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Short Communication

Carbapenem-resistant *Pseudomonas aeruginosa* carrying bla_{VIM-36} assigned to ST308: Indicated non-virulence in a *Galleria mellonella* model

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ABSTRACT

Objectives: Based on pulsed-field gel electrophoresis (PFGE) profile, whole-genome sequencing (WGS) of eight carbapenem-resistant *Pseudomonas aeruginosa* isolates from a bone marrow transplant unit in São Paulo, Brazil, was performed to investigate the presence of resistance and virulence genes as well as to determine the sequence type (ST) by multilocus sequence typing (MLST).
Methods: The initial phenotypic susceptibility pattern of the isolates was determined by VITEK®2. Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method for amikacin, meropenem and colistin. WGS was performed using an Illumina MiSeq system. A *Galleria mellonella* infection model was used to evaluate the virulence of the strains.
Results: WGS demonstrated that mutations in genes encoding outer membrane proteins and efflux pumps in an isolate harbouring bla_{VIM-36} (ST308) differed from those in isolates harbouring bla_{SPM} (ST277). The *mexT* gene harboured a mutation resulting in a frameshift in all isolates; in addition, the *oprD* gene of the bla_{VIM-36}-carrying isolate had an insertion leading to a frameshift. Virulence genes did not differ between ST277 and ST308 strains. Moreover, only two isolates harbouring bla_{SPM} showed virulence in the *G. mellonella* model, killing 100% of larvae after 18–24 h.
Conclusions: P. aeruginosa carrying bla_{VIM-36} belonging to ST308 was identified for the first time in our hospital. Although the virulence gene profiles were similar in isolates carrying bla_{SPM} and the isolate carrying bla_{VIM-36}, only two isolates harbouring bla_{SPM} showed virulence in the *G. mellonella* model.

1. Introduction

In recent years, carbapenem-resistant (CarbaR) *Pseudomonas aeruginosa* have disseminated around the world [1], mostly due to carbapenemase-producing isolates [2,3]. Many types of carbapenemases have been described in CarbaR *P. aeruginosa*, including metallo-β-lactamases such as imipenemase (IMP), Verona integron-encoded metallo-β-lactamase (VIM), São Paulo metallo-β-lactamase (SPM), Germany imipenemase (GIM) and New Delhi metallo-β-lactamase (NDM) [2,3]. In Brazil, SPM is the most frequent mechanism of resistance described in CarbaR *P. aeruginosa* [4]. An endemic clone of sequence type 277 (ST277) harbouring the bla_{SPM-1} metallo-β-lactamase gene has spread in the country [5].

In this study, whole-genome sequencing (WGS) of eight CarbaR *P. aeruginosa* clinical isolates from a bone marrow transplant unit in São Paulo, Brazil, was performed to determine the ST of the isolates by multilocus sequence typing (MLST) and to investigate the genes involved in the mechanisms of

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resistance and virulence. Virulence was also assessed in a *Galleria mellonella* model.

2. Materials and methods

2.1. Clinical isolates

This study was conducted at the Medical Research Laboratory 54 (LIM-54), Department of Infectious and Parasitic Diseases, Institute of Tropical Medicine of São Paulo, University of São Paulo (São Paulo, Brazil). WGS of eight CarbaR *P. aeruginosa* clinical isolates from haematopoietic stem cell transplant (HSCT) patients (one isolate per patient) collected from 2011–2014 and stored in the LIM-54 strain bank was carried out based on pulsed-field gel electrophoresis (PFGE) profile as described previously [4]. Three of the eight strains (strains 1303, 1410 and 1136) were identified in an outbreak of bloodstream infections that occurred in our hospital in 2012. The *P. aeruginosa* strains used in this study were isolated from blood ($n = 6$), faeces ($n = 1$) and gluteal abscess aspirate ($n = 1$) of HSCT patients. All isolates were placed in 20% glycerol and were stored at −80 °C. The reference strain *P. aeruginosa* ATCC 27853 was used as a control in all experiments.

This study was approved by the Ethical Committee of Hospital das Clínicas of the University of São Paulo (São Paulo, Brazil).

2.2. Antimicrobial resistance profile

The initial phenotypic susceptibility pattern was determined by VITEK®2 (bioMérieux, Marcy I'Étoile, France). Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method for meropenem, amikacin and colistin according to Clinical and Laboratory Standards Institute guidelines [6] based on the therapy guide of our hospital.

2.3. Whole-genome sequencing and bioinformatics analysis

WGS was performed by Nextera XT using Illumina MiSeq technology (Illumina Inc., San Diego, CA). De novo assembly of reads was performed using VelvetOptimiser v.2.2.5 (Victorian Bioinformatics Consortium, Australia) and contigs were ordered by ABACAS v.1.3.1 [7]. Annotation of the genome was performed by Prokka v.1:11. [8]. Analysis of antimicrobial resistance and virulence genes was performed using the following tools: ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/), Blast2Seq (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and MAFFT (http://mafft.cbrc.jp/alignment/server/). The results generated by these tools and the presence of the genes were subsequently confirmed by manual curation of coding sequences (CDS) and using the Artemis program v.16.0.0. MLST was performed using the MLST tool at the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/MLST/) and was analysed using eBURST software (http://eburst.mlst.net/). Clonal relatedness of the eight isolates was performed by dendrogram analysis generated using CSI Phylogeny tool (https://cge.cbs.dtu.dk/services/CSIPhylogeny/). Assembly of the circular genome map of the representative isolates of *P. aeruginosa* of each clone relative to *P. aeruginosa* strain PAO1 (GenBank accession no. **AE004091.2**) was carried out using the Blast Ring Image Generator (BRIG) tool (http://brig.sourceforge.net/). Single nucleotide polymorphisms (SNPs) were identified by mapping the sequencing reads of the isolates against the *P. aeruginosa* strain PAO1 using Burrows–Wheeler Aligner (BWA), SAMtools and Genome Analysis Toolkit (GATK). All SNPs were manually checked.

2.4. *G. mellonella* virulence assay

A *G. mellonella* (Greater wax moth) infection model was used to evaluate the virulence of the *P. aeruginosa* strains. Eggs of *G. mellonella* were kindly provided by the Laboratory of Molecular Biology of Nematodes (ICB II-USP, São Paulo, Brazil) and were maintained in the dark at 28 °C. After hatching, larvae were fed with pollen and beeswax (Sunyata, Atibaia, SP, Brazil). *Pseudomonas aeruginosa* strains were grown overnight in brain–heart infusion broth, were subsequently centrifuged and were washed and adjusted in phosphate-buffered saline (PBS) to reach a 0.5 McFarland standard as measured using a DensiCHEK™ Plus device (bioMérieux). The resulting bacterial suspension was used for infection of *G. mellonella*. To investigate the virulence of *P. aeruginosa* strains, bacterial suspensions were diluted in PBS to 1×10^4 CFU/mL and then 10 μL was injected into the haemocoel through the last left pro-leg of final instar stage larvae of *G. mellonella* (weight ca. 250–350 mg) using a 31 G × 6 mm BD Ultra-Fine™ 30U syringe (Becton, Dickinson & Co., Franklin Lakes, NJ). Groups of ten larvae for each *P. aeruginosa* strain were inoculated and were incubated at 37 °C, and the number of dead larvae was observed at 12–120 h after inoculation. Death was confirmed by melanisation (larvae change from cream to dark brown colour) and no movement in response to touch [9]. All experiments were performed in triplicate.

3. Results

The eight *P. aeruginosa* strains exhibited resistance to cefepime, ceftazidime, ciprofloxacin, amikacin, gentamicin, imipenem and meropenem by VITEK®2. Broth microdilution revealed the follow profiles: amikacin MIC $\geq$ 512 mg/L; meropenem MIC $>$ 256 mg/L; and susceptibility to colistin (MIC $<$ 1 mg/L). In addition, WGS analysis showed that one isolate harboured the *bla*$_{\text{VIM-36}}$ gene, which differs from *bla*$_{\text{VIM-2}}$ by only one amino acid alteration from glutamine to arginine at position 60 (Q60R), and seven isolates carried the *bla*$_{\text{SPM-1}}$ gene. The seven *bla*$_{\text{SPM}}$-positive strains were clustered in five clones designed I, II, IV, V and VI (Table 1). Moreover, WGS demonstrated the presence of resistance genes to aminoglycosides, β-lactams, fluoroquinolones, fosfomycin, phenicols and sulphonamides (Table 1). Mutations in carbapenem resistance genes, such as those encoding outer membrane proteins and efflux pumps, were found in *P. aeruginosa* strain 1030 carrying *bla*$_{\text{VIM-36}}$, which differed from those in isolates harbouring *bla*$_{\text{SPM}}$. In all of the isolates, the *mexT* gene harboured a mutation resulting in a frameshift, whilst the *oprD* gene had an insertion leading to a frameshift in *bla*$_{\text{VIM-36}}$-carrying *P. aeruginosa* strain 1030 (Table 1).

Regarding virulence genes, the strains carried at least 93 genes related to virulence factors, including toxins, flagella, fimbriae, lipopolysaccharide production, iron and zinc metabolism, siderophores and other mechanisms (Table 1). Clonal relatedness of the eight isolates in the dendrogram generated by CSI Phylogeny confirmed the results obtained by MLST and demonstrated the tight grouping of the isolates belong to ST277 and segregation of the isolate belong to ST308 (Fig. 1). Assembly of the circular genome map of representative isolates of each *P. aeruginosa* clone relative to the *P. aeruginosa* strain PAO1 is shown in Fig. 1B.

Investigation of virulence among groups of ten *G. mellonella* larvae inoculated with *P. aeruginosa* showed that two strains harbouring *bla*$_{\text{SPM}}$, namely strain 1166 (isolated from faeces in 2012) and strain 4285 (isolated from blood in 2014), killed 70–100% of larvae between 18–24 h post-infection with an inoculum of 1×10^4 CFU/mL (Fig. 2).

Table 1

Resistance profile, genotype and virulence pattern of eight carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates from a bone marrow transplant unit in São Paulo, Brazil, from 2011–2014.

Isolation year; strain no. (GenBank accession no.)	Isolation site	Tn*4371*	Resistance genes[a]	Whole genome sequencing analysis											
				Gene mutations					Virulence genes[b]					ST	PFGE
				OprD	MexB	OprM	OprE	MexT	Quorum-sensing	Biofilm	Adhesion	Cytotoxicity and invasion process T3SS	Phenazine operon		
2011, strain 1030 (**QHLT00000000**)	Gluteal abscess	+	*aph(3′)-IIb*, *aadA10*, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{VIM-36}}$, *fosA*, *catB7*, *cmx*, *sul1*	T103S, K115T, F170L, E185Q, P186G, V189T, R310E, A315G, G425A, Q327_Y328_insVTRFSSPTPCSK	G957D, S1041E, V1042A	None	S290_G294_del	F94I, I263F, D267E	*gacA*, *gacS*, *ladS*, *lasA*, *lasB*, *plcB*, *plcH*, *plcN*, *plcR*	*qscR*, *qteE*	*lecB*	*exoT*, *exoU*, *exoY*, *toxA*	*phzM*	308	III
2012, strain 1136 (**QJPA00000000**)	Blood	+	*aph(3′)*-IIb, *rmtD*, *aadA7*, *aacA4*, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{OXA-56}}$, *bla*$_{\text{SPM-1}}$, *aac(6′)-Ib*-cr, *fosA*, *cmx*, *catB7*, *sul1*	T103S, K115T, V118P, F170L	None	A261T	S213G, T377G, V378L, V380L, I388M, S391K, K394N, T397S, A398G, S410A, S434T, A438V, A439S, S443T, N444A, N445T, S441_S442insG, L106_D107_insGL	F94I, I263F, D267E	*gacA*, *gacS*, *ladS*, *lasB*	*bfmR*, *bfmS*, *qscR*, *qteE*	*lecB*	*exoS*, *exoY*, *toxA*	*phzM*	277	V
2012, strain 1166 (**QJOZ00000000**)	Faeces	+	*aph(3′)*-IIb, *aadA7*, *aacA4*, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{OXA-56}}$, *bla*$_{\text{SPM-1}}$, *aac(6′)*-Ib-cr, *fosA*, *cmx*, *catB7*, *sul1*	T103S, K115T, V118P, F170L	None	A261T	S213G, T377G, V378L, V380L, I388M, S391K, K394N, T397S, A398G, S410A, S434T, A438V, A439S, S443T, N444A, N445T, S441_S442insG, L106_D107_insGL	F94I, I263F, D267E	*ladS*, *lasA*, *lasB*	*bfmR*, *bfmS*, *qscR*	*lecB*	*exoS*, *exoY*, *toxA*	*phzM*	277	II
2012, strain 1209 (**QJPG00000000**)	Blood	+	*aph(3′)*-IIb, *aacA4*, *aadA7*, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{OXA-56}}$, *bla*$_{\text{SPM-1}}$, *aac(6′)*-Ib-cr, *fosA*, *cmx*, *catB7*, *sul1*	T103S, K115T, V118P, F170L	None	A261T	S213G, T377G, V378L, V380L, I388M, S391K, K394N, T397S, A398G, S410A, S434T, A438V, A439S, S443T, N444A, N445T, S441_S442insG, L106_D107_insGL	F94I, I263F, D267E	*gacA*, *gacS*, *ladS*, *lasA*, *lasB*	*bfmR*, *bfmS*, *qscR*, *qteE*	*lecB*	*exoS*, *exoY*, *toxA*	*phzM*	277	VI
2012, strain 1303 (**QHLR00000000**)	Blood	+	*aph(3′)*-IIb, *aacA4*, *aadA7*, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{OXA-56}}$, *bla*$_{\text{SPM-1}}$, *aac(6′)*-Ib-cr, *cmx*, *catB7*	T103S, K115T, V118P, F170L	None	A261T	S213G, T377G, V378L, V380L, I388M, S391K, K394N, T397S, A398G, S410A, S434T, A438V, A439S, S443T, N444A, N445T, S441_S442insG, L106_D107_insGL	F94I, I263F, D267E	*gacS*, *ladS*, *lasA*, *lasB*	*bfmS*, *qscR*	*lecB*	*exoS*, *toxA*	*phzM*	277	II
2012, strain 1410 (**QHLU00000000**)	Blood	+	*aph(3′)*-IIb, *rmtD*, *aacA4*, *aadA7*, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{SPM-1}}$, *bla*$_{\text{OXA-56}}$, *aac(6′)*-Ib-cr, *fosA*, *cmx*, *catB7*, *sul1*	T103S, K115T, V118P, F170L	None	A261T	S213G, T377G, V378L, V380L, I388M, S391K, K394N, T397S, A398G, S410A, S434T, A438V, A439S, S443T, N444A, N445T, S441_S442insG, L106_D107_insGL	F94I, I263F, D267E	*gacA*, *gacS*, *ladS*, *lasA*, *lasB*	*bfmR*, *bfmS*, *qscR*, *qteE*	*lecB*	*exoS*, *exoY*, *toxA*	*phzM*	277	VI
2013, strain 1680 (**QHLW00000000**)	Blood	+	*aph(3′)*-IIb, *rmtD*, *aacA4*, *aadA7*, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{OXA-56}}$, *bla*$_{\text{SPM-1}}$, *aac(6′)*-Ib-cr, *fosA*, *catB7*, *sul1*	T103S, K115T, V118P, F170L	None	A261T	S213G, T377G, V378L, V380L, I388M, S391K, K394N, T397S, A398G, S410A, S434T, A438V, A439S, S443T, N444A, N445T, S441_S442insG, L106_D107_insGL	F94I, I263F, D267E	*gacA*, *gacS*, *ladS*, *lasA*, *lasB*	*bfmR*, *bfmS*, *qscR*	*lecB*	*exoS*, *exoY*, *toxA*	*phzM*	277	I
2014, strain 4285 (**QHLS00000000**)	Blood	+	*aph(3′)*-IIb, *aacA4*, *aadA7*, *bla*$_{\text{PDC-1}}$, *bla*$_{\text{OXA-50}}$, *bla*$_{\text{SPM-1}}$, *bla*$_{\text{OXA-56}}$, *aac(6′)*-Ib-cr, *fosA*, *catB7*, *sul1*	T103S, K115T, V118P, F170L	None	A261T	S213G, T377G, V378L, V380L, I388M, S391K, K394N, T397S, A398G, S410A, S434T, A438V, A439S, S443T, N444A, N445T, S441_S442insG, L106_D107_insGL	F94I, I263F, D267E	*gacA*, *gacS*, *ladS*, *lasA*, *lasB*	*bfmR*, *bfmS*, *qscR*	*lecB*	*exoS*, *exoT*, *exoY*, *toxA*	*phzM*	277	IV

T3SS, type III secretion system; ST, sequence type; PFGE, pulsed-field gel electrophoresis.

[a] *aacA4*, aminoglycoside N(6′)-acetyltransferase type 1; *aac(6′)-Ib*-cr, aminoglycoside 6′-N-acetyltransferase type Ib-cr; *aadA7*, streptomycin 3″-adenylyltransferase; *aadA10*, streptomycin 3″-adenylyltransferase; *aph(3′)-IIb*, aminoglycoside phosphotransferase; *bla*$_{\text{PDC-1}}$, β-lactamase; *bla*$_{\text{OXA-50}}$, oxacillinase; *bla*$_{\text{OXA-56}}$, oxacillinase; *bla*$_{\text{SPM-1}}$, β-lactamase; *bla*$_{\text{VIM-36}}$, β-lactamase; *catB7*, chloramphenicol acetyltransferase; *cmx*, chloramphenicol resistance protein; *fosA*, glutathione transferase; *rmtD*, 16S rRNA methyltransferase; *sul1*, sulfonamide-resistant dihydropteroate synthase.

[b] *gacA*, response regulator; *gacS*, sensor protein; *ladS*, lost adherence sensor; *lasA*, protease; *lasB*, elastase; *plcB*, phospholipase C; *plcH*, haemolytic phospholipase C; *plcN*, non-haemolytic phospholipase C; *plcR*, phospholipase C accessory protein; *qscR*, quorum-sensing control repressor; *qteE*, quorum threshold expression element; *bfmR*, response regulator; *bfmS*, sensor kinase; *lecB*, fucose-binding lectin PA-IIL; *exoT*, exoenzyme T; *exoY*, adenylate cyclase; *exoU*, phospholipase; *toxA*, exotoxin A; *exoS*, exoenzyme S; *phzM*, phenazine-specific methyltransferase.

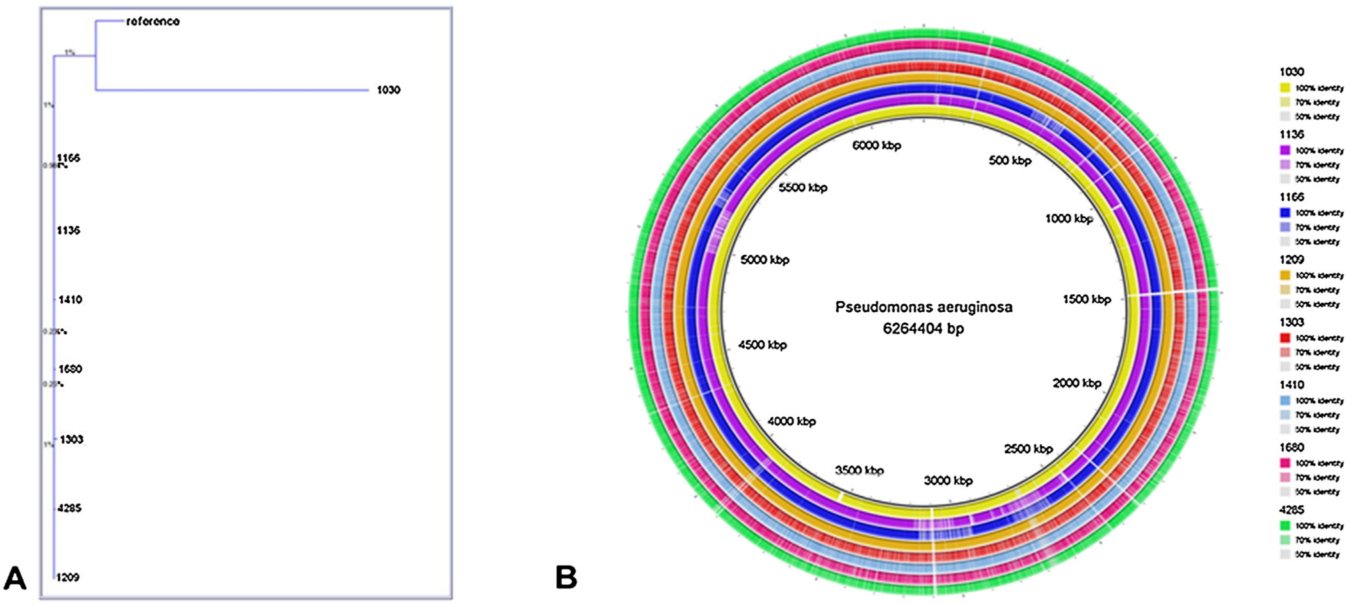

Fig. 1. Whole-genome sequencing analysis of eight *Pseudomonas aeruginosa* clinical isolates. (A) Phylogenetic relationship identified by single nucleotide polymorphism (SNP) mapping of the eight clinical isolates against the *P. aeruginosa* strain PAO1 constructed with CSI Phylogeny. (B) Circular map of the genomes of the eight clinical isolates and the reference strain *P. aeruginosa* PAO1 (represented by the black ring in the inner part of the figure). Non-related regions of the genome are represented by white spaces.

4. Discussion

MLST analysis revealed that the seven isolates carrying *bla*$_{\text{SPM}}$ belonged to ST277 and the isolate harbouring *bla*$_{\text{VIM-36}}$ clustered as ST308. An interesting finding is that no differences were observed in the virulence genes present between the *bla*$_{\text{SPM}}$-positive isolates with the *bla*$_{\text{VIM-36}}$-positive isolate. However, expression of these virulence genes is likely altered as two strains harbouring *bla*$_{\text{SPM}}$ were virulent in the *G. mellonella* model.

VIM-producing *P. aeruginosa* have been reported in Latin America, including in Brazilian hospitals, being the second most frequent carbapenemase in the country [10,11]. Generally, *P. aeruginosa* carrying *bla*$_{\text{VIM}}$ belong to ST111, ST244 and ST640 [11,12]. Unusually, the strain harbouring *bla*$_{\text{VIM-36}}$ in the current study was assigned as ST308. This finding contrasts with previous reports in Europe and Australia which demonstrated that *P. aeruginosa* strains assigned as ST308 used to carry *bla*$_{\text{IMP}}$ instead of *bla*$_{\text{VIM}}$ and presented great polymorphism in OprD [13,14]. The carbapenemase-encoding gene *bla*$_{\text{VIM-36}}$ was described in 2014 in a *P. aeruginosa* strain originally described in Belgium [15]. As previously described, *bla*$_{\text{VIM-36}}$ in the current study differs only by one amino acid from VIM-2.

These findings demonstrate that besides carbapenemases, mutations in carbapenem resistance genes such as those encoding outer membrane proteins and efflux pumps in the isolate harbouring *bla*$_{\text{VIM-36}}$ (ST308) are different from the *bla*$_{\text{SPM}}$-carrying isolates belonging to ST277. The *mexT* gene harboured a mutation resulting in a frameshift in all isolates tested, whilst the *oprD* gene of strain 1030 carrying *bla*$_{\text{VIM-36}}$ had an insertion leading to a frameshift.

In Brazil, dissemination of a CarbaR epidemic clone of *P. aeruginosa* producing *bla*$_{\text{SPM-1}}$ has been reported in several hospitals [3,4,16], being recurrently associated with outbreaks of nosocomial infection. More recently, *bla*$_{\text{SPM-1}}$ was described in Europe and was associated with transposon Tn*4371* [17].

The STs identified in our hospital harboured several resistance genes to aminoglycosides, phenicols and fluoroquinolones as well as Tn*4371*. All clones were resistant to aminoglycosides and carried the *aph(3′)-IIb* gene. The 16S rRNA methyltransferase gene *rmtD1*, which confers high-level resistance to all aminoglycosides and has been associated with ST277, was present in three of seven strains belonging to ST277 but was not identified in the *bla*$_{\text{VIM-36}}$-positive strain belonging to ST308.

Regarding virulence, WGS demonstrated that ST277 and ST308 strains carried virulence genes involved in adhesion, quorum sensing, biofilm production and type III secretion system [18,19], and no differences were observed comparing *bla*$_{\text{SPM}}$-positive isolates and the *bla*$_{\text{VIM-36}}$-positive isolate. Three of the strains studied (strains 1303, 1410 and 1136) were identified during a high-mortality outbreak of bloodstream infections caused by *P. aeruginosa* that occurred in our hospital in 2012 [20], but we cannot directly associate virulence with mortality since many factors interfere with this outcome, such as the clinical status of patients, their underlying diseases, co-morbidities and treatment-related issues. Only two isolates, strain 1166 (isolated from faeces in 2012) and strain 4285 (isolated from blood in 2014), showed virulence in the *G. mellonella* model, killing 100% of larvae after 18–24 h. *Galleria mellonella* is a suitable method that has been validated as an invertebrate infection model for virulence studies [9]. Strains 1166 and 4285 carried *bla*$_{\text{SPM}}$, belonged to ST277 and were clustered as different PFGE types (II and IV, respectively). Unfortunately, we could not assess epidemiological information regarding patient travel before the identification of the *bla*$_{\text{VIM-36}}$-positive isolate.

Although *bla*$_{\text{SPM-1}}$ remains a frequent carbapenemase in our hospital, *bla*$_{\text{VIM-36}}$ belonging to ST308 was identified in a HSCT patient. WGS demonstrated that virulence genes did not differ among the strains harbouring *bla*$_{\text{SPM}}$ and the strain carrying *bla*$_{\text{VIM-36}}$. Moreover, only two isolates harbouring *bla*$_{\text{SPM}}$ showed virulence in the *G. mellonella* model. Finally, the *P. aeruginosa* isolates carried carbapenemases and presented mutations in the genes encoding outer membrane proteins and efflux pumps, highlighting the importance of association of mechanisms of resistance in our hospital.

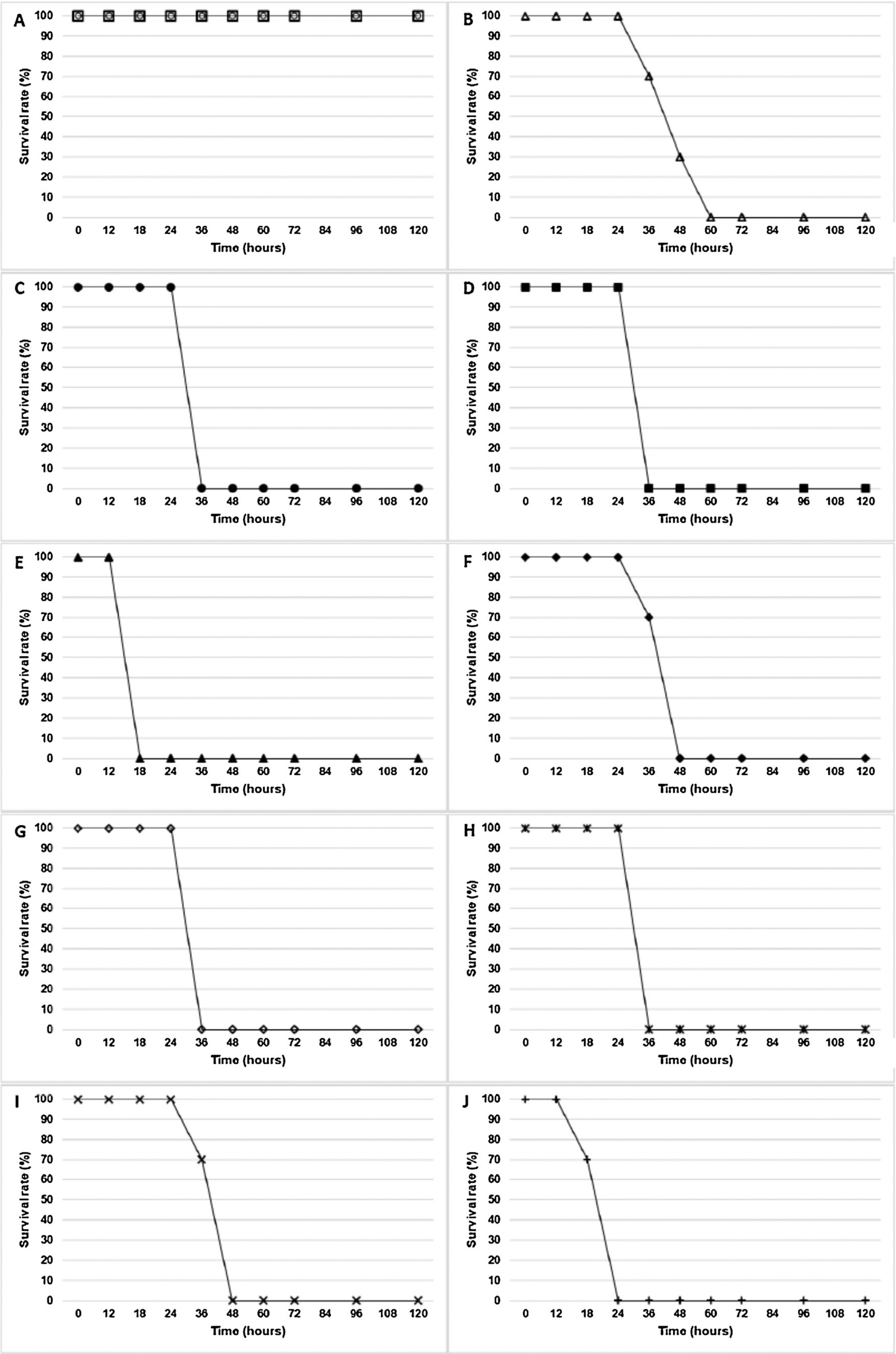

Fig. 2. Virulence assay for eight *Pseudomonas aeruginosa* clinical isolates in a *Galleria mellonella* model. *Galleria mellonella* larvae were infected with 1×10^4 CFU/mL of *P. aeruginosa* and were incubated at 37 °C. Dead larvae were scored 12–120 h after inoculation. (A) Uninoculated larvae (□) and larvae inoculated with phosphate-buffered saline (PBS) (○) (negative controls); (B) reference strain *P. aeruginosa* ATCC 27853; (C) strain 1030; (D) strain 1136; (E) strain 1166; (F) strain 1209; (G) strain 1303; (H) strain 1410; (I) strain 1680; and (J) strain 4285. Two isolates harbouring *bla*$_{\text{SPM}}$ (strains 1166 and 4285) killed 100% of the larvae at 18–24 h.

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Competing interests

None declared.

Ethical approval

This study was approved by the Ethical Committee of Hospital das Clínicas of the University of São Paulo (São Paulo, Brazil).

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