



Guidelines

Pseudomonas aeruginosa antimicrobial susceptibility profiles, resistance mechanisms and international clonal lineages: update from ESGARS-ESCMID/ISARPAE Group

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ABSTRACT

Scope: *Pseudomonas aeruginosa*, a ubiquitous opportunistic pathogen considered one of the paradigms of antimicrobial resistance, is among the main causes of hospital-acquired and chronic infections associated with significant morbidity and mortality. This growing threat results from the extraordinary capacity of *P. aeruginosa* to develop antimicrobial resistance through chromosomal mutations, the increasing prevalence of transferable resistance determinants (such as the carbapenemases and the extended-spectrum β -lactamases), and the global expansion of epidemic lineages. The general objective of this initiative is to provide a comprehensive update of *P. aeruginosa* resistance mechanisms, especially for the extensively drug-resistant (XDR)/difficult-to-treat resistance (DTR) international high-risk epidemic lineages, and how the recently approved β -lactams and β -lactam/ β -lactamase inhibitor combinations may affect resistance mechanisms and the definition of susceptibility profiles.

Methods: To address this challenge, the European Study Group for Antimicrobial Resistance Surveillance (ESGARS) from the European Society of Clinical Microbiology and Infectious Diseases launched the 'Improving Surveillance of Antibiotic-Resistant *Pseudomonas aeruginosa* in Europe (ISARPAE)' initiative in 2022, supported by the Joint programming initiative on antimicrobial resistance network call and included a panel of over 40 researchers from 18 European Countries. Thus, a ESGARS-ISARPAE position paper was designed and the final version agreed after four rounds of revision and discussion by all panel members.

Questions addressed in the position paper: To provide an update on (a) the emerging resistance mechanisms to classical and novel anti-pseudomonal agents, with a particular focus on β -lactams, (b) the susceptibility profiles associated with the most relevant β -lactam resistance mechanisms, (c) the impact of the novel agents and resistance mechanisms on the definitions of resistance profiles, and (d) the globally expanding XDR/DTR high-risk lineages and their association with transferable resistance mechanisms.

Implication: The evidence presented herein can be used for coordinated epidemiological surveillance and decision making at the European and global level. **Antonio Oliver, Clin Microbiol Infect 2024;30:469**

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Scope and context

Pseudomonas aeruginosa, a ubiquitous opportunistic pathogen considered one of the paradigms of antimicrobial resistance, is among the main causes of hospital-acquired and chronic infections associated with significant morbidity and mortality [1]. Accordingly, *P. aeruginosa* infections are estimated to be associated with over 300 000 annual deaths and are at the top of the WHO priority list for the need for research and development of new antibiotics [2,3]. This growing threat results from the extraordinary capacity of this pathogen to develop antimicrobial resistance through chromosomal mutations and from the increasing prevalence of transferable resistance determinants, particularly those encoding carbapenemases or extended-spectrum β -lactamases (ESBLs) [4,5]. Combinations of such mechanisms lead to concerning and complex resistance profiles, defined by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control

and Prevention as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR), whereas the Infectious Diseases Society of America/National Institutes of Health (IDSA/NIH) defines them as difficult-to-treat resistance (DTR) [6,7]. *P. aeruginosa* possesses a non-clonal epidemic population structure, comprising a limited number of widespread lineages, selected from a background of numerous rare and unrelated genotypes recombined at high frequency [8]. In fact, several surveys have provided evidence for the existence of XDR/DTR international high-risk clonal lineages, which have disseminated in hospitals worldwide [9–11]. Beyond classical molecular epidemiology analysis and phenotypic assessment of resistance mechanisms, whole-genome sequencing studies are providing pertinent information to elucidate the complex and evolving resistome of MDR/XDR/DTR *P. aeruginosa* high-risk lineages [12–15].

The recent introduction of novel β -lactam/ β -lactamase inhibitor combinations (BLBLIs), such as ceftolozane/tazobactam,

ceftazidime/avibactam, meropenem/vaborbactam or imipenem/relebactam, and the siderophore-cephalosporin cefiderocol, has contributed to mitigate, to some extent, the problem of XDR/DTR *P. aeruginosa* [16–19]. These agents exhibit enhanced stability against intrinsically and chromosomally encoded β -lactam resistance mechanisms in *P. aeruginosa*, such as overexpression of the AmpC β -lactamase encoding gene, overproduction of efflux pumps, or inactivation of the OprD porin. However, they are not exempt from resistance development through emerging mutational mechanisms [20–24]. These include modification (quantitative or qualitative) of AmpC hydrolytic activity or efflux pumps substrate specificity, which were observed shortly after their introduction into clinical practice. Moreover, BLBLs are not currently effective against the most potent transferable carbapenemases, particularly class B or metallo- β -lactamases (MBLs) such as VIM, IMP, or NDM enzymes [25]. Consequently, use of BLBLs could lead to the selection of these concerning resistance mechanisms [26]. Besides the approved options, several novel BLBLs are undergoing clinical trials [25]. These agents, such as aztreonam/avibactam, cefepime/zidebactam, or cefepime/taniborbactam, promise additional therapeutic choices and the ability to counteract already established resistance mechanisms [17].

The introduction of novel BLBLs is therefore significantly broadening the range of treatment options for XDR/DTR *P. aeruginosa* infections [17,25]. However, this expansion will also have a major impact on antimicrobial resistance epidemiology, including both novel and existing mutation-driven resistance mechanisms, transferable resistance determinants and epidemic high-risk clonal lineages. A comprehensive understanding of *P. aeruginosa* resistance mechanisms and susceptibility profiles, especially of the XDR/DTR high-risk lineages, and how these promising novel agents may affect resistance mechanisms and, in turn, the definition of resistance profiles, is needed to have a common ground and may help to anticipate and coordinate epidemiological information in the future.

Questions addressed in the position paper

To address this challenge, the European Study Group for Antimicrobial Resistance Surveillance (ESGARS) from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) launched the 'Improving Surveillance of Antibiotic-Resistant *Pseudomonas aeruginosa* in Europe' (ISARPAE) initiative in 2022, supported by the Joint programming initiative on antimicrobial resistance (JPIAMR) network. Thus, this position document from the ESGARS-ISARPAE Group aimed to provide an update on (a) the emerging resistance mechanisms to classical and novel anti-pseudomonal agents, with a particular focus on β -lactams, (b) the susceptibility profiles associated with the most relevant β -lactam resistance mechanisms, (c) the impact of the novel agents and resistance mechanisms on the definitions of resistance profiles, and (d) the globally expanding XDR/DTR high-risk lineages and their association with transferable β -lactamases.

Methods

All ESGARS-ESCMID members were contacted and invited to participate in the ISARPAE initiative, according to their interest and experience in the topic. This resulted in the generation of a panel of over 40 researchers from 18 European countries in June 2022. The panel agreed the above objectives to be addressed in the position paper and AO and ERM prepared a first draft of the document after extensive literature review helped by other panel members. In July 2023, the first draft of the document was sent to all ISARPAE members for revision and specific contributions, leading to a

second draft version that was extensively revised and discussed during an ISARPAE hybrid (onsite/online) meeting that took place at Hospital Son Espases-IdISBa (Mallorca, Spain) on 8 September 2023. The third resulting draft was then sent for review by panel members and final version of the document was approved on 6 October 2023.

Emerging resistance mechanisms to classical and novel anti-pseudomonal agents and associated susceptibility profiles

Table 1 shows the main categories and agents showing anti-pseudomonal activity, including those recently introduced and those that will be clinically available in the next few years, and presents the respective mutation-driven and horizontally acquired resistance mechanisms. On the other hand, Fig. 1 shows the susceptibility profiles associated with the most relevant β -lactam resistance mechanisms in *P. aeruginosa*.

Pseudomonas aeruginosa β -lactam resistance

Pseudomonas aeruginosa is intrinsically resistant to aminopenicillins, alone and combined with clavulanic acid, as well as to most of the older cephalosporins, notably including the third-generation cephalosporin cefotaxime, because of the production of an inducible AmpC β -lactamase [27]. Moreover, AmpC plays a major role in the basal resistance level (MIC) of *P. aeruginosa* to the potent AmpC inducer imipenem. On the other hand, the constitutive expression of the efflux pump MexAB-OprM plays a major role in the basal resistance level to most other β -lactams except imipenem.

The most frequent mutation-driven resistance mechanism to classical anti-pseudomonal penicillins (such as piperacillin) and cephalosporins (such as ceftazidime or cefepime) is the overproduction of the chromosomal cephalosporinase AmpC, involving a large number of genes belonging to cell-wall recycling regulatory pathways [28]. Notably, among these genes, the mutational inactivation of *dacB*, encoding the non-essential penicillin-binding protein (PBP) PBP4 and *ampD*, encoding a N-acetyl-muramyl-L-alanine amidase, have been found to be the most frequent cause of depressed *ampC* gene expression, and subsequent broad-spectrum β -lactam resistance [29,30]. In addition, specific point mutations causing a conformational change in the transcriptional regulator AmpR, leading to *ampC* upregulation and resistance to broad-spectrum β -lactams, have been noted among clinical strains. These mutations include the D135N amino acid replacement, described in several species [28] and the G154R mutation linked to the disseminated MDR/XDR ST175 high-risk lineage [14]. Mutation of several other genes, including those encoding amidases (AmpDh2 and AmpDh3), PBPs, such as PBP5 or PBP7, lytic transglycosylases, MPL, or NuoN, has also been shown to enhance *ampC* expression, either alone or in combination with other mutations. Nevertheless, their impact on β -lactam resistance among clinical strains still needs to be further analysed [28].

In addition to *ampC* overexpression, recent studies have revealed that increased levels of β -lactam resistance, involving the novel BLBLs ceftolozane/tazobactam and ceftazidime/avibactam, may result from mutations leading to the modification of the catalytic centre of AmpC, currently mainly occurring in (up to 10%–15%) patients treated with these agents [20,31–33]. Additional studies identified diverse AmpC variants associated with high-level resistance to BLBLs, including the above-mentioned ceftolozane/tazobactam and ceftazidime/avibactam, in a small proportion (around 1%) of clinical *P. aeruginosa* isolates [34]. Over 500 variants of those AmpC enzymes, also called *Pseudomonas*-derived cephalosporinases (PDC), have been described so far, including those

Table 1
Main resistance mechanisms to classical and novel antibiotics in *Pseudomonas aeruginosa*

Anti-pseudomonal categories	Anti-pseudomonal agents	Main mutational resistance mechanisms	Alternative mutational resistance mechanisms	Mutational resistance on horizontally acquired determinants	Horizontally acquired resistance mechanisms
Penicillins + β -lactamase inhibitors	Piperacillin/tazobactam	AmpC \uparrow	PBP3, GalU		ESBLs, class A and B carbapenemases
Cephalosporins	Ceftazidime	AmpC \uparrow	PBP3, GalU	OXA-2/10	ESBLs, class A and B carbapenemases
Monobactams	Cefepime	MexXY \uparrow , AmpC \uparrow	PBP3, GalU	OXA-2/10	ESBLs, class A and B carbapenemases
Carbapenems	Aztreonam	MexAB \uparrow , AmpC \uparrow	PBP3, GalU	OXA-2/10	ESBLs and class A carbapenemases
	Imipenem	OprD-	MexST, PBP2, PBP1a		Class A and B carbapenemases
	Meropenem	OprD-, MexAB \uparrow	PBP3, GalU		Class A and B carbapenemases
Fifth-generation cephalosporins + classical β -lactamase inhibitors	Ceftolozane/tazobactam	AmpC Ω -loop	PBP3, GalU Efflux pumps	OXA-2/10	ESBLs, class A and B carbapenemases
Cephalosporins + diazabicycloctanes β -lactamase inhibitors	Ceftazidime/avibactam	AmpC Ω -loop, MexAB \uparrow	PBP3, GalU	OXA-2/10, GES, KPC	Class B carbapenemases
Carbapenems + diazabicycloctanes β -lactamase inhibitors	Imipenem/relebactam	OprD-, MexAB \uparrow ^b	MexST, ParRS PBP2, PBP1a		Class A and B carbapenemases
Carbapenems + boronic acid β -lactamase inhibitors	Meropenem/vaborbactam	OprD-, MexAB \uparrow	PBP3, GalU		Class A and B carbapenemases
Siderophore cephalosporins	Cefiderocol	Iron transporters AmpC Ω -loop	PBP3, GalU	OXA-2/10 ^b	ESBLs, class A and B carbapenemases ^b
Monobactams + diazabicycloctanes β -lactamase inhibitors	Aztreonam/avibactam ^a	MexAB \uparrow	PBP3, GalU		ESBLs and class A carbapenemases ^b
Cephalosporins + diazabicycloctanes β -lactamase and PBP2 inhibitors	Cefepime/zidebactam ^a	MexXY \uparrow , MexAB \uparrow	PBP3, GalU PBP2		ESBLs, class A and B carbapenemases ^b
Cephalosporins + boronic acid β -lactamase inhibitors including MBLs	Cefepime/taniborbactam ^a	MexXY \uparrow , MexAB \uparrow	PBP3, GalU		IMPs
Fluoroquinolones	Ciprofloxacin, levofloxacin	QRDR	MexAB/XY/CD/EF \uparrow		Qnr
Aminoglycosides	Tobramycin, amikacin	MexXY \uparrow ^b	FusA1		Aminoglycoside modifying enzymes, 16S rRNA methylases
Polymyxins	Colistin, polymyxin B	PmrAB/PhoPQ/ParRS			MCR (Very uncommon)
Fosfonic acids	Fosfomicin	GlpT			FosA

ESBL, extended-spectrum β -lactamase; MBL, metallo- β -lactamase; QRDR, quinolone resistance-determining region.

\uparrow Hyperproduction.

^a Not yet approved.

^b Low-level resistance. Clinical resistance requires additional mechanisms.

Antibiotic	AmpC ↑	MexAB↑	OprD-	AmpC Ω-loop*	OXA ESBL	ESBL	CarbA	CarbA Mut**	CarbB	Iron transp.
Piperacillin/tazobactam	R	r	S	S/r	R	R	R	R	R	S
Ceftazidime	R	r	S	R	R	R	R	R	R	S
Cefepime	r/R	r/R	S	R	R	R	R	R	R	S
Aztreonam	r/R	R	S	R	r/R	R	R	R	S	S
Imipenem	S	S	r/R	S	S	S	R	S	R	S
Meropenem	S	r	r	S	S	S	R	S	R	S
Ceftolozane/tazobactam	S	S	S	R	R	r/R	R	R	R	S
Ceftazidime/avibactam	S/r	r	S	r/R	r/R	S/r	S	R	R	S
Meropenem/vaborbactam	S	r	r	S	S	S	r/R	S	R	S
Imipenem/relebactam	S	r	r	S	S	S	r/R	S	R	S
Cefiderocol	S	S	S	S/r	S/r	S/r	S/r	S/r	S/r	r
Aztreonam/avibactam	S	R	S	r/R	r/R	S/r	S/r	r/R	S	S
Cefepime/zidebactam	S	r/R	S	S/r	S/r	S/r	S/r	S/r	r/R	S
Cefepime/taniborbactam	S	r/R	S	S/r	S/r	S/r	S/r	S/r	r/R	S

Fig. 1. Antimicrobial spectrum expected for classical and novel β -lactams and β -lactam- β -lactamase inhibitor combinations against most relevant *P. aeruginosa* resistance mechanisms when present alone in clinical strains. To reduce complexity, combinations of resistance mechanisms are not considered, but acknowledged to be frequent among clinical strains. S (green), fully susceptible; r (orange), reduced susceptibility; R (red) clinical resistance. For some antibiotics-mechanisms combinations a range of effect S/r or r/R is considered depending on the specific mechanism or mutation; in such cases, the specific colour chosen was that of the most likely phenotype. It is noted, however, that variation in the quantitative effect on resistance does occur according to the specific nature of the mechanisms or their expression. *AmpC (PDC) variants associated with ceftolozane/tazobactam and/or ceftazidime/avibactam resistance. **KPC or GES mutations associated with ceftazidime/avibactam resistance and collateral carbapenem susceptibility.

associated with increased ceftolozane/tazobactam and ceftazidime/avibactam resistance. Moreover, some of these variants, such as those showing the L320P substitution, have a significant impact on cefiderocol MICs, but only a marginal effect on susceptibility to ceftolozane/tazobactam and ceftazidime/avibactam [35]. An updated database of PDC variants is maintained at IdISBa and is freely available at <https://arpbigidisba.com/pseudomonas-aeruginosa-derived-cephalosporinase-pdc-database/> and at the Beta-Lactamase Data Base (<http://www.blbd.eu/BLDB.php?prot=C#PDC>) [36]. Typically, the strains producing these AmpC variants show collateral susceptibility to imipenem (decreased MICs) and also to anti-pseudomonal penicillins such as piperacillin. In addition, resistance development to ceftolozane/tazobactam and/or ceftazidime/avibactam may involve mutations leading to the structural modification of narrow-spectrum OXA-2- and OXA-10-acquired oxacillinases [20,37,38]. Interestingly, these mutations may lead to collateral susceptibility to meropenem. Thus, imipenem/relebactam, and to a lesser extent, cefiderocol, meropenem/vaborbactam, and the novel combinations under development cefepime/zidebactam and cefepime/taniborbactam might be interesting options to treat infections by strains that have developed ceftolozane/tazobactam and/or ceftazidime/avibactam resistance through mutations in AmpC or OXA-2/10 [39].

Horizontally acquired β -lactamase genes are obviously a major source of resistance, including to the novel β -lactams and BLBLI (Fig. 1). An extensive revision of the nature and prevalence of the different horizontally acquired β -lactamases detected in *P. aeruginosa* is beyond the scope of this document. However, globally, MBLs are arguably the most frequent carbapenemases in *P. aeruginosa*, but very large geographical differences in prevalence and nature have been documented [40,41]. At European level, VIM, and particularly VIM-2, are likely the most frequently reported enzymes, but with major differences across different countries, and with an increasing prevalence of NDM enzymes [42,43]. Moreover, guiana extended-spectrum beta-lactamase (GES) class A

carbapenemases variants such as GES-5 are also increasingly reported in European countries [43,44]. Classical anti-pseudomonal penicillins, cephalosporins, and carbapenems lack significant activity and should be avoided against strains producing class A or MBL carbapenemases, even if MICs close to the clinical breakpoints are obtained for piperacillin/tazobactam, cefepime, or even carbapenems for some VIM-2-producing isolates [12]. Moreover, the production of MBLs is a frequent mechanism of resistance to ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam, and imipenem/relebactam [26]. However, with a few exceptions such as some NDM variants, cefiderocol retains activity because of its higher stability against hydrolysis and efficient uptake through the iron transport systems [45]. The combination of aztreonam with avibactam may also be a useful future alternative for MBL-producing strains, particularly when additionally hyper-producing AmpC and/or coproducing acquired class A enzymes [46,47]. Likewise, the novel combinations under development cefepime/zidebactam and cefepime/taniborbactam also remain active. The underlying mechanism for cefepime/zidebactam activity against MBL-producing strains is based on the fact that zidebactam has direct anti-pseudomonal activity by targeting PBP2, and therefore provides synergy with β -lactams targeting PBP3 such as the cephalosporins [48]. On the other hand, the activity of cefepime/taniborbactam relies on the fact that taniborbactam inhibits MBL hydrolytic activity, except for IMPs [49]. In addition to these three antimicrobials (cefiderocol, cefepime/zidebactam, and cefepime/taniborbactam), ceftazidime/avibactam, and to a lower extent imipenem/relebactam and meropenem/vaborbactam, show activity against producers of Ambler class A carbapenemases (such as GES-5 and klebsiella pneumoniae carbapenemase (KPC)s) [50–52]. However, the frequent concomitant OprD deficiency and/or MexAB-OprM overexpression limits the activity of imipenem/relebactam and meropenem/vaborbactam against clinical *P. aeruginosa* strains producing class A carbapenemases [52,53]. On the other hand, resistance development to ceftazidime/avibactam

caused by the selection of mutations within the catalytic site of KPC and GES enzymes has been described [54–56]. Interestingly, these mutations restore carbapenem susceptibility (if the strain is not oprD deficient) leading to an ESBL phenotype [54]. In addition to those of classes A and B, a few cases of class D carbapenemase production have been reported in *P. aeruginosa*, including the epidemic dissemination OXA-198 in a hospital from Belgium [57].

In addition to β -lactamases, there is growing evidence on the role of target modification in *P. aeruginosa* β -lactam resistance. Of particular relevance are the mutations in *ftsI*, encoding PBP3, an essential class B PBP with transpeptidase activity [58]. Indeed, data from patients with cystic fibrosis (CF) [59,60], epidemic high-risk clonal lineages [12,14] as well as from *in vitro* studies [61] have shown that PBP3 is under strong mutational pressure, with specific mutations in this PBP contributing to β -lactam resistance development. R504C/H and F533L mutations are those being most commonly reported and located within the protein domains implicated in the formation and stabilization of the inactivating complex β -lactam-PBP3 [62]. Moreover, these specific mutations have been documented to emerge *in vivo* during chronic respiratory infection in patients with CF [59,60] and upon exposure to meropenem [61], aztreonam [63], and ceftazidime [64] *in vitro*. However, the detailed effect of PBP3 mutations on β -lactam resistance phenotypes needs to be further investigated using isogenic strains. Likewise, despite unique polymorphisms having been detected in some clinical strains for other PBPs, their potential role in β -lactam resistance still needs to be experimentally determined. Also noteworthy are the specific PBP2 mutations involved in resistance to zidebactam [65], which obviate the β -lactam enhancer activity of this BLI.

Other relevant components of the β -lactam mutational resistome are the genes encoding OprD and efflux pumps. The inactivation of OprD is known to be the most frequent imipenem resistance mechanisms in *P. aeruginosa* [27,66]. OprD inactivation typically results from indels or nonsense mutations, including the Q142X mutation, characteristic of the widespread ST175 high-risk clonal lineage [14]. In addition, some amino acid replacements have been associated with OprD-driven resistance, particularly in the CF setting [67]. However, it should be noted that the presence of OprD inactivating mutations has also been identified in some carbapenem-susceptible isolates [68]. On the other hand, imipenem resistance may also result from repression of *oprD* caused by mutations in the MexEF-OprN efflux pump regulators (*mexS/T*) or the ParRS two-component system [69]. Overexpression of MexAB-OprM, caused by mutation of several genes involved in its regulation (*mexR*, *nalC*, or *nalD*) increases MICs of most β -lactams including meropenem but not imipenem, whereas overexpression of genes encoding MexXY (*mexZ*, *parRS*, *amgS* mutations) is involved in cefepime resistance [69].

Efflux pumps may also play a major role in resistance to the novel BLBLIs, not only because of their capacity to extrude the β -lactam components but, particularly, for their capacity to accommodate their partner β -lactamase inhibitor. Indeed MexAB-OprM overexpression plays a role in resistance to ceftazidime/avibactam, aztreonam/avibactam, cefepime/zidebactam, imipenem/relebactam, and meropenem/vaborbactam [65,70–72]. Likewise, MexXY overexpression should also impact cefepime combinations with zidebactam or taniborbactam [65]. Moreover, mutations leading to the modification of the substrate recognition domain of the efflux pump MexCD-OprJ have been shown to drive ceftolozane/tazobactam resistance development *in vivo* [23].

In addition, another potentially relevant mutational β -lactam resistance mechanism is the selection of large (up to 600 kb) deletions affecting specific parts of the chromosome [61,64]. Although the basis of the conferred resistance phenotype still needs to be

further clarified, these mutants can be recognized by the characteristic brown pigment (pyomelanine) caused by the deletion of one of the included genes, *hmgA*, coding for a homogentisate-1,2-dioxygenase. These deletions have been documented in both *in vitro* evolved β -lactam-resistant mutants and CF isolates [61,73]. However, the deletion of *hmgA* is not responsible for the resistance phenotype, which could be linked to the deletion of another of the affected genes, *galU*. This gene codes for a UDP-glucose pyrophosphorylase involved in the synthesis of the lipopolysaccharide (LPS) core. Indeed, analysis of transposon mutant libraries has revealed that inactivation of *galU* increases the MICs of ceftazidime and meropenem [74,75].

Lastly, specific cefiderocol resistance development mechanisms involve the selection of mutations in iron uptake systems, particularly in TonB-dependent receptors such as *piuA/piuC*, *pirA/pirR* or *ftpA* (pyochelin receptor) [35]. Among these, mutations seem to be particularly frequent in *piuC*, an iron-dependent oxygenase involved in the expression of the adjacent *piuA* (or its homolog *piuD* depending on the strain) iron receptor. On the other hand, mutations in the *ftpA* gene, despite being frequent, do not seem to have a direct significant impact on cefiderocol MICs, and thus selection might reflect adaptive mutations for growing in the presence of cefiderocol.

Pseudomonas aeruginosa aminoglycoside resistome

Primary aminoglycoside resistance is typically linked to the production of horizontally acquired aminoglycoside modifying enzymes, including acetyltransferases, adenylyltransferases, and phosphoryltransferases, frequently co-transferred with ESBLs or carbapenemases [76]. The specific pattern of aminoglycoside resistance depends on the specific enzymes involved, with amikacin showing an overall higher activity than tobramycin [77]. However, the more recently described transferable 16S rRNA methylases, which modify the cellular target of aminoglycosides, are further concerning because they confer resistance to all clinically available members of this antibiotic family and are also co-transferred with ESBLs or carbapenemases [78–80].

On the other hand, the development of resistance to aminoglycosides has been particularly linked to the overexpression of genes encoding the MexXY-OprM system upon some mutations in the regulatory machinery. Indeed, mutational overexpression of this pump, mainly caused by *mexZ*, *amgS*, or *parRS* mutations, is very frequent among clinical isolates, from both patients with CF and nosocomial infections [81,82]. Moreover, recent studies show that the epidemic high-risk clone ST175 hyperproduces MexXY because of a specific mutation in *mexZ* (G195E) [14]. However, recent data suggest that the aminoglycoside mutational resistome extends far beyond MexXY hyperproduction, and high-level resistance may result from the accumulation of multiple mutations. The involvement of several novel resistance determinants has been documented [83–85]. Among them is noteworthy *fusA1*, coding for the elongation factor G. Indeed, specific *fusA1* mutations have been linked to aminoglycoside resistance *in vitro* [4,85] and among clinical strains, particularly from patients with CF [4,60,86–88]. Moreover, the implication of *fusA1* mutations in aminoglycoside resistance has been demonstrated through site-directed mutagenesis [89].

Pseudomonas P. aeruginosa fluoroquinolone resistome

Fluoroquinolone resistance in *P. aeruginosa* is primarily driven by mutational mechanisms. The fluoroquinolone mutational resistome generally includes specific missense mutations in DNA gyrase (*gyrA* and/or *gyrB*) and topoisomerase IV (*parC* and/or *parE*)

quinolone resistance-determining regions [13,90]. High-level fluoroquinolone resistance in *P. aeruginosa* high-risk lineages is nearly universal, and typically involves combinations of mutations in GyrA T83 and ParC S87 [12]. Quinolone resistance-determining region mutations involved in fluoroquinolone resistance in CF might be more variable [60]. It is also well known that the mutational overexpression of efflux pumps modulates fluoroquinolone resistance (Table 1). Although the overexpression of MexAB-OprM and MexXY-OprM is globally frequent among clinical strains, its contribution to clinical fluoroquinolone resistance is likely to be modest [90]. On the other hand, the mutational overproduction of MexEF-OprN or MexCD-OprJ is associated with clinical fluoroquinolone resistance. Although their prevalence has been considered low, except in the settings of CF chronic infections, recent data show that it might be higher than expected [67]. Lastly, the transferable quinolone resistance determinant QnrVC has also been reported, linked to some epidemic strains producing acquired carbapenemases such as ST175 and ST244 [91,92].

Pseudomonas aeruginosa polymyxin resistome

Because of its limited efficacy, toxicity, and high epidemiological cut-offs (ECOFF) values (4 mg/L), colistin is not considered an optimal treatment for wild-type *P. aeruginosa*, at least in monotherapy (www.eucast.org). Moreover, whereas the prevalence of polymyxin (colistin and polymyxin B) resistance is still globally low (<5%), it has increased in the last years because of the frequent use of these last-resource antibiotics for the treatment of MDR/XDR/DTR nosocomial and CF isolates, particularly in countries with no access to novel BLBLIs [93]. Polymyxin resistance results most frequently from the modification of the LPS caused by the addition of a 4-amino-4-deoxy-L-arabinose moiety in the lipid A structure [94,95]. The involved mutations are frequently located in the PmrAB or PhoPQ two-component regulators, which lead to the activation of the *arnBCADTEF* operon [96]. More recent studies have revealed that mutations in the ParRS two-component regulator not only produce polymyxin resistance because of the activation of the *arnBCADTEF* operon, but also lead to a MDR phenotype determined by the hyperproduction of MexXY and the repression of *oprD* [97]. Moreover, two additional two-component regulators, ColRS and CprRS, have also been determined to be involved in colistin resistance [98]. The analysis of colistin resistance mechanisms among clinical strains is not always straightforward, because the presence of mutations in these two-component regulators is not always associated with clinical colistin resistance, probably denoting partial complementation between the different regulators [60,98,99]. Moreover, recent *in vitro* evolution assays have revealed the implication of additional mutations in high-level colistin resistance, facilitated by the emergence of *mutS*-deficient mutator (phenotypes such as those occurring in LptD, LpxC, or MigA) [100]. On the other hand, the role of phosphoethanolamine modification of LPS in *P. aeruginosa* seems marginal, including both, that are driven by intrinsic *eptA* gene expression [101] and that are driven by transferable determinants [102].

Pseudomonas aeruginosa fosfomycin resistome

Although not classified as an anti-pseudomonal agent (ECOFF of 256 mg/L), fosfomycin has been considered in the last decade as a potentially useful antibiotic in urinary tract infections and as combined therapy for MDR/XDR/DTR *P. aeruginosa* in other infection sites [103]. However, spontaneous mutation rates for fosfomycin resistance are high and the mechanism involved is typically the mutational inactivation of *glpT*, coding for a glycerol-3-phosphate permease required for fosfomycin uptake [104,105].

Mutations in *glpT* are also frequently found among MDR/XDR/DTR strains [106]. Certain specific mutations, such as T211P, have become fixed in some widespread lineages as described for ST175 [14].

Definitions of resistance profiles in *Pseudomonas aeruginosa*

According to established recommendations by ECDC [6], the MDR profile is defined as resistance to at least one agent in at least three of eight antibiotic categories. These categories include anti-pseudomonal penicillins + β -lactamase inhibitor combinations (ticarcillin/clavulanate, piperacillin/tazobactam), anti-pseudomonal cephalosporins (ceftazidime and cefepime), monobactams (aztreonam), anti-pseudomonal carbapenems (imipenem, meropenem, doripenem), fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (gentamicin, tobramycin, amikacin, netilmicin), polymyxins (colistin, polymyxin B), and fosfonic acids (fosfomycin). The XDR profile is defined as resistance to at least one agent in all antibiotic classes except one or two. Likewise, PDR profile is defined as resistance to all agents in the eight antibiotic categories. The eighth category (fosfonic acids, fosfomycin) included in the ECDC recommendations should be likely not considered, given the lack of current EUCAST clinical breakpoints. Likewise, the inclusion of gentamicin as anti-pseudomonal agents is questionable according to current EUCAST breakpoints, and the activity of ticarcillin/clavulanate likely not comparable with that of piperacillin/tazobactam in *P. aeruginosa*. On the other hand, the DTR profile is defined according to IDSA/NIH recommendations as resistance to all first-line (classical) agents: anti-pseudomonal penicillins + β -lactamase inhibitor combinations, cephalosporins, monobactams, carbapenems, and fluoroquinolones [7]. Thus, if fosfomycin is not considered, all DTR isolates would meet the XDR criteria, because they are resistant to at least five of seven categories, but not the other way around.

However, neither the ECDC nor IDSA/NIH definitions take into consideration the novel β -lactams and BLBLIs. The inclusion of these novel agents is challenging, starting by grouping them into meaningful 'categories' because their properties, spectrum, and mechanisms of resistance show similarities but also marked differences. As shown in Table 1, at least five novel categories could be considered to include the novel β -lactams already approved: fifth-generation anti-pseudomonal cephalosporins + classical β -lactamase inhibitors (ceftolozane/tazobactam), anti-pseudomonal cephalosporins + diazabicycloctanes β -lactamase inhibitors (ceftazidime/avibactam), anti-pseudomonal carbapenems + diazabicycloctanes β -lactamase inhibitors (imipenem/relebactam), anti-pseudomonal carbapenems + boronic acid β -lactamase inhibitors (meropenem/vaborbactam), and siderophore anti-pseudomonal cephalosporins (cefiderocol). In addition, there are at least three further classes to be considered in the future if the corresponding antibiotics are approved: monobactams + diazabicycloctanes β -lactamase inhibitors (aztreonam/avibactam), anti-pseudomonal cephalosporins + diazabicycloctanes β -lactamase and PBP2 inhibitors (cefepime/zidebactam), and anti-pseudomonal cephalosporins + boronic acid β -lactamase inhibitors including MBLs (cefepime/taniborbactam).

Within the framework of the ECDC definitions, these novel categories could potentially align with MDR implying resistance to at least three classes (of up to 13), XDR indicating resistance to all but one or two and PDR indicating resistance to all. Regarding DTR definition, it would imply resistance to all the novel β -lactams approved. However, the practical application of this definition is likely to encounter challenges because of limited access to these antibiotics for treatment and to the capacity to perform antimicrobial susceptibility testing in several countries. Moreover, the classification of the resistance profiles for the novel agents under

ST	Clonal Complex	O-antigen Serotype	T3SS	CONTINENT						CARBAPENEMASE							
				N. America	S. America	Europe	Africa	Asia	Oceania	<i>bla</i> GES	<i>bla</i> KPC	<i>bla</i> FIM	<i>bla</i> GIM	<i>bla</i> IMP	<i>bla</i> NDM	<i>bla</i> SPM	<i>bla</i> VIM
ST235	CC235	O11	ExoU+														
ST111	CC111	O12 (O4)	ExoS+														
ST233	CC233	O6	ExoS+														
ST244	CC244	O2	ExoS+														
ST357	CC357	O11	ExoU+														
ST308	CC308	O11	ExoU+														
ST175	CC175	O4	ExoS+														
ST277	CC277	O2	ExoS+														
ST654	CC654	O11	ExoS+														
ST298	CC446	O11	ExoU+														

Fig. 2. Summary of the main characteristics of the top 10 *P. aeruginosa* high-risk clones. Updated in July 2023 from Del Barrio-Tofio et al. [10]. Novel descriptions since 2020 are shown in red.

development into clinical susceptibility categories will need to consider PK/PD data, not yet available in some cases, in addition to existing phenotypic and genomic information.

Update on *Pseudomonas aeruginosa* high-risk lineages and their association with transferable β -lactamases

In a recent review [10], according to their prevalence, global spread and association with MDR/XDR/DTR profiles, and specially with concerning horizontally acquired β -lactamases such as ESBLs and carbapenemases, the worldwide top ten *P. aeruginosa* high-risk lineages were established to be, by order of relevance, ST235, ST111, ST233, ST244, ST357, ST308, ST175, ST277, ST654, and ST298. Fig. 2 shows updated information for these top ten high-risk lineages, including their virulence profile (presence of the genes coding the type III secretion system exotoxins ExoS and/or ExoU), worldwide distribution and association with acquired carbapenemases from key publications in the last 3 years [40–42,92,107–111]. Particularly noteworthy is the expansion of KPC enzymes in several of these lineages (ST233, ST277, and ST654 in addition to the previous detection in ST235, ST111, and ST244), followed by NDM (ST244 and ST357 in addition to ST235, ST233, ST308, and ST654). Moreover, coproduction of various carbapenemases is not infrequent among those lineages [43]. Besides these top ten lineages, a few others have gained relevance in the last few years, including globally expanding ST309, associated with the production of VIM-2, ST773 linked to NDM-1, or ST463 associated with the production of KPC-2, particularly in China [112–117].

Concluding remarks and future challenges

P. aeruginosa infections rank among the foremost global resistance threats, associated with significant morbidity and mortality. *P. aeruginosa* resistance mechanisms and epidemiology are complex and ever-evolving, with a significant impact on novel and forthcoming β -lactams. The interplay between novel antibiotics and resistance is notably challenging, as certain mechanisms can lead to cross-resistance to multiple agents, whereas others may confer collateral susceptibility to relevant anti-pseudomonals such as carbapenems. The global dissemination of XDR/DTR high-risk lineages is also a major challenge, particularly when coupled with increased virulence and capacity to acquire exogenous resistance elements as documented for ST235 [11]. In this sense, a recent nation-wide survey of *P. aeruginosa* susceptibility profiles and resistance genomics has revealed, on the one hand, a significant generalized decrease of resistance rates and XDR/DTR profiles in Spain in the last 5 years, but on the other, a significant increase in the proportion of the concerning carbapenemase-producing ST235 high-risk lineage [44].

Therefore, there is a major need for establishing comprehensive resistance surveillance initiatives, integrating both phenotypic and genomic data, and metadata. However, our current capacity to predict the susceptibility profiles and emerging high-risk clonal lineages from genomic sequences still needs to be improved, potentially through the incorporation of machine learning, knowledge-based approaches, or so-called artificial intelligence tools [43,118,119]. Nevertheless, current achievable surveillance strategies at European level should at least integrate: (a) monitoring of concerning high-risk lineages (particularly ST235); (b) analyses of resistance prevalence trends to recently introduced agents (such as the novel BLBLIs) in addition to classical anti-pseudomonals; (c) monitoring of strains producing horizontally acquired resistance mechanisms (particularly carbapenemases and ESBLs); and (d) monitoring of noteworthy chromosomal resistance mechanisms such as the AmpC (PDC) derivatives involved in

resistance to the novel BLBLIs. Likewise, in this scenario, antimicrobial stewardship and infection control are of paramount importance. Nevertheless, these aspects are equally challenging and should be guided by rapid diagnostics and antimicrobial susceptibility testing, including the detection of resistance mechanisms and specific high-risk clonal lineages [120]. Thus, efforts should also be directed to the implementation and scaling of personalized precision medicine that allows us to establish early targeted treatments and specific epidemiological control measures adapted to the strain/mechanism involved.

Author contributions

All authors agreed the questions to be addressed in the position paper. AO and ER-M drafted the first version of the document that was extensively revised by all other authors.

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References

- [1] Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa* infections. Clin Microbiol Rev 2019;32: e00031–19. <https://doi.org/10.1128/CMR.00031-19>.
- [2] Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 2022;399:629–55. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0).
- [3] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318–27. [https://doi.org/10.1016/S1473-3099\(17\)30753-3](https://doi.org/10.1016/S1473-3099(17)30753-3).
- [4] López-Causapé C, Cabot G, Del Barrio-Tofiño E, Oliver A. The versatile mutational resistome of *Pseudomonas aeruginosa*. Front Microbiol 2018;9: 685. <https://doi.org/10.3389/fmicb.2018.00685>.
- [5] Tenover FC, Nicolau DP, Gill CM. Carbapenemase-producing *Pseudomonas aeruginosa*—an emerging challenge. Emerg Microbe. Infect 2022;11:811–4. <https://doi.org/10.3389/fcimb.2022.823684>.
- [6] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>.

- [7] Kadri SS, Adjemian J, Lai YL, Spaulding AB, Ricotta E, Prevots DR, et al. Difficult-to-treat resistance in gram-negative bacteremia at 173 US hospitals: Retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. *Clin Infect Dis* 2018;67:1803–14. <https://doi.org/10.1093/cid/ciy378>.
- [8] Pelegrin AC, Palmieri M, Mirande C, Oliver A, Moons P, Goossens H, et al. *Pseudomonas aeruginosa*: a clinical and genomics update. *FEMS Microbiol Rev* 2021;45:fuab026. <https://doi.org/10.1093/femsre/fuab026>.
- [9] Oliver A, Mulet X, López-Causapé C, Juan C. The increasing threat of *Pseudomonas aeruginosa* high-risk clones. *Drug Resist Updat* 2015;21–22:41–59. <https://doi.org/10.1016/j.drup.2015.08.002>.
- [10] del Barrio-Tofiño E, López-Causapé C, Oliver A. *Pseudomonas aeruginosa* epidemic high-risk clones and their association with horizontally-acquired β -lactamases: 2020 update. *Int J Antimicrob Agents* 2020;56:106196. <https://doi.org/10.1016/j.ijantimicag.2020.106196>.
- [11] Treepong P, Kos VN, Guyeux C, Blanc DS, Bertrand X, Valot B, et al. Global emergence of the widespread *Pseudomonas aeruginosa* ST235 clone. *Clin Microbiol Infect* 2018;24:258–66. <https://doi.org/10.1016/j.cmi.2017.06.018>.
- [12] Del Barrio-Tofiño E, Zamorano L, Cortes-Lara S, López-Causapé C, Sánchez-Diener I, Cabot G, et al. Spanish nationwide survey on *Pseudomonas aeruginosa* antimicrobial resistance mechanisms and epidemiology. *J Antimicrob Chemother* 2019;74:1825–35. <https://doi.org/10.1093/jac/dkz147>.
- [13] Kos VN, Déraspe M, McLaughlin RE, Whiteaker JD, Roy PH, Alm RA, et al. The resistome of *Pseudomonas aeruginosa* in relationship to phenotypic susceptibility. *Antimicrob Agents Chemother* 2015;59:427–36. <https://doi.org/10.1128/AAC.03954-14>.
- [14] Cabot G, López-Causapé C, Ocampo-Sosa AA, Sommer LM, Domínguez MÁ, Zamorano L, et al. Deciphering the resistome of the widespread *Pseudomonas aeruginosa* sequence type 175 international high-risk clone through whole-genome sequencing. *Antimicrob Agents Chemother* 2016;60:7415–23. <https://doi.org/10.1128/AAC.01720-16>.
- [15] Jaillard M, van Belkum A, Cady KC, Creely D, Shortridge D, Blanc B, et al. Correlation between phenotypic antibiotic susceptibility and the resistome in *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 2017;50:210–8. <https://doi.org/10.1016/j.ijantimicag.2017.02.026>.
- [16] Wright H, Bonomo RA, Paterson DL. New agents for the treatment of infections with Gram-negative bacteria: restoring the miracle or false dawn? *Clin Microbiol Infect* 2017;23:704–12. <https://doi.org/10.1016/j.cmi.2017.09.001>.
- [17] Yahav D, Giske CG, Gramatniece A, Abodakpi H, Tam VH, Leibovici L. New β -lactam- β -lactamase inhibitor combinations. *Clin Microbiol Rev* 2020;34:1–61. <https://doi.org/10.1128/CMR.00115-20>.
- [18] Bassetti M, Echols R, Matsunaga Y, Ariyasu M, Doi Y, Ferrer R, et al. Efficacy and safety of ceftiderocol or best available therapy for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria (CREDIBLE-CR): a randomised, open-label, multicentre, pathogen-focused, descriptive, phase 3 trial. *Lancet Infect Dis* 2021;21:226–40. [https://doi.org/10.1016/S1473-3099\(20\)30796-9](https://doi.org/10.1016/S1473-3099(20)30796-9).
- [19] Viale P, Sandrock CE, Ramirez P, Rossolini GM, Lodise TP. Treatment of critically ill patients with ceftiderocol for infections caused by multidrug-resistant pathogens: review of the evidence. *Ann Intensive Care* 2023;13:52. <https://doi.org/10.1186/s13613-023-01146-5>.
- [20] Fraile-Ribot PA, Cabot G, Mulet X, Peñañez L, Martín-Pena ML, Juan C, et al. Mechanisms leading to in vivo ceftolozane/tazobactam resistance development during the treatment of infections caused by MDR *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2018;73:658–63. <https://doi.org/10.1093/jac/dkx424>.
- [21] Mojica MF, De La Cadena E, García-Betancur JC, Porras J, Novoa-Cacedo I, Páez-Zamora L, et al. Molecular mechanisms of resistance to Ceftazidime/Avibactam in clinical isolates of enterobacteriales and *Pseudomonas aeruginosa* in Latin American Hospitals. *mSphere* 2023;8:e0065122. <https://doi.org/10.1128/msphere.00651-22>.
- [22] Alonso-García I, Vázquez-Ucha JC, Lasarte-Monterrubio C, González-Mayo E, Lada-Salvador P, Vela-Fernández R, et al. Simultaneous and divergent evolution of resistance to cephalosporin/ β -lactamase inhibitor combinations and imipenem/relebactam following ceftazidime/avibactam treatment of MDR *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother* 2023;78:1195–200. <https://doi.org/10.1093/jac/dkad062>.
- [23] Gomis-Font MA, Pitart C, del Barrio-Tofiño E, Zboromyrska Y, Cortes-Lara S, Mulet X, et al. Emergence of resistance to novel cephalosporin- β -lactamase inhibitor combinations through the modification of the *Pseudomonas aeruginosa* MexCD-Opri efflux pump. *Antimicrob Agents Chemother* 2021;65:e0008921. <https://doi.org/10.1128/AAC.00089-21>.
- [24] Shields RK, Stellfox ME, Kline EG, Samanta P, Van Tyne D. Evolution of imipenem-relebactam resistance following treatment of multidrug-resistant *Pseudomonas aeruginosa* pneumonia. *Clin Infect Dis* 2022;75:710–4. <https://doi.org/10.1093/cid/ciac097>.
- [25] Bahr G, González LJ, Vila AJ. Metallo- β -lactamases in the age of multidrug resistance: from structure and mechanism to evolution, dissemination, and inhibitor design. *Chem Rev* 2021;121:7957–8094. <https://doi.org/10.1021/acs.chemrev.1c00138>.
- [26] Ruedas-López A, Alonso-García I, Lasarte-Monterrubio C, Guijarro-Sánchez P, Gato E, Vázquez-Ucha JC, et al. Selection of AmpC β -lactamase variants and metallo- β -lactamases leading to Ceftolozane/Tazobactam and Ceftazidime/Avibactam resistance during treatment of MDR/XDR *Pseudomonas aeruginosa* infections. *Antimicrob Agents Chemother* 2022;66:e0206721. <https://doi.org/10.1128/AAC.02067-21>.
- [27] Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev* 2009;22:582–610. <https://doi.org/10.1128/CMR.00040-09>.
- [28] Juan C, Torrens G, González-Nicolau M, Oliver A. Diversity and regulation of intrinsic β -lactamases from non-fermenting and other Gram-negative opportunistic pathogens. *FEMS Microbiol Rev* 2017;41:781–815. <https://doi.org/10.1093/femsre/fux043>.
- [29] Juan C, Maciá MD, Gutiérrez O, Vidal C, Pérez JL, Oliver A. Molecular mechanisms of beta-lactam resistance mediated by AmpC hyperproduction in *Pseudomonas aeruginosa* clinical strains. *Antimicrob Agents Chemother* 2005;49:4733–8. <https://doi.org/10.1128/AAC.49.11.4733-4738.2005>.
- [30] Moya B, Dötsch A, Juan C, Blázquez J, Zamorano L, Haussler S, et al. Beta-lactam resistance response triggered by inactivation of a nonessential penicillin-binding protein. *PLoS Pathog* 2009;5:e1000353. <https://doi.org/10.1371/journal.ppat.1000353>.
- [31] Cabot G, Bruchmann S, Mulet X, Zamorano L, Moyá B, Juan C, et al. *Pseudomonas aeruginosa* ceftolozane-tazobactam resistance development requires multiple mutations leading to overexpression and structural modification of AmpC. *Antimicrob Agents Chemother* 2014;58:3091–9. <https://doi.org/10.1128/AAC.02462-13>.
- [32] Lahiri SD, Johnstone MR, Ross PL, McLaughlin RE, Olivier NB, Alm RA. Avibactam and class C β -lactamases: mechanism of inhibition, conservation of the binding pocket, and implications for resistance. *Antimicrob Agents Chemother* 2014;58:5704–13. <https://doi.org/10.1128/AAC.03057-14>.
- [33] Haidar G, Phillips NJ, Shields RK, Snyder D, Cheng S, Potoski BA, et al. Ceftolozane-Tazobactam for the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections: clinical effectiveness and evolution of resistance. *Clin Infect Dis* 2017;65:110–20. <https://doi.org/10.1093/cid/cix182>.
- [34] Berrazeg M, Jeannot K, Ntsogo Enguéné VY, Broutin I, Loeffert S, Fournier D, et al. Mutations in β -lactamase AmpC increase resistance of *Pseudomonas aeruginosa* isolates to antipseudomonal cephalosporins. *Antimicrob Agents Chemother* 2015;59:6248–55. <https://doi.org/10.1128/AAC.00825-15>.
- [35] Gomis-Font MA, Sastre-Femenia MÁ, Taltavull B, Cabot G, Oliver A. In vitro dynamics and mechanisms of ceftiderocol resistance development in wild-type, mutator and XDR *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2023;78:1785–94. <https://doi.org/10.1093/jac/dkad172>.
- [36] Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A, Dortet L, et al. Beta-lactamase database (BLDB) – structure and function. *J Enzym Inhib Med Chem* 2017;32:917–9. <https://doi.org/10.1080/14756366.2017.1344235>.
- [37] Fraile-Ribot PA, Mulet X, Cabot G, Del Barrio-Tofiño E, Juan C, Pérez JL, et al. In Vivo emergence of resistance to novel cephalosporin- β -lactamase inhibitor combinations through the duplication of amino acid D149 from OXA-2 β -lactamase (OXA-539) in sequence type 235 *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2017;61:e01117-17. <https://doi.org/10.1128/AAC.01117-17>.
- [38] Arca-Suárez J, Lasarte-Monterrubio C, Rodiño-Janeiro BK, Cabot G, Vázquez-Ucha JC, Rodríguez-Iglesias M, et al. Molecular mechanisms driving the in vivo development of OXA-10-mediated resistance to ceftolozane/tazobactam and ceftazidime/avibactam during treatment of XDR *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother* 2021;76:91–100. <https://doi.org/10.1093/jac/dkaa396>.
- [39] Lasarte-Monterrubio C, Fraile-Ribot PA, Vázquez-Ucha JC, Cabot G, Guijarro-Sánchez P, Alonso-García I, et al. Activity of ceftiderocol, imipenem/relebactam, cefepime/taniborbactam and cefepime/zidebactam against ceftolozane/tazobactam- and ceftazidime/avibactam-resistant *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2022;77:2809–15. <https://doi.org/10.1093/jac/dkac241>.
- [40] Reyes J, Komarow L, Chen L, Ge L, Hanson BM, Cober E, et al. Global epidemiology and clinical outcomes of carbapenem-resistant *Pseudomonas aeruginosa* and associated carbapenemases (POP): a prospective cohort study. *Lancet Microbe* 2023;4:e159–70. [https://doi.org/10.1016/S2666-5247\(22\)00329-9](https://doi.org/10.1016/S2666-5247(22)00329-9).
- [41] Wang MG, Liu ZY, Liao XP, Sun RY, Li RB, Liu Y, et al. Retrospective data insight into the global distribution of carbapenemase-producing *Pseudomonas aeruginosa*. *Antibiotics (Basel)* 2021;10:548. <https://doi.org/10.3390/antibiotics10050548>.
- [42] Fortunato G, Vaz-Moreira I, Gajic I, Manaia CM. Insight into phylogenomic bias of blaVIM-2 or blaNDM-1 dissemination amongst carbapenem-resistant *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 2023;61:106788. <https://doi.org/10.1016/j.ijantimicag.2023.106788>.
- [43] Torrens G, Van Der Schalk TE, Cortes-Lara S, Timbermont L, Del Barrio-Tofiño E, Xavier BB, et al. Susceptibility profiles and resistance genomics of *Pseudomonas aeruginosa* isolates from European ICUs participating in the ASPIRE-ICU trial. *J Antimicrob Chemother* 2022;77:1862–72. <https://doi.org/10.1093/jac/dkac122>.
- [44] Àngel Sastre-Femenia M, Fernández-Muñoz A, Gomis-Font MA, Taltavull B, López-Causapé C, Arca-Suárez J, et al. *Pseudomonas aeruginosa* antibiotic susceptibility profiles, genomic epidemiology and resistance mechanisms: a nation-wide five-year time lapse analysis. *Lancet Reg Health Eur* 2023;34:100736. <https://doi.org/10.1016/j.lanepe.2023.100736>.
- [45] Takemura M, Wise MG, Hackel MA, Sahn DF, Yamano Y. In vitro activity of ceftiderocol against MBL-producing Gram-negative bacteria collected in

- North America and Europe in five consecutive annual multinational SIDERO-WT surveillance studies (2014–2019). *J Antimicrob Chemother* 2023;78: 2019–27. <https://doi.org/10.1093/jac/dkad200>.
- [46] Le Terrier C, Nordmann P, Poirel L. In vitro activity of aztreonam in combination with newly developed β -lactamase inhibitors against MDR Enterobacterales and *Pseudomonas aeruginosa* producing metallo- β -lactamases. *J Antimicrob Chemother* 2022;78:101–7. <https://doi.org/10.1093/jac/dkac360>.
- [47] Le Terrier C, Nordmann P, Freret C, Seigneur M, Poirel L. Impact of acquired broad spectrum β -Lactamases on susceptibility to novel combinations made of β -lactams (Aztreonam, Cefepime, Meropenem, and Imipenem) and novel β -lactamase inhibitors in *Escherichia coli* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2023;67:e0033923. <https://doi.org/10.1128/aac.00339-23>.
- [48] Moya B, Barceló IM, Bhagwat S, Patel M, Bou G, Papp-Wallace KM, et al. WCK 5107 (Zidebactam) and WCK 5153 are novel inhibitors of PBP2 showing potent “ β -lactam enhancer” activity against *Pseudomonas aeruginosa*, including multidrug-resistant metallo- β -lactamase-producing high-risk clones. *Antimicrob Agents Chemother* 2017;61:e02529-16. <https://doi.org/10.1128/AAC.02529-16>.
- [49] Meletiadiis J, Paranos P, Georgiou PC, Vourli S, Antonopoulou S, Michelaki A, et al. In vitro comparative activity of the new beta-lactamase inhibitor taniborbactam with cefepime or meropenem against *Klebsiella pneumoniae* and cefepime against *Pseudomonas aeruginosa* metallo-beta-lactamase-producing clinical isolates. *Int J Antimicrob Agents* 2021;58:106440. <https://doi.org/10.1016/j.ijantimicag.2021.106440>.
- [50] Poirel L, De la Rosa JMO, Sadek M, Nordmann P. Impact of Acquired broad-spectrum β -lactamases on susceptibility to ceftiderocol and newly developed β -lactam/ β -lactamase inhibitor combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2022;66:e0003922. <https://doi.org/10.1128/aac.00039-22>.
- [51] Shortridge D, Carvalhaes C, Deshpande L, Castanheira M. Activity of meropenem/vaborbactam and comparators against Gram-negative isolates from Eastern and Western European patients hospitalized with pneumonia including ventilator-associated pneumonia (2014–19). *J Antimicrob Chemother* 2021;76:2600–5. <https://doi.org/10.1093/jac/dkab252>.
- [52] Mushtaq S, Meunier D, Vickers A, Woodford N, Livermore DM. Activity of imipenem/relebactam against *Pseudomonas aeruginosa* producing ESBLs and carbapenemases. *J Antimicrob Chemother* 2021;76:434–42. <https://doi.org/10.1093/jac/dkaa456>.
- [53] Fraile-Ribot PA, Zamorano L, Orellana R, Del Barrio-Tofiño E, Sánchez-Diener I, Cortes-Lara S, et al. Activity of imipenem-relebactam against a large collection of *Pseudomonas aeruginosa* clinical isolates and isogenic β -lactam-resistant mutants. *Antimicrob Agents Chemother* 2020;64:e02165-19. <https://doi.org/10.1128/AAC.02165-19>.
- [54] Fraile-Ribot PA, Fernández J, Gomis-Font MA, Forcelledo L, Mulet X, López-Causapé C, et al. In Vivo evolution of GES β -lactamases driven by Ceftazidime/Avibactam treatment of *Pseudomonas aeruginosa* infections. *Antimicrob Agents Chemother* 2021;65:e0098621. <https://doi.org/10.1128/AAC.00986-21>.
- [55] Faccione D, de Mendieta JM, Albornoz E, Chavez M, Genero F, Echegorry M, et al. Emergence of KPC-31, a KPC-3 variant associated with Ceftazidime-Avibactam resistance, in an extensively drug-resistant ST235 *Pseudomonas aeruginosa* clinical isolate. *Antimicrob Agents Chemother* 2022;66:e0064822. <https://doi.org/10.1128/aac.00648-22>.
- [56] Recio R, Villa J, González-Bodí S, Brañas P, Orellana MÁ, Mancheno-Losa M, et al. Genomic analysis of Ceftazidime/Avibactam-resistant GES-producing sequence type 235 *Pseudomonas aeruginosa* isolates. *Antibiotics (Basel)* 2022;11:871. <https://doi.org/10.3390/antibiotics111070871>.
- [57] Bonnín RA, Bogaerts P, Girlich D, Huang TD, Dortet L, Glupczynski Y, et al. Molecular characterization of OXA-198 carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemother* 2018;62:e02496-17. <https://doi.org/10.1128/AAC.02496-17>.
- [58] Chen W, Zhang YM, Davies C. Penicillin-binding Protein 3 is essential for growth of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2016;61:e01651-16. <https://doi.org/10.1128/AAC.01651-16>.
- [59] Caballero JD, Clark ST, Coburn B, Zhang Y, Wang PW, Donaldson SL, et al. Selective sweeps and parallel pathoadaptation drive *Pseudomonas aeruginosa* evolution in the cystic fibrosis lung. *mBio* 2015;6:e00981-15. <https://doi.org/10.1128/mBio.00981-15>.
- [60] López-Causapé C, Sommer LM, Cabot G, Rubio R, Ocampo-Sosa AA, Johansen HK, et al. Evolution of the *Pseudomonas aeruginosa* mutational resistome in an international Cystic Fibrosis clone. *Sci Rep* 2017;7:5555. <https://doi.org/10.1038/s41598-017-05621-5>.
- [61] Cabot G, Zamorano L, Moyá B, Juan C, Navas A, Blázquez J, et al. Evolution of *Pseudomonas aeruginosa* antimicrobial resistance and fitness under low and high mutation rates. *Antimicrob Agents Chemother* 2016;60:1767–78. <https://doi.org/10.1128/AAC.02676-15>.
- [62] Han S, Zaniewski RP, Marr ES, Lacey BM, Tomaras AP, Evdokimov A, et al. Structural basis for effectiveness of siderophore-conjugated monocarbams against clinically relevant strains of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 2010;107:22002–7. <https://doi.org/10.1073/pnas.1013092107>.
- [63] Jorth P, McLean K, Ratjen A, Secor PR, Bautista GE, Ravishanker S, et al. Evolved aztreonam resistance is multifactorial and can produce hypervirulence in *Pseudomonas aeruginosa*. *mBio* 2017;8:e00517-17. <https://doi.org/10.1128/mBio.00517-17>.
- [64] Cabot G, Florit-Mendoza L, Sánchez-Diener I, Zamorano L, Oliver A. Deciphering β -lactamase-independent β -lactam resistance evolution trajectories in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2018;73:3322–31. <https://doi.org/10.1093/jac/dky364>.
- [65] Barceló I, Cabot G, Palwe S, Joshi P, Takalkar S, Periasamy H, et al. In vitro evolution of cefepime/zidebactam (WCK 5222) resistance in *Pseudomonas aeruginosa*: dynamics, mechanisms, fitness trade-off and impact on in vivo efficacy. *J Antimicrob Chemother* 2021;76:2546–57. <https://doi.org/10.1093/jac/dkab213>.
- [66] Castanheira M, Deshpande LM, Costello A, Davies TA, Jones RN. Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* collected during 2009–11 in 14 European and Mediterranean countries. *J Antimicrob Chemother* 2014;69:1804–14. <https://doi.org/10.1093/jac/dku048>.
- [67] Richardot C, Plésiat P, Fournier D, Monlezun L, Broutin I, Llanes C. Carbapenem resistance in cystic fibrosis strains of *Pseudomonas aeruginosa* as a result of amino acid substitutions in porin OprD. *Int J Antimicrob Agents* 2015;45: 529–32. <https://doi.org/10.1016/j.ijantimicag.2014.12.029>.
- [68] Ocampo-Sosa AA, Cabot G, Rodríguez C, Roman E, Tubau F, Maciá MD, et al. Alterations of OprD in carbapenem-intermediate and -susceptible strains of *Pseudomonas aeruginosa* isolated from patients with bacteremia in a Spanish multicenter study. *Antimicrob Agents Chemother* 2012;56:1703–13. <https://doi.org/10.1128/AAC.05451-11>.
- [69] Li XZ, Plésiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin Microbiol Rev* 2015;28:337–418. <https://doi.org/10.1128/CMR.00117-14>.
- [70] Gomis-Font MA, Cabot G, Sánchez-Diener I, Fraile-Ribot PA, Juan C, Moya B, et al. In vitro dynamics and mechanisms of resistance development to imipenem and imipenem/relebactam in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2020;75:2508–15. <https://doi.org/10.1093/jac/dkaa206>.
- [71] Gomis-Font MA, Cabot G, López-Argüello S, Zamorano L, Juan C, Moyá B, et al. Comparative analysis of in vitro dynamics and mechanisms of ceftolozane/tazobactam and imipenem/relebactam resistance development in *Pseudomonas aeruginosa* XDR high-risk clones. *J Antimicrob Chemother* 2022;77: 957–68. <https://doi.org/10.1093/jac/dkab496>.
- [72] Sanz-García F, Hernando-Amado S, Martínez JL. Mutation-driven evolution of *Pseudomonas aeruginosa* in the presence of either Ceftazidime or Ceftazidime-Avibactam. *Antimicrob Agents Chemother* 2018;62:e01379-18. <https://doi.org/10.1128/AAC.01379-18>.
- [73] Hocquet D, Petitjean M, Rohmer L, Valot B, Kulasekara HD, Bedel E, et al. Pyomelanin-producing *Pseudomonas aeruginosa* selected during chronic infections have a large chromosomal deletion which confers resistance to pyocins. *Environ Microbiol* 2016;18:3482–93. <https://doi.org/10.1111/1462-2920.13336>.
- [74] Dötsch A, Becker T, Pommerenke C, Magnowska Z, Jänsch L, Häußler S. Genomewide identification of genetic determinants of antimicrobial drug resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2009;53:2522–31. <https://doi.org/10.1128/AAC.00035-09>.
- [75] Alvarez-Ortega C, Wiegand I, Olivares J, Hancock REW, Martínez JL. Genetic determinants involved in the susceptibility of *Pseudomonas aeruginosa* to beta-lactam antibiotics. *Antimicrob Agents Chemother* 2010;54:4159–67. <https://doi.org/10.1128/AAC.00257-10>.
- [76] Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *Int J Antimicrob Agents* 2015;45:568–85. <https://doi.org/10.1016/j.ijantimicag.2015.03.001>.
- [77] Costello SE, Deshpande LM, Davis AP, Mendes RE, Castanheira M. Aminoglycoside-modifying enzyme and 16S ribosomal RNA methyltransferase genes among a global collection of Gram-negative isolates. *J Glob Antimicrob Resist* 2019;16:278–85. <https://doi.org/10.1016/j.jgar.2018.10.020>.
- [78] Yokoyama K, Doi Y, Yamane K, Kurokawa H, Shibata N, Shibayama K, et al. Acquisition of 16S rRNA methylase gene in *Pseudomonas aeruginosa*. *Lancet* 2003;362:1888–93. [https://doi.org/10.1016/S0140-6736\(03\)14959-8](https://doi.org/10.1016/S0140-6736(03)14959-8).
- [79] Choi YJ, Kim YA, Junglim K, Jeong SH, Shin JH, Shin KS, et al. Emergence of NDM-1-producing *Pseudomonas aeruginosa* sequence type 773 clone: shift of carbapenemase molecular epidemiology and spread of 16S rRNA methylase genes in Korea. *Ann Lab Med* 2023;43:196–9. <https://doi.org/10.3343/alm.2023.43.2.196>.
- [80] Mc Gann PT, Lebreton F, Jones BT, Dao HD, Martin MJ, Nelson MJ, et al. Six extensively drug-resistant bacteria in an injured soldier, Ukraine. *Emerg Infect Dis* 2023;29:1692–5. <https://doi.org/10.3201/eid2908.230567>.
- [81] Guénard S, Muller C, Monlezun L, Benas P, Broutin I, Jeannot K, et al. Multiple mutations lead to MexXY-OprM-dependent aminoglycoside resistance in clinical strains of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2014;58:221–8. <https://doi.org/10.1128/AAC.01252-13>.
- [82] Prickett MH, Hauser AR, McColley SA, Cullina J, Potter E, Powers C, et al. Aminoglycoside resistance of *Pseudomonas aeruginosa* in cystic fibrosis results from convergent evolution in the mexZ gene. *Thorax* 2017;72:40–7. <https://doi.org/10.1136/thoraxjnl-2015-208027>.
- [83] El'Garch F, Jeannot K, Hocquet D, Llanes-Barakat C, Plésiat P. Cumulative effects of several nonenzymatic mechanisms on the resistance of *Pseudomonas aeruginosa* to aminoglycosides. *Antimicrob Agents Chemother* 2007;51:1016–21. <https://doi.org/10.1128/AAC.00704-06>.

- [84] Schurek KN, Marr AK, Taylor PK, Wiegand I, Semenek L, Khaira BK, et al. Novel genetic determinants of low-level aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2008;52:4213–9. <https://doi.org/10.1128/AAC.00507-08>.
- [85] Feng Y, Jonker MJ, Moustakas I, Brul S, Ter Kuile BH. Dynamics of mutations during development of resistance by *Pseudomonas aeruginosa* against five antibiotics. *Antimicrob Agents Chemother* 2016;60:4229–36. <https://doi.org/10.1128/AAC.00434-16>.
- [86] Chung JCS, Becq J, Fraser L, Schulz-Trieglaff O, Bond NJ, Foweraker J, et al. Genomic variation among contemporary *Pseudomonas aeruginosa* isolates from chronically infected cystic fibrosis patients. *J Bacteriol* 2012;194:4857–66. <https://doi.org/10.1128/JB.01050-12>.
- [87] Markussen T, Marvig RL, Gómez-Lozano M, Aanæs K, Burleigh AE, Høiby N, et al. Environmental heterogeneity drives within-host diversification and evolution of *Pseudomonas aeruginosa*. *mBio* 2014;5:e01592-14. <https://doi.org/10.1128/mBio.01592-14>.
- [88] Greipel L, Fischer S, Klockgether J, Dorda M, Mielke S, Wiehlmann L, et al. Molecular epidemiology of mutations in antimicrobial resistance loci of *Pseudomonas aeruginosa* isolates from airways of cystic fibrosis patients. *Antimicrob Agents Chemother* 2016;60:6726–34. <https://doi.org/10.1128/AAC.00724-16>.
- [89] Bolard A, Plésiat P, Jeannot K. Mutations in Gene fusA1 as a Novel mechanism of aminoglycoside resistance in clinical strains of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2018;62:e01835-17. <https://doi.org/10.1128/AAC.01835-17>.
- [90] Bruchmann S, Dötsch A, Nouri B, Chaberny IF, Häussler S. Quantitative contributions of target alteration and decreased drug accumulation to *Pseudomonas aeruginosa* fluoroquinolone resistance. *Antimicrob Agents Chemother* 2013;57:1361–8. <https://doi.org/10.1128/AAC.01581-12>.
- [91] Belotti PT, Thabet L, Laffargue A, André C, Coulange-Mayonnove L, Arpin C, et al. Description of an original integron encompassing blaVIM-2, qnrVC1 and genes encoding bacterial group II intron proteins in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2015;70:2237–40. <https://doi.org/10.1093/jac/dkv103>.
- [92] Pérez-Vázquez M, Sola-Campoy PJ, Zurita AM, Ávila A, Gómez-Bertomeu F, Solís S, et al. Carbapenemase-producing *Pseudomonas aeruginosa* in Spain: interregional dissemination of the high-risk clones ST175 and ST244 carrying blaVIM-2, blaVIM-1, blaIMP-8, blaVIM-20 and blaKPC-2. *Int J Antimicrob Agents* 2020;56:106026. <https://doi.org/10.1016/j.ijantimicag.2020.106026>.
- [93] Shortridge D, Gales AC, Streit JM, Huband MD, Tsakris A, Jones RN. Geographic and temporal patterns of antimicrobial resistance in *Pseudomonas aeruginosa* over 20 years from the SENTRY Antimicrobial Surveillance Program, 1997–2016. *Open Forum Infect Dis* 2019;6:S63–8. <https://doi.org/10.1093/ofid/ofy343>.
- [94] Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol* 2014;5:643. <https://doi.org/10.3389/fmicb.2014.00643>.
- [95] Jeannot K, Bolard A, Plésiat P. Resistance to polymyxins in Gram-negative organisms. *Int J Antimicrob Agents* 2017;49:526–35. <https://doi.org/10.1016/j.ijantimicag.2016.11.029>.
- [96] Barrow K, Kwon DH. Alterations in two-component regulatory systems of phoPQ and pmrAB are associated with polymyxin B resistance in clinical isolates of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2009;53:5150–4. <https://doi.org/10.1128/AAC.00893-09>.
- [97] Muller C, Plésiat P, Jeannot K. A two-component regulatory system interconnects resistance to polymyxins, aminoglycosides, fluoroquinolones, and β -lactams in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2011;55:1211–21. <https://doi.org/10.1128/AAC.01252-10>.
- [98] Gutu AD, Sgambati N, Strasbourger P, Brannon MK, Jacobs MA, Haugen E, et al. Polymyxin resistance of *Pseudomonas aeruginosa* phoQ mutants is dependent on additional two-component regulatory systems. *Antimicrob Agents Chemother* 2013;57:2204–15. <https://doi.org/10.1128/AAC.02353-12>.
- [99] Moskowitz SM, Brannon MK, Dasgupta N, Pier M, Sgambati N, Miller AK, et al. PmrB mutations promote polymyxin resistance of *Pseudomonas aeruginosa* isolated from colistin-treated cystic fibrosis patients. *Antimicrob Agents Chemother* 2012;56:1019–30. <https://doi.org/10.1128/AAC.05829-11>.
- [100] Döselmann B, Willmann M, Steglich M, Bunk B, Nübel U, Peter S, et al. Rapid and consistent evolution of colistin resistance in extensively drug-resistant *Pseudomonas aeruginosa* during morbidostat culture. *Antimicrob Agents Chemother* 2017;61:e00043-17. <https://doi.org/10.1128/AAC.00043-17>.
- [101] Cervoni M, Sposato D, Lo Sciuto A, Imperi F. Regulatory landscape of the *Pseudomonas aeruginosa* phosphoethanolamine transferase gene eptA in the context of colistin resistance. *Antibiotics (Basel)* 2023;12:200. <https://doi.org/10.3390/antibiotics12020200>.
- [102] Snesrud E, Maybank R, Kwak YI, Jones AR, Hinkle MK, McGann P. Chromosomally encoded mcr-5 in colistin-nonsusceptible *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2018;62:e00679-18. <https://doi.org/10.1128/AAC.00679-18>.
- [103] Michalopoulos AS, Livaditis IG, Gougoutas V. The revival of fosfomycin. *Int J Infect Dis* 2011;15:e732–9. <https://doi.org/10.1016/j.ijid.2011.07.007>.
- [104] Castañeda-García A, Rodríguez-Rojas A, Guelfo JR, Blázquez J. The glycerol-3-phosphate permease GlpT is the only fosfomycin transporter in *Pseudomonas aeruginosa*. *J Bacteriol* 2009;191:6968–74. <https://doi.org/10.1128/JB.00748-09>.
- [105] Rodríguez-Rojas A, Maciá MD, Couce A, Gómez C, Castañeda-García A, Oliver A, et al. Assessing the emergence of resistance: the absence of biological cost in vivo may compromise fosfomycin treatments for *P. aeruginosa* infections. *PLOS ONE* 2010;5:e10193. <https://doi.org/10.1371/journal.pone.0010193>.
- [106] Del Barrio-Tofiño E, López-Causapé C, Cabot G, Rivera A, Benito N, Segura C, et al. Genomics and susceptibility profiles of extensively drug-resistant *Pseudomonas aeruginosa* isolates from Spain. *Antimicrob Agents Chemother* 2017;61:e01589-17. <https://doi.org/10.1128/AAC.01589-17>.
- [107] Silveira MC, Rocha-de-Souza CM, de Oliveira Santos IC, Pontes L da S, Oliveira TRTE, Tavares-Teixeira CB, et al. Genetic basis of antimicrobial resistant gram-negative bacteria isolated from bloodstream in Brazil. *Front Med (Lausanne)* 2021;8:635206. <https://doi.org/10.3389/fmed.2021.635206>.
- [108] Takahashi T, Tada T, Shrestha S, Hishinuma T, Sherchan JB, Tohya M, et al. Molecular characterisation of carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates in Nepal. *J Glob Antimicrob Resist* 2021;26:279–84. <https://doi.org/10.1016/j.jgar.2021.07.003>.
- [109] Lebreton F, Snesrud E, Hall L, Mills E, Galac M, Stam J, et al. A panel of diverse *Pseudomonas aeruginosa* clinical isolates for research and development. *JAC Antimicrob Resist* 2021;3:dlab179. <https://doi.org/10.1093/jacamr/dlab179>.
- [110] Cejas D, Elena A, González-Espinosa FE, Pallecchi L, Vay C, Rossolini GM, et al. Characterisation of blaKPC-2-harbouring plasmids recovered from *Pseudomonas aeruginosa* ST654 and ST235 high-risk clones. *J Glob Antimicrob Resist* 2022;29:310–2. <https://doi.org/10.1016/j.jgar.2022.04.017>.
- [111] Pincus NB, Bachta KER, Ozer EA, Allen JP, Pura ON, Qi C, et al. Long-term persistence of an extensively drug-resistant subclone of globally distributed *Pseudomonas aeruginosa* clonal complex 446 in an academic medical center. *Clin Infect Dis* 2020;71:1524–31. <https://doi.org/10.1093/cid/ciz973>.
- [112] Hu Y, Peng W, Wu Y, Li H, Wang Q, Yi H, et al. A potential high-risk clone of *Pseudomonas aeruginosa* ST463. *Front Microbiol* 2021;12:670202. <https://doi.org/10.3389/fmicb.2021.670202>.
- [113] Martak D, Gbaguidi-Haore H, Meunier A, Valot B, Conzelmann N, Eib M, et al. High prevalence of *Pseudomonas aeruginosa* carriage in residents of French and German long-term care facilities. *Clin Microbiol Infect* 2022;28:1353–8. <https://doi.org/10.1016/j.cmi.2022.05.004>.
- [114] Fonseca ÉL, Morgado SM, Caldart RV, Freitas F, Vicente ACP. Emergence of a VIM-2-producing extensively drug-resistant (XDR) *Pseudomonas aeruginosa* ST309 in South America: a comparative genomic analysis. *Int J Antimicrob Agents* 2022;59:106507. <https://doi.org/10.1016/j.ijantimicag.2021.106507>.
- [115] Chilam J, Argimón S, Limas MT, Masim ML, Gayeta JM, Lagrada ML, et al. Genomic surveillance of *Pseudomonas aeruginosa* in the Philippines, 2013–2014. *West. Pac Surveill Response J* 2021;12:4–18. <https://doi.org/10.5365/wpsar.2020.11.1.006>.
- [116] Morales-Espinosa R, Delgado G, Espinosa LF, Isselo D, Méndez JL, Rodríguez C, et al. Fingerprint analysis and identification of strains ST309 as a potential high risk clone in a *Pseudomonas aeruginosa* population isolated from children with bacteremia in Mexico City. *Front Microbiol* 2017;8:313. <https://doi.org/10.3389/fmicb.2017.00313>.
- [117] Khan A, Tran TT, Rios R, Hanson B, Shropshire WC, Sun Z, et al. Extensively Drug-resistant *Pseudomonas aeruginosa* ST309 harboring tandem guiana extended spectrum β -lactamase enzymes: a newly emerging threat in the United States. *Open Forum Infect Dis* 2019;6:ofz273. <https://doi.org/10.1093/ofid/ofz273>.
- [118] Cortes-Lara S, Barrio-Tofiño E del, López-Causapé C, Oliver A, GEMARA-SEIMC/REIPI *Pseudomonas* study Group. Predicting *Pseudomonas aeruginosa* susceptibility phenotypes from whole genome sequence resistome analysis. *Clin Microbiol Infect* 2021;27:1631–7. <https://doi.org/10.1016/j.cmi.2021.05.011>.
- [119] Khaledi A, Weimann A, Schniederjans M, Asgari E, Kuo T, Oliver A, et al. Predicting antimicrobial resistance in *Pseudomonas aeruginosa* with machine learning-enabled molecular diagnostics. *EMBO Mol Med* 2020;12:e10264. <https://doi.org/10.15252/emmm.201910264>.
- [120] Cabot G, Lara-Esbri P, Mulet X, Oliver A. Whole-genome sequence-guided PCR for the rapid identification of the *Pseudomonas aeruginosa* ST175 high-risk clone directly from clinical samples. *J Antimicrob Chemother* 2021;76:945–9. <https://doi.org/10.1093/jac/dkaa528>.