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Short Communication

Inactivation of the *oprD* porin gene by a novel insertion sequence IS*Pa195* associated with large deletion in a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate

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ABSTRACT

Objectives: Alteration of the porin-encoding gene *oprD* by insertion sequences (ISs) is one mechanism conferring carbapenem resistance in *Pseudomonas aeruginosa.* Here we describe a carbapenem-resistant clinical *P. aeruginosa* isolate 36-989 harbouring a novel IS (IS*Pa195*) in *oprD*.
Methods: Minimum inhibitory concentrations (MICs) of antimicrobial agents were determined by the broth microdilution method. Carbapenemase activity was assessed using a MALDI-TOF/MS-based assay of meropenem hydrolysis. Efflux-dependent carbapenem resistance was evaluated using an assay with carbonyl cyanide 3-chlorophenylhydrazone (CCCP). The *oprD* gene and IS sequence were analysed by the Sanger method. Whole-genome sequencing was performed on an Illumina HiSeq 2500 platform.
Results: Antimicrobial susceptibility testing demonstrated that *P. aeruginosa* 36-989 was resistant to imipenem (MIC = 32 mg/L) and meropenem (MIC = 16 mg/L). No carbapenemase activity was detected, however an efflux-mediated component of carbapenem resistance was revealed. A new IS element (IS*Pa195*) was found in the *oprD* gene of *P. aeruginosa* 36-989. IS*Pa195* was 1190 bp in length, belonging to the IS*3* family, and contained two open reading frames that overlapped through a ribosomal slippage to translate the full-size transposase enzyme. There was an IS-associated 284-bp deletion in the *oprD* gene; no direct repeats at flanking regions of the IS were detected.
Conclusion: The absence of direct repeats at flanking regions in combination with the IS-associated deletion distinguished IS*Pa195* from other ISs previously detected in *oprD*. Carbapenem resistance in *P. aeruginosa* 36-989 was conferred by a combination of *oprD* alteration and carbapenem efflux.

1. Introduction

Pseudomonas aeruginosa is an important opportunistic pathogen demonstrating a worrying trend of increasing resistance to many antimicrobial agents, including carbapenems [1]. Inactivation of porin channels, primarily the OprD porin, is one mechanism conferring carbapenem resistance in *P. aeruginosa* [2]. Alterations in the *oprD* gene sequence are common among carbapenem-resistant isolates and include point mutations as well as insertion of mobile genetic elements such as insertion sequences (ISs) [3,4]. ISs are short DNA segments encoding only the transposase enzyme and containing imperfect terminal inverted repeats at both ends of the sequence. They also form flanking direct repeats of the target upon insertion [5]. Here we report a novel IS disrupting the structure of the *oprD* gene in a carbapenem-resistant *P. aeruginosa* clinical isolate.

2. Materials and methods

The carbapenem-resistant *P. aeruginosa* isolate described here was isolated in 2013 from the nasopharynx of a 6-month-old child treated in an intensive care unit at a paediatric hospital in Moscow, Russia. Species identification of the isolate was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using a microflex® MALDI Biotyper® (Bruker Daltonik GmbH, Bremen, Germany) and was confirmed by 16S rRNA gene sequencing. Minimum inhibitory

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concentrations (MICs) of antimicrobial agents were determined by the broth microdilution method and the results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [6,7]. Carbapenemase activity was assessed using a MALDI-TOF/MS-based assay of meropenem hydrolysis as described by Hrabak et al. [8]. Efflux-dependent carbapenem resistance was evaluated using an assay with carbonyl cyanide 3-chlorophenylhydrazone (CCCP) [9]. A $\geq$4-fold reduction in the meropenem MIC in the presence of 2.5 mg/L CCCP was considered as relevant efflux activity.

The *oprD* gene was sequenced using primers described by Ocampo-Sosa et al. [10]. The *oprD* sequence of *P. aeruginosa* ATCC 27853 (GenBank accession no. KF649209.1) was used as the reference. Additional primers were designed (ISPa195_1F, 5′-AAGTCATCAATGACGTTGAGCAG-3′; ISPa195_2F, 5′-TCATGCATAAAGTCCATTGACCAA-3′) for sequencing of IS*Pa195*. A paired-end library was prepared from genomic DNA of the isolate using a Nextera^{TM} DNA Sample Prep Kit (Illumina Inc., San Diego, CA), followed by whole-genome sequencing (WGS) on the Illumina HiSeq 2500 platform (Illumina Inc.) with a read length of 250 bp. The bacterial genome was assembled using SPAdes software v.3.11.1 [11]. Prokka software, BLAST and ResFinder were used for analysis of WGS data. The sequence type (ST) of the isolate was determined according to the standard *P. aeruginosa* multilocus sequence typing (MLST) scheme [https://pubmlst.org/paeruginosa/; accessed 15 October 2018] using WGS data.

3. Results and discussion

While investigating carbapenem resistance mechanisms among *P. aeruginosa* paediatric clinical isolates, carbapenem-resistant isolate 36-989 of ST313 was detected carrying a new IS element in the *oprD* gene at nucleotide position 195 (Fig. 1). According to the ISfinder database, this 1190-bp IS belonged to the IS*3* family and was designated IS*Pa195*. The sequence of IS*Pa195* has been deposited in the GenBank database under accession no. MF770250.

Examination of antimicrobial susceptibility demonstrated that *P. aeruginosa* 36-989 was resistant to imipenem (MIC = 32 mg/L), meropenem (MIC = 16 mg/L), ceftazidime (MIC = 256 mg/L) and cefepime (MIC = 32 mg/L) but remained susceptible to amikacin (MIC = 8 mg/L), gentamicin (MIC = 4 mg/L), netilmicin (MIC = 4 mg/L), tobramycin (MIC = 4 mg/L), ciprofloxacin (MIC = 0.25 mg/L) and colistin (MIC = 1 mg/L). A number of antimicrobial resistance genes were detected in the isolate including *aph(3′)-IIb*, *bla*$_{\text{AmpC}}$, *bla*$_{\text{OXA-50}}$ and *fosA*, but no carbapenemase-encoding genes were found. A lack of detectable carbapenemase activity as evidenced by the absence of meropenem hydrolysis corroborated this finding.

Usually in *P. aeruginosa*, inactivation of *oprD* in itself confers a moderate level of carbapenem resistance [3,10]. However, IS*Pa195*-positive *P. aeruginosa* 36-989 displayed relatively high imipenem and meropenem MICs (32 mg/L and 16 mg/L, respectively). Examination of efflux activity using the ionophore CCCP demonstrated a 4-fold reduction in the meropenem MIC, indicating the contribution of an efflux-mediated mechanism to carbapenem resistance in isolate 36-989.

Sequence analysis revealed two open reading frames (ORFs) in IS*Pa195* that overlapped through a ribosomal slippage to translate the full-size transposase enzyme that resembled the IS*As22* transposase from *Aeromonas salmonicida* (73.2% identity) [12]. IS*Pa195* contained terminal imperfect inverted repeats (Fig. 1); no direct repeats at flanking regions of the IS were detected. In addition, a 284-bp deletion in the *oprD* gene was found in proximity to the left inverted repeat. WGS analysis of *P. aeruginosa* 36-989 revealed that all contigs containing the sequence of IS*Pa195* mapped to a single locus representing the *oprD* gene. Thus, the examined isolate harboured one copy of IS*Pa195* in the genome.

A number of IS elements that disrupt functioning of *oprD* and mediate carbapenem resistance have been reported [13]. Overlapping ORFs are typical to IS3 family representatives, and such ISs have been previously described in *oprD* [14,15]. However, the absence of direct repeats in the flanking regions of IS*Pa195* was an unusual feature for IS elements disrupting *oprD*. Another remarkable finding was a 284-bp *oprD* deletion associated with IS*Pa195*. ISs are known to promote rearrangements in the bacterial genome mediating large deletions and inversions, as has been shown for the *Escherichia coli* genome [16]. In that report, IS-associated deletions related to elements of IS*1*, IS*2* and IS*5* families. In the *P. aeruginosa* genome, inversions were associated with IS*3* and IS*5* family elements [17]. ISs combined with a deletion in the *oprD* gene are rarely reported. For instance, IS*Pa133* (GenBank accession no. FJ387166) is associated with a 762-bp deletion in the *oprD* sequence. Interestingly, in the original publication on IS*Pa133* the deletion was not described [18], and we revealed it analysing the raw data of the IS*Pa133*-disrupted *oprD* sequence available in the GenBank database. In contrast to IS*Pa195* described in the present report, IS*Pa133* harbours direct repeats in the flanking regions [19]. Thus, the presence of overlapping ORFs harbouring no flanking direct repeats in association with a large deletion in *oprD* distinguishes IS*Pa195* from other ISs previously detected in *oprD*.

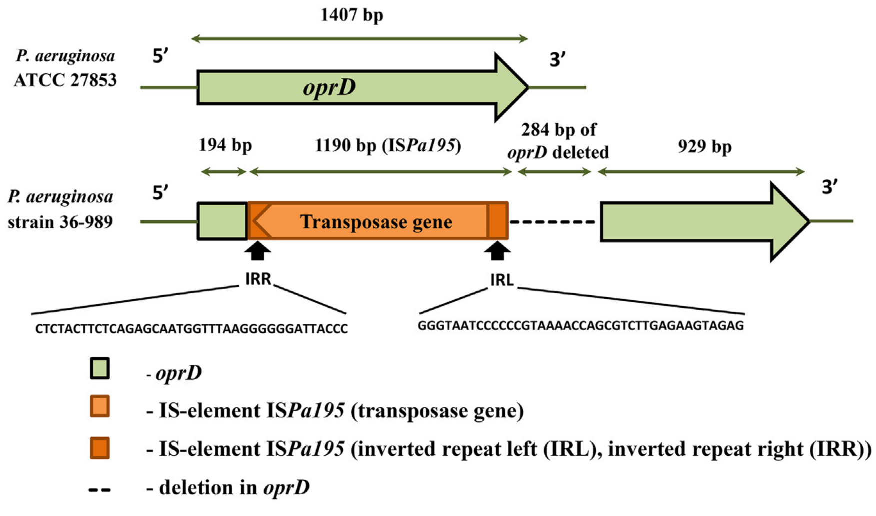

Fig. 1. The *oprD* gene structure of the IS*Pa195*-carrying *Pseudomonas aeruginosa* isolate in comparison with *P. aeruginosa* ATCC 27853.

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Competing interests

None declared.

Ethical approval

Not required.

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