemination of several bla_{CTX-M} variants, the occurrence of $bla_{CTX-M-14}$ in IncN plasmids is rare and the few existing reports correspond to uncharacterised plasmids from Asia [3].

The second resistance region, located downstream of *uvp1*, consisted of a complex class 1 integron carrying *dfrA25*, followed by *sul1* as part of the 3′-CS; the second variable region of this integron, downstream of IS*CR1*, featured genes for a peptide transport periplasmic protein (*sapA*), a hypothetical protein, the quinolone resistance gene *qnrB2*, a putative transcriptional factor (*pspF*), and a secondary 3′-CS (3′-CS2), albeit with a truncated copy of *qacEΔ1* and flanked by IS*15D*. The same complex integron was described in the multidrug resistance region of SGI1-X, a *Salmonella* genomic island 1 present in *Proteus mirabilis* [4].

Finally, the third resistance region consisting of the tetracycline resistance genes *tetR–tet(A)* was inserted between *nuc* and *traI* within the fertility inhibition gene *fipA*, and has already been described in plasmid pKPI-6 (**AB616660**) [5].

Plasmid pUY_STM96 belonged to the IncI group, sequence type 80 (pST80), clonal complex 31 (CC31) and was 96 476 bp in size with an average G + C content of 49.3%. The number of predicted CDS was 115, and three ncRNAs were detected.

The replication region was organised like other IncI plasmids. The origin of replication (*oriV*), located at genomic position 92 651–96 422, showed four single nucleotide polymorphisms (SNPs) in relation to IncI plasmids R64 (**AP005147.1**) and ColIb-P9 (**AB021078**).

Several stability-related genes were identified (e.g. *parAB*, *ibfA*, *resD* and the *rfsF* site, and *stbAB*) as well as a single toxin–antitoxin system (*pndAC*).

The transfer region was similar to other IncI plasmids: (i) *traABC* regulatory genes; (ii) type IV pilus biogenesis gene (*pil*); (iii) conjugation *tra*/*trb* genes; and (iv) an *oriT* operon.

The resistance region, inserted between the *resD* gene and the *rfsF* site, was composed of the azithromycin resistance gene *ermB* and its regulator *ermBL*. It was flanked on both sides by copies of IS*26* in direct orientation (Fig. 1B). Again, the closest match between this resistance region and public databases corresponded to plasmid pUR-EC07, although in the latter the copies of IS*26* flanking *ermB–ermBL* are in diverging orientation (i.e. facing outwards). The presence of *ermB* in *Salmonella* is rather infrequent and the only matching results in nucleotide databases correspond to IncA/C plasmids pSE12-01738-2 (**CP027679.1**) and pRH-1238 (**KR091911.1**), obtained from *S. enterica* serovar Corvallis of animal origin. However, in such plasmids *ermB* was flanked by IS*91* and IS*15D*.

The similarity between Tn*6652* and Tn*6651* in pUR-EC07, the β-lactam resistance region of pUY_STM62, and the macrolide

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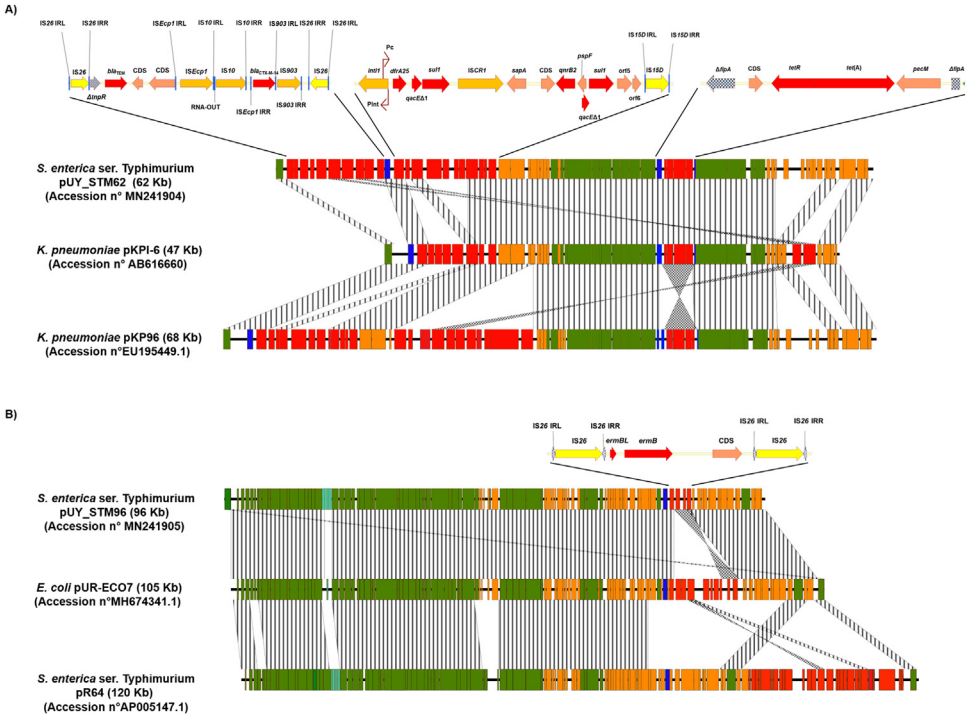

Fig. 1. Genetic context of the antimicrobial resistance regions of the plasmidome of *Salmonella enterica* serovar Typhimurium STM3224 and comparison with similar plasmids. (A) Resistance regions found in plasmid pUY_STM62 and comparison with other sequence type 5 (pST5) IncN plasmids available in public databases. (B) Genetic context of *ermB* in pUY_STM96 and comparison with similar pST80 IncI plasmids. Resistance regions are represented by solid arrows indicating the direction of transcription; resistance genes are shown in red; mobile genetic elements are depicted in tones of yellow, and miscellaneous coding sequences (CDS) are in orange; textured arrows represent pseudogenes; integron-related promoters (Pc and PInt; cassette and integrase promoter, respectively) are indicated by small red arrows. Plasmid comparison: green, replication/stability and conjugal transfer; blue, recombination hotspots; red, resistance region; orange, miscellaneous. Regions of homology (BLASTn ≥ 99% identity) are shown as striped blocks; inverted DNA regions are depicted as chequered blocks.

resistance region of pUY_STM96, respectively, could be accounted for by: (i) transfer of Tn*6652* as a translocatable unit via cointegrative transposition between pUR-EC07 and an IncN plasmid at a pre-existing copy of IS*26*; and eventually (ii) an intramolecular transposition event in trans, generating an inversion of Tn*6651*. Alternatively, the presence of such transposons in pUY_STM62 and pUY_STM96 could be derived from other sources, although the lack of similar conserved genetic structures in public databases suggests otherwise.

To the best of our knowledge, this is the first report of a *Salmonella* Typhimurium clinical isolate displaying transferable resistance to oxyimino-cephalosporins, fluroquinolones and azithromycin encoded by $bla_{\text{CTX-M-14}}$, *qnrB2* and *ermB*, respectively.

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Competing interests

None declared.

Ethical approval

Not required.

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