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

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

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96

97 **Abstract**

98

99 **Scope:** *Pseudomonas aeruginosa*, a ubiquitous opportunistic pathogen
100 considered one of the paradigms of antimicrobial resistance, is among the main
101 causes of hospital-acquired and chronic infections associated with significant
102 morbidity and mortality. This growing threat results from the extraordinary
103 capacity of *P. aeruginosa* to develop antimicrobial resistance through
104 chromosomal mutations, the increasing prevalence of transferable resistance
105 determinants (such as the carbapenemases and the extended spectrum β -
106 lactamases), and the global expansion of epidemic lineages. The general
107 objective of this initiative is to provide a comprehensive update of *P. aeruginosa*
108 resistance mechanisms, especially for the extensively drug-resistant (XDR)/
109 difficult to treat resistance (DTR) international high-risk epidemic lineages, and
110 how the recently approved β -lactams and β -lactam/ β -lactamase inhibitor
111 combinations may affect resistance mechanisms and the definition of
112 susceptibility profiles. **Methods:** To address this challenge, the European Study
113 Group for Antimicrobial Resistance Surveillance (ESGARS) from the European
114 Society of Clinical Microbiology and Infectious Diseases (ESCMID) launched
115 the “Improving Surveillance of Antibiotic-Resistant *Pseudomonas aeruginosa* in
116 Europe” (ISARPAE) initiative in 2022, supported by the Joint programming
117 initiative on antimicrobial resistance (JPIAMR) network call and included a panel
118 of over 40 researchers from 18 European Countries. Thus, an ESGARS-
119 ISARPAE position paper was designed and the final version agreed after four
120 rounds of revision and discussion by all panel members. **Questions addressed**
121 **in the position paper:** To provide an update on (i) the emerging resistance
122 mechanisms to classical and novel antipseudomonal agents, with a particular
123 focus on β -lactams, (ii) the susceptibility profiles associated with the most
124 relevant β -lactam resistance mechanisms, (iii) the impact of the novel agents
125 and resistance mechanisms on the definitions of resistance profiles and (iv)
126 the globally expanding XDR/DTR high-risk lineages and their association with
127 transferable resistance mechanisms. **Implication:** The evidence presented
128 herein can be used for coordinated epidemiological surveillance and decision-
129 making at the European and global level.

130

131 **Scope and context**

132 *Pseudomonas aeruginosa*, a ubiquitous opportunistic pathogen
133 considered one of the paradigms of antimicrobial resistance, is among the main
134 causes of hospital-acquired and chronic infections associated with significant
135 morbidity and mortality (1). Accordingly, *P. aeruginosa* infections are estimated
136 to be associated with over 300,000 annual deaths and are at the top of the
137 WHO priority list for the need for research and development of new antibiotics
138 (2,3). This growing threat results from the extraordinary capacity of this
139 pathogen to develop antimicrobial resistance through chromosomal mutations
140 and from the increasing prevalence of transferable resistance determinants,
141 particularly those encoding carbapenemases or extended-spectrum β -
142 lactamases (ESBLs) (4,5). Combinations of such mechanisms lead to
143 concerning and complex resistance profiles, defined by the European Centre for
144 Disease Prevention and Control (ECDC) and the Centers for Disease Control
145 and Prevention (CDC) as multidrug-resistant (MDR), extensively drug-resistant
146 (XDR), and pandrug-resistant (PDR), while the Infectious Diseases Society of
147 America/National Institutes of Health (IDSA/NIH) defines them as difficult-to-
148 treat resistance (DTR) (6,7). *P. aeruginosa* possesses a non-clonal epidemic
149 population structure, comprising a limited number of widespread lineages,
150 selected from a background of numerous rare and unrelated genotypes
151 recombined at high frequency (8). In fact, several surveys have provided
152 evidence for the existence of XDR/DTR international high-risk clonal lineages,
153 which have disseminated in hospitals worldwide (9–11). Beyond classical
154 molecular epidemiology analysis and phenotypic assessment of resistance
155 mechanisms, whole genome sequencing (WGS) studies are providing pertinent
156 information to elucidate the complex and evolving resistome of MDR/XDR/DTR
157 *P. aeruginosa* high-risk lineages (12–15).

158 The recent introduction of novel β -lactam/ β -lactamase inhibitor
159 combinations (BLBLIs) such as ceftolozane/tazobactam, ceftazidime/avibactam,
160 meropenem/vaborbactam or imipenem/relebactam and the siderophore-
161 cephalosporin cefiderocol, has contributed to mitigate, to some extent, the
162 problem of XDR/DTR *P. aeruginosa* (16–19). These agents exhibit enhanced
163 stability against intrinsically- and chromosomally-encoded β -lactam resistance

164 mechanisms in *P. aeruginosa*, such as overexpression of the AmpC β -
165 lactamase encoding gene, overproduction of efflux pumps, or inactivation of the
166 OprD porin. However, they are not exempt from resistance development
167 through emerging mutational mechanisms (20–24). These include modification
168 (quantitative or qualitative) of AmpC hydrolytic activity or efflux pumps
169 substrate specificity, which were observed shortly after their introduction into
170 clinical practice. Moreover, BLBLIs are not currently effective against the most
171 potent transferable carbapenemases, particularly class B or metallo- β -
172 lactamases [MBLs] such as VIM, IMP or NDM enzymes (25). Consequently,
173 use of BLBLIs could lead to the selection of these concerning resistance
174 mechanisms (26). Besides the approved options, several novel BLBLIs are
175 undergoing clinical trials (25). These agents, such as aztreonam/avibactam,
176 cefepime/zidebactam or cefepime/taniborbactam, promise additional
177 therapeutic choices and the ability to counteract already established resistance
178 mechanisms (17).

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

190 **Questions addressed in the position paper**

191 To address this challenge, the European Study Group for Antimicrobial
192 Resistance Surveillance (ESGARS) from the European Society of Clinical
193 Microbiology and Infectious Diseases (ESCMID) launched the “Improving
194 Surveillance of Antibiotic-Resistant *Pseudomonas aeruginosa* in Europe”
195 (ISARPAE) initiative in 2022, supported by the Joint programming initiative on

196 antimicrobial resistance (JPIAMR) network. Thus, this position document from
197 the ESGARS-ISARPAE Group aimed to provide an update on (i) the emerging
198 resistance mechanisms to classical and novel anti-pseudomonal agents, with a
199 particular focus on β -lactams, (ii) the susceptibility profiles associated with the
200 most relevant β -lactam resistance mechanisms, (iii) the impact of the novel
201 agents and resistance mechanisms on the definitions of resistance profiles, and
202 (iv) the globally expanding XDR/DTR high-risk lineages and their association
203 with transferable β -lactamases.

204 **Methods**

205 All ESGARS-ESCMID members were contacted and invited to participate in the
206 ISARPAE initiative, according to their interest and experience in the topic. This
207 resulted in the generation of a panel of over 40 researchers from 18 European
208 countries in June 2022. The panel agreed the above objectives to be addressed
209 in the position paper and AO and ERM prepared a first draft of the documented
210 after extensive literature review helped by other panel members. In July 2023
211 the first draft of the document was sent to all ISARPAE members for revision
212 and specific contributions, leading to a second draft version that was
213 extensively revised and discussed during an ISARPAE hybrid (onsite/online)
214 meeting that took place at Hospital Son Espases-IdISBa (Mallorca, Spain) on
215 September 8th 2023. The third resulting draft was then sent for review by panel
216 members and final version of the document was approved in October 6th2023.

217 **Emerging resistance mechanisms to classical and novel anti-** 218 **pseudomonal agents and associated susceptibility profiles**

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

225

226 ***Pseudomonas aeruginosa* β -lactam resistome**

227

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

236

237 The most frequent mutation-driven resistance mechanism to classical
238 antipseudomonal penicillins (such as piperacillin) and cephalosporins (such as
239 ceftazidime or cefepime) is the overproduction of the chromosomal
240 cephalosporinase AmpC, involving a large number of genes belonging to cell-
241 wall recycling regulatory pathways (28). Notably, among these genes, the
242 mutational inactivation of *dacB*, encoding the nonessential penicillin-binding
243 protein (PBP] PBP4 and *ampD*, encoding a N-acetyl-muramyl-L-alanine
244 amidase, have been found to be the most frequent cause of derepressed *ampC*
245 gene expression, and subsequent broad-spectrum β -lactam resistance (29,30).
246 Additionally, specific point mutations causing a conformational change in the
247 transcriptional regulator AmpR, leading to *ampC* upregulation and resistance to
248 broad-spectrum β -lactams, have been noted among clinical strains. These
249 mutations include the D135N amino acid replacement, described in several
250 species (28) and the G154H mutation linked to the disseminated MDR/XDR
251 ST175 high-risk lineage (14). Mutation of several other genes, including those
252 encoding amidases (AmpDh2 and AmpDh3), PBPs, such as PBP5 or PBP7,
253 lytic transglycosylases, MPL, or NuoN have also been shown to enhance *ampC*
254 expression, either alone or in combination with other mutations. Nevertheless,
255 their impact on β -lactam resistance among clinical strains still needs to be
256 further analysed (28).

257 In addition to *ampC* overexpression, recent studies have revealed that
258 increased levels of β -lactam resistance, involving the novel BLBLIs
259 ceftolozane/tazobactam and ceftazidime/avibactam, may result from mutations
260 leading to the modification of the catalytic center of AmpC, currently mainly

261 occurring in (up to 10-15%) patients treated with these agents (20,31–33).
262 Additional studies identified diverse AmpC variants associated with high-level
263 resistance to BLBLIs, including the above mentioned ceftolozane/tazobactam
264 and ceftazidime/avibactam, in a small proportion (around 1%) of clinical *P.*
265 *aeruginosa* isolates (34). Over 500 variants of those AmpC enzymes, also
266 called *Pseudomonas* Derived Cephalosporinases (PDC), have been described
267 so far, including those associated with increased ceftolozane/tazobactam and
268 ceftazidime/avibactam resistance. Moreover, some of these variants, such as
269 those showing the L320P substitution, have a significant impact on cefiderocol
270 MICs, but only a marginal effect on susceptibility to ceftolozane/tazobactam and
271 ceftazidime/avibactam (35). An updated database of PDC variants is
272 maintained at IdISBa and is freely available at
273 ([https://arpbigidisba.com/pseudomonas-aeruginosa-derived-cephalosporinase-](https://arpbigidisba.com/pseudomonas-aeruginosa-derived-cephalosporinase-pdc-database/)
274 [pdc-database/](https://arpbigidisba.com/pseudomonas-aeruginosa-derived-cephalosporinase-pdc-database/)) and at the Beta-Lactamase Data Base
275 (<http://www.blbd.eu/BLDB.php?prot=C#PDC>) (36). Typically, the strains
276 producing these AmpC variants show collateral susceptibility to imipenem
277 (decreased MICs) and also to antipseudomonal penicillins such as piperacillin.
278 Additionally, resistance development to ceftolozane/tazobactam and/or
279 ceftazidime/avibactam may involve mutations leading to the structural
280 modification of narrow spectrum OXA-2 and OXA-10 acquired oxacillinases
281 (20,37,38). Interestingly, these mutations may lead to collateral susceptibility to
282 meropenem. Thus, imipenem/relebactam, and to a lesser extent, cefiderocol,
283 meropenem/vaborbactam and the novel combinations under development
284 cefepime/zidebactam and cefepime/taniborbactam might be interesting options
285 to treat infections by strains that have developed ceftolozane/tazobactam and/or
286 ceftazidime/avibactam resistance through mutations in AmpC or OXA-2/10 (39).

287 Horizontally-acquired β -lactamase genes are obviously a major source of
288 resistance, including to the novel β -lactams and BLBLI (**Figure 1**). An extensive
289 revision of the nature and prevalence of the different horizontally-acquired β -
290 lactamases detected in *P. aeruginosa* is beyond the scope of this document.
291 However, globally, MBLs are arguably the most frequent carbapenemases in *P.*
292 *aeruginosa*, but very large geographical differences in prevalence and nature
293 have been documented (40,41). At European level, VIM, and particularly VIM-2,

294 are likely the most frequently reported enzymes, but with major differences
295 across different countries, and with an increasing prevalence of NDM enzymes
296 (42,43). Moreover, GES class A carbapenemases variants such as GES-5 are
297 also increasingly reported in European countries (43,44). Classical
298 antipseudomonal penicillins, cephalosporins and carbapenems lack significant
299 activity and should be avoided against strains producing class A or MBL
300 carbapenemases, even if MICs close to the clinical breakpoints are obtained for
301 piperacillin/tazobactam, cefepime or even carbapenems for some VIM-2-
302 producing isolates (12). Moreover, the production of MBLs is a frequent
303 mechanism of resistance to ceftolozane/tazobactam, ceftazidime/avibactam,
304 meropenem/vaborbactam and imipenem/relebactam (26). However, with a few
305 exceptions such as some NDM variants, cefiderocol retains activity due to its
306 higher stability against hydrolysis and efficient uptake through the iron transport
307 systems (45). The combination of aztreonam with avibactam may also be a
308 useful future alternative for MBL producing strains, particularly when additionally
309 hyperproducing AmpC and/or coproducing acquired class A enzymes (46,47).
310 Likewise, the novel combinations under development cefepime/zidebactam and
311 cefepime/taniborbactam also remain active. The underlying mechanism for
312 cefepime/zidebactam activity against MBL producing strains is based on the
313 fact that zidebactam has direct antipseudomonal activity by targeting PBP2, and
314 therefore provides synergy with β -lactams targeting PBP3 such as the
315 cephalosporins (48). On the other hand, the activity of cefepime/taniborbactam
316 relies on the fact that taniborbactam inhibits MBL hydrolytic activity, except for
317 IMPs (49). In addition to these three antimicrobials (cefiderocol,
318 cefepime/zidebactam and cefepime/taniborbactam), ceftazidime/avibactam, and
319 to a lower extent imipenem/relebactam and meropenem/vaborbactam show
320 activity against producers of Ambler class A carbapenemases (such as GES-5
321 and KPCs) (50–52). However, the frequent concomitant OprD deficiency and/or
322 MexAB-OprM overexpression limits the activity of imipenem/relebactam and
323 meropenem/vaborbactam against clinical *P. aeruginosa* strains producing class
324 A carbapenemases (52,53). On the other hand, resistance development to
325 ceftazidime/avibactam caused by the selection of mutations within the catalytic
326 site of KPC and GES enzymes has been described (54–56). Interestingly, these
327 mutations restore carbapenem susceptibility (if the strain is not oprD deficient)

328 leading to an ESBL phenotype (54). In addition to those of classes A and B, a
329 few cases of class D carbapenemase production have been reported in *P.*
330 *aeruginosa*, including the epidemic dissemination OXA-198 in a hospital from
331 Belgium (57).

332 In addition to β -lactamases, there is growing evidence on the role of
333 target modification in *P. aeruginosa* β -lactam resistance. Of particular relevance
334 are the mutations in *ftsI*, encoding PBP3, an essential class B PBP with
335 transpeptidase activity (58). Indeed, data from cystic fibrosis (CF) patients
336 (59,60), epidemic high-risk clonal lineages (12,14) as well as from *in vitro*
337 studies (61) have shown that PBP3 is under strong mutational pressure, with
338 specific mutations in this PBP contributing to β -lactam resistance development.
339 R504C/H and F533L mutations are those being most commonly reported and
340 located within the protein domains implicated in the formation and stabilization
341 of the inactivating complex β -lactam-PBP3 (62). Moreover, these specific
342 mutations have been documented to emerge *in vivo* during chronic respiratory
343 infection in CF patients (59,60) and upon exposure to meropenem (61),
344 aztreonam (63) and ceftazidime (64) *in vitro*. However, the detailed effect of
345 PBP3 mutations on β -lactam resistance phenotypes needs to be further
346 investigated using isogenic strains. Likewise, despite unique polymorphisms
347 having been detected in some clinical strains for other PBPs, their potential role
348 in β -lactam resistance still needs to be experimentally determined. Also
349 noteworthy are the specific PBP2 mutations involved in resistance to
350 zidebactam (65), that obviate the β -lactam enhancer activity of this BLI.

351 Other relevant components of the β -lactam mutational resistome are the
352 genes encoding OprD and efflux pumps. The inactivation of OprD is known to
353 be the most frequent imipenem resistance mechanisms in *P. aeruginosa*
354 (66,67). OprD inactivation typically results from indels or nonsense mutations,
355 including the Q142X mutation, characteristic of the widespread ST175 high-risk
356 clonal lineage (14). Additionally, some amino-acid replacements have been
357 associated with OprD-driven resistance, particularly in the CF setting (68).
358 However, it should be noted that the presence of OprD inactivating mutations
359 has also been identified in some carbapenem-susceptible isolates (69). On the
360 other hand, imipenem resistance may also result from repression of *oprD*

361 caused by mutations in the MexEF-OprN efflux pump regulators (*mexS/T*) or
362 the ParRS two-component system (70). Overexpression of MexAB-OprM,
363 caused by mutation of several genes involved in its regulation (*mexR*, *nalC* or
364 *nalD*) increases MICs of most β -lactams including meropenem but not
365 imipenem, whereas overexpression of genes encoding MexXY (*mexZ*, *parRS*,
366 *amgS* mutations) is involved in cefepime resistance (70)

367 Efflux pumps may also play a major role in resistance to the novel
368 BLBLIs, not only because of their capacity to extrude the β -lactam components
369 but, particularly, for their capacity to accommodate their partner β -lactamase
370 inhibitor. Indeed MexAB-OprM overexpression plays a role in resistance to
371 ceftazidime/avibactam, aztreonam/avibactam, cefepime/zidebactam,
372 imipenem/relebactam, and meropenem/vaborbactam (65,71–73). Likewise,
373 MexXY overexpression should also impact cefepime combinations with
374 zidebactam or taniborbactam (65). Moreover, mutations leading to the
375 modification of the substrate recognition domain of the efflux pump MexCD-
376 OprJ have been shown to drive ceftolozane/tazobactam resistance
377 development *in vivo* (23)

378 Additionally, another potentially relevant mutational β -lactam resistance
379 mechanism is the selection of large [up to 600 kb] deletions affecting specific
380 parts of the chromosome (61,64). Although the basis of the conferred resistance
381 phenotype still needs to be further clarified, these mutants can be recognized by
382 the characteristic brown pigment (pyomelanine) caused by the deletion of one
383 of the included genes, *hmgA*, coding for a homogentisate-1,2-dioxygenase.
384 These deletions has been documented in both *in vitro* evolved β -lactam-
385 resistant mutants and CF isolates (61,74). However, the deletion of *hmgA* is not
386 responsible for the resistance phenotype, which could be linked to the deletion
387 of another of the affected genes, *galU*. This gene codes for a UDP-glucose
388 pyrophosphorylase involved in the synthesis of the lipopolysaccharide (LPS)
389 core. Indeed, analysis of transposon mutant libraries has revealed that
390 inactivation of *galU* increases the MICs of ceftazidime and meropenem (75,76).

391 Lastly, specific cefiderocol resistance development mechanisms involve
392 the selection of mutations in iron uptake systems, particularly in TonB-

393 dependent receptors such as *piuA/piuC*, *pirA/pirR* or *fptA* [pyochelin receptor]
394 (35). Among these, mutations seem to be particularly frequent in *piuC*, an iron-
395 dependent oxygenase involved in the expression of the adjacent *piuA* [or its
396 homolog *piuD* depending on the strain] iron receptor. On the other hand,
397 mutations in the *fptA* gene, despite being frequent, do not seem to have a direct
398 significant impact on cefiderocol MICs, and thus selection might reflect adaptive
399 mutations for growing in the presence of cefiderocol.

400

401 ***Pseudomonas aeruginosa* aminoglycoside resistome**

402 Primary aminoglycoside resistance is typically linked to the production of
403 horizontally-acquired aminoglycoside modifying enzymes, including
404 acetyltransferases, adenylyltransferases and phosphoryltransferases, frequently
405 co-transferred with ESBLs or carbapenemases (77). The specific pattern of
406 aminoglycoside resistance depends on the specific enzymes involved, with
407 amikacin showing an overall higher activity than tobramycin (78). However, the
408 more recently described transferable 16S rRNA methylases, which modify the
409 cellular target of aminoglycosides, are further concerning since they confer
410 resistance to all clinically available members of this antibiotic family and are
411 also cotransferred with ESBLs or carbapenemases (79–81).

412 On the other hand, the development of resistance to aminoglycosides
413 has been particularly linked to the overexpression of genes encoding the
414 MexXY-OprM system upon some mutations in the regulatory machinery.
415 Indeed, mutational overexpression of this pump, mainly caused by *mexZ*,
416 *amgS*, or *parRS* mutations, is very frequent among clinical isolates, from both
417 CF patients and nosocomial infections (82,83). Moreover, recent studies show
418 that the epidemic high-risk clone ST175 hyperproduces MexXY due to a
419 specific mutation in *mexZ* (G195E) (14). However, recent data suggests that the
420 aminoglycoside mutational resistome extends far beyond MexXY
421 hyperproduction, and high-level resistance may result from the accumulation of
422 multiple mutations. The involvement of several novel resistance determinants
423 has been documented (84–86). Among them, is noteworthy *fusA1*, coding for
424 the elongation factor G. Indeed, specific *fusA1* mutations have been linked to

425 aminoglycoside resistance *in vitro* (4,86) and among clinical, strains, particularly
426 from CF patients (4,60,87–89). Moreover, the implication of *fusA1* mutations in
427 aminoglycoside resistance has been demonstrated through site-directed
428 mutagenesis (90).

429

430 ***Pseudomonas P. aeruginosa* fluoroquinolone resistome**

431

432 Fluoroquinolone resistance in *P. aeruginosa* is primarily driven by
433 mutational mechanisms. The fluoroquinolone mutational resistome generally
434 includes specific missense mutations in DNA gyrase (*gyrA* and/or *gyrB*) and
435 topoisomerase IV (*parC* and/or *parE*) Quinolone Resistance-Determining
436 Regions (QRDRs) (13,91). High-level fluoroquinolone resistance in *P.*
437 *aeruginosa* high-risk lineages is nearly universal, and typically involves
438 combinations of mutations in GyrA T83 and ParC S87 (12)QRDR mutations
439 involved in fluoroquinolone resistance in CF might be more variable (60). It is
440 also well-known that the mutational overexpression of efflux pumps modulates
441 fluoroquinolone resistance [Table 1]. While the overexpression of MexAB-OprM
442 and MexXY-OprM is globally frequent among clinical strains, its contribution to
443 clinical fluoroquinolone resistance is likely to be modest (91)On the other hand,
444 the mutational overproduction of MexEF-OprN or MexCD-OprJ is associated
445 with clinical fluoroquinolone resistance. Although their prevalence has been
446 considered low, except in the settings of CF chronic infections, recent data
447 show that it might be higher than expected (68). Lastly, the transferable
448 quinolone resistance determinant QnrVC has also been reported, linked to
449 some epidemic strains producing acquired carbapenemases such as ST175
450 and ST244 (92,93).

451

452 ***Pseudomonas aeruginosa* polymyxin resistome**

453

454 Due to its limited efficacy, toxicity and high ECOFF values (4 mg/L),
455 colistin is not considered an optimal treatment for wild-type *P. aeruginosa*, at
456 least in monotherapy [www.eucast.org]. Moreover, whereas the prevalence of
457 polymyxin [colistin and polymyxin B] resistance is still globally low (<5%), it has

458 increased in the last years because of the frequent use of these last-resource
459 antibiotics for the treatment of MDR/XDR/DTR nosocomial and CF isolates,
460 particularly in countries with no access to novel BLBLIs (94). Polymyxin
461 resistance results most frequently from the modification of the LPS caused by
462 the addition of a 4-amino-4-deoxy-L-arabinose moiety in the lipid A structure
463 (95,96). The involved mutations are frequently located in the PmrAB or PhoPQ
464 two-component regulators, which lead to the activation of the *arnBCADTEF*
465 operon (97). More recent studies have revealed that mutations in the ParRS
466 two-component regulator, not only produce polymyxin resistance due to the
467 activation of the *arnBCADTEF* operon, but also lead to a MDR phenotype
468 determined by the hyperproduction of MexXY and the repression of *oprD* (98).
469 Moreover, two additional two-component regulators, ColRS and CprRS, have
470 also been determined to be involved in colistin resistance (99). The analysis of
471 colistin resistance mechanisms among clinical strains is not always
472 straightforward, since the presence of mutations in these two-component
473 regulators is not always associated with clinical colistin resistance, probably
474 denoting partial complementation between the different regulators (60,99,100).
475 Moreover, recent *in vitro* evolution assays have revealed the implication of
476 additional mutations in high level colistin resistance, facilitated by the
477 emergence of *mutS* deficient mutator (phenotypes such as those occurring in
478 LptD, LpxC or MigA (101). On the other hand, the role of phosphoethanolamine
479 modification of LPS in *P. aeruginosa* seems marginal, including both, that are
480 driven by intrinsic *eptA* gene expression (102) as well as that are driven by
481 transferable determinants (103).

482

483 ***Pseudomonas aeruginosa* fosfomicin resistome**

484

485 Although not classified as an antipseudomonal agent (ECOFF of 256
486 mg/L), fosfomicin has been considered in the last decade as a potentially
487 useful antibiotic in urinary tract infections and as combined therapy for
488 MDR/XDR/DTR *P. aeruginosa* in other infection sites (104). However,
489 spontaneous mutation rates for fosfomicin resistance are high and the
490 mechanism involved is typically the mutational inactivation of *glpT*, coding for a
491 glycerol-3-phosphate permease required for fosfomicin uptake (105,106).

492 Mutations in *gfpT* are also frequently found among MDR/XDR/DTR strains
493 (107). Certain specific mutations, like T211P, have become fixed in some
494 widespread lineages as described for ST175 (14)

495

496 **Definitions of resistance profiles in *Pseudomonas aeruginosa***

497 According to established recommendations by ECDC (6) the MDR profile
498 is defined as resistance to at least one agent in at least three of eight antibiotic
499 categories. These categories include antipseudomonal penicillins + β -lactamase
500 inhibitor combinations (ticarcillin/clavulanate, piperacillin/tazobactam),
501 antipseudomonal cephalosporins (ceftazidime and cefepime), monobactams
502 (aztreonam), antipseudomonal carbapenems (imipenem, meropenem,
503 doripenem), fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides
504 (gentamicin, tobramycin, amikacin, netilmicin), polymyxins (colistin, polymyxin
505 B) and fosfonic acids (fosfomicin). The XDR profile is defined as resistance to
506 at least one agent in all antibiotic classes except one or two. Likewise, PDR
507 profile is defined as resistance to all agents in the eight antibiotic categories.
508 The eighth category (fosfonic acids, fosfomicin) included in the ECDC
509 recommendations should be likely not considered, given the lack of current
510 EUCAST clinical breakpoints. Likewise, the inclusion of gentamicin as
511 antipseudomonal agents is questionable according to current EUCAST
512 breakpoints, and the activity of ticarcillin/clavulanate likely not comparable to
513 that of piperacillin/tazobactam in *P. aeruginosa*. On the other hand, the DTR
514 (difficult to treat resistance) profile is defined according to IDSA/NIH
515 recommendations as resistance to all first line (classical) agents:
516 antipseudomonal penicillins + β -lactamase inhibitor combinations,
517 cephalosporins, monobactams, carbapenems and fluoroquinolones (7). Thus, if
518 fosfomicin is not considered, all DTR isolates would meet the XDR criteria,
519 since they are resistant to at least five of seven categories, but not the other
520 way around.

521 However, neither the ECDC or IDSA/NIH definitions take into
522 consideration the novel β -lactams and BLBLIs. The inclusion of these novel
523 agents is challenging, starting by grouping them into meaningful “categories”

524 since their properties, spectrum and mechanisms of resistance show similarities
525 but also marked differences. As shown in **Table 1**, at least 5 novel categories
526 could be considered to include the novel β -lactams already approved: fifth
527 generation antipseudomonal cephalosporins + classical β -lactamase inhibitors
528 (ceftolozane/tazobactam), antipseudomonal cephalosporins +
529 diazabicycloctanes β -lactamase inhibitors (ceftazidime/avibactam),
530 antipseudomonal carbapenems + diazabicycloctanes β -lactamase inhibitors
531 (imipenem/relebactam), antipseudomonal carbapenems + boronic acid β -
532 lactamase inhibitors (meropenem/vaborbactam) and siderophore
533 antipseudomonal cephalosporins (cefiderocol). Additionally, there are at least
534 three further classes to be considered in the future if the corresponding
535 antibiotics are approved: monobactams + diazabicycloctanes β -lactamase
536 inhibitors (aztreonam/avibactam), antipseudomonal cephalosporins+
537 diazabicycloctanes β -lactamase and PBP2 inhibitors (cefepime/zidebactam)
538 and antipseudomonal cephalosporins + boronic acid β -lactamase inhibitors
539 including MBLs (cefepime/taniborbactam).

540 Within the framework of the ECDC definitions, these novel categories
541 could potentially align with MDR implying resistance to at least three classes (of
542 up to 13), XDR indicating resistance to all but one or two and PDR indicating
543 resistance to all. Regarding DTR definition, it would imply resistance to all the
544 novel β -lactams approved. However, the practical application of this definition is
545 likely to encounter challenges due to limited access to these antibiotics for
546 treatment and to the capacity to perform antimicrobial susceptibility testing in
547 several countries. Moreover, the classification of the resistance profiles for the
548 novel agents under development into clinical SIR categories will need to
549 consider PK/PD data, not yet available in some cases, in addition to existing
550 phenotypic and genomic information.

551

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

554 In a recent review (10), according to their prevalence, global spread and
555 association with MDR/XDR/DTR profiles, and specially with concerning
556 horizontally-acquired β -lactamases such as ESBLs and carbapenemases, the
557 worldwide top ten *P. aeruginosa* high-risk lineages were established to be, by
558 order of relevance, ST235, ST111, ST233, ST244, ST357, ST308, ST175,
559 ST277, ST654 and ST298. **Figure 2** shows updated information for these top
560 ten high-risk lineages, including their virulence profile (presence of the genes
561 coding the type III secretion system exotoxins ExoS and/or ExoU), worldwide
562 distribution and association with acquired carbapenemases from key
563 publications in the last three years (40–42,93,108–112). Particularly noteworthy
564 is the expansion of KPC enzymes in several of these lineages (ST233, ST277
565 and ST654 in addition to the previous detection in ST235, ST111 and ST244),
566 followed by NDM (ST244 and ST357 in addition to ST235, ST233, ST308 and
567 ST654). Moreover, coproduction of various carbapenemases is not infrequent
568 among those lineages (43). Besides these top ten lineages, a few others have
569 gained relevance in the last few years, including globally expanding ST309,
570 associated with the production of VIM-2, ST773 linked to NDM-1, or ST463
571 associated with the production of KPC-2, particularly in China (113–118).

572

573 **Concluding remarks and future challenges**

574 *P. aeruginosa* infections rank among the foremost global resistance
575 threats, associated with significant morbidity and mortality. *P. aeruginosa*
576 resistance mechanisms and epidemiology are complex and ever-evolving, with
577 a significant impact on novel and forthcoming β -lactams. The interplay between
578 novel antibiotics and resistance is notably challenging, as certain mechanisms
579 can lead to cross-resistance to multiple agents, while others may confer
580 collateral susceptibility to relevant antipseudomonals such as carbapenems.
581 The global dissemination of XDR/DTR high-risk lineages are also a major
582 challenge, particularly when coupled with increased virulence and capacity to
583 acquire exogenous resistance elements as documented for ST235 (11). In this
584 sense, a recent nation-wide survey of *P. aeruginosa* susceptibility profiles and
585 resistance genomics has revealed in one hand a significant generalized

586 decrease of resistance rates and XDR/DTR profiles in Spain in the last five
587 years, but in the other, a significant increase in the proportion of the concerning
588 carbapenemase-producing ST235 high-risk lineage (44).

589 Therefore, there is a major need for establishing comprehensive
590 resistance surveillance initiatives, integrating both phenotypic and genomic
591 data, as well as metadata. However, our current capacity to predict the
592 susceptibility profiles and emerging high-risk clonal lineages from genomic
593 sequences still needs to be improved, potentially through the incorporation of
594 machine learning, knowledge-based approaches, or so-called artificial
595 intelligence tools (43,119,120). Nevertheless, current achievable surveillance
596 strategies at European level should at least integrate: (1) monitoring of
597 concerning high-risk lineages (particularly ST235); (2) analyses of resistance
598 prevalence trends to recently introduced agents (like the novel BLBLIs) in
599 addition to classical antipseudomonals; (3) monitoring of strains producing
600 horizontally-acquired resistance mechanisms (particularly carbapenemases and
601 ESBLs); and (4) monitoring of noteworthy chromosomal resistance mechanisms
602 such as the AmpC (PDC) derivatives involved in resistance to the novel BLBLIs.
603 Likewise, in this scenario, antimicrobial stewardship and infection control are of
604 paramount importance. Nevertheless, these aspects are equally challenging
605 and should be guided by rapid diagnostics and antimicrobial susceptibility
606 testing, including the detection of resistance mechanisms and specific high-risk
607 clonal lineages (121). Thus, efforts should also be directed to the
608 implementation and scaling of personalized precision medicine that allows us to
609 establish early targeted treatments and specific epidemiological control
610 measures adapted to the strain/mechanism involved.

611

612 **Conflict of interests**

613

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620

621 **Authors contributions.**

622

623 All authors agreed the questions to be addressed in the position paper. AO and
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626

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1144 *Chemother.* 2021 Apr 1 ;76(4):945–9.

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

Antipseudomonal categories	Antipseudomonal 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
	Cefepime	MexXY \uparrow , AmpC \uparrow	PBP3, GalU	OXA-2/10	ESBLs, class A and B carbapenemases
Monobactams	Aztreonam	MexAB \uparrow , AmpC \uparrow	PBP3, GalU	OXA-2/10	ESBLs and class A carbapenemases
Carbapenems	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 **	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**	ESBLs, class A and B carbapenemases**
Monobactams + diazabicycloctanes β -lactamase inhibitors	Aztreonam/avibactam*	MexAB \uparrow	PBP3, GalU		ESBLs and class A carbapenemases**
Cephalosporins+	Cefepime/zidebactam*	MexXY \uparrow , MexAB \uparrow	PBP3, GalU		ESBLs, class A and B

diazabicyclooctanes β -lactamase and PBP2 inhibitors			PBP2		carbapenemases**
cephalosporins+ boronic acid β -lactamase inhibitors including MBLs	Cefepime/taniborbactam*	MexXY \uparrow , MexAB \uparrow	PBP3, GalU		IMPs
Fluoroquinolones	Ciprofloxacin, levofloxacin	QRDR	MexAB/XY/CD/EF \uparrow		Qnr
Aminoglycosides	Tobramycin, amikacin	MexXY \uparrow **	FusA1		Aminoglycoside modifying enzymes, 16S rRNA methylases
Polymyxins	Colistin, polymyxin B	PmrAB/PhoPQ/ParRS			MCR (Very uncommon)
Fosfonic acids	Fosfomicin	GlpT			FosA

* Not yet approved

**Low level resistance. Clinical resistance requires additional mechanisms

\uparrow Hyperproduction

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

Figure 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 color 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.

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+														

Figure 2. Summary of the main characteristics of the top 10 *P. aeruginosa* high-risk clones. Updated in July 2023 from Del Barrio-Tofiño 2020 (10). Novel descriptions since 2020 are shown in red.