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Abstract

Whole genome sequencing (WGS) was used for the characterization of the, frequently extensively-drug resistant (XDR), *P. aeruginosa* high-risk clone ST175. A total of eighteen ST175 isolates recovered from 8 different Spanish hospitals were analyzed; four isolates from four different French hospitals were included for comparison. The typical resistance profile of ST175 included penicillins, cephalosporins, monobactams, carbapenems, aminoglycosides, and fluoroquinolones. In the phylogenetic analysis, the four French isolates clustered together with the two isolates from one of the Spanish regions. Sequence variation was analyzed for 146 chromosomal genes related to antimicrobial resistance and horizontally-acquired genes were explored using online databases. The resistome of ST175 was mainly determined by mutational events, with resistance traits common to all or nearly all of the strains, including specific *ampR* mutations leading to *ampC* overexpression, specific mutations in *oprD* conferring carbapenem resistance or a *mexZ* mutation leading to MexXY overexpression. All isolates additionally harbored an *aadB* gene conferring gentamicin and tobramycin resistance. Several other resistance traits were specific to certain geographic areas such as a streptomycin resistance *aadA13* gene detected in all four isolates from France and in the 2 isolates from the Cantabria region or a *glpT* mutation conferring fosfomycin resistance detected in all but these six isolates. Finally, several unique resistance mutations were detected in single isolates; particularly interesting among them were those in genes encoding PBPs (PBP1A, PBP3 and PBP4). Thus, these results provide valuable information for understanding the genetic basis of resistance and the dynamics of dissemination and evolution of high-risk clones.

55

56 **Introduction**

57 *Pseudomonas aeruginosa*, a ubiquitous microorganism, is one of the most
58 relevant pathogens causing human opportunistic infections (1). Due to its impressive
59 metabolic plasticity and versatility, *P. aeruginosa* is capable of infecting/colonizing a
60 wide range of ecological niches, including aquatic and soil habitats, animals and plants
61 (2). *P. aeruginosa* is one of the most frequent and severe causes of acute nosocomial
62 infections, particularly affecting immunocompromised (especially neutropenic) patients
63 or those admitted to the Intensive Care Unit (ICU). Indeed, *P. aeruginosa* is the number
64 one pathogen causing ventilator associated pneumonia (VAP) and burn wound
65 infections, both associated with a very high (>30%) mortality rate (3). Likewise, *P.*
66 *aeruginosa* is the most frequent and severe driver of chronic respiratory infections in
67 patients suffering from cystic fibrosis (CF) or other chronic underlying diseases such as
68 bronchiectasis and chronic obstructive pulmonary disease (COPD) (4).

69 The increasing prevalence of nosocomial infections produced by multidrug-
70 resistant (MDR) or extensively drug-resistant (XDR) *P. aeruginosa* strains severely
71 compromises the selection of appropriate treatments and is therefore associated with
72 significant morbidity and mortality (5, 6). This growing threat results from the
73 extraordinary capacity of this pathogen for developing resistance to nearly all available
74 antibiotics by the selection of mutations in chromosomal genes and from the increasing
75 prevalence of transferable resistance determinants, particularly those encoding class B
76 carbapenemases (metallo-β-lactamases [MBLs]) or extended-spectrum β-lactamases
77 (ESBLs), frequently cotransferred with genes encoding aminoglycoside-modifying
78 enzymes (7, 8, 9).

79 The global success of bacterial pathogens is expected to be determined by a
80 complex interplay between fitness, pathogenicity, epidemicity, and antibiotic resistance
81 (10-15). For decades, multiple reports have warned of the occurrence of epidemic
82 outbreaks caused by MDR/XDR strains within the hospital environment. More recently,
83 concerning reports have provided evidence of the existence of MDR/XDR global clones
84 disseminated in several hospitals worldwide that have been denominated high-risk
85 clones (16, 17). One such clone is ST175, widely distributed in multiple European
86 countries, especially Spain and France (18, 19, 20, 21). Some of the genetic markers
87 responsible of the MDR/XDR profile of this clone have been delineated through Sanger
88 sequencing in some ST175 isolates from a Spanish multicenter study of bloodstream
89 infections (18). In this work, we used whole genome sequencing (WGS) for the
90 characterization of the phylogeny and the resistome of this major clone, defining
91 common and variable resistance traits, using for this purpose a collection of isolates
92 recovered from 8 different Spanish hospitals as well as French isolates for comparative
93 purposes.

94

Materials and methods

Bacterial strains, molecular typing, and susceptibility testing. A total of 14 ST175 *P. aeruginosa* isolates recovered from bloodstream infections in a 2008-2009 multicenter study in Spain were studied (18, 22). The isolates selected included up to two ST175 representatives per each of the 10 hospitals participating in the multicenter study. Since ST175 was detected in 8 of the 10 hospitals (from 4 different geographic regions: Catalonia, Cantabria, Andalusia and Balearic Islands), and two of them provided only one case, the collection studied included 14 isolates. Additionally, 4 isolates recovered from bloodstream infections in one of the hospitals (Hospital Son Espases, Balearic Islands) six years later (2015) were included for comparative purposes. Likewise, 4 ST175 isolates recovered from hospitals in 4 different French cities in 2010 were included for comparison. The MICs of ceftazidime, cefepime, aztreonam, piperacillin-tazobactam, imipenem, meropenem, gentamicin, tobramycin, amikacin, levofloxacin, ciprofloxacin, colistin and fosfomycin were determined by broth microdilution according to CLSI guidelines and breakpoints (23). When needed, sequence types were determined by Multilocus Sequence Typing (MLST) analysis according to previously described protocols (24).

Characterization of resistance mechanisms. The presence of horizontally-acquired β-lactamases was ruled out through previously established phenotypic and molecular (PCR) methods (18). The expression of the genes encoding the chromosomal β-lactamase AmpC (*ampC*) and four *P. aeruginosa* efflux pumps, MexAB-OprM (*mexB*), MexCD-OprJ (*mexD*), MexEF-OprN (*mexF*), and MexXY-OprM (*mexY*), were determined from late-log-phase Luria-Bertani (LB) broth cultures at 37ºC and 180 rpm by real-time reverse transcription-PCR (RT-PCR) with an Illumina (Eco Real-Time PCR System), as previously described (25). When needed, the sequence of genes

involved in antibiotic resistance was confirmed through PCR amplification followed by Sanger sequencing using previously described protocols (25).

Library preparation and whole genome sequencing. Genomic DNA was isolated by the use of a commercial capture system (High Pure PCR Template Preparation Kit, Roche diagnostics) and indexed paired-end libraries were generated by using a commercial library preparation kit (NexteraXT DNA Library Preparation Kit, Illumina). Libraries were then sequenced on an Illumina MiSeq® bench-top sequencer with MiSeq reagent kit v2 (Illumina Inc., USA), generating 250-bp paired-end reads and using a multiplexed protocol (to obtain an average of 195.595 reads (range of 49.923-518.714) per isolate).

Variant calling. Each isolate reads were mapped against the genome of the *P. aeruginosa* reference strain PAO1 (RefSeq accession number: NC_002516.2) using the Bowtie2 software (v2.2.4, http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) (26). Pileups and raw files of the mapped reads were obtained by using the SAMtools (v.0.1.16, https://sourceforge.net/projects/samtools/files/samtools/) (27) and the PicardTools (v1.140, https://github.com/broadinstitute/picard). Read alignments surrounding all putative InDels were realigned using GATK (v3.4-46, https://www.broadinstitute.org/gatk/) (28). The list of Single Nucleotide Polymorphisms (SNPs) were obtained from the raw files meeting the following criteria: a quality score >50, a root-mean-square (RMS) mapping quality >25 and a coverage depth > 3; moreover, we checked manually all the positions in which at least one of the isolates differs from *P. aeruginosa* PAO1 in all the others raw files without any filtering. InDels were extracted from the total pileup files using the following criteria: a quality score >250, a RMS mapping quality >25 and a coverage depth >3.

144 **De novo assembly and phylogenetic analysis.** Genomes from all isolates were
145 *de novo* assembled using the *de Bruijn* graph-based assembler Velvet (v1.2.10,
146 https://www.ebi.ac.uk/~zerbino/velvet/) (29). The 'velveth' command was executed
147 using a k-mer size of 31 and the 'velvetg' command was run using the following
148 parameters: scaffolding = no, ins_length = 500, cov_cutoff = 3 and min_contig_lgth =
149 500. The size range of the *de novo* assembled genomes was 4.9-6.9 Mbp, and each
150 genome shared 93.5-96.9 % of the content of the *P. aeruginosa* reference strain PAO1.
151 The *de novo* assembled genomes were aligned against each other using parSNP from
152 the Harvest Suite package (v1.2,
153 http://harvest.readthedocs.io/en/latest/content/parsnp.html) (30) and a core-genome
154 phylogeny tree was obtained and displayed with FigTree. The default parameters
155 recommended by the program were followed during the whole analysis. SNP
156 differences among isolates were calculated from the lists of SNP previously obtained,
157 excluding all common and ambiguous positions.

158 **BEAST analysis.** Bayesian analysis of divergence times was performed using
159 BEAST (v2.4.2, http://beast2.org/) (31). For this purpose a nexus file was constructed
160 including all the positions in which at least one of the isolates differs from the *P.*
161 *aeruginosa* reference strain PAO1, and converted into an .xml file with BEAUTi. These
162 positions fulfilled the criteria used to calculate the SNP distances among isolates.
163 BEAST was run under the following user-determined settings; a lognormal relaxed
164 molecular clock model and a general time-reversible substitution model with gamma
165 correction (32). Results were produced from one chain with 50 million steps, sampled
166 every 1000 steps. The first 5 million steps were discarded as a burn-in. The maximum
167 clade credibility tree was generated (using TreeAnnotator program from BEAST
168 package), and displayed using FigTree (v1.4.2,

http://tree.bio.ed.ac.uk/software/figtree/). Tree parameters were calculated by Tracer (v1.6, http://beast.bio.ed.ac.uk/Tracer).

Antibiotic resistance genes profiling. Based on literature review we established a set of 146 genes known to be related with antibiotic resistance mechanisms in *P. aeruginosa*, in order to correlate the documented resistance genotypes with the observed resistance phenotypes. SNP and InDels were annotated by using the snpEff software (v4.2, http://snpeff.sourceforge.net/index.html) (33) with the default options, and the output file containing the nucleotidic changes and the predicted effect at protein level were analyzed. Finally we used the on-line Comprehensive Antibiotic Resistance Data Base (CARD, v3.1.1, https://card.mcmaster.ca/home) (34) to complete the analysis, and the online tool Resfinder (v2.1, https://cge.cbs.dtu.dk//services/ResFinder/) (35) in order to identify horizontally-acquired antimicrobial resistance genes.

Nucleotide sequence accession numbers. Sequence files were deposited in the European Nucleotide Archive under study number ERP016726 and accession numbers ERS1280254 to ERS1280271 and ERS1280273 to ERS1280276.

Results and discussion

Phylogenetic analysis of ST175 isolates. A total of 453 high-quality SNPs were detected among ST175 isolates using PAO1 genome as reference. Accordingly, ST175 isolates differed from each other in 18 to 168 SNPs. As shown in Figure 1, the four French isolates clustered together with the two isolates from one of the Spanish regions (Cantabria). In some of the cases, but not always, both studied isolates from a single hospital clustered closely (e.g. isolates from hospitals 1 and 5). BEAST analysis (Figure 2) indicated that all the isolates were linked by a common ancestor from approximately 25 years ago (1990s).

ST175 resistome. The complete list of genes investigated and the variations detected in each of the 22 studied isolates is reported in Table S1; Table 1 shows the list of genes showing nonsynonymous SNPs and the number of isolates involved; insertions or deletions were not detected in any of the genes. Up to 83 (56.8%) of the 146 genes showed nonsynonymous SNPs in at least one of the studied isolates. Of those genes, 55 (66.3%) showed the same nonsynonymous SNPs in all 22 studied isolates, 23 (25.3%) showed SNPs in just one of the isolates and 5 (6.0%) showed SNPs in at least 2 (but not all) of the studied isolates.

Table 2 shows the MICs obtained for the panel of antibiotics tested and a selection of the main mutations and genes known or expected to be involved in the resistance profiles, mainly including penicillin-β-lactamase inhibitor combinations, cephalosporins, monobactams, carbapenems, aminoglycosides (gentamicin and tobramycin), and fluoroquinolones.

AmpC-driven resistance. All except one (PAmb75) of the 22 studied isolates showed *ampC* overexpression (mean 263.6 ± 215.8-fold higher respect wild-type

PAO1). *ampC* overexpression correlated well with ceftazidime and piperacillin-tazobactam resistance, except for isolate PAmb258 (from the bloodstream infections multicenter study) which was susceptible to these antibiotics. A possible explanation is the presence of a nonsense mutation in *mexA* (Q183X), component of the constitutive efflux pump MexAB-OprM, well known to play a major role in intrinsic resistance to many antibiotics including most β-lactams (36). Consistently with this observation, isolate PAmb258 showed also increased susceptibility to aztreonam and cefepime. Regarding the genetic basis of *ampC* overexpression, all of the 14 isolates from the bloodstream infections multicenter study contained the G154R *ampR* mutation previously demonstrated to cause the hyperproduction of AmpC (18). Moreover, the *ampR* mutation was also evidenced in the four recent isolates from Hospital Son Espases, and in 2 of the 4 French ST175 isolates. The other 2 French isolates, also showing *ampC* overexpression, contained a nonsynonymous mutation in *ampD*. Moreover, 2 isolates from the bloodstream infections multicenter study showed and additional nonsynonymous SNPs in *ampD* or *dacB* (PBP4). Additionally, as shown in Table 1, all 22 isolates shared nonsynonymous SNPs in genes recently related to *ampC* expression and β-lactam resistance such as *nuoG*, *mltB1*, *mltD*, *creB*, *creC* or *creD* (37, 38, 39), but in all cases these SNPs were documented in other available *P. aeruginosa* genomes suggesting that they are natural polymorphism not involved in resistance (www.pseudomonas.com). However, the role in *ampC* expression and β-lactam resistance of unique nonsynonimous SNPs in *sltB1*, *creB*, *creC*, *ampP* or *ampG*, detected in single isolates (Table 1), needs to be explored in future studies.

Role of porins. Carbapenem resistance correlated well with the presence of nonsense mutations in *oprD* (encoding the porin for basic aminoacids and carbapenems OprD), evidenced in all isolates except the single one that was imipenem susceptible

(Table 2). All imipenem resistant Spanish isolates, and one of the French isolates, contained the previously described Q142X mutation in *oprD* (18); the 3 remaining French isolates showed an alternative *oprD* mutation, W138X. Low meropenem MICs were also seen in the isolate showing wild-type *oprD* sequence, and, in agreement with its substrate profile (36) in the isolate showing a nonsense mutation in *mexA* (Table 2). Finally, as shown in Table 1, a single isolate contained a nonsense mutation in the porin OprF, although its contribution to antimicrobial resistance is still uncertain (40).

Contribution of PBP mutations to the resistance profile. Another potentially relevant target for β-lactam resistance are the PBPs. *pbpC* (PBP3A), *ponA* (PBP1A) and *pbpG* (PBP7) showed (the same) nonsynonimous SNPs in all 22 studied isolates (Table 1) and thus they might just well be natural polymorphisms seen in ST175 lineage not affecting the function of these PBPs. Indeed, most of these SNPs were documented in other available *P. aeruginosa* genomes (www.pseudomonas.com). On the other hand, *ftsI* (PBP3) mutations emerged independently in three different ST175 isolates (Table 1, Table 2). These findings suggest that PBP3 is under strong mutational pressure, consistently with recent data showing that PBP3 mutations emerge frequently during the course of CF chronic infection (41). Likewise, recent studies show that PBP3 mutations emerge frequently upon meropenem exposure in vitro (42). The documented R504C mutation support these findings, since (i) it was detected in the isolate showing the highest level of resistance to meropenem, (ii) it is located close to the β-lactam binding site (43), and (iii) mutations in this precise residue have been shown to be selected upon meropenem exposure in vitro (42). However, the involvement of the other two mutations in resistance is less obvious, both at phenotypic and structural level. Finally, one isolate (PAmb440) showed a unique nonsynonymous substitution in PBP1B (R746H). The effect of this mutation still needs to be experimentally addressed, but it is

located in a highly conserved residue and is documented in the isolate showing overall highest resistance to cefalosporins, penicillins and monobactams (Table 2). The effect, if any, of a unique mutation in PBP5 documented in one of the isolates is uncertain (Table 2), although PBP5 has been shown to contribute to some extent through its role in AmpC induction, β-lactam trapping and/or β-lactamase activity (44, 45).

Involvement of efflux pumps. Another major relevant aspect to consider when analyzing *P. aeruginosa* resistance profiles is the expression of efflux pumps. Interestingly, all 22 isolates showed the previously identified G195E substitution in MexZ (18). Indeed, this substitution has been previously demonstrated to be involved in MexXY overexpression (46), as seen in all studied isolates (12.9 ± 11.0-fold higher with respect to PAO1). According to existing literature, the unique mutations, found in single isolates, in ParS, AmgS or AmgR could also modulate MexXY expression (46). MexXY overexpression is well known to contribute to cefepime and aminoglycoside resistance (47), although the profiles documented for the latter agents are largely caused by aminoglycoside modifying enzymes (see below). The four French and two of the Spanish isolates showed nonsynonymous SNPs in *mexX* (W358R); whether this mutation affects the substrate profile needs to be further investigated. As commented above, one isolate was found to harbor a nonsense mutation in *mexA*, associated with hypersusceptibility to most β-lactams due to the impairment of MexAB-OprM efflux pump. On the other hand, all 22 studied isolates showed two nonsynonimous mutations the MexAB-OprM regulator *nalC* (G71E and A186T) (Table 1). However, these substitutions are likely polymorphisms because they are frequent among diverse available *P. aeruginosa* genomes (www.pseudomonas.com) and because overall *mexB* expression was not much different among ST175 isolates compared to wild-type PAO1 (2.4±1.4). Finally, a number of isolates showed unique or common substitutions in other

efflux pump components, including MexCD-OprJ or MexEF-OprN (Table 1), but overexpression of either efflux pumps was not documented in any of the isolates (not shown).

Mutations leading to fluoroquinolone resistance. All eighteen Spanish isolates studied showed high-level fluoroquinolone resistance (ciprofloxacin MICs 32-128 mg/L) and contained 3 previously described QRDR mutations: T83I and D87N in *gyrA* and S87W in *parC*. However, the four French isolates showed a slightly lower resistance level (ciprofloxacin MICs 8-16 mg/L) and lacked the D87N mutation in *gyrA* (Table 2).

Horizontally acquired resistance elements-aminoglycoside modifying enzymes. The presence of horizontally-acquired resistance determinants was explored through PCR amplification of the most relevant ESBL and carbapenemase genes and by using the Resfinder tool on WGS. While genes coding for horizontally-acquired β-lactamases were ruled out in all the strains, the 22 isolates harbored an *aadB* gene coding for an adenyltransferase previously shown to be responsible of the aminoglycoside resistance profile (resistant to gentamicin and tobramycin and susceptible to amikacin) of ST175 isolates (18). Moreover,the four French isolates and the 2 isolates from one of the Spanish regions (Cantabria) harbored and additional adenyltransferase gene *aadA13*, known to be involved in streptomycin resistance (48). Indeed, streptomycin MICs in isolates positive for *aadA13* were >64 mg/L whereas in those lacking this gene the MIC were ≤8 mg/L. In all isolates *aad* genes were located in a class 1 integron, containing either *aadB* alone or both *aadB* and *aadA13*.

Mechanisms of fosfomycin resistance. All studied isolates except the four French isolates and the two isolates from Cantabria showed a nonsynonimous SNP

(T211P) in *glpT*, coding for a glycerol-3-phospate permease known to be involved in *P. aeruginosa* fosfomycin resistance (49). Indeed, as shown in Table 2, the presence of the mutation was associated with extremely high fosfomycin MICs (>1024 mg/L) whereas those strains lacking the mutation showed much lower MICs (8-16 mg/L).

Concluding remarks. In the last few years WGS has consolidated as a highly useful tool for the investigation of the molecular epidemiology and genetic basis of resistance in MDR pathogens, including *P.aeruginosa*. For instance, Kos *et al* have recently analyzed the correlation between the resistome and the phenotype of resistance to three antibiotics in a large collection of diverse *P. aeruginosa* strains (50). Likewise, a recent study by Turton *et al* have used WGS to analyze the molecular epidemiology and evolution of the MDR/XDR MBL-producing high-risk clone ST111 in UK (51). In this work we have analyzed through WGS the molecular epidemiology and the resistome of the international MDR/XDR high-risk clone ST175. Indeed, the resistome of ST175 was found to be mainly determined by mutational events, with resistance traits ranging from those common to all (e.g. specific QRDR and *mexZ* mutations) or most (e.g. specific *ampR* and *oprD* mutations) of the studied isolates from Spain and France, to those found in certain geographic areas (e.g. *glpT* mutation) or unique strains (e.g. PBP3 mutations). Phylogenetic analysis revealed two distinct clades, one containing the isolates from France and Cantabria and the other one all remaining isolates from Spain; at least two clear resistance features separated the French-Cantabria clade from the other one, the presence of the streptomycin resistance gene *aadA13* and the absence of a specific GlpT (fosfomycin resistance) mutation. Moreover, we analyzed the WGS available for the nine ST175 isolates from the Kos *et al* collection (50), recovered from Spain, France, Germany and Italy, and confirmed the existence of common and variable ST175 resistance mutations. Likewise, comparative genome

analysis of our isolates with that of a ST175 strain that produced a large outbreak in a hospital from Madrid (19, 52) revealed the same QRDR and *mexZ* mutations, despite in this case, instead of the β-lactam resistance mutations (*oprD* and *ampR*), the strain harbored a VIM-2 MBL. Thus, tracking down these mutations provides valuable information for understanding both, the genetic basis of resistance and the dynamics of dissemination and evolution of MDR/XDR strains. Much further work is however needed to understand the basis for the global success of this and other high-risk clones. Recent works have suggested that high-risk clones are associated with defined biological markers, including reduced fitness, motility (Swimming, swarming and twitching), and pigment production but increased biofilm formation and mutation rates (53). Thus, ongoing WGS analysis will add valuable data to correlate genotype with phenotype and to decipher the underlying factors driving the global success of high-risk clones.

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References

1. **Gellatly SL, Hancock RE.** 2013. *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. Pathog Dis. **67:**159-73.
2. **Silby MW, Winstanley C, Godfrey SA, Levy SB, Jackson RW.** 2011. *Pseudomonas* genomes: diverse and adaptable. FEMS Microbiol Rev. **35:**652-80.
3. **Vincent JL.** 2003. Nosocomial infections in adult intensive-care units. Lancet. **361:**2068-77.
4. **López-Causapé C, Rojo-Molinero E, Macià MD, Oliver A.** 2015. The problems of antibiotic resistance in cystic fibrosis and solutions. Expert Rev Respir Med. **9:**73-88.
5. **Mesaros N, Nordmann P, Plésiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F.** 2007. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect. **13:**560-78.
6. **Peña C, Suarez C, Gozalo M, Murillas J, Almirante B, Pomar V, Aguilar M, Granados A, Calbo E, Rodríguez-Baño J, Rodríguez F, Tubau F, Martínez-Martínez L, Oliver A; Spanish Network for Research in Infectious Diseases REIPI.** 2012. Prospective multicenter study of the impact of carbapenem resistance on mortality in *Pseudomonas aeruginosa* bloodstream infections. Antimicrob Agents Chemother. **56:**1265-72.

7. **Lister PD, Wolter DJ, Hanson ND.** 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev. **22:**582-610.
8. **Livermore DM.** 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin Infect Dis. **34:**634-40.
9. **Poole K. 2011.** *Pseudomonas aeruginosa*: resistance to the max. Front Microbiol. **5;**2:65.
10. **Martínez JL, Baquero F.** 2002. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev. **15:**647-79.
11. **Andersson DI, Hughes D.** 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol. **8:**260-71.
12. **Balasubramanian D, Schneper L, Kumari H, Mathee K.** 2013. A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. Nucleic Acids Res. **41:**1-20.
13. **Gooderham WJ, Hancock RE.** 2009. Regulation of virulence and antibiotic resistance by two-component regulatory systems in *Pseudomonas aeruginosa*. FEMS Microbiol Rev. **33:**279-94.
14. **Baquero F.** 2004. From pieces to patterns: evolutionary engineering in bacterial pathogens. Nat Rev Microbiol. **2:**510-8.
15. **Beceiro A, Tomás M, Bou G.** 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev. **26:**185-230.

16. **Woodford N, Turton JF, Livermore DM.** 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev. **35:**736-55.

17. **Oliver A, Mulet X, López-Causapé C, Juan C.** 2015. The increasing threat of *Pseudomonas aeruginosa* high-risk clones. Drug Resist Updat. **21-22:**41-59.

18. **Cabot G, Ocampo-Sosa AA, Domínguez MA, Gago JF, Juan C, Tubau F, Rodríguez C, Moyà B, Peña C, Martínez-Martínez L, Oliver A; Spanish Network for Research in Infectious Diseases (REIPI).** 2012. Genetic markers of widespread extensively drug-resistant *Pseudomonas aeruginosa* high-risk clones. Antimicrob Agents Chemother. **56:**6349-57.

19. **Viedma E, Juan C, Villa J, Barrado L, Orellana MA, Sanz F, Otero JR, Oliver A, Chaves F.** 2012. VIM-2-producing multidrug-resistant *Pseudomonas aeruginosa* ST175 clone, Spain. Emerg Infect Dis. **18:**1235-41.

20. **García-Castillo M, Del Campo R, Morosini MI, Riera E, Cabot G, Willems R, van Mansfeld R, Oliver A, Cantón R.** 2011. Wide dispersion of ST175 clone despite high genetic diversity of carbapenem-nonsusceptible *Pseudomonas aeruginosa* clinical strains in 16 Spanish hospitals. J Clin Microbiol. **49:**2905-10.

21. **Cholley P, Thouverez M, Hocquet D, van der Mee-Marquet N, Talon D, Bertrand X.** 2011. Most multidrug-resistant *Pseudomonas aeruginosa* isolates from hospitals in eastern France belong to a few clonal types. J Clin Microbiol. **49:**2578-83.

22. **Peña C, Cabot G, Gómez-Zorrilla S, Zamorano L, Ocampo-Sosa A, Murillas J, Almirante B, Pomar V, Aguilar M, Granados A, Calbo E, Rodríguez-Baño J, Rodríguez-López F, Tubau F, Martínez-Martínez L,**

Oliver A; Spanish Network for Research in Infectious Diseases (REIPI). 2015. Influence of virulence genotype and resistance profile in the mortality of Pseudomonas aeruginosa bloodstream infections. Clin Infect Dis. **60:**539-48.

23. **Clinical and Laboratory Standards Institute (CLSI).** 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. CLSI Document M07-A10. ISBN 1-56238-988-2
24. **Curran B, Jonas D, Grundmann H, Pitt T and Dowson CG.** 2004. Development of a Multilocus Sequence Typing Scheme for the Opportunistic Pathogen *Pseudomonas aeruginosa*. J Clin Microbiol. **42:**5644–5649.
25. **Cabot G, Ocampo-Sosa AA, Tubau F, Macia MD, Rodríguez C, Moya B, Zamorano L, Suárez C, Peña C, Martínez-Martínez L, Oliver A; Spanish Network for Research in Infectious Diseases (REIPI).** 2011. Overexpression of AmpC and efflux pumps in *Pseudomonas aeruginosa* isolates from bloodstream infections: prevalence and impact on resistance in a Spanish multicenter study. Antimicrob Agents Chemother. **55:**1906-11.
26. **Langmead B, Trapnell C, Pop M, Salzberg SL.** Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol **10:**R25.
27. **Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R and 1000 Genome Project Data Processing Subgroup.** 2009. The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics, **25:**2078-9.
28. **McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA.** 2010. The

Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Research. **20:**1297-303.

29. **Zerbino DR, and Birney E.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Research. **18:**821-829.

30. **Treangen TJ, Ondov BD, Koren S, Phillippy AM.** 2014. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biology. **15**, 1-15

31. **Drummond AJ, Suchard MA, Xie D & Rambaut A.** 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7 Molecular Biology And Evolution. **29:**1969-1973

32. **Marvig RL, Johansen HK, Molin S, Jelsbak L.** 2013. Genome analysis of a transmissible lineage of *Pseudomonas aeruginosa* reveals pathoadaptive mutations and distinct evolutionary paths of hypermutators. PLoS Genet. e1003741.

33. **Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM.** 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain. Fly (Austin). **6:**80-92.

34. **McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJ, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD.** 2013. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother. **57:**3348-57.

35. **Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV.** 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. **67**:2640-4.

36. **Li XZ, Nikaido H, Poole K.** 1995. Role of mexA-mexB-oprM in antibiotic efflux in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. **39:**1948-53.

37. **Cavallari JF, Lamers RP, Scheurwater EM, Matos AL, Burrows LL.** 2013. Changes to its peptidoglycan-remodeling enzyme repertoire modulate β-lactam resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. **57:**3078-84.

38. **Lamers RP, Nguyen UT, Nguyen Y, Buensuceso RN, Burrows LL.** 2015. Loss of membrane-bound lytic transglycosylases increases outer membrane permeability and β-lactam sensitivity in *Pseudomonas aeruginosa*. Microbiologyopen. **4:**879-95.

39. **Moya B, Dötsch A, Juan C, Blázquez J, Zamorano L, Haussler S, Oliver A.** 2009. Beta-lactam resistance response triggered by inactivation of a nonessential penicillin-binding protein. PLoS Pathog. **5:**e1000353

40. **Bratu S, Landman D, Gupta J, Quale J.** 2007. Role of AmpD, OprF and penicillin-binding proteins in beta-lactam resistance in clinical isolates of *Pseudomonas aeruginosa*. J Med Microbiol. **56:**809-14.

41. Diaz Caballero J, Clark ST, Coburn B, Zhang Y, Wang PW, Donaldson SL, **Tullis DE, Yau YC, Waters VJ, Hwang DM, Guttman DS.** 2015. Selective Sweeps and Parallel Pathoadaptation Drive *Pseudomonas aeruginosa* Evolution in the Cystic Fibrosis Lung. MBio. **6:**e00981-15.

42. **Cabot G, Zamorano L, Moyà B, Juan C, Navas A, Blázquez J, Oliver A.** 2016. Evolution of *Pseudomonas aeruginosa* Antimicrobial Resistance and

Fitness under Low and High Mutation Rates. Antimicrob Agents Chemother. **60:**1767-78.

43. **Han S, Zaniewski RP, Marr ES, Lacey BM, Tomaras AP, Evdokimov A, Miller JR, Shanmugasundaram V.** 2010. Structural basis for effectiveness of siderophore-conjugated monocarbams against clinically relevant strains of *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA. **107:**22002-7.
44. **Ropy A, Cabot G, Sánchez-Diener I, Aguilera C, Moya B, Ayala JA, Oliver A.** 2015. Role of *Pseudomonas aeruginosa* low-molecular-mass penicillin-binding proteins in AmpC expression, β-lactam resistance, and peptidoglycan structure. Antimicrob Agents Chemother. **59:**3925-34.
45. **Smith JD, Kumarasiri M, Zhang W, Hesek D, Lee M, Toth M, Vakulenko S, Fisher JF, Mobashery S, Chen Y.** 2013. Structural analysis of the role of *Pseudomonas aeruginosa* penicillin-binding protein 5 in β-lactam resistance. Antimicrob Agents Chemother. **57:**3137-46.
46. **Guénard S, Muller C, Monlezun L, Benas P, Broutin I, Jeannot K, Plésiat P.** 2014. Multiple mutations lead to MexXY-OprM-dependent aminoglycoside resistance in clinical strains of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. **58:**221-8.
47. **Hocquet D, Nordmann P, El Garch F, Cabanne L, Plésiat P.** 2006. Involvement of the MexXY-OprM efflux system in emergence of cefepime resistance in clinical strains of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. **50:**1347-51.
48. **Fling ME, Kopf J, Richards C.** 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase. Nucleic Acids Res. **13:**7095-106.

49. **Castañeda-García A, Rodríguez-Rojas A, Guelfo JR, Blázquez J.** 2009. The glycerol-3-phosphate permease GlpT is the only fosfomycin transporter in *Pseudomonas aeruginosa*. J Bacteriol. **191:**6968-74.

50. **Kos VN, Déraspe M, McLaughlin RE, Whiteaker JD, Roy PH, Alm RA, Corbeil J, Gardner H.** 2015. The resistome of *Pseudomonas aeruginosa* in relationship to phenotypic susceptibility. Antimicrob Agents Chemother. **59:**427-36.

51. **Turton JF, Wright L, Underwood A, Witney AA, Chan YT, Al-Shahib A, Arnold C, Doumith M, Patel B, Planche TD, Green J, Holliman R, Woodford N.** 2015. High-Resolution Analysis by Whole-Genome Sequencing of an International Lineage (Sequence Type 111) of *Pseudomonas aeruginosa* Associated with Metallo-Carbapenemases in the United Kingdom. J Clin Microbiol. **53:**2622-31.

52. **Viedma E, Juan C, Otero JR, Oliver A, Chaves F**. 2013. Draft Genome Sequence of VIM-2-Producing Multidrug-Resistant *Pseudomonas aeruginosa* ST175, an Epidemic High-Risk Clone. Genome Announc. **1**:e0011213

53. **Mulet X, Cabot G, Ocampo-Sosa AA, Domínguez MA, Zamorano L, Juan C, Tubau F, Rodríguez C, Moyà B, Peña C, Martínez-Martínez L, Oliver A; Spanish Network for Research in Infectious Diseases (REIPI).** 2013. Biological markers of *Pseudomonas aeruginosa* epidemic high-risk clones. Antimicrob Agents Chemother. **57:**5527-35.

548 **Legends**

549 **Figure 1.** *Core-genome* phylogenetic tree, based on SNPs analysis by default
550 parameters of parSNPs. Isolates from the Spanish multicenter study are labeled by:
551 Sample name-Hospital number (H1-H8)-city-year of isolation. French isolates are
552 labeled by: Sample name – France – year of isolation.

553 **Figure 2.** Bayesian evolutionary analysis sampling trees (BEAST) analysis of ST175
554 isolates from Spain and France calculated using a relaxed molecular clock. Isolates from
555 the Spanish multicenter study are labeled by: Sample name – Hospital number (H1-H8)-
556 city-year of isolation. French isolates are labeled by: Sample name – France – year of
557 isolation. The horizontal position of isolates on the tree is according to the date of
558 isolation. Calculated times for putative common ancestors (at the nodes) can be read
559 from the scale (in years). Time zero is that of the most recent isolate (2015).

560

Table 1. Nonsynonymous SNPs detected for the 146 genes related to antibiotic resistance in the 22 ST175 studied isolates using PAO1 as reference genome

Gene Locus	Name	SNP (number of isolates)	Gene Locus	Name	SNP (number of isolates)
PA0018	***fmt***	S54N (22)	**PA2642**	***nuoG***	A433T (22), T484A (22)
PA0058	***dsbM***	G32C (22)	**PA2798**		S280C (22), E297D (22), G301S (22), T317S (22), S353L (22)
PA0425	***mexA***	Q183X (1)	**PA2809**	***copR***	F25L (1)
PA0426	***mexB***	V45L (1)	**PA2810**	***copS***	N97S (22), R130Q (22)
PA0427	***oprM***	R404H (1)	**PA3047**	***dacB***	P59S (1)
PA0463	***creB***	E128G (22), N152T (1)	**PA3078**	***cprS***	I348M (1)
PA0464	***creC***	G157A (22), S437C (1), Q457L (22)	**PA3168**	***gyrA***	T83I (22), D87N (18)
PA0465	***creD***	V394A (22)	**PA3677**	***mexJ***	F13L (22)
PA0486		D210E (22), D258E (22), L313Q (1)	**PA3678**	***mexL***	S4P (22)
PA0487		R118C (1), H191Q (22)	**PA3721**	***nalC***	G71E (22), A186T (22)
PA0610	***prtN***	S4T (22), S8T (22)	**PA3999**	***dacC***	D308E (1)
PA0611	***prtR***	L16H (1), A75T (22), G251A (22)	**PA4001**	***sltB1***	D225E (1)
PA0807	***ampDh$_3$***	A219T (22)	**PA4109**	***ampR***	G154R (19)
PA0869	***pbpG***	S250N (22)	**PA4119**	***aph***	A42V (22)
PA0958	***oprD***	Q142X (18), W138X (3)	**PA4207**	***mexI***	A561V (1), A603S (22)
PA1343	***pagP***	G149A (22)	**PA4218**	***ampP***	S329A (1)
PA1345	***gshB***	L17P (22), A460T (1)	**PA4270**	***rpoB***	V51I (22)
PA1375	***pdxB***	H71Q (1), Q365R (22)	**PA4381**	***colR***	D69Y (1)
PA1409	***aphA***	L62I (22), E236X (1)	**PA4393**	***ampG***	A583T (22)
PA1777	***oprF***	E149X (1)	**PA4418**	***ftsI***	W13R (1), D40V (1), R504C (1)
PA1796	***folD***	G159V (22)	**PA4444**	***mltB1***	H64R (22), Q303R (22)
PA1797		N171S (22), H195Y (22), L575Q (22)	**PA4462**	***rpoN***	R257W (1)
PA1798	***parS***	H398R (22)	**PA4522**	***ampD***	H77Y (2), T139M (1), G148A (22), N183Y (22)
PA1799	***parR***	E214D (1)	**PA4597**	***oprJ***	M69V (22), Y91X (1)
PA1812	***mltD***	T466P (22)	**PA4598**	***mexD***	E257Q (22), S845A (22), A963V (1)
PA1886	***polB***	D176G (22)	**PA4599**	***mexC***	E26A (22), H310R (22), S330A (22), P383S (22)
PA2006		E232Q (22)	**PA4700**	***mrcB***	R746H (1)
PA2018	***mexY***	T543A (22)	**PA4748**	***tpiA***	E21V (1)
PA2019	***mexX***	K329Q (22), W358R (7)	**PA4773**		E21X (1)
PA2020	***mexZ***	G195E (22)	**PA4774**		H208N (1)
PA2023	***galU***	K274N (1)	**PA4777**	***pmrB***	Y345H (22), M356T (1)
PA2050		C142G (22)	**PA4964**	***parC***	S87W (22), L168Q (22)
PA2272	***pbpC***	E37K (22), A104P (22)	**PA5000**	***wapR***	T85A (22)
PA2489		A244T (22)	**PA5038**	***aroB***	V85A (22), A200E (22)
PA2490		T48S (22), R104C (22)	**PA5045**	***ponA***	E245D (22)
PA2491	***mexS***	D249N (22)	**PA5199**	***amgS***	R389S (1)
PA2492	***mexT***	P60S (22), F172I (22)	**PA5200**	***amgR***	Q180X (1)
PA2494	***mexF***	S65Y (1), L799V (1), T1003S (1)	**PA5235**	***glpT***	T211P (16)
PA2525	***opmB***	S139R (1), N311K (1), P495S (22)	**PA5297**	***poxB***	N243T (1)
PA2526	***muxC***	D716N (1)	**PA5366**	***pstB***	Q20H (1)
PA2528	***muxA***	T261A (22), V420M (22)	**PA5471**	***armZ***	L88P (22), D161G (22), H182Q (22), V243A (22), V266M (22)
PA2615	***ftsK***	S52P (22), S287P (22), G729S (22)			

Table 2. Susceptibility profiles and antibiotic resistance genes and mutations detected in the studied collection of ST175 isolates.

Strain	MIC[a]													*ampC* hyperproduction[b]	*oprD*	PBPs[b]	MexAB/XY-OprM[b]	QRDR	*glpT*	Aminoglycoside Modifying Enzymes
	CAZ (S≤8)	FEP (S≤8)	AZT (S≤8)	PTz (S≤16)	IMI (S≤4)	MER (S≤4)	GEN (S≤4)	TOB (S≤4)	AMK (S≤16)	LEV (S≤2)	CIP (S≤1)	COL (S≤2)	FOS							
PAmb12	16	16	16	32	16	16	>64	16	4	64	32	0.125	>1024	*ampR* (G154R)	Q142X	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb27	64	128	32	64	8	64	>64	32	2	128	64	0.5	>1024	*ampR* (G154R)	Q142X	PBP3 (R504C)	mexB (V45L) + mexZ (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb43	64	32	16	128	32	16	>64	32	4	128	64	0.5	16	*ampR* (G154R) + *ampD* (T139M)	Q142X	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	-	*aadB* + *aadA13*
PAmb75	4	16	16	16	0.5	1	>64	32	4	128	64	0.25	16	-	-	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	-	*aadB* + *aadA13*
PAmb93	64	32	32	128	16	16	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	-	*mexX* (W358R) + *mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb123	32	32	32	128	16	32	>64	32	4	256	128	0.5	>1024	*ampR* (G154R) + *dacB* (P59S)	Q142X	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb147	32	32	16	64	32	16	>64	16	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb179	32	32	16	128	32	16	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	-	*mexZ* (G195E)	*gyrA1* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb193	32	16	16	64	16	8	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	PBP5 (D308E)	*mexD* (A963V) + *mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb207	32	16	16	64	16	16	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb258	4	4	2	8	32	1	>64	16	4	32	32	0.5	>1024	*ampR* (G154R)	Q142X	PBP3 (W13R)	*mexA* (Q183X) + *mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb440	128	64	64	256	32	8	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	PBP1B (R746H)	*mexX* (W358R) + *mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb482	32	32	16	128	64	32	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	PBP3 (D40V)	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
PAmb516	32	8	8	16	8	4	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
HUSE_F028	64	16	32	128	16	16	>64	16	4	64	64	0.5	>1024	*ampR* (G154R)	Q142X	-	*oprM* (R404H) + *mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
HUSE_F034	64	32	32	256	64	32	>64	16	2	64	32	0.125	>1024	*ampR* (G154R)	Q142X	-	*mexX* (W358R) + *mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*

HUSE_F036	16	16	16	64	64	16	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X		*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
HUSE_F041	16	16	16	64	64	16	>64	32	4	128	64	0.5	>1024	*ampR* (G154R)	Q142X	-	*mexZ* (G195E)	*gyrA* (T83I, D87N) + *parC* (S87W)	T211P	*aadB*
FRA_BOR	8	8	8	32	32	16	>64	32	4	16	16	0.5	8	*ampD* (H77Y)	W138X	-	*mexX* (W358R) + *mexZ* (G195E)	*gyrA* (T83I) + *parC* (S87W)	-	*aadB + aadA13*
FRA_CLI	8	8	8	64	32	16	>64	>32	4	8	8	0.5	16	*ampD* (H77Y)	W138X	-	*mexX* (W358R) + *mexZ* (G195E)	*gyrA* (T83I) + *parC* (S87W)	-	*aadB + aadA13*
FRA_DIJ	16	8	8	64	32	8	>64	32	4	16	16	0.125	16	*ampR* (G154R)	Q142X	-	*mexX* (W358R) + *mexZ* (G195E)	*gyrA* (T83I) + *parC* (S87W)	-	*aadB + aadA13*
FRA_BES	8	4	8	32	16	4	>64	16	4	16	16	0.5	8	*ampR* (G154R)	W138X	-	*mexX* (W358R) + *mexZ* (G195E)	*gyrA* (T83I) + *parC* (S87W)	-	*aadB + aadA13*

[a] CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; PTz, piperacillin-tazobactam; IMI, imipenem, MER, meropenem; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; LEV, levofloxacin, CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin.

[b] Except for the *mexZ* G195E substitution, changes respect PAO1 sequences documented in all 22 isolates are not included in this table