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

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Source / Izvornik: **Clinical Microbiology and Infection, 2024, 30**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.1016/j.cmi.2023.12.026

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:127:735057

Clinical Microbiology and Infection 30 (2024) 469–480

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Guidelines

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https://doi.org/10.1016/j.cmi.2023.12.026

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ARTICLE INFO

Article history:
Received 13 October 2023
Received in revised form
18 December 2023
Accepted 25 December 2023
Available online 30 December 2023

Editor: J. Rodríguez-Baño

Keywords:
Antimicrobial resistance surveillance
Carbapenemase
High-risk clones
Pseudomonas aeruginosa
Resistome
Whole genome sequencing

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**

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, BLBLIs 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 BLBLIs could lead to the selection of these concerning resistance mechanisms [26]. Besides the approved options, several novel BLBLIs 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 BLBLIs 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 documented 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 resistome

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 of 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 derepressed *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 BLBLIs 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 BLBLIs, 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↑	PBP3, GalU		ESBLs, class A and B carbapenemases
Cephalosporins	Ceftazidime	AmpC↑	PBP3, GalU	OXA-2/10	ESBLs, class A and B carbapenemases
	Cefepime	MexXY↑, AmpC↑	PBP3, GalU	OXA-2/10	ESBLs, class A and B carbapenemases
Monobactams	Aztreonam	MexAB↑, AmpC↑	PBP3, GalU	OXA-2/10	ESBLs and class A carbapenemases
Carbapenems	Imipenem	OprD-	MexST, PBP2, PBP1a		Class A and B carbapenemases
	Meropenem	OprD-, MexAB↑	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 + diazabicyclooctanes β-lactamase inhibitors	Ceftazidime/avibactam	AmpC Ω-loop, MexAB↑	PBP3, GalU	OXA-2/10, GES, KPC	Class B carbapenemases
Carbapenems + diazabicyclooctanes β-lactamase inhibitors	Imipenem/relebactam	OprD-, MexAB↑[b]	MexST, ParRS PBP2, PBP1a		Class A and B carbapenemases
Carbapenems + boronic acid β-lactamase inhibitors	Meropenem/vaborbactam	OprD-, MexAB↑	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 + diazabicyclooctanes β-lactamase inhibitors	Aztreonam/avibactam[a]	MexAB↑	PBP3, GalU		ESBLs and class A carbapenemases[b]
Cephalosporins + diazabicyclooctanes β-lactamase and PBP2 inhibitors	Cefepime/zidebactam[a]	MexXY↑, MexAB↑	PBP3, GalU PBP2		ESBLs, class A and B carbapenemases[b]
Cephalosporins + boronic acid β-lactamase inhibitors including MBLs	Cefepime/taniborbactam[a]	MexXY↑, MexAB↑	PBP3, GalU		IMPs
Fluoroquinolones	Ciprofloxacin, levofloxacin	QRDR	MexAB/XY/CD/EF↑		Qnr
Aminoglycosides	Tobramycin, amikacin	MexXY↑[b]	FusA1		Aminoglycoside modifying enzymes, 16S rRNA methylases
Polymyxins	Colistin, polymyxin B	PmrAB/PhoPQ/ParRS			MCR (Very uncommon)
Fosfonic acids	Fosfomycin	GlpT			FosA

ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase; QRDR, quinolone resistance-determining region.
↑ 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.bldb.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 hyperproducing 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 ß-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 has 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 fptA* (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 + diazabicy cloctanes 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

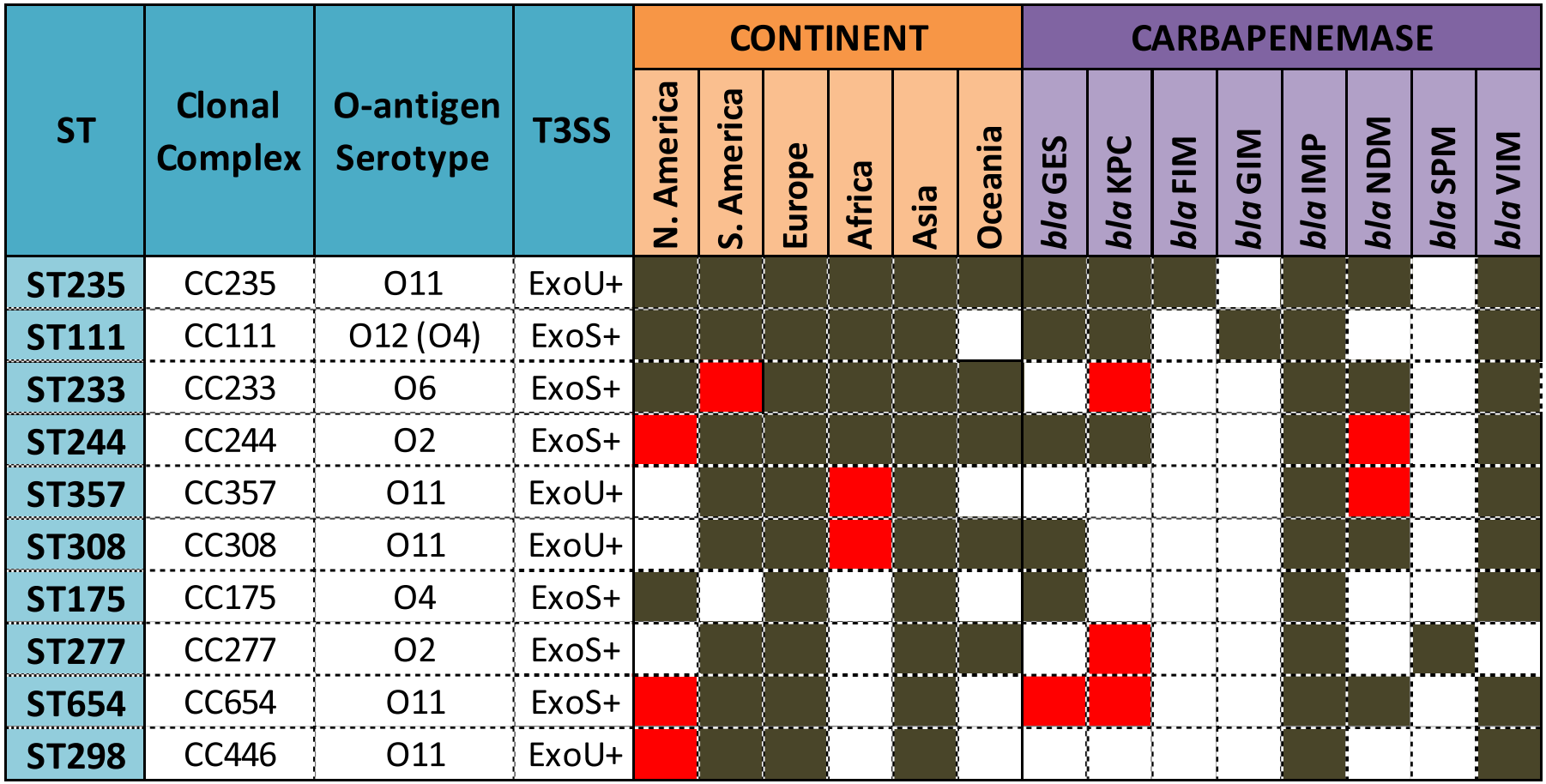

Fig. 2. Summary of the main characteristics of the top 10 *P. aeruginosa* high-risk clones. Updated in July 2023 from Del Barrio-Tofiio et al. [10]. Novel descriptions since 2020 are shown in red.

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) derivates 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.

Transparency declaration

AO received grants, consulting fees and honoraria for lectures from MSD, Shionogi, Pfizer and Wockhardt. ER-M received honoraria for lectures from Menarini and Shionogi. JA-S received honoraria for lectures from Shionogi. RC received grants and honoraria for lectures from MSD, Menarini and Shionogi. PDC received grants from MSD and Shionogi. All other authors declare no conflict of interests. This work was supported by an EU JPIAMR Grant (ISAR-PAE) financed by Instituto de Salud Carlos III (AC22/00032). AO, CL-C, SM-K, AT, and DS are supported by the EU Horizon 2020 research and innovation programme (952491-AmReSu). AO, ER-M, JA-S, CL-C, RC, and JO-I are supported by the Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación and Unión Europea – NextGenerationEU through the Centres for network research I Personalized and precision medicine grant (MePRAM Project, PMP22/00092). BK was supported by János Bolyai Research Scholarship (BO/00286/22/5) of the Hungarian Academy of Sciences. PP is supported by the projects UIDP/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology.

Acknowledgements

This work was performed under the auspices of the European Study Group on Antimicrobial Resistance Surveillance (ESGARS) from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

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