LETTER TO THE EDITOR

First detection of insertion sequence element IS*Pa*1328 in the *oprD* porin gene of an imipenem-resistant *Pseudomonas aeruginosa* isolate from an idiopathic pulmonary fibrosis patient in Marseille, France

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Abstract

We report here the first case of a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate harboring the insertion sequence (IS) element IS*Pa*1328 in the *oprD* gene in an idiopathic pulmonary fibrosis patient in France previously treated with imipenem.

Keywords: Carbapenem resistance, insertion sequence, *oprD* gene, *Pseudomonas aeruginosa*, pulmonary fibrosis
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Carbapenems are frequently used as last drug choice for the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections [1], but the emergence of carbapenem resistance is increasingly reported. Carbapenem resistance in *P. aeruginosa* may be due to low permeability, multidrug efflux pumps or the production of class B β-lactamases (metallo-β-lactamases, MBLs) [2]. However, the main mechanism of carbapenem resistance in *P. aeruginosa* remains the loss or the alteration of the outer membrane porin (oprD) through mutations, deletions or insertions in the *oprD* gene [3]. Clinical isolate of *P. aeruginosa* exhibiting high-level resistance to carbapenems was isolated from a sputum sample of a 69-year-old man with idiopathic pulmonary fibrosis treated by the association of either ciprofloxacin or ceftazidime or tazocillin with tobramycin or colimycin and later by imipenem. Strain (PA 461) was cultured on trypticase soy agar plate at 37°C for 24 hours, and identification was confirmed by matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Microflex; Bruker Daltonics, Bremen, Germany) with FlexControl software, as previously described [4]. Antibiotic susceptibility testing performed on Mueller-Hinton agar by standard disk diffusion method as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/), showed that the isolate was resistant to almost all antibiotics, including β-lactams, as well as fluoroquinolones and rifampicin, but remained susceptible to aminoglycosides and colistin. Phenotypic detection of carbapenemase by modified Hodge test and imipenem–ethylene diamine tetra-acetic acid test, performed as previously described [5], were negative. The presence of MBLs genes, investigated by PCR as previously described [6], confirmed that this isolate did not produce a carbapenemase. Amplification of the *oprD* gene using previously described primers [3,6] resulted in PCR product of 2.6 kb instead of 1332 bp. Sequence analysis of the 2668 bp product revealed insertion of a sequence of 1336 bp at position 610 in *oprD*. Within the sequence, an open reading frame of 1227 bp was found and corresponded to IS*Pa*1328 from *P. aeruginosa* (GenBank accession AY539833). This insertion sequence (IS) was bordered by two terminal imperfect repeats and flanked on both sides by direct repeat sequences of 7 bp (CCAAGAG) (Fig. 1). Multilocus sequence typing, performed as previously described (http://pubmlst.org/paeruginosa), showed a novel sequence type (ST1797) and thus a novel clone of *P. aeruginosa*.

Random transposition of IS elements is known to be a form of adaptation of bacteria to environmental changes. To date, the presence of IS elements disrupting the *oprD* gene has been reported in South Africa (IS*Pa*26) [7], Spain (IS*Pa*133) [8], China (IS*Pa*1328, IS*Pre2*) [9], the United States (IS*Pa*8 [1] and IS*Pa*1328 [10]) and France (IS*Pa*46) [6]. The occurrence of multidrug-

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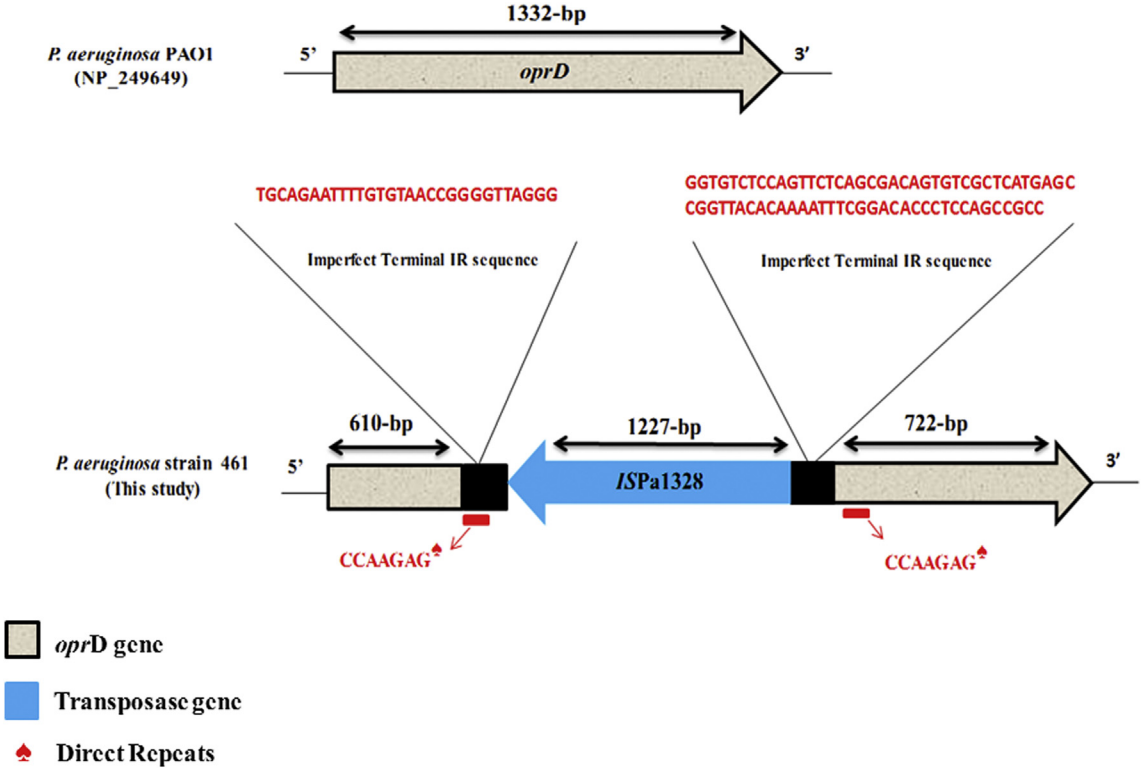

FIG. 1. Schematic representation of *oprD* gene of PA461 disrupted by IS*Pa*1328 compared to reference strain PAO1.

resistant bacteria is associated with the extensive use of broad-spectrum antimicrobial drugs in treating humans. This could explain the emergence of imipenem-resistant *P. aeruginosa* in this patient, the result of direct and specific antibiotic selective pressure created by the use of imipenem.

In conclusion, we report for the first time in France the emergence of IS*Pa*1328 in a patient with idiopathic pulmonary fibrosis associated with carbapenem resistance.

Conflict of interest

None declared.

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