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

Beatriz Rojo-Bezares,¹ Laurent Cavalié,^{2,3} Damien Dubois,^{2,3} Eric Oswald,^{2,3} Carmen Torres^{1,4} and Yolanda Sáenz¹

¹Centro de Investigación Biomédica de La Rioja (CIBIR), Área de Microbiología Molecular, Logroño, Spain

²CHU Toulouse, Hôpital Purpan, Service de Bactériologie-Hygiène, Toulouse, France

³Centre de Physiopathologie de Toulouse Purpan (CPTP), Inserm UMR1043 – CNRS UMR5282 – INRA USC1360, Université Toulouse III, Toulouse, France

⁴Universidad de La Rioja, Área de Bioquímica y Biología Molecular, Logroño, Spain

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 blavIII-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 ISPa56 identified in two strains. Class 1 integrons were detected in 24 (42.9%) IRPA strains. The blaVIM-2 gene was found inside the class 1 integron arrangements. The new integrons In1054 (intl1-aacA56-gacE∆1-sul1) and In1160 (intl1-aacA4-aacC1d-ISKpn4gcuE- $gacE\Delta 1$ -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.

Received 29 October 2015 Accepted 29 January 2016

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

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

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

Correspondence Yolanda Sáenz ysaenz@riojasalud.es

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

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 (PA β N, 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 PA β N, respectively.

Molecular typing. The clonal relationship among IRPA isolates was evaluated by PFGE using *SpeI* 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 ($qacE\Delta 1 + sul1$) 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_{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, $PA\beta N$ 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 without premature terminations. changes with or

The presence of insertion sequences (ISs) truncating the oprD gene was detected in eight IRPA strains. ISPa26 was detected truncating oprD in two clonally related strains (Ps402 and Ps410) in which a novel IS, named ISPa56, was also found upstream of the oprD gene. The ISPa56 element belongs to the IS3 family IS2 group, with 72% amino acid similarity to ISRso10. ISPa56 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. ISPa26, ISPa45 and ISPa1328, found truncating the oprD gene in our strains, as well as other ISs (ISPa8, ISPa27, ISPa45, ISPa46, ISPa47, ISPa133, ISPa1328, ISPa1635, ISPre2, ISPst12 and ISPpu21, 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 $qacE\Delta I$ at the 3' conserved segment (Table 2). This atypical and infrequent structure was been reported previously (Rosser & Young, 1999).

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

Diddition 150 43 Nonet PM01 25000 - - Standard Stad PM01 2000 - - Standard Stad Standard Stad PM01 2000 - - Standard Stad Standard Stad <th>Strain(s)*</th> <th>Amplicon PCR size (bp)</th> <th>OprD siz (aa)</th> <th>e Amino acid changes in OprD sequence†</th> <th>Insertion/deletion</th> <th>Disruption of OprD by ISs</th>	Strain(s)*	Amplicon PCR size (bp)	OprD siz (aa)	e Amino acid changes in OprD sequence†	Insertion/deletion	Disruption of OprD by ISs
PM01, Pedito, PM01, Pedito, PM01, Pedito, PM01, Pedito, PM01, PM01, PM01, PM0 S000 - Non- transmission Streads Streads PM41, PM1, PM1 200 - PM0, PM01, P	Ps441	1500	443	None‡		
Polity, Prolity 2000 - Number of the polity of the pol	Ps402 (Ps410)	≥3000	I		I	ISPa56§ and ISPa26
Pudt. Pad3 Pudt. Pad3 Pid5 2300 - Pid67 Pid67 <td< td=""><td>Ps403, Ps404 (Ps406),</td><td>≥3000</td><td>I</td><td></td><td>I</td><td>ISPa1328</td></td<>	Ps403, Ps404 (Ps406),	≥3000	I		I	ISPa1328
#9439 = 2000 0 #9439 = 2000 0 Hars, Warry Fant, Boo Definition of 1 bp (A) at nt 23401 Definition of 1 bp (A) at nt 23401 #9405, Paul 2, Fant, Fant, Boo 270 Daty, Sark, Sagk, Eznet, Azio, Azio, Xuo, Yazar, Azors, LOOP L7-doot Definition of 1 bp (A) at nt 23401 Paul 2, Fant, Fant, Boo 411 Daty, Sark, Sagk, Eznet, Azio, Azio, Xuo, Yazar, Azors, LOOP L7-doot Definition of 1 bp (A) at nt 23401 Paul 2, Fant, Fant, Boo 130 411 Daty, Sark, Sagk, Eznet, Azio, Azio, Xuo, Yuzar, Xuors, LOOP L7-doot Definition of 1 bp (A) at nt 23401 Paul 2, Fant, Fant, Boo 23016, Kasoo, Qauli, Ratio, Vasar, Mazer, Azors, LOOP L7-doot Definition of 1 bp (A) at nt 23401 Paul 2, Fant, Boo 341 Daty, Sark, Segk, Eznet, Azio, Xaor, Xuors, Suor, Loot Definition of 1 bp (A) at nt 23401 Paul 2, Fant, Bars, Paul 2, Fant, Bars, Paul 2, Azio, Xuor,	Ps447, Ps428					
Bold Deletion of 1 bp (A) at nt 2941 Peroff Full, 150 20 Days, Ser, Sen, Ezrod, AJDI, K20E, SJJT, N2CT, ACT, AGT, ALM, FMIT, 150 Deletion of 1 bp (A) at nt 2941 Peroff Full, 1510 100 21 Adris, W27510P COP L7-bott Peroff Full, 1510 101 Adris, W27510P COP L7-bott Adris, W27510P Peroff Full, 1510 101 Days, SSF, SSN, Ezrod, AJDI, K20E, SJJT, N2CT, AZ65, LOOP L7-bott Pacing 1500 411 Days, SSF, SSN, Ezrod, AJDI, WAST, AZ65, LOOP L7-bott Pacing 1500 21 Days, SSF, SSN, Ezrod, AJDI, WAST, AZ65, LOOP L7-bott Pacing 1500 21 Days, SSF, SSN, Ezrod, AJDI, WAST, AZ65, LOOP L7-bott Pacing 1500 242 Days, SSF, SSN, ZDO, AJDI, SPIG, VISH, WAST, AZ65, LOOP L7-bott Pacing 1500 234 Dass, SSN, Pacing, SJJT, WAST, AZ65, LOOP L7-bott Pacing 1500 234 Dass, SSN, Pacing, SJJT, WAST, AZ65, LOOP L7-bott Pacing 2300 2301 RUG, SSN, VIST, TA327, A265, LOOP L7-bott Pacing	Ps439	≥3000	I		I	ISPa45
Page, Pati Patid, Page, Pati Patid, Page, Pati Patid, Pade, Patid, Patid, Pade, Patid, Patid, Patil, Patid, Pade, Patid, Patid, Patid, Patil, Patil, Patid, Pade, Patid,	Ps432	1500	109		Deletion of 1 bp (A) at nt 294ll	
Public Media Ansist Matrix Matrix Ansist Matrix Matrix IOOP L7-short Public Media 150 41 DANIS STR, SSR, EDOQ, A210, K230E, S40T, N26T, A56S, A00P L7-short Public Media 150 41 DANIS STR, SSR, EDOQ, A210, K230E, S40T, N26T, A56S, A00P L7-short Public Media 150 41 DANIS, SSR, EDOQ, A201E, R210C, V39E, Q30E, R117CDP Public Media 150 42 DANIS, SSR, EDOQ, Q30E, R310C, V39E, Q30E, R310C, V39E, Q34570P Public Media 150 22 TONS, K115, F170L, B18G, V189T, V2575 IOOP L7-short Public Media 150 23 TONS, K115, F170L, B18G, V189T, K205E, S30G, A207, V38T, Q475 IOOP L7-short Public Media 150 23 TONS, K115, F170L, B18G, V189T, K205E, S30G, A207, V38T, Q56S, V189T, K205E, S30G, A207, V38T, Q50S, A204, V38T, P30S, V187T, P30S, P30G, A304, V38T, P30S, V187T, P30S, P30G, V187T, P30S, P30G, V187T, P30S, P30G, A304, V38T, P30S, V187T, P30S, P30G, V38T, P30S, P30G, V38T, P30S, V38T, P30S, P30G, V38T, P30S, V38T, P30S, V38T, P30S, V38T, P30S, V38T, P30S, V38T, P30S, P30G, V38T, P30S, V38T, P30S, P30G, V38T,	Ps396, Ps413 Ps414,	1500	276	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T,		
Perform Perform Description Cold Trabor PadG, Fradd, PadT 150 441 DAN, SSTE, SSRE, REDQ, AZDII, K30E, SJUT, NGET, AJSTS, LOOP 17-abort PadG, Fradd, PadT 150 414 DAN, SSTE, SSRE, REDQ, AZDII, K30E, SJUT, NGET, AJSTS, LOOP 17-abort PadG, Fradd, PadT 150 414 DAN, SSTE, SSRE, REDQ, AZDII, K30E, SJUT, NGET, AJSTS, LOOP 17-abort PadJ 1500 414 DANS, SSTE, SSRE, REDQ, AZDII, S20E, SJUT, NGET, AJSTS, LOOP 17-abort PadJ 1500 34 DANS, SSTE, SSRE, REDQ, AZDII, S20E, SJUT, NGET, AJSTS, LOOP 17-abort PadJ 1500 34 DANS, SSTE, SSRE, REDQ, AZDII, S20E, SJUT, NGET, AJSTS, LOOP 17-abort PadJ 1500 23 TOSK, K1157, F170L, E185Q, V1877, V235570P LOOP 17-abort PadJ 250 TOSK, K1157, F170L, E185Q, V1897, V1877, N275570P LOOP 17-abort PadJ 230 TOSK, K1157, F170L, E185Q, V1897, V1927, R151, F170L, E185Q, V1897, V1927, R151, F170L, E185Q, V1897, R217, A2655, A2047, A215G, A215G, A215G, A216G,	Ps425, Ps431, Ps438			A267S, W277STOP		
Pe43b, Pe44b, Pe44b A281, Gr. S990, Q301, R3106, V3591, WATSTI, A2675, L0OP L7-short Pe420 130 41 D438, S57E, S598, E3020, A2101, K230E, S240T, NASTI, A2675, LOOP L7-short Pe451 1300 421 D438, S57E, S598, E3020, A2101, K230E, S240T, NASTI, A2675, LOOP L7-short Pe451 1300 421 D438, S57E, S598, E3020, A2101, K230E, S2497, NASTI, A2675, LOOP L7-short Pe4101, Pe416 1500 348 D438, S57E, S598, E3020, A101, K230E, S44770P LOOP L7-short Pe4101, Pe416 1500 348 D438, S57E, S598, E3020, A101, K2015, K2045, S1056, A1077 LOOP L7-short Pe430 1500 225 T1038, K1137, F1704, E1850, V1897, K205E, S2066, A3077 LOOP L7-short Pe431 2301 D235 T1038, K1137, F1704, E1850, V1897, K205E, S2066, A2077 L2056 Pe451 2300 205 T1038, K1137, F1704, E1850, V1897, K205E, S2066, A2077 L2056 Pe451 2301 N517, F1704, E1850, V1897, K205E, S206, A2077 L2056 L2004 L7-short Pe451 2301 N517, F1704, E1850, V1897, K205E, N206, V1897, K205E, S2066, A2077 L2024 L2024 Pe451 2301 N21	Ps407¶ Ps411, Ps417,	1500	441	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,	LOOP L7-short	
Pei20 1300 414 D-Ni, Siris, Sisy, Rango, Aloi, K2ofd, Yaoff, Nacr, Alor S, LoOP L7-short Pei43 1300 421 D-Ni, Siris, Saye, Qaotf, Nacr, Alor S, LoOP L7-short Pai01, Path 150 421 D-Ni, Siris, Saye, Qaotf, Nacr, Alor S, LoOP L7-short Pad01, Path 150 421 D-Ni, Siris, Saye, Qaotf, Nacr, Alor S, LoOP L7-short Pad01, Path 150 232 T1038, K1157, F1701, E185Q, V1897, V2337, OP Pad01 1500 224 T1038, K1157, F1701, E185Q, V1897, V2337, OP Pad1 223 T1038, K1157, F1701, E185Q, P186G, V1897, K210E, A1315G, Pad1 239 T1038, K1157, F1701, E185Q, P186G, V1897, K210E, A1315G, Pad3 236 T1038, K1157, F1701, E185Q, P186G, V1897, K210E, A1315G, Pad4 2300 205 T1038, K1157, F1701, E185Q, P186G, V1897, K210E, A1315G, Pad5 2300 1301 411 V221, V221, U221, E185Q, P186G, V1897, E2020, I210A, E2306, Pad5 1500 441 V221, V127, E185Q, P186G, V1897, E2020, I210A, E2306, A2017, Pad6 1500 441 V221, V127, E185Q, P186G, V1897, E2020, I210A, E2306, A2104,	Ps426, Ps446, Ps448			A281G, K296Q, Q301E, R310G, V359L		
Best Land, K.S.F., S.S.M, LENGO, ADII, K.S.M, S.A.M., MATSTOP MASIG	Ps420	1500	414	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,	LOOP L7-short	
Ps454 1500 421 D438, S75, S598, E2020, A210, K236, Q434TOP LOOP L7-short Ps401, P416 1500 348 D438, S75, S598, E3020, A101, K236, S40T, N26T, A267S, LOOP L7-short Ps423 1500 328 D138, K157, F1704, E1830, V1837, T235TOP D438, S57, S598, E3020, V1837, T235TOP Ps420 1500 324 T1035, K1157, F1704, E1830, V1857, V1877, W37T, W37T, M257, V1877, W37T, W37 D438, S157, F1704, E1830, P1866, V1877, W37T, W37T				A281G, K296Q, Q301E, R310G, V359L, W417STOP		
Pach, Path AzBIG, K296Q, 300L; R310G, V359L, Q245TOP P4423 1500 34 D43N, S57E, S59R, E2020, A101C, K20E, S40T, N267T, A267S, A267S, A267S, A267S, A267S, P36G, Q30L; R310G, S495TOP P5431 1500 223 T103S, K115T, F170L, E18SQ, V189T, Y2335TOP P4451 2301 T103S, K115T, F170L, E18SQ, P186G, V189T, R310E, A315G, G35A, L255TOP P4451 2300 205 T103S, K115T, F170L, E18SQ, P186G, V189T, K205E, S206G, A207V, C3125TOP P4451 2300 205 T103S, K115T, F170L, E18SQ, P186G, V189T, K205E, Z306G, A207V, C3125TOP P4450 1500 1500 2103S, K115T, T276A, A281G, K296Q, N180L, G312R, A315G, C3125TOP P4460 1500 1500 241 V2210B, R520, P186G, V189T, E2020, 1210A, C311R, E230K, LOOP L7-short P5440 1500 137 S57L, S99R, V127L, E18SQ, P186G, V189T, E202A, A315G, K296Q, V391E, G312R, A315G, L347M, S37E, S98R, V127L, W183STOP 1500 P5440 1500 137 S57L, S98R, V127L, E18SQ, P186G, V189T, E202A, A15G, K296Q, Q301E, N310E, A315G, L347M, S75F, S98R, P122A, A316G, K296Q, Q301E, N310E, A315G, L347M, A15G, K394A, N30E, A31G, K296Q, Q301E, N30E, A31G, K230E, A30T, N262T, A367S, A316G, K230E, A320T, N262T, A367S, A316G, K230E, A320T, N262T, A367S, A316G, K230E, A320T, N262T, A367S, A315G, K230G, A301G, K230E, S240T, N262T, A367S, A316G, K	Ps454	1500	421	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,	LOOP L7-short	
Ps401, Ps416 1500 348 D43N, S5TE, S59R, E30Q, AJOI, K13CT, A267S, Ps423 1500 223 T103S, K115T, F170L, E18SQ, V189T, V3275TOP Ps430 1500 224 T103S, K115T, F170L, E18SQ, V189T, V3275TOP Ps440 1500 225 T103S, K115T, F170L, E18SQ, V189T, V3275TOP Ps451 2300 226 T103S, K115T, F170L, E18SQ, P186G, V189T, K20E, X206G, A3DTV Ps451 2300 205 T103S, K115T, F170L, E18SQ, P186G, V189T, K20E, S206G, A3DTV Ps450 1500 205 T103S, K115T, F170L, E18SQ, P186G, V189T, K20E, S206G, A3DTV Ps450 1500 205 T103S, K115T, F170L, E18SQ, P186G, V189T, R205L, S206G, A3DTV Ps460 1500 206 T103S, K115T, F170L, E18SQ, P186G, V189T, R205L, S206G, A3DTV Ps440 1500 441 V221, V12TA, M3STOP Ps440 1500 141 S24T, S26A, V13TA, W13STOP Ps441 1500 141 S75T, S59R, V12TA, W13STOP Ps440 1500 141 S75T, S26G, V18TA, V12A,				A281G, K296Q, Q301E, R310G, V359L, Q424STOP		
P4423 1500 234 TUOSK, K1157, F170L, EISGO, VI89T, Y233STOP P4400 1500 276 TIOSK, K1157, F170L, EISGO, VI89T, Y233STOP P4411 1500 354 TIOSK, K1157, F170L, EISGO, PISGC, V189T, R310E, A315G, P4451 ≥3000 205 TIOSK, K1157, F170L, EISGO, PISGC, V189T, R310E, A315G, P4451 ≥3000 205 TIOSK, K1157, F170L, EISGO, PISGC, V189T, R205E, S206G, A207V, P4450 1500 441 V221, V127L, EISGO, PISGC, V189T, E202O, 1210A, G211R, E306K, P5440 1500 441 V221, V127L, EISGO, PISGC, V189T, E202O, 1210A, G212R, A315G, P5440 1500 137 S57E, S59R, V127L, W13STOP P5441 1500 137 S57E, S59R, V127L, W13STOP P5441 1500 441 S57E, S59R, V127L, W13STOP P5443 1500 137 S57E, S59R, V127L, W13STOP P5444 1500 441 S57E, S59R, V127L, W13STOP <td< td=""><td>Ps401, Ps416</td><td>1500</td><td>348</td><td>D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,</td><td></td><td></td></td<>	Ps401, Ps416	1500	348	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,		
Ps423 1500 222 T103S, K115T, F170L, E18SQ, N189T, Y233STOP Ps400 26 T103S, K115T, F170L, E18SQ, P186G, V189T, W277STOP Ps401 1500 276 T103S, K115T, F170L, E18SQ, P186G, V189T, W277STOP Ps451 = 3300 205 T103S, K115T, F170L, E18SQ, P186G, V189T, W275STOP Ps451 = 3300 205 T103S, K115T, F170L, E18SQ, P186G, V189T, W275TOP Ps450 1500 205 T103S, K115T, F170L, E18SQ, P186G, V189T, K205E, S206G, A207V, Ps450 1500 441 V221, V121L, E18SQ, P186G, V189T, E202Q, 1210A, G211R, E230K, Ps440 1500 441 V221, V126T, D202A, 1210A, G211R, E230K, Ps440 1500 441 S240T, N262T, T266A, A310G, K296Q, 301E, N310E, G312R, A315G, Ps440 1500 441 S27E, S59R, V127L, W138STOP Ps440 1500 441 S37E, S59R, V127L, E18SQ, P186G, V180T, E202Q, 1210A, E230K, Ps441 1500 441 S37E, S59R, V127L, E18SQ, P186G, V180T, E202Q, 1210A, E230K, Ps441 1500 137 S57E, S59R, V127L, W138STOP Ps442 1500 137 <				A281G, K296Q, Q301E, R310G, S349STOP		
Ps397 Ps398 I500 276 T103S, K115T, F170L, E18SQ, P186G, V189T, K210E, A315G, Ps400 1500 334 T103S, K115T, F170L, E18SQ, P186G, V189T, K205E, S206G, A207V, Ps451 ≥3000 205 T103S, K115T, F170L, E18SQ, P186G, V189T, K205E, S206G, A207V, Ps450 1500 441 V23L, V127L, E18SQ, P186G, V189T, K205E, S206G, A207V, Ps450 1500 441 V23L, V127L, E18SQ, P186G, V189T, E202Q, I210A, E31R, A315G, Ps440 1500 441 V23L, V127L, E18SQ, P186G, V189T, E202Q, I210A, E30K, Ps440 1500 137 S57E, S59R, V127L, M13STOP Ps440 1500 137 S57E, S59R, V127L, M13STOP Ps440 1500 137 S57E, S59R, V127L, M13STOP Ps440 1500 441 S57E, S59R, V127L, M13STOP Ps440 1500 441 S57E, S59R, V127L, M13STOP Ps440 1500 441 S57E, S59R, V127L, M13STOP Ps441 1500 137 S57E, S59R, V127L, M13STOP Ps442 1500 137 S57E, S59R, V120TOP Ps443 <td>Ps423</td> <td>1500</td> <td>232</td> <td>T103S, K115T, F170L, E185Q, V189T, Y233STOP</td> <td></td> <td></td>	Ps423	1500	232	T103S, K115T, F170L, E185Q, V189T, Y233STOP		
Ps400 1500 354 T103S, K115T, F170L, E18SQ, P186G, V189T, K20ES, S206G, A207V, c254A, L255TOP C254A, L255TOP Ps451 ≥3000 205 T103S, K115T, F170L, E18SQ, P186G, V189T, K20ES, S206G, A207V, D208R, F209N, L10F, G211V, G212TOP C254A, L255TOP Ps450 1500 441 V21, V12T, E18SQ, P186G, V189T, E202Q, I210A, G211R, E230K, S240T, N262T, T276A, A281G, K296Q, N310E, G312R, A315G, L347M, S402A, Q424E L00P L7-short Ps440 1500 137 S57E, S99R, V127L, E18SQ, P186G, V189T, E202Q, I210A, E230K, L347M, S402A, Q424E L00P L7-short Ps440 1500 441 S57E, S99R, V127L, E18SQ, P186G, V189T, E202Q, I210A, E230K, L347M, S402A, Q424E L00P L7-short Ps445 1500 441 S57E, S99R, V127L, E18SQ, P186G, V189T, E202Q, I210A, E230K, L347M, S402A, Q424E L00P L7-short Ps445 1500 441 S57E, S59R, V127L, E18SQ, P186G, V189T, E202Q, I310A, L347M, S403A, Q424E L00P L7-short Ps445 1500 341 S57E, S59R, V127L, M138ETOP L00P L7-short Ps445 1500 341 V231, V27L, M33G, L347M, S403A, Q242F L00P L7-short Ps445 1500 341 D43N, S57E, S59R, P120C, A210L, K230E, S240T, N262T, A265G, A	Ps397 (Ps398)	1500	276	T103S, K115T, F170L, E185Q, P186G, V189T, W277STOP		
Ps451 ≥300 205 TI03S, K115T, F170I, E183Q, P186G, V189T, K205E, S206G, A207V, D208R, F209N, 121G, G211V, G212STOP Ps450 1500 411 V221, V127L, E183Q, P186G, V189T, E020Q, 1210A, G211R, E230K, D208R, P209N, 121G, E183Q, P186G, V189T, E020Q, 1210A, G211R, E230K, S240T, N262T, T276A, A281G, K296Q, N310E, G312R, A315G, L347M, S402A, Q424E L00P L7-short Ps430 1500 117 S57E, S58R, V127L, E185Q, P186G, V189T, E020Q, 1210A, E230K, S407K, N267T, T276A, A281G, K296Q, Q301E, N310E, A315G, L347M, S407A, Q424E L00P L7-short Ps445 1500 119 D43N, S57E, S58R, V127L, E185Q, P186G, V189T, E202Q, 1210A, E230K, S407A, Q424E L00P L7-short Ps442 1500 119 D43N, S57E, S58R, V120TP Insertion of 7 bp (ACTGATG) at nt 708 Ps442 1500 119 D43N, S57E, S59R, F202Q, A210I, K230E, S240T, N267T, A267S, Insertion of 1 bp (T) at nt 890 Ps442 Ps443, Ps4441 1500 364 D43N, S57E, S59R, F202Q, A210I, K230E, S240T, N267T, A267S, Insertion of 1 bp (T) at nt 107 Ps429 Ps430 1500 243 D43N, S57E, S59R, F202Q, A210I, K230E, S240T, N267T, A267S, Insertion of 1 bp (T) at nt 107 Ps442 Ps443, Ps4441 1500 340 D43N, S57E, S59R, F202Q, A210I, K230E, S240T, N267T, A267S, Insertion of 1 bp (T) at nt 107 Ps428 D43N, S57E,	Ps400	1500	354	T103S, K115T, F170L, E185Q, P186G, V189T, R310E, A315G,		
Ps451 ≥300 205 T1035, K1157, F1701, E185Q, P186G, V189T, K205E, S206G, A207V, Ps450 1500 441 V221, V127L, E185Q, P186G, V189T, E202Q, I210A, G211R, E230K, Ps440 1500 11 V221, V127L, E185Q, P186G, V189T, E202Q, I210A, G211R, A315G, Ps440 1500 137 S57E, S59R, V127L, W1385TOP Ps440 1500 441 S57E, S59R, V127L, W1385TOP Ps440 1500 441 S57E, S59R, V127L, W1385TOP Ps441 1500 441 S57E, S59R, V127L, W1385TOP Ps442 1500 441 S57E, S59R, V127L, W1385TOP Ps443 1500 441 S57E, S59R, V120T, W1365G, X130G, X240T, N262T, A267S, I1347M, Ps444 1500 119 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Ps445 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Ps445 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T,				G254A, L255STOP		
Ps450 D208K, F209N, I210F, G211V, G212TOP Ps440 1500 441 V221, V127L, E185Q, P186G, V189T, E202Q, I210A, G211R, E330K, C00P L7-short Ps440 1500 137 S247T, N262T, T276A, A281G, K296Q, N310E, G312R, A315G, L00P L7-short Ps440 1500 137 S37E, S59R, V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S315G, L347M, S402A, Q424E Ps435 1500 137 S37E, S59R, V127L, N1385TOP LOOP L7-short Ps435 1500 137 S37E, S59R, V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S31G, L347M, S402A, Q424E LOOP L7-short Ps405 1500 119 D43N, S57E, S59R, V1205TOP Insertion of 7 bp (ACTGATG) at nt 708 Ps414 1500 364 D43N, S57E, S59R, V1205TOP Insertion of 1 bp (T) at nt 890 Ps442 Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 1 bp (T) at nt 890 Ps442 Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 1 bp (T) at nt 890 Ps442 Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 1 bp (T) at nt 1002	Ps451	≥3000	205	T103S, K115T, F170L, E185Q, P186G, V189T, K205E, S206G, A207V,		
Ps450 1500 441 V22I, V127L, E185Q, P186G, V189T, E202Q, I210A, G211R, E230K, LOOP L7-short Ps440 1500 137 557L, 559R, V127L, W138STOP Ps4410 1500 137 557L, 559R, V127L, W138STOP Ps4436 1500 137 557L, 559R, V127L, W138STOP Ps436 1500 137 557L, 559R, V127L, W138STOP Ps436 1500 441 557L, 559R, V127L, W138STOP Ps436 1500 441 557L, 559R, V127L, W138STOP Ps434 1500 441 557L, 559R, V127L, W138STOP Ps405 1500 191 D43N, 557L, 556N, V120T, W130E, A315G, L347M, Ps405 1500 119 D43N, 557L, 556N, V120STOP Insertion of 7 bp (ACTGATG) at nt 708 Ps441 1500 340 D43N, 557L, 556N, E202Q, A210I, K230E, S240T, N262T, A267S, Insertion of 1 bp (T) at mt 890 Ps442 Ps443, Ps444) 1500 340 D43N, 557L, 556N, E202Q, A210I, K230E, S240T, N262T, A267S, Ps442 Ps443, Ps444) 1500 340 D43N, 557L, 556N, E202Q, A210I, K230E, S240T, N262T, A267S, Ps442 Ps443, Ps444) 1500 340 D43N, 557L, 559R, E				D208R, F209N, I210F, G211V, G212STOP		
Ps440 150 137 S240T, N262T, T276A, A281G, K296Q, N310E, G312R, A315G, L347M, S402A, Q424E Ps436 1500 137 S57E, S59R, V127L, W1385TOP Ps436 1500 137 S57E, S59R, V127L, W1385TOP Ps436 1500 441 S57E, S59R, V127L, W1385TOP Ps436 1500 441 S57E, S59R, V127L, W1385TOP Ps405 1500 441 S57E, S59R, V127L, W1385TOP Ps405 1500 441 S57E, S59R, V127L, W1385TOP Ps405 1500 19 D43N, S57E, S59R, V120T, M310E, A315G, L347M Ps434 1500 364 D43N, S57E, S59R, Y1205TOP Ps445 1500 364 D43N, S57E, S59R, P202Q, A210I, K230E, S240T, N262T, A267S, Iastron of 7 bp (ACTGATG) at nt 708 Ps442 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Iastron of 1 bp (T) at nt 890 Ps442 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Iastron of 1 3 bp (GGCGAGAAATCCT) Ps442 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Iastron of 1 3 bp (GGCGAGAAATCCT) Ps429 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T	Ps450	1500	441	V22I, V127L, E185Q, P186G, V189T, E202Q, I210A, G211R, E230K,	LOOP L7-short	
Ps440 L347M, S402A, Q424E Ps440 L30 137 S57E, S59R, V127L, W138STOP Ps436 1500 441 S57E, S59R, V127L, W138STOP Ps436 1500 441 S57E, S59R, V127L, W138STOP Ps436 1500 441 S57E, S59R, V127L, W138STOP Ps437 Loop L7 Loop L7 Ps405 1500 441 S57E, S59R, V127L, W138STOP Ps405 1500 19 D43N, S57E, S59R, V120STOP Insertion of 7 bp (ACTGATG) at nt 708 Ps434 1500 364 D43N, S57E, S59R, Y120STOP Insertion of 7 bp (ACTGATG) at nt 890 Ps443 1500 364 D43N, S57E, S59R, P202Q, A2101, K230E, S240T, N262T, A26S, Insertion of 1 bp (T) at nt 890 Ps443 1500 340 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A26S, Deletion of 13 bp (GGCGAGAAATCCT) Ps443 1500 2401 A281G, K296Q, Q301E, R310G at nt 1002 Ps429 Ps430 1500 243N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A26S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 Ps430 1500 243N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A26S, Deletion				S240T, N262T, T276A, A281G, K296Q, N310E, G312R, A315G,		
Ps440 1500 137 S57E, S59R, V127L, W138STOP Loop Loop L7-short Ps436 1500 441 S57E, S59R, V127L, E18SQ, P186G, V189T, E202Q, I210A, E230K, LOOP L7-short Ps436 1500 441 S57E, S59R, V127L, E18SQ, P186G, V189T, E202Q, I210A, E230K, LOOP L7-short Ps405 1500 441 S57E, S59R, V127L, E18SQ, P186G, V189T, E202Q, I210A, E230K, Incertion of 7 bp (ACTGATG) at nt 708 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, Insertion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 1 3 bp (GGCGAGAAATCCT) Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 1 3 bp (GGCGAGAAATCCT) Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 1 3 bp (GGCGAGAAATCCT) Ps442 (Ps443, Ps444) 1500 >443 D43N, S57E, S59R, E				L347M, S402A, Q424E		
Ps436 1500 441 S57E, S59R, V127L, E185Q, P186G, V189T, E202Q, 1210A, E230K, LOOP L7-short S407t, N262T, T276A, A281G, K296Q, Q301E, N310E, A315G, L347M, S402A, Q424E S402A, Q424E Ps405 1500 119 D43N, S57E, S59R, V1205TOP Ps414 1500 364 D43N, S57E, S59R, V1205TOP Ps442 1500 340 D43N, S57E, S59R, F202Q, A210I, K230E, S240T, N262T, A267S, Insertion of 7 bp (ACTGATG) at nt 708 Ps442 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Insertion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Insertion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Insertion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Int 1002 Ps429 (Ps430) 1500 >433N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Int 1002 Int 1002 Ps429 (Ps430) 1500 >433N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Int 1107 Int 1002 Ps429 (Ps430) 1500 >433N, S57E, S59R,	Ps440	1500	137	S57E, S59R, V127L, W138STOP		
S240T, N26ZT, T276A, A281G, K296Q, Q301E, N310E, A315G, L347M, Ps405 1500 119 D43N, S57E, S59R, Y120STOP Insertion of 7 bp (ACTGATG) at nt 708 Ps434 1500 364 D43N, S57E, S59R, F202Q, A2101, K230E, S240T, N262T, A267S, Insertion of 7 bp (ACTGATG) at nt 708 Ps442 1500 340 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Insertion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107	Ps436	1500	441	S57E, S59R, V127L, E185Q, P186G, V189T, E202Q, I210A, E230K,	LOOP L7-short	
Ps405 5402A, Q424E S402A, Q424E Insertion of 7 bp (ACTGATG) at nt 708 Ps405 1500 119 D43N, S57E, S59R, Y120STOP Insertion of 7 bp (ACTGATG) at nt 708 Ps434 1500 364 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Insertion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 1 bp (T) at nt 890 Ps429 (Ps430) 1500 340 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107				S240T, N262T, T276A, A281G, K296Q, Q301E, N310E, A315G, L347M,		
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, N26ZT, A267S, Insertion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N26ZT, A267S, Insertion of 1 bp (T) at nt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N26ZT, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N26ZT, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N26ZT, A267S, Deletion of 2 bp (CA) at nt 1107 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N26ZT, A267S, Deletion of 2 bp (CA) at nt 1107				S402A, Q424E		
Ps434 1500 364 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Insertion of 1 bp (T) at mt 890 Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at mt 1107 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at mt 1107	P_{s405}	1500	119	D43N, S57E, S59R, Y120STOP	Insertion of 7 bp (ACTGATG) at nt 708	
A281G A281G Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 13 bp (GGCGAGAAATCCT) Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107	Ps434	1500	364	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,	Insertion of 1 bp (T) at nt 890	
Ps442 (Ps443, Ps444) 1500 340 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 13 bp (GGCGAGAAATCCT) A281G, K296Q, Q301E, R310G at nt 1002 at nt 1002 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107				A281G		
A281G, K296Q, Q301E, R310G at nt 1002 Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A2101, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107 A281G, K296Q, Q301E, R310G, V359L	Ps442 (Ps443, Ps444)	1500	340	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,	Deletion of 13 bp (GGCGAGAAATCCT)	
Ps429 (Ps430) 1500 >443 D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S, Deletion of 2 bp (CA) at nt 1107 A281G, K296Q, Q301E, R310G, V359L				A281G, K296Q, Q301E, R310G	at nt 1002	
A281G, K296Q, Q301E, R310G, V359L	Ps429 (Ps430)	1500	>443	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T, A267S,	Deletion of 2 bp (CA) at nt 1107	
				A281G, K296Q, Q301E, R310G, V359L		

	Amplicon	OprD size			Disruption of
Strain(s)*	PCR size (bp)	(aa)	Amino acid changes in OprD sequence [†]	Insertion/deletion	OprD by ISs
Ps449	1500	431	D43N, S57E, S59R, E202Q, A210I, K230E, S240T, N262T,	Deletion of 1 bp (C) at nt 1091	
D-101 D-102		277 277	A2675, A281G, K296Q, Q301E, R310G, V359L‡		
FS421, FS423	00001	C##~	D451N, 557E, 559K, E202Q, AZ1UI, N20E, 52401, N2021, A2075, A281G, K296O, O301E, R310G, V359L,	LOUF L/-snort insertion of 1 pp (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	
				(CCGGGTTTTTTTTTCTCGGCGGC	
				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 pare *The substitutions were for a No mutations we	entheses has the se re determined by o ound in the porin	ume PFGE pi comparison ¹ OprD of <i>P</i> .	attern as the previous indicated strain. with the sequence of <i>P. aeruginosa</i> PAO1 control strain (GenBank acc <i>aeruginosa</i> Ps441 in comparison with the sequence of <i>P. aeruginosa</i> P	:ession no. AE004091), which encodes a protein o	of 443 aa.
IIThe ORF changed aft	ter the deletion, ar	upsuction of the protein	n OprD was completely different to the OprD of <i>P. aeruginosa</i> PAOI		
MBL-producing strai	ins.				

Table 1. cont.

Strain type	Strain(e)*	MIST	Gene cassettes inside the variable region of class 1 integrons	Promotor	
otrain type	otram(s)	MLOI	integrons	Tomoter	
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-ISKpn4-gcuE ‡	PcS	
MBL-producing strains	Ps422 (Ps455)	ST654	aacA56 $bla_{VIM-2} + ND $	PcH1 PcW-TG	
	Ps407	ST233	aacA4-cmlA1 $bla_{VIM-2}+ND $	PcS PcW	

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

ND, Not determined.

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

An empty class 1 integron with a sull gene but without qacE $\Delta 1$ 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.

IThese 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, $intI1-aacA56-qace\Delta1-sul1$, was named In1054 by Integrall (http://integrall.bio.ua.pt/), and the second, intI1-aacA4-aacC1d-ISKpn4-gcuE-qace $\Delta1$ -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	exo Y	exoT	exoA	lasA-lasB	aprA	rhlAB	rhlI	rhlR	lasI-lasR	No. of strains
+	_	+	+	+	+	+	+	+	+	+	14
+	_	+	+	+	+	+	+	_	_	+	3
+	_	+	+	+	+	+	+	+	_	+	1
+	_	-	+	+	+	+	+	+	+	+	4
-	+	+	+	+	+	+	+	+	+	+*	14
-	+	+	+	+	+	+	+	+	+	_	4
_	+	+	+	+	+	$+\dagger$	+	_	_	+	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, ISPa1635-like, which belongs to the IS4 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 IS5 family group, not to IS4 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_{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.

ACKNOWLEDGEMENTS

This work was supported by the REFBIO-Pyrenees Biomedical Network from Programa de Cooperación Transfronterizo

España-Francia-Andorra (POCTEFA) (project ARVINET-BAC). This work was also financed by the Instituto de Salud Carlos III of Spain (project FIS PI12/01276). Part of this study was presented at the 25th ECCMID Congress (Copenhagen, Denmark, 25–28 April 2015). We thank Patricia Siguier for analysing the IS*Pa56* sequence (https://www-is.biotoul.fr/) and Thomas Jové for studying the integrons In1054 and In1160 (http://integrall.bio.ua.pt/). The authors declare they have no conflicts of interest.

REFERENCES

Cabot, G., Ocampo-Sosa, A. A., Domínguez, M. A., Gago, J. F., Juan, C., Tubau, F., Rodríguez, C., Moyà, B., Peña, C. & other authors (2012). Genetic markers of widespread extensively drugresistant *Pseudomonas aeruginosa* high-risk clones. *Antimicrob Agents Chemother* 56, 6349–6357.

Cabrol, S., Olliver, A., Pier, G. B., Andremont, A. & Ruimy, R. (2003). Transcription of quorum-sensing system genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. *J Bacteriol* **185**, 7222–7230.

Cornaglia, G., Giamarellou, H. & Rossolini, G. M. (2011). Metallo- β -lactamases: a last frontier for β -lactams? *Lancet Infect Dis* **11**, 381–393.

Curran, B., Jonas, D., Grundmann, H., Pitt, T. & Dowson, C. G. (2004). Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J Clin Microbiol 42, 5644–5649.

Ellington, M. J., Kistler, J., Livermore, D. M. & Woodford, N. (2007). Multiplex PCR for rapid detection of genes encoding acquired metallo- β -lactamases. *J Antimicrob Chemother* **59**, 321–322.

Evans, J. C. & Segal, H. (2007). A novel insertion sequence, ISPA26, in *oprD* of *Pseudomonas aeruginosa* is associated with carbapenem resistance. *Antimicrob Agents Chemother* **51**, 3776–3777.

Feltman, H., Schulert, G., Khan, S., Jain, M., Peterson, L. & Hauser, A. R. (2001). Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. *Microbiology* 147, 2659–2669.

Fournier, D., Jeannot, K., Robert-Nicoud, M., Muller, E., Cholley, P., van der Mee-Marquet, N. & Plésiat, P. (2012). Spread of the *bla*_{IMP-13} gene in French *Pseudomonas aeruginosa* through sequence types ST621, ST308 and ST111. *Int J Antimicrob Agents* **40**, 571–573.

Fournier, D., Richardot, C., Müller, E., Robert-Nicoud, M., Llanes, C., Plésiat, P. & Jeannot, K. (2013). Complexity of resistance mechanisms to imipenem in intensive care unit strains of *Pseudomonas aeruginosa*. *J Antimicrob Chemother* **68**, 1772–1780.

Francisco, A. P., Vaz, C., Monteiro, P. T., Melo-Cristino, J., Ramirez, M. & Carriço, J. A. (2012). PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. *BMC Bioinformatics* 13, 87.

Franco, M. R., Caiaffa-Filho, H. H., Burattini, M. N. & Rossi, F. (2010). Metallo- β -lactamases among imipenem-resistant *Pseudomonas aeruginosa* in a Brazilian university hospital. *Clinics* (*Sao Paulo*) **65**, 825–829.

Gutiérrez, O., Juan, C., Cercenado, E., Navarro, F., Bouza, E., Coll, P., Pérez, J. L. & Oliver, A. (2007). Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Spanish hospitals. *Antimicrob Agents Chemother* 51, 4329–4335.

Hauser, A. R. (2009). The type III secretion system of *Pseudomonas* aeruginosa: infection by injection. Nat Rev Microbiol 7, 654–665.

Kaszab, E., Szoboszlay, S., Dobolyi, C., Háhn, J., Pék, N. & Kriszt, B. (2011). Antibiotic resistance profiles and virulence markers of *Pseudomonas aeruginosa* strains isolated from composts. *Bioresour Technol* **102**, 1543–1548.

CA-SFM (2011). Comité de l'antibiogramme de la Société Française de Microbiologie. Recommendations 2011. http://www.sfm-microbiologie.org/UserFiles/files/casfm/casfm_2011.pdf

Lee, K., Chong, Y., Shin, H. B., Kim, Y. A., Yong, D. & Yum, J. H. (2001). Modified Hodge and EDTA-disk synergy tests to screen metallo- β -lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 7, 88–91.

Lee, J. Y., Peck, K. R. & Ko, K. S. (2013). Selective advantages of two major clones of carbapenem-resistant *Pseudomonas aeruginosa* isolates (CC235 and CC641) from Korea: antimicrobial resistance, virulence and biofilm-forming activity. *J Med Microbiol* 62, 1015–1024.

Lin, H. H., Huang, S. P., Teng, H. C., Ji, D. D., Chen, Y. S. & Chen, Y. L. (2006). Presence of the *exoU* gene of *Pseudomonas aeruginosa* is correlated with cytotoxicity in MDCK cells but not with colonization in BALB/c mice. *J Clin Microbiol* 44, 4596–4597.

Lister, P. D., Wolter, D. J. & Hanson, N. D. (2009). Antibacterialresistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev* 22, 582–610.

Maatallah, M., Cheriaa, J., Backhrouf, A., Iversen, A., Grundmann, H., Do, T., Lanotte, P., Mastouri, M., Elghmati, M. S. & other authors (2011). Population structure of *Pseudomonas aeruginosa* from five Mediterranean countries: evidence for frequent recombination and epidemic occurrence of CC235. *PLoS One* **6**, e25617.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G. & other authors (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18, 268–281.

Moyo, S., Haldorsen, B., Aboud, S., Blomberg, B., Maselle, S. Y., Sundsfjord, A., Langeland, N. & Samuelsen, Ø. (2015). Identification of VIM-2-producing *Pseudomonas aeruginosa* from Tanzania is associated with sequence types 244 and 640 and the location of bla_{VIM-2} in a TniC integron. Antimicrob Agents Chemother 59, 682–685.

Pesci, E. C., Pearson, J. P., Seed, P. C. & Iglewski, B. H. (1997). Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa. J Bacteriol* 179, 3127–3132.

Petit, S. M., Lavenir, R., Colinon-Dupuich, C., Boukerb, A. M., Cholley, P., Bertrand, X., Freney, J., Doléans-Jordheim, A., Nazaret, S. & other authors (2013). Lagooning of wastewaters favors dissemination of clinically relevant *Pseudomonas aeruginosa. Res Microbiol* 164, 856–866.

Poole, K. (2011). *Pseudomonas aeruginosa*: resistance to the max. *Front Microbiol* **2**, 65.

Rojo-Bezares, **B.**, **Estepa**, **V.**, **de Toro**, **M.**, **Undabeitia**, **E.**, **Olarte**, **I.**, **Torres**, **C. & Sáenz**, **Y. (2011)**. A novel class 1 integron array carrying *bla*_{VIM-2} genes and a new insertion sequence in a *Pseudomonas aeruginosa* strain isolated from a Spanish hospital. *J Med Microbiol* **60**, 1053–1054.

Rojo-Bezares, B., Estepa, V., Cebollada, R., de Toro, M., Somalo, S., Seral, C., Castillo, F. J., Torres, C. & Sáenz, Y. (2014). Carbapenemresistant *Pseudomonas aeruginosa* strains from a Spanish hospital: characterization of metallo- β -lactamases, porin OprD and integrons. *Int J Med Microbiol* **304**, 405–414.

Rosser, S. J. & Young, H. K. (1999). Identification and characterization of class 1 integrons in bacteria from an aquatic environment. *J Antimicrob Chemother* **44**, 11–18.

Sáenz, Y., Briñas, L., Domínguez, E., Ruiz, J., Zarazaga, M., Vila, J. & Torres, C. (2004). Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrob Agents Chemother* **48**, 3996–4001.

Samuelsen, O., Toleman, M. A., Sundsfjord, A., Rydberg, J., Leegaard, T. M., Walder, M., Lia, A., Ranheim, T. E., Rajendra, Y. & other authors (2010). Molecular epidemiology of metallo- β lactamase-producing *Pseudomonas aeruginosa* isolates from Norway and Sweden shows import of international clones and local clonal expansion. *Antimicrob Agents Chemother* 54, 346–352.

Shaver, C. M. & Hauser, A. R. (2004). Relative contributions of *Pseudomonas aeruginosa* ExoU, ExoS, and ExoT to virulence in the lung. *Infect Immun* 72, 6969–6977.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33, 2233–2239.

Vatcheva-Dobrevska, R., Mulet, X., Ivanov, I., Zamorano, L., Dobreva, E., Velinov, T., Kantardjiev, T. & Oliver, A. (2013). Molecular epidemiology and multidrug resistance mechanisms of *Pseudomonas aeruginosa* isolates from Bulgarian hospitals. *Microb Drug Resist* 19, 355–361.

Wang, J., Zhou, J. Y., Qu, T. T., Shen, P., Wei, Z. Q., Yu, Y. S. & Li, L. J. (2010). Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Chinese hospitals. *Int J Antimicrob Agents* 35, 486–491.

Wiehlmann, L., Wagner, G., Cramer, N., Siebert, B., Gudowius, P., Morales, G., Köhler, T., van Delden, C., Weinel, C. & other authors (2007). Population structure of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* **104**, 8101–8106.

Willmann, M., Bezdan, D., Zapata, L., Susak, H., Vogel, W., Schröppel, K., Liese, J., Weidenmaier, C., Autenrieth, I. B. & other authors (2015). Analysis of a long-term outbreak of XDR *Pseudomonas aeruginosa*: a molecular epidemiological study. *J Antimicrob Chemother* **70**, 1322–1330.

Wolter, D. J., Hanson, N. D. & Lister, P. D. (2004). Insertional inactivation of *oprD* in clinical isolates of *Pseudomonas aeruginosa* leading to carbapenem resistance. *FEMS Microbiol Lett* 236, 137–143.

Woodford, N., Turton, J. F. & Livermore, D. M. (2011). Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev* **35**, 736–755.

Wright, L. L., Turton, J. F., Livermore, D. M., Hopkins, K. L. & Woodford, N. (2015). Dominance of international 'high-risk clones' among metallo- β -lactamase-producing *Pseudomonas aeruginosa* in the UK. *J Antimicrob Chemother* **70**, 103–110.