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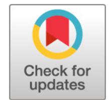
LETTER TO THE EDITOR



Plasmid Carrying $bla_{CTX-M-2}$ and bla_{GES-1} in Extensively Drug-Resistant *Pseudomonas aeruginosa* from Cerebrospinal Fluid

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Extended-spectrum β-lactamases (ESBL) are spread worldwide in the order *Enterobacterales* (1, 2) but are less common in *Pseudomonas aeruginosa*; consequently, little is known regarding the genetic environment and plasmid-carrying bla_{ESBL} genes in this species (3). The predominant ESBL enzymes are those in the CTX-M family (1). The GES family is a less common group of ESBL enzymes comprising 40 members, which have been found in various Gram-negative bacilli (4).

One *P. aeruginosa* strain, clinical strain 1206/13 (here called Pa1206/13), isolated from cerebrospinal fluid at a hospital in São Paulo State, Brazil, from 2007 to 2014 and resistant to third- and fourth-generation cephalosporins, aztreonam, or carbapenems, was studied. The antimicrobial resistance genes were investigated by PCR (5–9). Plasmid incompatibility groups were investigated by the PCR-based replicon typing (PBRT) (10, 11) and *Acinetobacter baumannii* PBRT (AB-PBRT) (12) methods. Pa1206/13, displaying an extensively drug-resistant (XDR) phenotype (13) (Table 1), carried $bla_{CTX-M-2}$ and bla_{GES-1} genes. S1 and I-Ceu-I nuclease digestion followed by pulsed-field gel electrophoresis (PFGE) and Southern blot hybridization with specific probes was performed to determine the locations of the *bla* genes. Based on S1-PFGE, Pa1206/13 possessed a single ~340-kb plasmid (p1206/13), which was nontypeable by PBRT, IncU, IncR, or AB-PBRT. Although these methodologies are not optimized for the typing of *Pseudomonas aeruginosa* plasmids, they are the most commonly used plasmid-typing methodologies. Southern blotting followed by hybridization with $bla_{CTX-M-2}$- and bla_{GES-1}-specific probes revealed that both *bla* genes were carried by p1206/13. Hybridization with probes for a *Pseudomonas* sp. 16S rRNA gene and the two *bla* genes after I-Ceu-I-PFGE further excluded a chromosomal location. Whole-genome sequencing of Pa1206/13 was then performed using Illumina NextSeq 250-bp paired-end sequencing. *De novo* assembly was carried out using CLC Genomics Workbench, version 8.0 (CLC bio, Aarhus, Denmark), and generated 565 contigs, with a contig N_{50} of 125,375 bp, an average coverage of 84×, and an assembled genome of approximately 7.1 Mb (draft sequence). Gene prediction was performed for the draft sequence using the RAST server (http://rast.nmpdr.org/).

According to multilocus sequence typing (http://pubmlst.org/paeruginosa/), Pa1206/13 belongs to sequence type 1602 (ST1602), which was recently characterized in two *P. aeruginosa* clinical isolates from Brazil (14), and Pa1206/13 seems to be the first reported ST1602 isolate producing ESBL. The sequencing data revealed bla_{GES-1} as a gene cassette on a previously unreported class 1 integron, In1600 (http://integrall.bio.ua.pt/) (Fig. 1). Furthermore, $bla_{CTX-M-2}$ was found downstream of IS*CR1* associated with

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TABLE 1 *In vitro* evaluation of activities of antimicrobial drugs against *P. aeruginosa* 1206/13

Drug[a]	Susceptibility profile[b]	MIC (μg/ml)[c]
TZP	I	
TIM	R	
CZA	S	
C/T	R	
CAZ	R	≥256 (R)
CPM	R	≥256 (R)
ATM	R	16 (I)
IPM	R	≥32 (R)
MER	R	≥32 (R)
GEN	R	
TOB	R	
AMK	R	
CIP	R	
LVX	R	

[a]TZP, piperacillin-tazobactam; TIM, ticarcillin-clavulanate; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam; CAZ, ceftazidime; CPM, cefepime; ATM, aztreonam; IPM, imipenem; MER, meropenem; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CIP, ciprofloxacin; LVX, levofloxacin.
[b]S, susceptible; I, intermediate; R, resistant.
[c]MIC testing was performed by Etest (bioMérieux). MIC breakpoints were evaluated according to CLSI guidelines (19).

In1600, resulting in a complex class 1 integron of ~11,680 bp (15). Additional antimicrobial resistance genes were predicted using ResFinder, version 3.1 (https://cge.cbs.dtu.dk/services/ResFinder/), which showed a resistome consisting of 15 resistance genes [*aadA2*, *aphA-6*, *aph(3′)-IIb*, *aacA4*, $bla_{OXA-395}$, $bla_{CTX-M-2}$, bla_{PAO}, bla_{OXA-2}, bla_{GES-1}, *crpP*, *fosA*, *cmlA4*, *catB7*, *sul1*, *dfrB5*]. PlasmidFinder was also used to determine the type of plasmid and, again, confirmed it as nontypeable. *In silico* analysis of the draft sequence showed that the plasmid was closely related to IncP2 plasmids (GenBank accession numbers KC543497.1 and KY494864.1). IncP2 plasmids have been found in environmental bacteria and have been observed carrying a tellurite resistance determinant (16). p1206/13 possessed conjugation (*tra* family; TraV, TraB, TraG) and partitioning (*par* family; ParA and ParB) genes, showing that *in vivo* conjugation may occur. Furthermore, p1206/13 carried diverse virulence determinants, including *pil* proteins (PilT and PilG), which govern twitching motility, as well as type IV pili and biofilm formation, and the *che* operon, which is known to be essential for flagellum chemotaxis in *P. aeruginosa* (17). These virulence factors have also been detected in other IncP2 plasmids from *P. aeruginosa* (pBJ37 [18] and pOZ176 [16]). However, the *mer* operon present in those plasmids was not detected in p1206/13.

$bla_{CTX-M-2}$ inserted into the *P. aeruginosa* chromosome has been described previously; however, this is the first report of an IncP2 plasmid coharboring two ESBL genes, $bla_{CTX-M-2}$ and bla_{GES-1}, in *P. aeruginosa*.

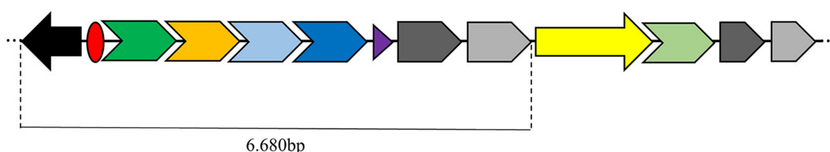

FIG 1 Schematic representation of the complex class 1 integron characterized in the GES-1- and CTX-M-2-producing *Pseudomonas aeruginosa* 1206/13 isolate. Arrows indicate the gene orientations. The black arrow represents *intI* (the class I integron integrase gene); the red circle, *attI* (the integron-associated recombination site). The four cassette genes/proteins that follow are bla_{GES-1}/β-lactamase (dark green arrow), *aacA4*/aminoglycoside-modifying enzyme (orange arrow), *cmlA4*/chloramphenicol exporter (light blue arrow), and *aadA2*/aminoglycoside-modifying enzyme (dark blue arrow). The purple triangle represents *attC* (the cassette-associated recombination sites). The 3′ conserved segment consists of fused genes for disinfectant and sulfonamide resistance (*qacE*Δ [dark gray arrow] and *sul1* [light gray arrow], respectively). Downstream of *sul1* are IS*CR1* (yellow arrow) associated with $bla_{CTX-M-2}$ (light green arrow) and duplicate *qacE*Δ/*sul1* genes.

Accession number(s). This sequence has been deposited in the DDBJ/ENA/GenBank database under BioSample accession number SAMN08384001.

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We have no conflicts of interest to declare.

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