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Characterization of carbapenem resistance mechanisms and integrons in *Pseudomonas aeruginosa* strains from blood samples in a French hospital

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Metallo-β-lactamases (MBLs), porin OprD, integrons, virulence factors and the clonal relationships were characterized in imipenem-resistant *Pseudomonas aeruginosa* (IRPA) isolates. Fifty-six IRPA strains were recovered from blood samples of different patients at a Toulouse teaching hospital from 2011 to 2013. Susceptibility testing of 14 antibiotics was performed by the disc diffusion method. Detection and characterization of MBLs, the *oprD* gene and integrons were studied by PCR and sequencing. Thirteen genes involved in the virulence of *P. aeruginosa* were analysed. Molecular typing of IRPA strains was performed by PFGE and multilocus sequence typing. In this study, 61 % of the IRPA isolates showed a multi-resistance phenotype. The MBL phenotype, detected in three isolates (5.4 %), was linked to the bla_{VIM-2} gene. The *oprD* gene was amplified in 55 (98.2 %) IRPA strains, and variations were observed in 54 of them. Insertion sequences (IS) truncating *oprD* were detected in eight IRPA strains, with the novel IS*Pa56* identified in two strains. Class 1 integrons were detected in 24 (42.9 %) IRPA strains. The bla_{VIM-2} gene was found inside the class 1 integron arrangements. The new integrons In1054 (*intI1-aacA56-qacEΔ1-sul1*) and In1160 (*intI1-aacA4-aacC1d*-IS*Kpn4*-*gcuE-qacEΔ1-sul1*) have been described for the first time, to the best of our knowledge, in this study. A high clonal diversity was found in our strains. Among the variety of sequence types (STs) found, ST175, ST233, ST235, ST244 and ST654 were noteworthy as epidemic clones. In conclusion, 5.4 % of IRPA strains showed an MBL phenotype linked to the bla_{VIM-2} gene. The identified *oprD* high polymorphism could be implicated in the variable resistance to carbapenems in IRPA strains. The dissemination of high-risk clones is a cause of concern.

INTRODUCTION

Pseudomonas aeruginosa is a common opportunistic and nosocomial pathogen that causes severe infections with a high mortality rate (Poole, 2011). Intensive clinical use of carbapenems has caused an increase in carbapenem resistance by acquisition of different mechanisms, such as hyperproduction of chromosomal AmpC β-lactamase, overexpression of efflux systems, carbapenemase production, and alterations or loss of the porin OprD (Lister *et al.*, 2009). The *P. aeruginosa* OprD protein is a substrate-specific porin that facilitates the diffusion of basic amino acids, small peptides and carbapenems into the cell. Alterations or loss of OprD significantly decrease the susceptibility to available carbapenems in *P. aeruginosa* (Lister *et al.*, 2009). Metallo-β-lactamases (MBLs), particularly VIM and IMP types, are among the most widespread and globally reported carbapenemases (Cornaglia *et al.*, 2011). MBL genes are often located in mobile or

Abbreviations: CC, clonal complex; IRPA, imipenem-resistant *Pseudomonas aeruginosa*; IS, insertion sequence; MBL, metallo-β-lactamase; MLST, multilocus sequence typing; ST, sequence type.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are KR258747 (IS*Pa56*), KM201605 (integron In1054) and KR184824 (integron In1160).

mobilizable genetic elements (plasmids, insertion sequences and integrons), which can contribute to the acquisition of new resistance mechanisms, and may increase the plasticity of the *P. aeruginosa* genome and improve the environmental adaptation of *P. aeruginosa.* MBLs are also commonly associated with multidrug-resistant epidemic high-risk clones, such as sequence types (STs) ST111, ST235 and ST175, which have become disseminated in hospitals worldwide (Woodford *et al.*, 2011; Cabot *et al.*, 2012).

In contrast, the success of *P. aeruginosa* in infecting the host cell and evading the host immune system is due to a broad arsenal of pathogenicity factors such as biofilm production and secretion of adhesins, toxins, proteases and pigments. The type III secretion system is used to inject toxic effector proteins into the cytoplasm of eukaryotic cells, thus promoting severe illness (Shaver & Hauser, 2004). Four effector proteins of *P. aeruginosa* (ExoU, ExoS, ExoT and ExoY) have been described. ExoU is a potent cytotoxin with phospholipase A2 activity. ExoS and ExoT are enzymes that have 76% amino acid identity and encode both GTPase-activating protein and ADP-ribosyltransferase activities. ExoY is an adenylate cyclase (Hauser, 2009). Moreover, the transcription of genes encoding several virulence factors of *P. aeruginosa* is controlled by quorum-sensing systems, mainly *las* and *rhl* (Pesci *et al.*, 1997). These systems control the expression of elastases (LasB and LasA), alkaline protease (AprA), exotoxin A (ToxA), autoinductor synthase (LasI), rhamnosyltransferase (RhlAB) and pyocyanin, among others (Cabrol *et al.*, 2003).

The objective of this study was to characterize the MBLs, porin OprD, integrons and virulence factors in imipenem-resistant *P. aeruginosa* (IRPA) strains isolated from clinical blood cultures.

METHODS

Bacterial isolates. During 2011–2013, the bacteriology laboratory of a 2850-bed teaching hospital in Toulouse (France) recovered 542 *P. aeruginosa* isolates from 311 patients from approximately 150 000 received blood cultures (0.36% of blood cultures). The mean age of patients was 44 years (range 0–99 years) and 191 were males (61.4%).

A total of 434 *P. aeruginosa* isolates were analysed, comprising one isolate per patient and also those isolates from the same patient that showed different resistance phenotypes. The hospital services from which they were recovered were resuscitation (18.2%), oncology (15.7%), haematology (9.4%), emergency (8.7%), transplant units (6.2%) and others (41.8%, which include a burns unit, different intensive care units, and surgical and medical wards).

Among the 434 isolates, 64 (14.7%) were IRPA, and 56 of these from 48 patients (mean age 32 years, 70.8% males) were further characterized in this study (eight isolates could not be recovered).

Susceptibility testing. Susceptibility testing against 14 antipseudomonal antibiotics (ticarcillin, ticarcillin-clavulanate, piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, gentamicin, tobramycin, amikacin, ciprofloxacin and colistin) was performed using the disc diffusion method (Oxoid). The zone diameters were interpreted according to the criteria published by Le comité de l'antibiogramme de la société française de microbiologie (CA-SFM, 2011). The MBL phenotype was determined by a double disc diffusion method (A disc of 0.5 M EDTA (pH 8) was located in the middle between a disc of imipenem and another of meropenem) (Lee *et al.*, 2001).

AmpC and efflux pump overexpression were determined by phenotypic methods. AmpC hyperproduction was analysed among the ceftazidime-resistant IRPA strains using ceftazidime discs and plates in the presence or absence of cloxacillin (250 mg l^{-1}). Efflux pump overexpression was studied using imipenem, meropenem and ciprofloxacin discs and plates in the presence or absence of the inhibitor Phe-Arg-β-naphthylamide (PAβN, 40 mg l^{-1}). Isolates were defined as AmpC or efflux pump overproducers when there was more than a 5 mm difference between the antibiotic inhibition zone in the presence or absence of cloxacillin or PAβN, respectively.

Molecular typing. The clonal relationship among IRPA isolates was evaluated by PFGE using *Spe*I for genomic DNA restriction, as described previously (Rojo-Bezares *et al.*, 2011). PFGE patterns were analysed and interpreted using BioNumerics software 2.0 (Applied Maths) with a Dice similarity coefficient with a 1.0% band position tolerance, and as previously recommended by Tenover *et al.* (1995).

Multilocus sequence typing (MLST) was performed as recommended (Curran *et al.*, 2004). Internal fragments of the following seven housekeeping genes were amplified and subsequently sequenced: *acsA* (acetyl coenzyme A synthetase), *aroE* (shikimate dehydrogenase), *guaA* (GMP synthase), *mutL* (DNA mismatch repair protein), *nuoD* (NADH dehydrogenase I chain C, D), *ppsA* (phosphoenolpyruvate synthase) and *trpE* (anthralite synthetase component I). The nucleotide sequences of alleles were compared with those of the MLST database (http://pubmlst.org/paeruginosa/) to obtain the specific ST. The clonal complex (CC) was defined using the software PHYLOViZ (Francisco *et al.*, 2012).

Characterization of MBLs, porin OprD and integron structures. The presence of MBL-encoding genes was carried out by multiplex PCR and subsequent sequencing (Ellington *et al.*, 2007).

Mutations in the *oprD* gene were analysed in all IRPA isolates by PCR, sequencing and comparison with the sequence of the *P. aeruginosa* PAO1 reference strain (GenBank accession no. AE004091) (Wolter *et al.*, 2004; Gutiérrez *et al.*, 2007).

The presence of genes encoding type 1 and 2 integrases, as well as the 3′ conserved segment of class 1 integrons ($qacE\Delta1 + sul1$) was studied by PCR. The characterization of class 1 integron variable regions was performed by PCR mapping and sequencing (Sáenz *et al.*, 2004).

Detection of virulence factors. Molecular characterization of 13 genes involved in virulence (*exoU*, *exoS*, *exoY*, *exoT*, *exoA*, *lasA*, *lasB*, *aprA*, *rhlAB*, *rhlI*, *rhlR*, *lasI* and *lasR*) was performed by PCR as described previously (Petit *et al.*, 2013).

RESULTS AND DISCUSSION

Antibiotic susceptibility, MBL presence, and AmpC and efflux pump overexpression

The percentages of resistance to different antibiotics in the 56 IRPA isolates are shown in Fig. 1. Sixty-one per cent of the IRPA isolates from blood samples exhibited a multidrug resistance phenotype (according to the criteria of Magiorakos *et al.*, 2012). The global data for *P. aeruginosa* from the European Centre for Disease Prevention and Control

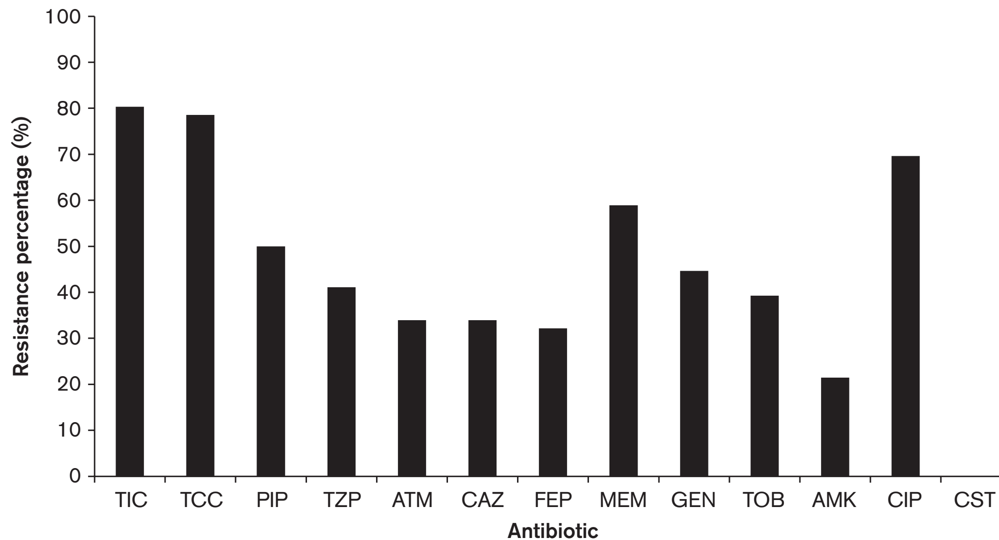

Fig. 1. Percentages of resistance to different antibiotics in the 56 IRPA isolates. TIC, ticarcillin; TCC, ticarcillin-clavulanate; PIP, piperacillin; TZP, piperacillin-tazobactam; ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; MEM, meropenem; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CIP, ciprofloxacin; CST, colistin.

(http://ecdc.europa.eu/) show a relatively low level of IRPA strains in France (17.1% in 2013), similar to our data (14.7%).

The MBL phenotype was detected in only three isolates (5.4%), in which the $bla_{\text{VIM-2}}$ gene was found. As other studies have reported previously, the MBL rates are lower in France than in other countries (Franco *et al.*, 2010; Fournier *et al.*, 2013; Rojo-Bezares *et al.*, 2014; Wright *et al.*, 2015).

Almost one-third of our 18 ceftazidime-resistant IRPA isolates exhibited AmpC hyperproduction (28%). Regarding efflux pump overexpression, PAβN showed an effect on imipenem, meropenem and ciprofloxacin inhibition zones in 34, 50 and 93% of the 56 IRPA isolates, respectively.

Characterization of porin OprD

The *oprD* gene was amplified in 55 IRPA (98.2%) isolates with the following amplicon sizes: 750 bp (three isolates), 1500 bp (43 isolates) and $\geq$3000 bp (nine isolates) (Table 1). Variations in the *oprD* gene (presence of insertions, deletions and/or premature stop codons) were detected in 54/55 IRPA isolates, while the predicted porin OprD of one isolate (Ps441) was identical to porin OprD of *P. aeruginosa* PAO1. In a recent study in *P. aeruginosa* from intensive care units, no mutations in porin OprD were described, including one MBL-positive *P. aeruginosa* (Fournier *et al.*, 2013). Thirty-one isolates (55.4%) harboured amino acid changes and insertions and/or deletions; another isolate underwent only deletions in the *oprD* gene. However, 14 isolates only presented amino acid changes with or without premature terminations.

The presence of insertion sequences (ISs) truncating the *oprD* gene was detected in eight IRPA strains. IS*Pa26* was detected truncating *oprD* in two clonally related strains (Ps402 and Ps410) in which a novel IS, named IS*Pa56*, was also found upstream of the *oprD* gene. The IS*Pa56* element belongs to the IS*3* family IS*2* group, with 72% amino acid similarity to IS*Rso10*. IS*Pa56* is composed of ORFs (ORFA and ORFB), which can join to form the third ORF, which is the putative ORFAB transposase reconstructed *in silico*, by a possible −1 frameshift. IS*Pa26*, IS*Pa45* and IS*Pa1328*, found truncating the *oprD* gene in our strains, as well as other ISs (IS*Pa8*, IS*Pa27*, IS*Pa45*, IS*Pa46*, IS*Pa47*, IS*Pa133*, IS*Pa1328*, IS*Pa1635*, IS*Pre2*, IS*Pst12* and IS*Ppu21*, among others) described in a large number of works in recent years, have led to an increase in carbapenem resistance as described previously by other authors (Wolter *et al.*, 2004; Evans & Segal, 2007; Gutiérrez *et al.*, 2007; Wang *et al.*, 2010; Rojo-Bezares *et al.*, 2014).

Characterization of integron structure

Class 1 integrons were detected in 24 IRPA isolates (42.9%), whereas no class 2 integrons were found. Twenty-one non-MBL-producing isolates contained class 1 integrons, and the majority of them harboured genes that conferred resistance to aminoglycosides regulated by PcH1 promoters (Table 2). It was remarkable that one strain harboured an empty class 1 integron with a *sul1* gene but without *qacE*Δ*1* at the 3′ conserved segment (Table 2). This atypical and infrequent structure was been reported previously (Rosser & Young, 1999).

The $bla_{\text{VIM-2}}$ gene was found inside the class 1 integron arrangements of the three MBL-producing isolates.

Table 1. Molecular characterization of porin OprD in the IRPA strains

Strain(s)*	Amplicon PCR size (bp)	OprD size (aa)	Amino acid changes in OprD sequence†	Insertion/deletion	Disruption of OprD by ISs
Ps441	1500	443	None‡		
Ps402 (Ps410)	≥3000	–			IS*Pa56*§ and IS*Pa26*
Ps403, Ps404 (Ps406), Ps447, Ps428	≥3000	–			IS*Pa1328*
Ps439	≥3000	–			IS*Pa45*
Ps432	1500	109		Deletion of 1 bp (A) at nt 294‖	
Ps396, Ps413 Ps414, Ps425, Ps431, Ps438	1500	276	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, W277STOP		
Ps407¶ Ps411, Ps417, Ps426, Ps446, Ps448	1500	441	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L	LOOP L7-short	
Ps420	1500	414	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L, W417STOP	LOOP L7-short	
Ps454	1500	421	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L, Q424STOP	LOOP L7-short	
Ps401, Ps416	1500	348	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, S349STOP		
Ps423	1500	232	T103S, K115T, F170L, E185Q, V189T, Y233STOP		
Ps397 (Ps398)	1500	276	T103S, K115T, F170L, E185Q, P186G, V189T, W277STOP		
Ps400	1500	354	T103S, K115T, F170L, E185Q, P186G, V189T, R310E, A315G, G254A, L255STOP		
Ps451	≥3000	205	T103S, K115T, F170L, E185Q, P186G, V189T, K205E, S206G, A207V, D208R, F209N, I210F, G211V, G212STOP		
Ps450	1500	441	V22I, V127L, E185Q, P186G, V189T, E202Q, I210A, G211R, E230K, S240T, N262T, T276A, A281G, K296Q, N310E, G312R, A315G, L347M, S402A, Q424E	LOOP L7-short	
Ps440	1500	137	S57E, S59R, V127L, W138STOP		
Ps436	1500	441	S57E, S59R, V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S240T, N262T, T276A, A281G, K296Q, Q301E, N310E, A315G, L347M, S402A, Q424E	LOOP L7-short	
Ps405	1500	119	D43N, S57E, S59R, Y120STOP	Insertion of 7 bp (ACTGATG) at nt 708	
Ps434	1500	364	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G	Insertion of 1 bp (T) at nt 890	
Ps442 (Ps443, Ps444)	1500	340	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G	Deletion of 13 bp (GGCGAGAAATCCT) at nt 1002	
Ps429 (Ps430)	1500	>443	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L	Deletion of 2 bp (CA) at nt 1107	

Table 1. cont.

Strain(s)*	Amplicon PCR size (bp)	OprD size (aa)	Amino acid changes in OprD sequence†	Insertion/deletion	Disruption of OprD by ISs
Ps449	1500	431	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L‡	Deletion of 1 bp (C) at nt 1091	
Ps421, Ps433	1500	>443	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L,	LOOP L7-short insertion of 1 bp (C) at nt 1199	
Ps452 (Ps453)	1500	>443	T103S, K115T, F170L	Insertion of 1 bp (C) at nt 1205	
Ps409	1500	295	T103S, K115T, F170L, E185Q, P186G, V189T	Deletion of 23 bp (TCAGCAACACCACTTGGTCCCTG) at nt 814	
Ps412	1500	372	T103S, K115T, F170L, E185Q, P186G, V189T, R310E, A315G	Deletion of 5 bp (TTTCA) at nt 1067	
Ps419	1500	353	T103S, K115T, F170L, E185Q, P186G, V189T, R310E, A315G	Deletion of 4 bp (TCCT) at nt 1044	
Ps424	1500	164	S57E, S59R, V127L	Deletion of 11 bp (AGACCGCGACC) at at 472	
Ps427	750	202	S57E, S59R, V127L, E185Q, P186G, V189T	Insertion of 36 bp (CCGGGTTTTTTCTTCTCGGCGGC AACGCGCCTATAA) at nt 573	
Ps422 (Ps455)¶	1500	258	V127L, E185Q, P186G, V189T	Deletion of 14 bp (GGCGAGACCGCCAA) at nt 600	
Ps408 (Ps418)	750	164	G425A	Deletion of 837 bp at nt 255	

*The strain inside parentheses has the same PFGE pattern as the previous indicated strain.
†The substitutions were determined by comparison with the sequence of *P. aeruginosa* PAO1 control strain (GenBank accession no. AE004091), which encodes a protein of 443 aa.
‡No mutations were found in the porin OprD of *P. aeruginosa* Ps441 in comparison with the sequence of *P. aeruginosa* PAO1.
§A novel IS named IS*Pa56* was detected upstream of the *oprD* gene and first described in this study.
‖The ORF changed after the deletion, and the protein OprD was completely different to the OprD of *P. aeruginosa* PAO1.
¶MBL-producing strains.

Table 2. Type of class 1 integrons detected in the integron-positive *P. aeruginosa* strains

Strain type	Strain(s)*	MLST	Gene cassettes inside the variable region of class 1 integrons	Promoter
Non-MBL-producing strains	Ps401, Ps414, Ps416, Ps417, Ps421, Ps429, Ps430, Ps431, Ps433, Ps434, Ps449	ST175	*aadB*	PcH1
	Ps441	ST244	*aadB*	PcH1
	Ps436	ST1977	*aadB*	PcH1
	Ps399, Ps402 (Ps410)	ST235	*aadA6-orfD*	PcH1
	Ps419	ST235	*aadB-orf1-aadA11*	PcH1, P2 active
	Ps423	ST235	dfrB1	PcH1
	Ps426	ST175	None†	PcH1
	Ps397 (Ps398)	ST308	*aacA4-aacC1d*-IS*Kpn4-gcuE* ‡	PcS
MBL-producing strains	Ps422 (Ps455)	ST654	*aacA56*§ $bla_{\text{VIM-2}}$+ND‖	PcH1 PcW-TG
	Ps407	ST233	*aacA4-cmlA1* $bla_{\text{VIM-2}}$+ND‖	PcS PcW

ND, Not determined.

*The strain inside parentheses has the same PFGE pattern as the previous indicated strain.

†An empty class 1 integron with a *sul1* gene but without *qacE*Δ*1* at the 3′ conserved segment was observed in this strain.

‡This integron was named In1160 by Integrall (the integron database: http://integrall.bio.ua.pt/).

§This integron was named In1054 by Integrall.

‖These integrons could not be completely determined by PCR.

In addition, these three isolates contained a second class 1 integron whose resistance gene cassettes were *aacA4*+ *cmlA1* in one isolate, and the *aacA56* gene in the remaining two isolates. Two new integrons were described for the first time, to the best of our knowledge, in this study. The first, *intI1-aacA56-qacE*Δ*1-sul1*, was named In1054 by Integrall (http://integrall.bio.ua.pt/), and the second, *intI1-aacA4-aacC1d*-IS*Kpn4-gcuE-qacE*Δ*1-sul1*, was named In1160 by Integrall (Table 2).

Clonal relationship

A high clonal diversity was found in our isolates, with a total of 44 different PFGE patterns detected among the 56 IRPA isolates (78.6% of the isolates were different). Most of the indistinguishable PFGE patterns were found among isolates from the same patient (five PFGE patterns, 12 isolates and five patients), whereas one patient harboured two isolates with different PFGE patterns, and five PFGE patterns were related to 10 isolates from 10 patients.

The STs were determined by MLST in the 24 class 1 integron-positive selected strains (Table 2). MBL-producing strains belonged to ST233 and ST654, and non-MBL-producing strains were ascribed to ST175, ST235, ST244, ST308 and a novel ST named ST1977. This ST1977 is related to CC313, which is constituted by ST174, ST313, ST648, ST678, ST1462, ST1628 and ST1691 according to the software program PHYLOViZ. In France, ST244 and ST308 have been described among *P. aeruginosa* isolates from water samples (MLST database: http://pubmlst.org/paeruginosa/) or from clinical outbreaks, and even among *P. aeruginosa* strains producing the IMP-type MBL (Fournier *et al.*, 2012; Willmann *et al.*, 2015). However, to the best of our knowledge, there are no previous descriptions of these STs among *P. aeruginosa* isolates from blood samples. Among the diversity of STs found, ST175, ST233, ST235, ST244 and ST654 were noteworthy as high-risk clones that are frequently associated with carbapenemase producers (Samuelsen *et al.*, 2010; Vatcheva-Dobrevska *et al.*, 2013; Moyo *et al.*, 2015; Wright *et al.*, 2015).

Detection of virulence factors

Table 3 shows the different patterns of virulence genes studied in the 56 IRPA strains. The genes of the type III secretion system, *exoU*, *exoS*, *exoY* and *exoT*, were found in 39.3, 58.9, 89.3 and 100% of our IRPA strains, respectively. No IRPA strains contained *exoU* and *exoS* together, as reported previously (Feltman *et al.*, 2001; Wiehlmann *et al.*, 2007; Maatallah *et al.*, 2011; Lee *et al.*, 2013). In contrast, only one strain lacked both the *exoU* and *exoS* genes. This fact is unusual, as confirmed by the few similar reported descriptions (Kaszab *et al.*, 2011). According to other authors, *exoU* and *exoS* are important virulence markers, with *exoS* found more frequently than *exoU* (Feltman *et al.*, 2001; Kaszab *et al.*, 2011). The *exoU* gene is a major contributor to the potential pathogenesis of *P. aeruginosa*, and therefore it is found almost exclusively in clinical isolates (Lin *et al.*, 2006; Lee *et al.*, 2013; Petit *et al.*, 2013). All the IRPA strains amplified the *exoA*, *lasA*, *lasB* and *aprA* genes. The quorum-sensing

Table 3. Virulence genes studied in the 56 IRPA strains

Virulence gene											
exoU	*exoS*	*exoY*	*exoT*	*exoA*	*lasA-lasB*	*aprA*	*rhlAB*	*rhlI*	*rhlR*	*lasI-lasR*	No. of strains
+	−	+	+	+	+	+	+	+	+	+	14
+	−	+	+	+	+	+	+	−	−	+	3
+	−	+	+	+	+	+	+	+	−	+	1
+	−	−	+	+	+	+	+	+	+	+	4
−	+	+	+	+	+	+	+	+	+	+*	14
−	+	+	+	+	+	+	+	+	+	−	4
−	+	+	+	+	+	+†	+	−	−	+	1
−	+	+	+	+	+	+	−	−	−	+	10
−	+	+	+	+	+	+	−	+	−	+	2
−	+	−	+	+	+	+	+	+	+	+	1
−	+	−	+	+	+	+	−	−	−	+	1
−	−	+	+	+	+	+	+	+	−	+	1

*The *lasR* amplicon was 2500 bp in one IRPA strain.
†The amplicon size of the *aprA* gene was greater (250 bp) than the expected size (140 bp).

systems were also studied (the *las* and *rhl* systems), as they have great importance in the induction of biofilm formation and virulence factor production, such as elastase (*lasB* gene), staphylolysin or LasA protease (*lasA* gene), exotoxin A (*exoA* or *toxA* gene), alkaline protease (*aprA* gene) and pyocyanin (Pesci *et al.*, 1997; Cabrol *et al.*, 2003). Most of our IRPA strains harboured *lasI*+*lasR* (92.9%) or *rhlI*+*rhlR* (66.1%) (Table 3). More than half of the studied strains (58.9%) contained both quorum-sensing systems (Table 3). In one strain, a *lasR* PCR amplicon of approximately 2500 bp was found (Table 3). Its sequence revealed that the *lasR* gene was disrupted by an IS, IS*Pa1635*-like, which belongs to the IS*4* family group. IS elements disrupting the *lasR* gene have been described previously, but to the best of our knowledge, the described IS belonged to the IS*5* family group, not to IS*4* group (Cabrol *et al.*, 2003; Petit *et al.*, 2013).

In conclusion, a low prevalence of MBL-producing *P. aeruginosa* (5.4%) was found among the clinical IRPA isolates from blood in the studied hospital. Dissemination of the $bla_{\mathrm{VIM-2}}$ gene through mobilizable elements, such as class 1 integrons, and the inclusion of other resistance genes are causes of great concern because this constitutes an effective way of multiple antibiotic resistance dissemination. In addition, a high polymorphism was identified in the *oprD* gene in our IRPA strains, which could be implicated in the variable resistance to carbapenems in *P. aeruginosa* strains. The dissemination of high-risk clones (ST175, ST233, ST235, ST244 and ST654) among clinical *P. aeruginosa* is a cause for concern, as the incidence of these micro-organisms has been increasing in recent years.

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