Journal of Global Antimicrobial Resistance 31 (2022) 187–188

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

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 $bla_{\text{CTX-M-2}}$–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: $bla_{\text{CTX-M-9}}$, *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 $bla_{\text{SHV-12}}$ was identified only in HC193 (p296_193) (Fig. 1). In addition, *fosA* and $bla_{\text{ACT-7}}$ were identified on the chromosome of HC188 and HC193, respectively.

In p290_188 and p296_193, *mcr-9* is located downstream of the $bla_{\text{CTX-M-9}}$ gene and is surrounded by two insertion sequences, IS*903B* and IS*1R*. 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 IS*CR1*-$bla_{\text{CTX-M-9}}$ element and a Tn*21*-like transposon holding a class 1 integron In*293* and a complex class 1 integron In*36*.

The megaplasmids additionally carried heavy metal tolerance operons: tellurium, mercury, and arsenic. Silver/copper tolerance operon was present as part of a *Tn*7-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-

https://doi.org/10.1016/j.jgar.2022.09.002

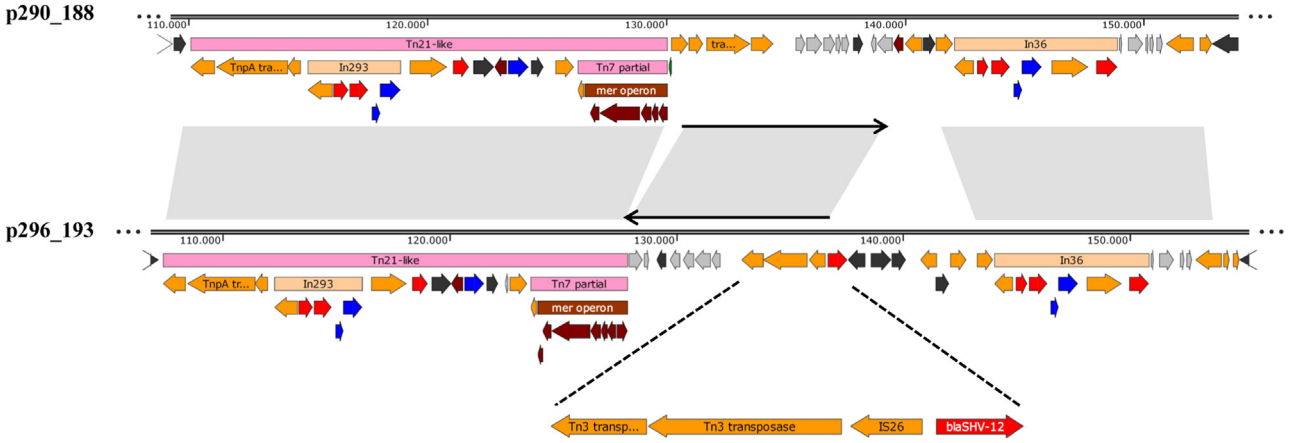

Fig. 1. Comparison between p290_188 and p296_193 megaplasmids. The regions in grey represent a common (100 % identical) region between the two megaplasmids and the arrows by the grey areas represent a sequence inversion. The dotted line highlights the bla_{SHV-12} gene in the transposase-rich region. 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.

Funding

This work was supported by the São Paulo Research Foundation (FAPESP) [grant number 2014/14494-8], Instituto Nacional de Pesquisa em Resistência Antimicrobiana [INPRA] INCT – MCTI/CNPQ/CAPES/FAPS N° 16/2014, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. Renata Galetti was supported by a postdoctoral fellowship from the FAPESP [grant 2015/11728-0 and 2017/26311-3]. Work in the laboratory of Lars Jelsbak is supported by The Danish Council for Independent Research (Grant number 9039-00350A).

Competing interests

None declared

Ethical approval

Not required

Acknowledgments

We thank Professor Roberto Martinez for providing the isolates studied. We also thank the National Council for Scientific and Technological Development (CNPq, Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), and São Paulo Research Foundation (FAPESP) for supporting our research.

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