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Genome Note

# SPM-1-producing *Pseudomonas aeruginosa* ST277 carries a chromosomal pack of acquired resistance genes: An example of high-risk clone associated with 'intrinsic resistome'

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ABSTRACT

*Objectives:* The purpose of this study was to investigate the resistome of an SPM-1-producing *Pseudomonas aeruginosa* ST277 isolate (HC84) from Brazil.
*Methods:* Whole-genome sequencing of *P. aeruginosa* HC84 was performed using an Ion Proton™ System. De novo assembly was carried out using CLC Genomics Workbench 8.0, and gene prediction was performed using the Prokka pipeline.
*Results and conclusion:* Here we describe the resistome of SPM-1-producing *P. aeruginosa* ST277 (HC84) consisting of 13 different antimicrobial resistance genes [$bla_{SPM-1}$, *rmtD*, *aacA4*, *aadA7*, $bla_{OXA-56}$, $bla_{OXA-396}$, $bla_{PAO}$, *aph(3′)-IIb*, *aac(6′)*-Ib-cr, *crpP*, *catB7*, *cmx* and *fosA*). This particular chromosomal pack of resistance genes is strongly associated with clonal dissemination and suggests an important role in the persistence of this clone in Brazilian nosocomial infections. For that reason, could we already consider the 'chromosomal pack of acquired resistance genes' like an 'ST277 intrinsic resistome'? This is an example of chromosomal accumulation of acquired resistance genes as well as integrative and conjugative elements into a successful bacterial pathogen and calls attention to the evolution of other species driving to insertion and persistence of multiple acquired resistance genes in the bacterial chromosome.


Carbapenem-resistant *Pseudomonas aeruginosa* causing nosocomial infections have been identified in several countries and are recognised critical pathogens according to the World Health Organization (WHO) (http://www.who.int). In Brazil, São Paulo metallo-β-lactamase-1 (SPM-1)-producing *P. aeruginosa* sequence type (ST) 277 is an endemic clone showing some genomic diversification [1–3] that is practically restricted to Brazil [4,5].

We have investigated the resistome (presence of antimicrobial resistance genes) and the associated mobile genetic elements (MGEs) of an SPM-1-producing *P. aeruginosa* ST277 isolate (HC84), an extensively drug-resistant clone frequently detected in a tertiary hospital of Ribeirão Preto, São Paulo State, Brazil, over the last 10 years. Moreover, in silico analysis was also performed to compare other ST277 isolates (from other Brazilian regions) as well as other *P. aeruginosa* STs, including several 'high-risk clones' (HiRC) detected worldwide.

Whole-genome sequencing of *P. aeruginosa* HC84 [1] was performed using an Ion Proton™ System (Thermo Fisher). De novo assembly was carried out using CLC Genomics Workbench 8.0 (CLC bio, Aarhus, Denmark) and generated 1286 contigs, with an $N_{50}$ value of 20.036 bp, 150× coverage and the assembled genome spanning ca. 6.6 Mb (draft sequence). Gene prediction was performed for the draft sequence of the isolate using the Prokka pipeline.

Antimicrobial resistance genes were predicted using ResFinder 3.1 (https://cge.cbs.dtu.dk/services/ResFinder/), which showed a resistome consisting of 13 different antimicrobial resistance genes [$bla_{SPM-1}$, *rmtD*, *aacA4*, *aadA7*, $bla_{OXA-56}$, $bla_{OXA-396}$, $bla_{PAO}$, *aph(3′)*-IIb, *aac(6′)*-Ib-cr, *crpP*, *catB7*, *cmx* and *fosA*) as detailed in Table 1. In addition, considering only the *P. aeruginosa* STs and HiRC analysed here, interestingly only ST277 showed $bla_{OXA-56}$. The genetic context of the resistance genes was characterised and matched with most *P. aeruginosa* ST277, highlighting the integrative and

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**Table 1**
Resistome of *Pseudomonas aeruginosa* ST277 strains.

| Antimicrobial category/agents | *P. aeruginosa* strains and resistance genes | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HC84 | PA1088 | PA3448 [a] | PA7790 | PA8281 [a] | PA11803 | PA12117 | CCBH4851 | AZPAE14819 | AZPAE14821 | AZPAE14853 | AZPAE14923 | 19BR | 213BR | 9BR |
| Aminoglycosides: gentamicin, tobramycin, amikacin, netilmicin | *rmtD* | *rmtD* | | | | *rmtD* | *rmtD* | *rmtD* | *rmtD* | *rmtD* | *rmtD* | *rmtD* | *rmtD* | *rmtD* | *rmtD* |
| Non-specific aminoglycosides | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* | *aacA4* *aadA7* *aph(3')-IIb* |
| Antipseudomonal carbapenems | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ |
| Antipseudomonal cephalosporins: ceftazidime, cefepime | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ |
| β-Lactams, non-specific | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ |
| | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ | $bla_{OXA-56}$ |
| | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ |
| Antipseudomonal | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* | *aac(6')-Ib-cr* |
| fluoroquinolones: ciprofloxacin, levofloxacin | *crpP* | *crpP* | *crpP* | *crpP* | *crpP* | | | *crpP* | *crpP* | *crpP* | | | *crpP* | *crpP* | |
| Antipseudomonal penicillins + β-lactamase inhibitor | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ | $bla_{SPM-1}$ |
| Monobactams | | | | | | | | | | | | | | | |
| Phosphonic acids | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* | *fosA* |
| Polymyxins: colistin, polymyxin B | | | | | | | | | | | | | | | |
| Phenicols | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* | *catB7* | *catB7* *cmx* | *catB7* *cmx* | *catB7* *cmx* | *catB7* |

[a] PA3448 and PA8281 have a double copy of $bla_{SPM-1}$ in the chromosome.

conjugative element (ICE)$_{Tn4371}$6061–$bla_{SPM-1}$ structure, IS*CR14*–*rmtD* structure and a class 1 integron In*163* carrying *aacA4*, *aadA7* and $bla_{OXA-56}$ organised as cassette genes. To confirm that strain HC84 does not carry any plasmid, *S1* nuclease pulsed field gel electrophoresis (S1-PFGE) and *I-Ceu*I-PFGE followed by hybridisation were performed. All resistance genes detected here were inserted in the chromosome, although most of them had been previously reported as commonly plasmid-mediated, mainly in Enterobacteriaceae [3,6] such as *aacA4 (Klebsiella pneumoniae)*, *aadA7 (Salmonella)*, *aac(6′)*-Ib-cr *(Escherichia coli)*, *fosA* (*Citrobacter freundii*, *Enterobacter* sp., *E. coli*) and *rmtD (K. pneumoniae*, *E. coli*, *Citrobacter* sp.). Moreover, different MGEs were found, including the ICE cited above and at least 11 full-length copies of different insertion sequences (ISs) (Supplementary Table S1). ISs were predicted by IS*saga* tool, however plasmids were not detected.

Strain HC84 showed $bla_{SPM-1}$ inserted on ICE$_{Tn4371}$6061 as described by Fonseca et al. for *P. aeruginosa* ST277 (PS106-RJ strain) [2], however strain HC84 showed only one copy of the $bla_{SPM-1}$ gene in the genome, which was different from the findings (two copies) of other authors [4]. Besides, in strain HC84 the ICE$_{Tn4371}$6061 was inserted in the same region in strain PS106-RJ compared with the *P. aeruginosa* PAO (**NC_002516.2**) genome. The ICE$_{Tn4371}$6061 genetic localisation was different from that found in some *P. aeruginosa* ST277 isolates studied by Nascimento et al. [7]. In addition, in silico analysis showed that ICE$_{Tn4371}$6061 was particularly related to *P. aeruginosa* ST277 since this MGE was not found in *P. aeruginosa* belonging to other STs, such as *P. aeruginosa* PAO-ST549, BL17-ST235, BH6-ST244, PA1R-ST782, B136-33-ST1024 and LES431-ST146, or at least was not found intact, as in *P. aeruginosa* SCV20265-ST299 (query coverage 74% and 83% of identity distributed in 35 different matches), PA7-ST1195 (query coverage 66% and 85% of identity distributed in 15 different matches) and DK2-ST386 (query coverage 5% and 80% of identity distributed in 6 different matches). The isolates cited above were chosen as representative STs, HiRC (ST235 and ST244) and non-HiRC using the PATRIC database (https://www.patricbrc.org/).

This particular chromosomal pack of acquired resistance genes and MGEs is strongly associated with *P. aeruginosa* ST277 clonal dissemination and suggest an important role in the adaptation and persistence of this clone in Brazilian nosocomial infections,

**Table 2**
Resistome of *Pseudomonas aeruginosa* strains of diverse sequence types (STs).

| Antimicrobial category/agents | *P. aeruginosa* strains and resistance genes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | BL17-ST235 [a] | BH6-ST244 [b] | PAO-ST549 | PA1R-ST782 | B136-33-ST1024 | LES431-ST146 | SCV20265-ST299 | PA7-ST1195 | DK2-ST386 |
| Aminoglycosides: gentamicin, tobramycin, amikacin, netilmicin | | | | | | | | | |
| Non-specific aminoglycosides | | | | | | | | *aacA4* | |
| | *aph(3')-IIb* | *aph(3')-IIb* | *aph(3')-IIb* | *aph(3')-IIb* | *aph(3')-IIb* | *aph(3')-IIb* | *aph(3')-IIb* | | |
| | | | | | | | | *aph(6)-Ic* | |
| | | | | | | | | *aph(3')-IIa* | |
| | | | | | | | | *strA* | |
| | | | | | | | | *strB* | |
| | *aadB* | | | | | | | | |
| | *aadA6* | | | | | | | | |
| Antipseudomonal carbapenems | | $bla_{KPC-2}$ | | | | | | | |
| Antipseudomonal cephalosporins: ceftazidime, cefepime | | $bla_{KPC-2}$ | | | | | | | |
| β-Lactams, non-specific | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-396}$ | $bla_{OXA-50}$ | $bla_{OXA-388}$ | | $bla_{OXA-396}$ | | $bla_{OXA-396}$ |
| | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | $bla_{PAO}$ | | $bla_{PAO}$ |
| Antipseudomonal fluoroquinolones: ciprofloxacin, levofloxacin | | | | | | | | *aac(6')-Ib-cr* | |
| | | | | | *crpP* | | | *crpP* | *crpP* |
| Antipseudomonal penicillins + β-lactamase inhibitor | | $bla_{KPC-2}$ | | | | | | | |
| Monobactams | | $bla_{KPC-2}$ | | | | | | | |
| Phosphonic acids | *fosA* | *fosA* | *fosA* | | *fosA* | | *fosA* | | *fosA* |
| Polymyxins: colistin, polymyxin B | | | | | | | | | |
| Phenicols | *catB7* | *catB7* | *catB7* | | *catB7* | | *catB7* | | *catB7* |
| | | | | | | | | *cmx* | |

[a] BL17 has a double copy of *aadB* in the chromosome.
[b] BH6 has a $bla_{KPC-2}$ gene located on a small plasmid, with no copy in the bacterial chromosome.

highlighting $bla_{SPM-1}$ and *rmtD* (Tables 1 and 2). The presence of this set of acquired resistance genes located in the chromosome, associated with additional mutational resistance mechanisms (e.g. to quinolones and polymyxins) and intrinsic resistance per se (e.g. impermeability), is extremely worrisome because they drastically reduce empirical therapeutic options for infections caused by these bacteria and impact clinical outcomes.

Moreover, *P. aeruginosa* ST277 shows a similar clinical impact compared with the resistome of other bacterial species, such as international HiRC *Acinetobacter baumannii* ST1, ST2 and ST3, *E. coli* ST131 and *K. pneumoniae* ST258 [6].

*Pseudomonas aeruginosa* ST277 appears to be extremely capable of genetic uptake and chromosomal recombination/integration of acquired resistance genes and MGEs. Considering the clinical impact of the resistome from different bacterial species and comparing the common genetic features of *P. aeruginosa* ST277, could we already consider this 'chromosomal pack of acquired resistance genes' like a 'ST277 intrinsic resistome'? This question is open to discussion, however due to increasing reports of multi-resistant bacteria, many hospital pathogens have shown increasing chromosomal integration of acquired adaptive traits, commonly plasmid-mediated, and this feature appears to lead to evolutionary step point to chromosome enlargement, with an advantageous fitness cost, contributing to boost the selection, dissemination and persistence of hospital pathogens.

Therefore, the 'ST277 intrinsic resistome' is an example of acquired resistance genes, usually carried by plasmids, totally inserted in the bacterial chromosome of successful HiRC bacteria.

## GenBank accession numbers

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. **MVOB00000000**. The version described in this paper is **MVOB01000000**. Other accession nos.: PA1088, **CP_015001**; PA3448, **LVWC01000000**; PA7790, **CP014999**; PA8281, **CP015002**; PA11803, **CP015003**; PA12117, **LVXB01000000**; PAO, **NC_002516.2**; PA1R, **NC_022806.1**; B136-33, **NC_020912.1**; LES431, **NC_023066**; SCV20265, **NC_023149.1**; PA7, **NC_009656.1**; DK2, **NC_018080**; BH6, **LGVH01000000**; BL17, **AXPJ01000000**; CCBH4851, **CP021380.1**; AZPAE14819, **NZ_JTVP01000000**; AZPAE14821, **NZ_JTVN01000000**; AZPAE14853, **NZ_JTUI01000000**; AZPAE14923, **NZ_JTRS01000000**; 213BR, **NZ_AFXK01000001**; 19BR, **NZ_AFXJ01000001**; and 9BR, **NZ_AFXI01000000**.

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## Competing interests

None declared.

## Ethical approval

Not required.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2018.12.009.

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