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Genome Note

Polymyxin-resistant *Pseudomonas aeruginosa* assigned as ST245: First report in an intensive care unit in São Paulo, Brazil

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ABSTRACT

Objectives: *Pseudomonas aeruginosa* is a Gram-negative bacterium that causes severe infections, especially in hospitalised and immunocompromised patients. Polymyxins are the last therapeutic option to treat infections caused by this micro-organism. Here we describe a polymyxin-resistant *P. aeruginosa* assigned as sequence type (ST) 245 for the first time in Brazil.
Methods: Antimicrobial susceptibility testing of the isolate was performed. In addition, whole-genome sequencing was performed and its virulence and resistance genes were analysed.
Results: The *P. aeruginosa* ST245 isolate was identified for the first time in Brazil in a patient with ventilator-associated pneumonia hospitalised at Hospital das Clínicas, São Paulo. Analysis of the genome showed the presence of several resistance and virulence genes. Mutations in β-lactam resistance genes were found in β-lactamases, outer membrane proteins, efflux pump and penicillin-binding proteins. Polymorphisms related to pathways leading to polymyxin resistance are also present, such as lipid A or keto-deoxyoctulosonate modification with aminoarabinose as well as activation of lipopolysaccharide (LPS).
Conclusion: Such findings may represent an alert for the spread of an unusual profile in the country.

Currently, resistance to carbapenems is increasing worldwide among Gram-negative micro-organisms, including *Pseudomonas aeruginosa*. In this scenario, polymyxins are the antimicrobial agents of choice. Although there are increasing reports of resistance to polymyxins in *P. aeruginosa*, this pattern of resistance is still rare in Brazil [1–3].

The present study was carried out on an isolate from a 62-year-old kidney-transplanted male patient admitted in 2015 to Hospital das Clínicas (São Paulo, Brazil) with community-acquired pneumonia. Initial empirical treatment was azithromycin, piperacillin/tazobactam and trimethoprim/sulfamethoxazole but, due to worsening of his clinical condition, other antimicrobials/antifungals were gradually added or substituted; amikacin, vancomycin, amphotericin B, fluconazole, micafungin and colistin were also administered empirically as the cultures were all negative until that point. After 13 days of colistin therapy the patient developed ventilator-associated pneumonia due to *P. aeruginosa*, isolated from blood, that was resistant to fluoroquinolones, carbapenems and polymyxins according to VITEK®2 (bioMérieux, Paris, France). Treatment with fosfomycin and meropenem was then started but the patient died less than 72 h later.

Minimum inhibitory concentrations (MICs) were determined for ciprofloxacin, levofloxacin, cefotaxime, ceftazidime, cefepime, ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanic acid, aztreonam, imipenem, meropenem, doripenem, polymyxin B and colistin by the microdilution method using GNX3F Sensititre™ plates (Thermo Fisher Scientific, Waltham, MA), for fosfomycin (Sigma-Aldrich, St Louis, MO) using the agar dilution method, and for ceftazidime/avibactam using disk diffusion (BD BBL, Franklin Lakes, NJ) and broth microdilution (GlaxoSmithKline, Verona, Italy). All results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI document M100, 28th ed).

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Genotypic characterisation was done by whole-genome sequencing (WGS). Total DNA was extracted using an illustra™ bacteria genomicPrep Mini Spin Kit (GE Healthcare Life Sciences, Amersham, UK). DNA quality was verified using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The DNA concentration (25–100 ng/mL) was determined using a Qubit® fluorometer (Thermo Fisher Scientific) and DNA integrity was ascertained using 1.5% Ultrapure Agarose gel (Invitrogen™; Life Technologies). Sequencing was performed using an Illumina MiSeq system (Illumina Inc. San Diego, CA), with coverage of ≥30×, using a paired-end technique. Paired reading segments with >500 bp were processed. The quality of the files was evaluated by FastQC v.0.11.3 (Babraham Institute, Cambridge, UK) and Trimmomatic v.0:33 (AG Usadel, Aachen, Germany). De novo genome assembly was performed using Velvet assembler v.1.2.10. The genome was annotated with Prokka v.1:11, and single nucleotide polymorphisms were identified using Burrows–Wheeler Aligner (BWA), SAMtools and Genome Analysis Toolkit (Broad Institute, Cambridge, MA). The sequence type (ST) of the isolate was identified using the MLST (Multi-Locus Sequence Typing) tool (https://cge.cbs.dtu.dk/services/MLST/).

The search for virulence and resistance genes was performed using the Virulence Factors Database (http://www.mgc.ac.cn/VFs), ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) and Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). In addition, the data were analysed with Artemis 16.0.0.

The isolate was susceptible to ceftazidime/avibactam using two methods (MIC = 2/0.5 μg/mL and zone diameter = 27 mm), had intermediate susceptibility to ceftazidime (MIC = 16 μg/mL), cefepime (MIC = 16 μg/mL), aztreonam (MIC = 16 μg/mL) and doripenem (MIC = 4 μg/mL) and was resistant to ciprofloxacin (MIC > 2 μg/mL), levofloxacin (MIC > 8 μg/mL), piperacillin/tazobactam (MIC > 64/4 μg/mL), ticarcillin/clavulanic acid (MIC = 128/2 μg/mL), imipenem (MIC > 8 μg/mL), meropenem (MIC = 8 μg/mL) and polymyxins (MIC > 4 μg/mL). The fosfomycin MIC was 64 μg/mL (no breakpoints available).

Regarding WGS, 730 contigs were assembled (6 823 948 bp), with a G + C content of 66.11%, and 6408 protein coding sequences, 71 tRNAs and 6 rRNAs were predicted. Virulence analysis of the isolate identified the presence of genes related to adherence [flagellum, lipopolysaccharide (LPS) and type IV pili], antiphagocytosis (alginate), iron uptake (pyochelin and pyoverdine), Xcp secretion system and quorum sensing as well as the production of biosurfactant (rhamnolipid), pigments (pyocyanin), toxins and proteases. In WGS, substitution and insertion mutations in some outer membrane proteins, efflux pumps, penicillin-binding proteins and β-lactamases were found. The São Paulo metallo-β-lactamase gene (bla_{SPM}), the most frequent carbapenemase in Brazilian *P. aeruginosa* strains, was not found by WGS (or PCR previously performed). Regarding polymyxin resistance, it was possible to identify substitution mutations in genes encoding the mechanisms of lipid A or keto-deoxyoctulosonate (Kdo) modification with aminoarabinose as well

Table 1
Description of β-lactam and polymyxin resistance genes by whole-genome sequencing of a polymyxin-resistant *Pseudomonas aeruginosa* ST245 isolate in Brazil.

Resistance class/gene	Polymorphism(s)[a]	Product/function
β-Lactam		
bla_{PAO}	R79Q; T105A	β-Lactamase
bla_{OXA-50}	D109E; R167H	Oxacillinase
oprD	M1E; T103S; K115T; F170L	Outer membrane porin
oprE	L106_D107insGL; S213G; T377G; V378L; V380L; I388M; S391K; K394N; T397S; A398G; S410A; S434T; A438V; A439S; S441_S442insG; S443T; N444A; N445T	
mexM	I47N; R160L; L230P; D325A; T381A; R384Q	Pump/efflux system
mexN	V480L	
mexV	A229G; Q321R	
mexW	Q511R	
mexX	V92L; K329Q; L331V; W358R	
mexY	T543A; Q840E	
oprM	A261T	
mexC	S330A	
mexD	E257Q; S845A	
oprJ	M69V; Q267R	
mexE	P397Q	
mexI	A68T; A782E	
opmD	S112N; A351T	
mexQ	D9A	
opmE	S175T	
triA	G12V	
triB	P24Q	
triC	V742M	
opmH	A75G; V77I; T80S; F84L; R87P; P88A; N94S; Q96H; T101S; K119R; R126K; Y310D; D312E; S315T	
mexT	F172I; I341F; D345E	
ponA/PBP1a	E53K; A615_D616insP; S632I	Penicillin-binding protein
mrcB/PBP1b	S25G	
Polymyxins		
arnB	V302A	Modification of lipid A or Kdo with aminoarabinose
arnT	G156R; A267S; R502Q; I509V	
pmrA	L71R	Activation of LPS; operon mutated in TCS
pmrB	D47G; Y345H	
cprS	R301H	Acquisition/adaptation of polymyxin resistance through LPS modification with aminoarabinose
parS	H398R	

Kdo, keto-deoxyoctulosonate; LPS, lipopolysaccharide; TCS, two-component system.
The reference sequence was *P. aeruginosa* PAO1 (GenBank no. **AE004091**).
[a] These polymorphisms have previously been associated with resistant phenotypes.

as activation of LPS (mutated operon in two-component system) (Table 1).

In Brazil, *P. aeruginosa* ST277 is the most common ST. However, the *P. aeruginosa* strain in this case was assigned as ST245, described for the first time in Brazil, which is phylogenetically distant from ST277 as they differ by four alleles; such a ST has rarely been described in the world [1,2,4,5]. The first report of this ST was in 2005 in Poland [4]. Since then it has been reported in Korea [1,2] and the UK [5]. Unfortunately, we do not know whether the patient had a history of any recent foreign travel.

This report of a *P. aeruginosa* ST245 isolate is important not only for Brazil but also for other countries as this ST is rare worldwide and has already presented itself in Brazil with a rare phenotype (more resistant than the isolates previously described and resistant to polymyxins) and with more virulence genes described than the other isolates [1,2,4,5], related to adhesion, LPS, type IV pili, rhamnolipid and alginate production, iron uptake, Xcp secretion system, and pyocyanin, toxin and protease production.

In conclusion, this study presents a concern for dissemination of a micro-organism with a new ST in the country with a rare phenotype (i.e. polymyxin-resistant). It was possible to identify mutations in genes encoding lipid A or Kdo modification with aminoarabinose as well as activation of LPS, mechanisms leading to polymyxin resistance in *P. aeruginosa*.

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Competing interests

None declared.

Ethical approval

Not required.

Nucleotide sequence accession no.

The Whole Genome Shotgun project of this *P. aeruginosa* strain has been deposited at DDBJ/EMBL/GenBank under the accession no. **QHLV00000000**. The version described in this paper is the first version: QHLV00000000.

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