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Acquisition of *bla*$_{IMP-13}$ on a novel IncP-7 plasmid in XDR VIM-2–positive *Pseudomonas aeruginosa* belonging to the global high-risk clone ST235 in an agricultural ecosystem

Edited by Stefania Stefa

Sir,

Carbapenem-resistant *Pseudomonas aeruginosa* strains are designated by the World Health Organization (WHO) as critical priority pathogens, which seriously limit the current treatment options. Sequence type (ST) 235 is the most prevalent and widespread clone associated with therapeutic failure [1]. In this regard, a number of genes encoding metallo-β-lactamases (MBLs) have become associated with ST235, which has promoted their dissemination [1,2]. VIM-2 and IMP-13 MBLs exhibit a broad-spectrum activity and their intercontinental spread has been reported in hospital outbreaks [2,3]. Nevertheless, neither co-production of VIM-2 and IMP-13 in a single bacteria species nor fully sequenced plasmids carrying *bla*$_{IMP-13}$ have been described so far.

During a surveillance study conducted to monitor the occurrence of priority pathogens in crops, one carbapenem-resistant *P. aeruginosa* strain, named S810, was isolated from an agricultural soil sample with a history of cattle manure use. Antimicrobial susceptibility testing was performed by disk diffusion, broth microdilution, and/or agar dilution methods according to Clinical and Laboratory Standards Institute (CLSI) guidelines (M100, 31st ed.). Strain S810 was classified as extensively drug-resistant (XDR) because it presented resistance to piperacillin-tazobactam (128/4 mg/L), ceftazidime (64 mg/L), cefepime (64 mg/L), aztreonam (32 mg/L), imipenem (128 mg/L), meropenem (128 mg/L), gentamicin (256 mg/L), tobramycin, amikacin, ciprofloxacin (128 mg/L), norfloxacin, lomefloxacin, ofloxacin, and levofloxacin, but it was susceptible to colistin (0.5 mg/L). Polymerase chain reaction (PCR) screening and Sanger sequencing revealed that the strain was positive for *bla*$_{VIM}$ and *bla*$_{IMP}$ genes.

Whole-genome sequencing of *P. aeruginosa* S810 was performed using an Illumina HiSeq platform (Illumina, San Diego, CA) and *de novo* assembly was conducted using SPAdes v. 3.15.2 and refined with Geneious v. 11.1.5. Bioinformatics tools available from the Center for Genomic Epidemiology (www.cge.dtu.dk) and the Virulence Factor Database (VFDB) database (http://www.mgc.ac.cn/VFs/) were used to analyse the data.

The S810 strain belonged to serotype O11 and ST235/CC235, a globally disseminated high-risk clone associated with high virulence and multiple resistance determinants [1,4]. In this regard, virulome analysis of S810 revealed the presence of *aprA* (alkaline protease), *exoU* (cytotoxin), *lasB* (elastase), *plcH* (hemolytic phospholipase C), *plcN* (non-hemolytic phospholipase C), and *toxA* (exotoxin A) genes. Antimicrobial resistance genes to β-lactams (*bla*$_{VIM-2}$, *bla*$_{IMP-13}$, *bla*$_{OXA-50}$, and *bla*$_{PAO}$), aminoglycosides [*aac(6′)-29b, aac(6′)-Ib3, aadA1, aph(3′′)-Ib, aph(3′)-IIb*, and *aph(6)-Id*], fluoroquinolones (*crpP*), fosfomycin (*fosA*), tetracyclines (*tetC*), phenicols (*catB7* and *cmlA1*), trimethoprim (*dfrA15*), and sulphonamides (*sul1*) were also identified. Additionally, mutations in the quinolone resistance-determining regions of *gyrA* (T83I), *gyrB* (S466F), and *parC* (S87L), in the outer membrane porin *oprD* gene (Δ588-603), and in efflux pumps expression regulators [*mexR* (V126E, Δ265-343), *mexZ* (C59W, A159G), *nalC* (F55L), and *armZ* (F61Y, G72A, Q176H, V243A, A262S)] were detected.

Pseudomonas aeruginosa S810 harbored two plasmids, ~140 kb (named pS810a) and ~240 kb (named pS810b), bearing *bla*$_{VIM-2}$ and *bla*$_{IMP-13}$, respectively, which were transferred separately by conjugation using a rifampicin-resistant mutant of *P. aeruginosa* PAO1 as recipient strain. The complete sequence of the plasmids was obtained from *de novo* assembly followed by gap closure.

The pS810a plasmid was 139,453 bp in length, containing 58.6% GC content and 159 coding regions (CDSs), and could not be assigned to any known incompatibility group. Besides *bla*$_{VIM-2}$, the plasmid co-harbored *aac(6′)-29b, aph(3′′)-Ib, aph(6)-Id, cmlA1, dfrA15*, and *sul1* genes, and showed a high similarity (71–76% query coverage and 98–99.9% nucleotide identity) to other unknown Inc group plasmids from clinical and environmental *P. aeruginosa* and *Pseudomonas putida*, respectively (GenBank accession numbers CP011370.1 and CP003589.1).

In contrast, pS810b was a novel 240,265 bp IncP-7 plasmid, containing 54.3% GC and 282 CDSs, and was most related to p1160-VIM (32% query coverage and 96.1% nucleotide identity), an ~205.4 kb plasmid harboring *blaVIM-24* of a carbapenem-resistant *P. aeruginosa* strain isolated from a human patient in China [5]. In addition to *bla*$_{IMP-13}$, pS810b co-harbored *aac(6′)-Ib3, aadA1, sul1*, and *tetC*, and genes encoding resistance to tellurite (*terY3Y2XY1W* and *terZABCDEF*), silver (*silESRCBAP*), and mercury (*merRTPCADE*). Variable regions of this plasmid were mostly composed of resistance genes, transposable elements, and genes encoding hypothetical proteins (Fig. 1A).

Analysis of the genetic context of the MBL genes revealed that *bla*$_{VIM-2}$ was associated with the In*1025* integron, carrying the *aac(6')-29b-bla*$_{VIM-2}$*-aac(6')-29b* gene cassette array. On the other hand, a novel Tn*402*-like class 1 integron, In*2113*, carrying the *bla*$_{IMP-13}$*-qacG2-aac(6′)-Ib3-aadA1* cassette array, was identified using INTEGRALL (http://integrall.bio.ua.pt/). In*2113* was also harbored by a novel Tn*21*-derivative transposon, Tn*7362* (18,431 bp), identified using the Transposon Registry (https://transposon.lstmed.ac.uk/). In addition to In*2113*, Tn*7362* carried genes related to transposition (*tnp*), EAL domain-containing protein (*urf2*), and mercury resistance (*merRTPCADE*) (Fig. 1B).

In conclusion, to the best of our knowledge, this is the first report of the *blaIMP-13* gene in Brazil, co-production of VIM-2 and IMP-13 in a single bacteria species, and a fully sequenced *bla*$_{IMP-13}$-carrying plasmid. Although the origin of *bla*$_{VIM-2}$ and *bla*$_{IMP-13}$ in agricultural soil was unclear, cattle manure and irrigation water might be sources. The detection of *bla*$_{IMP-13}$ and *bla*$_{VIM-2}$ in an XDR

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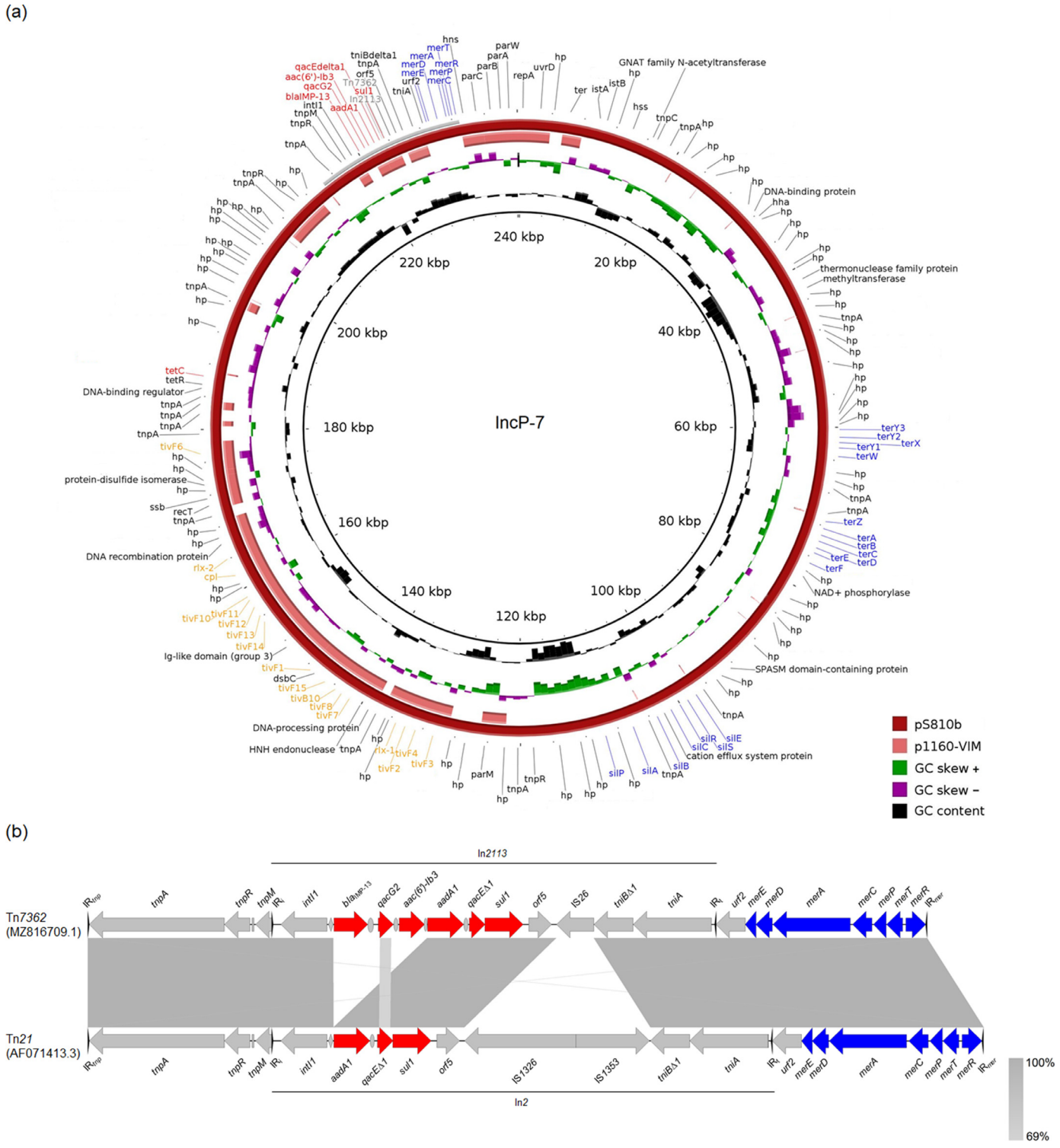

Fig. 1. Novel IncP-7 plasmid, pS810b, harboring bla_{IMP-13} on a novel Tn*21*-derivative transposon, Tn*7362*, in extensively drug-resistant *Pseudomonas aeruginosa* ST235 identified in Brazil. A) Comparison between IncP-7 plasmids from *P. aeruginosa* S810 (pS810b, GenBank accession number OL468818.1) isolated from agricultural soil in Brazil and *P. aeruginosa* 1160 (p1160-VIM, GenBank accession number MF144194.2) isolated from a human patient in China. Genes related to antimicrobial resistance, metal resistance, and conjugal transfer are indicated in red, blue, and orange, respectively; hp represents genes encoding hypothetical proteins. The gray arc indicates Tn*7362* harboring a novel bla_{IMP-13}-containing Tn*402*-like class 1 integron, In*2113*. Matches with less than 50% identity and no matches appear as blank spaces. B) Schematic representation of Tn*7362* and Tn*21*. The In*2113* integron, carrying the bla_{IMP-13}-*qacG2-aac(6′)-Ib3-aadA1* gene cassette array, is shown. Black filled arrows indicate flanking inverted repeats (IR) of transposons and integrons. The vertical bar, diamond, and ellipses represent resolution (*res*), *attI1*, and *attC* sites, respectively. The gray shading denotes shared regions of homology.

P. aeruginosa strain of the ST235 epidemic lineage in agricultural soil represents a threat to food and environmental safety. Therefore, continued monitoring of critical priority bacteria in crops remains necessary to effectively limit the spread of high-risk clones.

Nucleotide sequence accession numbers

Nucleotide sequences of *P. aeruginosa* S810 have been deposited at GenBank under accession numbers: JAJJQH000000000.1 (whole-genome shotgun project), MZ816709.1 (transposon Tn*7362*), OL468818.1 (plasmid pS810b), and OL468819.1 (plasmid pS810a).

Ethical approval

Not required

Competing interests

None declared

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Ralf Lopes
João Pedro Rueda Furlan
Eliana Guedes Stehling*
Department of Clinical Analyses, Toxicology and Food Science, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
*Corresponding author. Mailing address: Av. do Café, s/n, Monte Alegre, Ribeirão Preto, 14040-903, Brazil.
E-mail address: elianags@usp.br (E.G. Stehling)
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