



## **pST15-IncHI2 plasmids co-harboring *mcr-9* and several other antibiotic resistance genes in heavy metal tolerant *Enterobacter cloacae* complex isolates from hospital infections**

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## Letter to the Editor

**pST15-IncHI2 plasmids co-harboring *mcr-9* and several other antibiotic resistance genes in heavy metal tolerant *Enterobacter cloacae* complex isolates from hospital infections**

Editor: Prof Holger Rohde



Sir,

*Enterobacter cloacae* complex species have been accumulating antibiotic resistance mechanisms against both beta-lactam and non-beta-lactam antibiotics. Therefore, alternatives, such as metal compounds, have been used to treat bacterial infections and to prevent microbial contamination. However, this could in turn lead to acquisition of metal tolerance mechanisms. In this study, we identified and characterized antibiotic resistance and heavy metal tolerance genes in plasmids carried by multidrug-resistant (MDR) *Enterobacter* Spp.

*Enterobacter* Spp. HC188 and HC193 were isolated in 2007 from surgical wound and urine cultures, respectively, from an inpatient in a University Hospital in Brazil. The antimicrobial susceptibility and minimum inhibitory concentration (MIC) for fifteen antimicrobial agents were determined using Vitek 2 (bioMérieux, Brazil) and microdilution; after evaluation, both isolates were considered MDR [1].

Genomic DNA of HC188 and HC193 was extracted using Wizard Genomic DNA Purification Kit (Promega), and whole-genome sequencing (WGS) was performed using both Illumina MiSeq (Illumina, Inc., San Diego, California, United States of America) and MinION (Oxford Nanopore Technologies, Oxford, England, United Kingdom). A subsequent hybrid de novo genome assembly (Unicycler 0.4.0) [2] was done to resolve complete circularized sequences of chromosome and plasmids. Gene prediction was carried out using the RAST Server [3]. The annotation of plasmid sequences was manually curated using BLASTN and BLASTP (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The multilocus sequence typing (MLST), species, resistome, plasmidome, and plasmid double-locus sequence typing MLST (pDLST) were evaluated using bioinformatic tools (MLST, SpeciesFinder, ResFinder, PlasmidFinder, and pDLST, respectively) available from the Center for Genomic Epidemiology (<http://genomicepidemiology.org/>).

Assembled complete genomes had an average coverage of 140x. The isolate HC188 was identified as *E. cloacae* ST513 and the isolate HC193 as *E. hormaechei* subsp. *hormaechei*. The WGS of HC188 revealed a circular chromosome spanning 5,154,805 bp and one 291,773 bp plasmid (hereafter called p290\_188). The WGS of HC193 revealed a circular chromosome spanning 4,611,369 bp and a plasmidome consisting of four different plasmids ranging from 2,317 bp to 296,770 bp (hereafter called p2\_193, p3\_193, p15\_193 and p296\_193).

The plasmids p290\_188 and p296\_193 were low copy number (~1X) and highly similar to each other (98% query coverage and 99.91% nucleotide identity). They were both related to *bla*<sub>CTX-M-2</sub>-harbouring IncHI2 plasmid p280\_40A from *Escherichia fergusonii* (CP031284.1) isolated from healthy chicken in Brazil (~69% query coverage; 98.24% nucleotide identity). In addition, p290\_188 and p296\_193 presented over 92% query coverage and over 99.96% nucleotide similarity with plasmid pCTXM9\_020038 from an *E. hormaechei* (CP031724) isolated in 2016 in China, as well as plasmid pC45-VIM4 from *E. cloacae* (LT991958) isolated in a hospital in France in 2018. Interestingly, p280\_40A, pCTXM9\_020038 and pC45-VIM4 belong to the pST-1, while p290\_188 and p296\_193 belong to the new pST-15.

In both isolates, the megaplasmids carried almost all of the present antibiotic resistance genes: *bla*<sub>CTX-M-9</sub>, *aadB*, two copies of *aadA2*, and three copies of *sul1*, *dfrA16*, *qnrA1*, and *mcr-9*, conferring resistance to beta-lactams, aminoglycosides, sulfonamides, trimethoprim, quinolone, and possibly colistin, respectively.

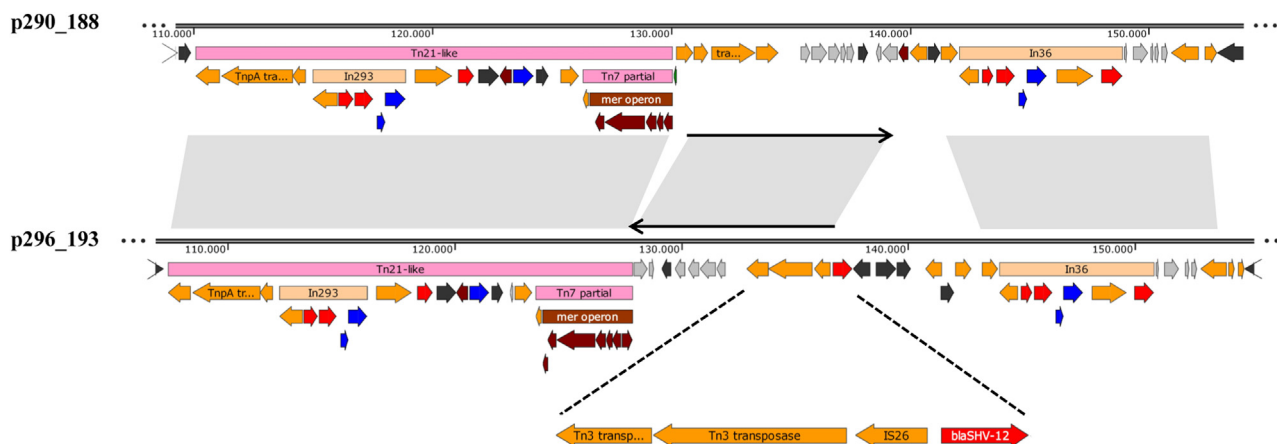
Beta-lactamase *bla*<sub>SHV-12</sub> was identified only in HC193 (p296\_193) (Fig. 1). In addition, *fosA* and *bla*<sub>ACT-7</sub> were identified on the chromosome of HC188 and HC193, respectively.

In p290\_188 and p296\_193, *mcr-9* is located downstream of the *bla*<sub>CTX-M-9</sub> gene and is surrounded by two insertion sequences, *IS903B* and *IS1R*. This organization is similar to what was recently found in a plasmid pME-1a in an *E. hormaechei* isolate from a pediatric patient in the United States [4]. However, amino acid alterations were found in both isolates in PhoP, PhoQ, PmrA, PmrB, and MgrB, modifications, which have been associated with colistin resistance.

It was noted that both megaplasmids possessed two conjugation regions, Tra1 and Tra2. In addition, both megaplasmids contained an *ISCR1-bla*<sub>CTX-M-9</sub> element and a Tn21-like transposon holding a class 1 integron In293 and a complex class 1 integron In36.

The megaplasmids additionally carried heavy metal tolerance operons: tellurium, mercury, and arsenic. Silver/copper tolerance operon was present as part of a Tn7-like transposon inserted in the chromosome of the isolates. This feature suggests a possible recombination between chromosome and plasmid, since other IncHI2 plasmids have been observed harboring silver/copper tolerance operon [5]. The chromosomal location of silver operon indicates that tolerance to silver provides an advantage to the isolates.

The present study provides the complete genome sequences of MDR *E. cloacae* and *E. hormaechei* from hospital inpatients, showing a variety of different plasmids, including megaplasmids. By utilizing two different sequencing technologies, we were able to resolve all plasmid sequences and, importantly, discern which clinically relevant genes are plasmid-borne and are, therefore, transmissible. The megaplasmids presented the complete transfer ma-



**Fig. 1.** Comparison between p290\_188 and p296\_193 megaplasmids. The regions in grey represent a common (100 % identical) region between the two megaplasmids and the arrows by the grey areas represent a sequence inversion. The dotted line highlights the *bla<sub>SHV-12</sub>* gene in the transposase-rich region. Graphic representations of the plasmids were performed using SnapGene Viewer (version 4.3.2.1).

chinery, suggesting that even a megaplasmid can spread among bacteria in a hospital. The presence of *mcr-9* and metal tolerance genes should be particularly highlighted, as they may enable the bacteria to survive under extremely unfavorable conditions.

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### Competing interests

None declared

### Ethical approval

Not required

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