Consensus Document

Antibiotic selection in the treatment of acute invasive infections by *Pseudomonas aeruginosa*: Guidelines by the Spanish Society of Chemotherapy

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ABSTRACT

Pseudomonas aeruginosa is characterized by a notable intrinsic resistance to antibiotics, mainly mediated by the expression of inducible chromosomic β-lactamases and the production of constitutive or inducible efflux pumps. Apart from this intrinsic resistance, *P. aeruginosa* possess an extraordinary ability to develop resistance to nearly all available antimicrobials through selection of mutations. The progressive increase in resistance rates in *P. aeruginosa* has led to the emergence of strains which, based on their degree of resistance to common antibiotics, have been defined as multidrug resistant, extended-resistant and panresistant strains. These strains are increasingly disseminated worldwide, progressively complicating the treatment of *P. aeruginosa* infections. In this scenario, the objective of the present guidelines was to review and update published evidence for the treatment of patients with acute, invasive and severe infections caused by *P. aeruginosa*. To this end, mechanisms of intrinsic resistance, factors favoring development of resistance during antibiotic exposure, prevalence of resistance in Spain, classical and recently appeared new antibiotics active against *P. aeruginosa*, pharmacodynamic principles predicting efficacy, clinical experience with monotherapy and combination therapy, and principles for antibiotic treatment were reviewed to elaborate recommendations by the panel of experts for empirical and directed treatment of *P. aeruginosa* invasive infections.

Key words: *Pseudomonas aeruginosa*, treatment, guidelines

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Elección del tratamiento antibiótico en la infección invasiva aguda por *Pseudomonas aeruginosa*: Guía de la Sociedad Española de Quimioterapia

RESUMEN

Pseudomonas aeruginosa se caracteriza por una notable resistencia intrínseca a los antibióticos mediada fundamentalmente por la expresión de β-lactamasas cromosómicas inducibles y la producción constitutiva o inducible de bombas de expulsión. Además de esta resistencia intrínseca, *P. aeruginosa* posee una extraordinaria capacidad para desarrollar resistencia a prácticamente todos los antimicrobianos disponibles a través de la selección de mutaciones. El aumento progresivo de la resistencia en *P. aeruginosa* ha llevado a la aparición de cepas que, de acuerdo con el grado de resistencia frente a los antibióticos habituales, se han definido como multirresistentes, extensamente resistentes y panresistentes. Estas cepas se están diseminando mundialmente, complicando progresivamente el tratamiento de las infecciones por *P. aeruginosa*. En este escenario, el objetivo de las presentes recomendaciones es la revisión y puesta al día de la evidencia publicada para el tratamiento de pacientes con infección aguda, invasiva y grave por *P. aeruginosa*. Con este fin, se han revisado los mecanismos de resistencia intrínseca, factores que favorecen el desarrollo de resistencia durante la exposición a antibióticos, prevalencia de la resistencia en España, antibióticos clásicos así como los de reciente introducción activos frente a *P. aeruginosa*, principios farmacodinámicos predictores de eficacia, experiencia clínica con tratamientos en monoterapia o terapia combinada y principios del tratamiento antibiótico para elaborar por un panel de expertos recomendaciones para el tratamiento empírico o dirigido de infecciones invasivas por *P. aeruginosa*.

Palabras clave: *Pseudomonas aeruginosa*, tratamiento, recomendaciones

Pseudomonas aeruginosa is not part of normal microbiota in healthy humans [1]. Significant and/or prolonged colonization by *P. aeruginosa* occurs following loss of resistance to colonization due to changes in the composition of normal microbiota as consequence of antibiotic treatment and/or pre-existence of severe disease. Clinical and experimental observations indicate that, in both cases, colonization occurs within the first 3-5 days of exposure to an environment with high exposure pressure, as in hospitals, mainly in Intensive Care Units (ICUs). In the 2016 ENVIN study, *P. aeruginosa* was the second most frequent isolated microorganism, just behind *Escherichia coli*, as cause of nosocomial infections in ICUs, and the third most frequent (after *E. coli* and *Staphylococcus aureus*) in community-acquired infections requiring ICU admission. Mortality of bacteremia by *P. aeruginosa* is 20-39% [2-11], values similar to or greater than those for bacteremia by *S. aureus* and candidemia episodes. In ventilator associated pneumonia (VAP), mortality is even higher, reaching 44% [12,13].

The progressive increase in resistance rates in *P. aeruginosa* has led to the emergence of strains which, based on their degree of resistance to common antibiotics, have been defined as multidrug resistant (MDR), extended-drug-resistant (XDR) and pan-drug-resistant (PDR) strains [14]. However, two new antibiotics active against *P. aeruginosa* have been introduced in the therapeutic armamentarium recently: 1) a new cephalosporin, ceftolozane, associated with tazobactam, active against most of the strains resistant to the remaining β-lactams [15,16], and 2) ceftazidime associated with a new β-lactamase inhibitor, avibactam, able to block AmpC β-lactamases, including those produced by *P. aeruginosa* [17,18]. Selection of the most appropriate antibiotic, dose, and route of administration, as well as potential association with other antibiotics, are critical decisions to obtain optimal clinical efficacy with the lowest risk of resistance increase and development of toxicity.

From the clinical point of view, infections caused by *P. aeruginosa* can be classified as: 1) acute superficial, noninvasive, infections in immunocompetent patients, 2) acute invasive infections in patients with significant comorbidities or immunodepression, and 3) chronic infections. The first group includes the following entities: external otitis (swimmer's ear), perichondritis, queratitis associated with the use of contact lens, hydromassage-associated folliculitis, paronychia (green nail syndrome), palmoplantar hidradenitis, foot bones osteomyelitis (secondary to puncture wounds by objects penetrating sport shoes), and interdigital intertrigo. In all these cases, the infection that follows the exposure to a high *P. aeruginosa* inocula could be self-limited or respond to topical or oral ciprofloxacin treatment, and only exceptionally could pose problems in relation to the presence or development of resistance. The second group includes, among others, bacteremia, nosocomial pneumonia or VAP, endocarditis in parenteral drug users, pacemaker infections, necrotizing enterocolitis in the neutropenic patient, post-surgical meningitis, cerebrospinal fluid shunt infection, necrotizing fasciitis, gangrenous ecthyma, tertiary peritonitis or peritonitis associated with ambulatory peritoneal dialysis, malignant external otitis, central venous catheter infection, burn wound infection, and urinary tract infection (pyelonephritis or prostatitis) in patients with vesical catheters. In all these circumstances, the severity of the infection and the risk of resistance in the infecting strain and of the empirical treatment resulting inadequate or generating higher degree of resistance, make important the knowledge of criteria guiding most appropriate treatment selection. The objective of the present guidelines is the treatment of this group of patients with acute, invasive, and usually severe infections by *P. aeruginosa*. In the third group, chronic infections are included. Usually, isolates of *P. aeruginosa* from patients with cystic fibrosis produce an extracellular polysaccharide, alginate, conferring mucoid-type colonies. The same phenotype could be observed in bronchial infections in patients with bronchiectasis, advanced COPD (GOLD IV) or panbronchiolitis. These strains are usually less virulent and rarely produce bacteremia or extend beyond the lung. However, growth within biofilms makes difficult its eradication, and in advanced stages it is not possible with current treatments.

The present document does not address the treatment of chronic infections observed in patients with cystic fibrosis or bronchiectasis since it was subject of two recently published consensus [19,20]. We have reviewed the mechanisms of intrinsic and acquired resistance in *P. aeruginosa*, and their prevalence in Spain, to review afterwards the principles of treatment, basis for the further analysis of the main antibiotics with activity against *P. aeruginosa*. Lastly, recommendations for empirical and directed treatments are formulated.

MECHANISMS OF INTRINSIC RESISTANCE IN *P. aeruginosa* AND RESISTANCE DEVELOPMENT DURING TREATMENT

P. aeruginosa is characterized by its notable intrinsic resistance to antibiotics, mainly determined by the expression of inducible chromosomic AmpC β-lactamase and the production of constitutive (MexAB-OprM) or inducible (MexXY) efflux pumps [21]. The expression of inducible AmpC is determinant in the natural resistance of *P. aeruginosa* to most penicillins and cephalosporins [22]. Besides, the constitutive expression of MexAB-OprM contributes to the reduced susceptibility of *P. aeruginosa* to all β-lactams (except imipenem) and fluoroquinolones [23]. In addition, the inducible expression of MexXY has a major role in the lower basal activity and adaptive (inducible) resistance to aminoglycosides in *P. aeruginosa* [24]. Similarly, the inducible expression of operon *arnBCADTEF,* responsible for the addition of a 4-aminoarabinose residual to lipid A of the lipopolysaccharide, is critical for the development of inducible/adaptive resistance to polymyxins [25].

Apart from its notable intrinsic resistance, *P. aeruginosa* possess an extraordinary ability to develop resistance to nearly all available antimicrobials, through the selection of mutations in a complex network of genes implicated in resistance and their regulation [21,26]. This fact has major consequences for the efficacy of treatments for *P. aeruginosa* infections, mainly among critical patients at the ICU or

those with chronic infections where the problem is magnified due to the high frequency of hypermutator strains, which present a spontaneous mutation rate up to 1000 times higher than normal [27]. The rate of spontaneous mutation for development of resistance usually ranges from 10^{-6} (1 mutant per million bacteria) to 10^{-8} (1 mutant per 100 millions) for most antibiotics. Therefore, in those infections linked to high bacterial load (as respiratory infections) the probability of resistance development is elevated for most classical antipseudomonal compounds, even for strains with normal rate of spontaneous mutation (non hypermutator strains). In fact, for most antipseudomonals mutant prevention concentrations (MPCs) [28] are frequently above concentrations achieved by systemic administration; colistin and ceftolozane/tazobactam being among the few exceptions [29]. Table 1 summarizes the characteristics of resistance development for the main antipseudomonals, including: a) main mechanisms of resistance developed through exposure to each antibiotic, b) the relatively frequency of spontaneous occurrence, c) the baseline minimal inhibitory concentrations (MICs) and MPCs, and d) development of cross-resistance to other antipseudomonals.

The main mechanism of development of resistance to penicillins (ticarcillin, piperacillin, piperacillin-tazobactam) and cephalosporins (ceftazidime and cefepime) active against *P. aeruginosa*, is the selection of mutants with constitutive hyperproduction (derepression) of inducible AmpC chromosomic cephalosporinase [30]. Although AmpC derepression also increases MIC of ceftolozane-tazobactam, clinical resistance to this new combination requires an additional structural modification of AmpC, thus explaining the lower development of resistance [31]. The new combination of ceftazidime with the β-lactamase inhibitor avibactam, equally preserves activity against AmpC hyperproducer strains [32]. Among the great number of mutational resistance mechanisms stand out the repression or inactivation of the OprD porine which, together with the inducible expression of AmpC, confers resistance to imipenem and reduced susceptibility to meropenem [22]. Frequently, inactivation of OprD also synergically acts with derepression of AmpC, confering resistance to all available β-lactams except ceftolozane/tazobactam [33] and ceftazidime/avibactam [32]. Finally, the hyperexpression of any of the multiple efflux pumps, mainly MexAB-OprM and MexXY-OprM and to a lesser entent MexEF-OprN and MexCD-OprJ, significantly contributes to the resistance phenotypes [23]. MexAB-OprM is the efflux pump presenting the larger substrate profile. Its constitutive expression plays an important role in intrinsic resistance and its hyperexpression by chromosomic mutations affects all classical β-lactams (except imipenem) and fluoroquinolones. Hyperexpression of MexAB-OprM plus OprD inactivation is one

Table 1 **Activity and frequency of individual and cross-resistance resistance development for the different antipseudomonals, according to mechanisms implicated**

Antimicrobial[a]	PIP-TZ	CAZ	FEP	TOL-TZ	ATM	IMP	MER	FQ	AMG	COL	FOS	MIC (mg/L)	MPC (mg/L)	Primary R MEC	Secondary R MEC
PIP/TZ	+++	+++	++	-	++	-	+	-/+	-	-	-	2	>32	↑ AmpC	↑ MexAB
CAZ	+++	+++	++	-/+	++	-	+	-/+	-	-	-	1	>32	↑ AmpC	↑ MexAB
FEP	++	++	+++	-/+	+++	-	++	+	+	-	-	1	>32	↑ MexAB/XY	↑ AmpC
TOL/TZ	-/+	+	+	+	-/+	-	-/+	-	-	-	-	0.5	2	↑ AmpC+mut AmpC	PBP3
ATM	++	++	+++	-/+	+++	-	++	+	-	-	-	4	>32	↑ MexAB/XY	↑ AmpC
IMP	-/+	-/+	-/+	-	-/+	+++	++	-/+	-	-	-	1	>32	OprD	MexST (↑ MexEF ↓ OprD)
MER	+	+	+	-	+	++	++	+	-	-	-	0.5	8	OprD	↑ MexAB, PBP3
FQ[b]	+	+	++	-	++	-/+	+	+++	+	-	-	0.12	2	QRDR	↑ MexAB/XY/CD/EF
AMG[c]	-	-	+	-	-	-	-	+	++	-	-	1	8	↑ MexXY	FusA
COL	-	-	-/+	-	-	-/+	-/+	-/+		+	-	0.5	2	*pmrAB/phoPQ*	*parRS*
FOS	-	-	-	-	-	-	-	-	-	-	++++	64	>1,024	GlpT	

PIP-TZ: piperacillin-tazobactam; CAZ: ceftazidime; FEP: cefepime; TOL-TZ: ceftolozane-tazobactam; ATM: aztreonam; IMP: imipenem; MER: meropenem; FQ: Fluoroquinolones; AMG: aminoglycosides; COL: colistin; FOS: fosfomycin. MIC: minimum inhibitory concentration. MPC: mutant prevention concentration (concentration preventing selection of resistant mutants). R MEC: resistance mechanism

[a]Frequency of spontaneous development of clinical resistance (EUCAST resistance breakpoints) to antibiotics in columns by exposure to antibiotics in rows. (++++) Extremely elevated resistance development, (+++) Very elevated resistance development, (++) Elevated resistance development, (+) Moderate resistance development, (-/+) Low or improbable resistance development, (-) Non expected resistance development. Data shown in the Table refer to wild-type strains without acquired mechanisms of resistance, using as reference strain PAO1 (28;236; A. Oliver data non published). [b]FQ resistance development: levofloxacin > ciprofloxacin (pumps hyperexpression). Data shown in the Table refer to ciprofloxacin. [c]Resistance development aminoglycosides: gentamicin > amikacin > tobramycin. Data shown in the Table refer to tobramycin.

of the most frequent causes of clinical resistance to meropenem [34]. The expression of inducible MexXY plays an important role in the intrinsic resistance to aminoglycosides, and its mutational hyperexpression in the acquired resistance to cefepime. Hyperexpression of MexEF-OprN and MexCD-OprJ is less frequent and mainly affects quinolones. However, mutations (*mexT*/*mexS*) leading to hyperexpression of MexEF-OprN also confer decreased susceptibility to carbapenems through repression of *oprD*. Quinolone resistance is frequently produced by mutations in topoisomerases including ADN gyrase (GyrA/GyrB) and type IV topoisomerases (ParC/ParE). Lastly, development of resistance to polymyxins generally implies the modification of lipopolysaccharide mediated by mutations in the two-component systems involving PmrAB, PhoPQ or ParRS [35]. Interactions between all these mutations are complex, but it should be taken into account that in many cases the selection of a first mutation facilitates the subsequent selection of others, frequently resulting in MDR/XDR phenotypes close to panresistance.

PREVALENCE AND MECHANISMS OF PRIMARY RESISTANCE IN SPAIN

Although there are local important differences that should be analyzed and considered at each institution, table 2 shows the estimated prevalence and resistance mechanisms in *P. aeruginosa* that could be expected in Spanish hospitals. Overall, resistance rates are over 20% for most antipseudomonal antibiotics, including penicillins (piperacillin, piperacillin-tazobactam), cephalosporins (ceftazidime, cefepime), monobactams (aztreonam), carbapenems (imipenem, meropenem), fluoroquinolones (ciprofloxacin, levofloxacin) and aminoglycosides (gentamicin and tobramycin). Among the available antipseudomonal antibiotics, only colistin, amikacin and the recently introduced combination ceftolozane-tazobactam exhibit an activity close to 95%. The prevalence of MDR strains is already above 30% worldwide, including Spanish hospitals; approximately half of MDR strains would be also XDR [9]. The increasing prevalence of MDR/XDR phenotypes results from the combination of the extraordinary ability of *P. aeruginosa* to develop resistance against nearly all available antimicrobials through selection of chromosomal mutations, together with the increasing frequency of exogenous resistance determinants, generally localized in integrons codified in transferable genetic elements (plasmids or transposons) [21]. Among these determinants, due to its clinical importance, the genes of β-lactamases with higher hydrolytic profile, class B carbapenemases (metallo-β-lactamases, MBLs) and extended-spectrum β-lactamases (ESBLs), usually associated with determinants of aminoglycoside resistance should be highlighted [36]. No doubt that intra-hospital dissemination, originating epidemic/endemic situations of MDR/XDR strains, plays an important role in the increasing magnitude of this problem. Even more important if posible is the alarming evidence of epidemic MDR/XDR strains widely disseminated worldwide, the so-called *high-risk clones*, mainly ST111, ST175 and ST235 [37]. A recent Spanish multicenter study (2015) showed that the most prevalent clone was by far ST175, being responsible for 68% cases of XDR *P. aeruginosa* in our country [38]. This study also showed that 20% of XDR strains were carbapenemase-producers (mainly VIM-type MBLs), while in the remaining 80% β-lactam resistance was mediated by chromosomal mutations (OprD inactivation + AmpC hyperproduction). It should be highlighted that although all XDR strains were resistant to all classical antipseudomonal β-lactams, only those carbapenemase-producing strains were highly resistant (MIC > 8 mg/L) to ceftolozane-tazobactam. In fact, 68% of XDR strains were susceptible to this combination, although in many cases MICs were close to EUCAST and CLSI breakpoints (4 mg/L) [38].

Table 2 **Prevalence and primary resistance mechanisms expected in *P. aeruginosa* in Spain.**

Antimicrobial[a]	% I+R (R)[a]	In order of frequency implicated mechanisms of resistance[b]
PIP-TZ	20-30	↑AmpC (++), ↑MexAB (+), MBL (+), OXAs and other ESBL (+)
CAZ	20-30	↑AmpC (++), ↑ MexAB (+), MBL (+), OXAs and other ESBL (+)
FEP	20-30	↑MexAB/XY (++), ↑AmpC (++), MBL (+), OXAs and other ESBL (+)
TOL-TZ	1-5	MBL (+), OXAs and other ESBL (+) ↑AmpC+mut AmpC (-/+)
ATM	>50 (20-30)	↑MexAB/XY (+++) ↑AmpC (++), OXAs and other ESBL (+)
IMP	20-30 (20-30)	OprD (+++), MBL (+)
MER	20-30 (5-20)	OprD (+++), ↑MexAB (++), MBL (+)
CIP	30-50	QRDR (+++), ↑MexAB/XY (++), ↑MexCD/EF (+)
TOB	20-30	Modified enzyme AMG (++) ↑MexXY (+)
AMK	5-20 (1-5)	↑MexXY (++),modified enzyme AMG (+)
COL	1-2	*pmrAB/phoPQ/parRS* (-/+)

PIP-TZ: piperacillin-tazobactam; CAZ: ceftazidime; FEP: cefepime; TOL-TZ: ceftolozane-tazobactam; ATM: aztreonam; IMP: imipenem; MER: meropenem; CIP: ciprofloxacin; TOB: tobramycin; AMK: amikacin; COL: colistin

[a]Prevalence of primary resistance expected in Spain, according to 2017 EUCAST breakpoints. When there is an intermediate susceptibility category, prevalence of non-susceptible strains (I+R) is shown and prevalence of resistant strains are in parenthesis. Data estimated using available information from EARS-Net (https://ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net), multicenter studies (29;33;101;237) and microbiology department in several Spanish hospitals (H. Son Espases, Palma de Mallorca; H. Clinic, Barcelona; H. A Coruña, A Coruña).

[b]Relative frequency of resistance mechanisms: +++ (20-30%), ++ (5-20%), + (1-5%), -/+ (<1%).

PRINCIPLES FOR THE TREATMENT OF INFECTIONS CAUSED BY *P. aeruginosa*

Principles guiding election of antibiotic treatment, whether empirical or directed treatment, in case of suspected or confirmed *P. aeruginosa* infections, are those also applying to any severe infection, but with some peculiarities as follows:

1) MIC of main antibiotics active against *P. aeruginosa*. The breakpoint used to categorize *P. aeruginosa* as resistant to one β-lactam or aminoglycoside is from 2-times (piperacillin-tazobactam, imipenem, tobramycin, gentamicin) to 8-times (ceftazidime, cefepime) higher than the one used to consider resistant an enterobacteria. Against most clinical isolates of *P. aeruginosa* susceptible to β-lactams, the MIC of an antibiotic is usually at or close to its breakpoint value (2-8 mg/L). For this reason, high doses of β-lactams are recommended, even if the strain has been categorized as susceptible in *in vitro* susceptibility tests.

Clinical and/or bacteriological efficacy of β-lactams is related with the time of exposure of the microorganism to the antibiotic or the percentage of time that the free fraction of the antibiotic exceeds the MIC (% *f*T>CMI). In the treatment of infections by Gram-negative bacilli, including *P. aeruginosa,* ceftazidime and cefepime exhibits bactericidal effect (reduction of 2-3 $\log_{10}$ CFU) when serum concentrations exceed the MIC for more than 60% of the dosing interval [39]. Clinical cure, especially in severe infections, has been related with exposure to antibiotic concentrations 4-times higher than the MIC for 100% of the dosing interval [40,41]. In an *in vitro P. aeruginosa* growth model, the PK/PD index predicting efficacy for piperacillin-tazobactam was a maintained antibiotic concentration 5-times above the MIC [42]. In another similar study performed with a *P. aeruginosa* inocula of 10^8 CFU/mL, the consecution of a Cmin/MIC index ≥3.8 avoided emergence of resistance [43].

Elimination half-lives for most β-lactams are 1-2 hours. After 30-minutes administration of standard doses at 8-hour intervals, serum concentrations decrease below 4-8 mg/L before the 4^{th}-6^{th} hours from administration, especially in septic patients with a volume of distribution (V_d) and renal clearance presumably elevated. For the treatment of severe or high bacterial load infections, produced by microorganisms exhibiting MIC ≥4 mg/L of the β-lactam, only elevated doses administered by continuous or extended infusion reach free antibiotic concentrations exceeding 4-times the MIC [44-46]. Nevertheless, serum antibiotic concentrations in the first hours (until the steady state is reached) are noticeably lower after continuous infusion than with a 30-min dose administration. Consequences of the delay may be important for critically ill patients or patients with severe immunodepression or severe infection. In these circumstances it is necessary to start antibiotic treatment with an additional loading dose, by bolus infusion, followed by the total daily dose administered as continuous infusion. The initial dose by bolus infusion allows early achievement of an elevated Cmax, favoring diffusion of the free fraction of the antibiotic to the infectious foci, on one side, and on the other, to relatively compensate the increased V_d and renal clearance in early phases of sepsis.

Several studies performed in patients with *P. aeruginosa* infections analyzed the potential advantages of maintaining serum β-lactam concentrations over MIC values for the maximum possible time through iv continuous or extended infusion administration. In a retrospective study including 87 patients with bacteremia and/or pneumonia by *P. aeruginosa*, the extended infusion of cefepime (MIC_{50}=4 mg/L) significantly decrease mortality and days in the ICU compared with the standard intermittent administration [47]. In other study, piperacillin-tazobactam was administered as intermittent doses or 4-hour extended infusion in the treatment of 194 patients with infections by *P. aeruginosa* [48]. For the extended infusion, doses were lower than those used for intermittent administration; however, both mortality was lower and mean hospital stay was shorter in the group of patients receiving extended infusion. The difference was significant only in the subgroup of more severe patients (APACHE II ≥17) [48]. In cystic fibrosis patients with acute exacerbations of *P. aeruginosa* infection, extended or continuous infusion of a β-lactam (generally ceftazidime) has shown to be better than intermittent dose administration, with respect to improvement of FEV1, forced vital capacity and extension of exacerbation-free intervals [49]. The potential greater efficacy of continuous infusion has also been shown in Montecarlo simulations with patients treated with meropenem [50] or piperacillin-tazobactam [51] and in one case of carbapenem-resistant *P. aeruginosa* infection well controlled by 12g of meropenem extended infusion [52]. In rabbit models of infectious endocarditis caused by *P. aeruginosa*, maintained ceftazidime concentrations 4-5 times over the MIC provided optimal clinical efficacy [53,54]. *In vitro* models of *P. aeruginosa* infection also indicate that continuous infusion is the most efficient administration for β-lactams [55-57].

In most clinical studies [58-66] but not in all [67-69], continuous or extended infusion of piperacillin-tazobactam, cefepime, ceftazidime or meropenem, for the treatment of infections by Gram-negative bacilli (including *P. aeruginosa*) was more efficacious than intermittent administration with respect to the one or more following parameters: clinical cure rate, microbiological eradication, days with fever, length of ICU or hospital stay and decrease in severity (measured by APACHE II) and/or mortality. Negative or non-conclusive results in some studies might be explained by one or more of these facts: 1) the infecting microorganism was highly susceptible to the antibiotic used (very low MIC) and the antibiotic administration as intermittent doses was enough to maintain a serum concentration over the MIC for most of the dosing interval [67], 2) patients were not critically ill and/or did not suffer a severe infection [69], 3) the dose used for intermittent administration was frequently higher than the dose used for continuous infusion [63,69], 4) a significant number of patients was treated with a concomitant antibiotic (aminoglycoside or fluoroquinolone) [69], and 5) other factors that might have attenuated potential advantages of continuous infusion

were absence of an initial loading dose, lack of consideration of the favorable effect of renal function impairment on the intermittent dosification and the recruitment of an insufficient number of patients to obtain significant differences [70]. The conclusion of three meta-analyses [71-73] including most of the above referred studies, was favorable to the use of extended or continuous infusion with respect to the risk of death. On the contrary, a third meta-analysis [74] including, among others, studies carried out in patients with COPD exacerbations, did not show differences in outcome in relation to ways of antibiotic administration.

A recent study [75] showed that in septic patients attended at the Emergency department, a first-to-second antibiotic dose delay of near 4h (for 6-hour dosing intervals) was seen in >50% patients. The delay in the second dose administration was associated with a significant increase in mortality. Antibiotic continuous infusion can preclude the risk of an eventual prolongation of the dosing interval.

The main determinant for clinical response to an aminoglycoside treatment is the Cmax/MIC value [76]. For the reasons exposed below, the greatest efficacy for a treatment is obtained when Cmax/MIC ≥10. For a MIC value for *P. aeruginosa* of 2-4 mg/L of tobramycin and gentamicin, the recommended Cmax is 30-40 mg/L and for amikacin MIC of 8 mg/L, Cmax should be between 60 and 80 mg/L [77]. As later commented, usually these values are not achieved with standard doses.

2) Importance of the bacterial load in the infectious foci. In *P. aeruginosa* infectious foci as pneumonia, purulent tracheobronchitis in the intubated patient, secondary peritonitis, neutropenic colitis and skin and soft tissue infections (gangrenous ecthyma, cellulitis in a diabetic foot wound or wound infection in severely burned patients), the bacterial load at antibiotic treatment initiation is usually high (≥10^7-10^8 CFU). This bacterial inocula is between 100 and 1000 higher than standard inocula used in *in vitro* susceptibility tests. The intrinsic activity of most antibiotics decreases when bacterial load is high. In the case of β-lactams, this effect could be due to a reduced growth rate and/or expression of different PBPs with reduced affinity to β-lactams in the stationary phase of growth or the increase of β-lactamase concentrations due to bacterial lysis. Piperacillin and piperacillin-tazobactam seems the most affected by the inoculum size followed by ceftazidime, with meropenem in the third place [78]. For β-lactams, time over MIC is the most important factor for low bacterial inocula or very susceptible microorganisms. However, when the microorganism is less susceptible or the inoculum is high, the β-lactam activity shows certain dependence of the antibiotic concentration [40].

The ability of granulocytes to eradicate microorganisms is saturable [79]. In rat models of pneumonia by *P. aeruginosa*, when the bacterial load was close to or higher than 2.5 x 10^6 CFU/g of tissue, the bacteriolytic ability of granulocytes was surpassed and bacterial growth occurred [80,81]. The authors of these studies suggested that in infections with high bacterial load, as VAP, an early and rapid ≥2 $\log_{10}$ CFU/mL decrease produced by the antibiotic treatment might decrease bacterial density below the cut-off level of granulocyte activity saturation, allowing an optimal contribution for microorganism eradication.

Another important consequence of the presence of a high bacterial load is the increased risk of selection of resistant mutants.

3) Mutation ability and development of resistance in *P. aeruginosa*. Frequency of emergence of resistant mutants within *P. aeruginosa* populations ranges from 10^{-6} to 10^{-8} depending on the antibiotic [82]. In the presence of agents damaging DNA (fluoroquinolones) and in biofilm-embedded bacterial growth, the basal rate of emergence of resistant mutants can be around 100 times increased. These are strains with mutations in genes involved in repair mechanisms of DNA replication errors. These hypermutants strains are usually seen in the mucoid phenotype present in patients with cystic fibrosis and other situations as chronic bronchial infections [83-87].

A bacterial density ≥ 10^7-10^8 CFU at treatment initiation involves high risk of selection and amplification of the resistant subpopulation under the selective antibiotic pressure. Measures to counter this risk include: a) reduction of the bacterial load through the control of the infectious foci (drainage, debridement, de-obstruction or removal of catheter or infected foreign body), b) initiation of treatment with associations of antibiotics not sharing the main resistance mechanism [88], and c) use of doses and/or routes of administration able to generate an antibiotic concentration higher than MIC for potential resistant mutants in the infectious foci.

If the *P. aeruginosa* infecting strain is susceptible to the antibiotics used and the dose and the administration schedule are appropriate, after 48-72 hours of treatment, the residual bacterial load in the infectious foci would presumably be lower to the one needed to generate a significant number of resistant mutants, i.e., lower than the inferior limit of the spontaneous mutation rate (10^{-6}). From then, the risk of development of resistance in the infectious foci could be considered as negligible and, if there are no other reasons justifying the association (see below), treatment can be continued as monotherapy with the β-lactam chosen based on the antibiogram.

Antibiotics (aminoglycoside, ciprofloxacin or levofloxacin) associated with the β-lactam during the first 48-72 h, among other purposes to avoid selection of resistant mutants, should be administered at doses achieving concentrations over the corresponding MPCs. Although MPCs are unknown and could not be predicted from MIC values, generally for these antibiotics they are from 8 to 12 times higher than the MIC. In any case, the activity of these antibiotics is concentration-dependent and, higher the concentration in the infection foci, higher the bactericidal effect and lower the number of resistant mutants surviving antibiotic exposure. *In vitro* studies carried out with *P. aeruginosa* strains have shown that exposure to high tobramycin concentrations for 1-4 h [89] and to high ciprofloxacin

concentrations along 1 and 10h [90] widely reduce bacterial population without selection/amplification of resistant mutants. However, in both experiments the addition of a second antibiotic was needed to prevent regrowth of the residual bacterial population that remained susceptible.

At the 2nd-3rd day of treatment, when deescalation to monotherapy is considered, most patients remain colonized by *P. aeruginosa* in mucosa and bronchial secretion (in case of pneumonia, tracheal intubation or previous bronchial pathology), especially if no inhaled antibiotic treatment with tobramycin, colistin or aztreonam had been administered. Persistence of bronchial colonization does not justify by itself prolongation of iv administration of the aminoglycoside more than 3-5 days. Despite reaching a Cmax in serum ≥10 times the MIC, there is a low probability that the concentration and the activity of the aminoglycoside in bronchial secretion exceeds the MPC, thus hardly precluding development of resistance at the expense of a higher risk of renal toxicity secondary to treatment prolongation. The same concept could be applied to colistin administered by systemic route, but not to ciprofloxacin and levofloxacin with better diffusion to bronchial secretion.

4) Importance of an appropriate empirical treatment. Studies performed in patients with VAP [13,91] or bacteremia [2-4,8,11,92-94] caused by *P. aeruginosa* showed high mortality rates if the initial empirical antibiotic treatment is not appropriate. Non appropriate antibiotics are those for which the microorganism shows resistance in *in vitro* susceptibility tests. Early administration of an appropriate antibiotic treatment has special relevance when the infection presents clinical or biological severity criteria, the patient suffers important immunodepression or comorbidities or has advanced age. These are particularly frequent clinical situations in patients with *P. aeruginosa* infections [3,95-97]. Given the current high prevalence of *P. aeruginosa* strains resistant to β-lactams, treatment initiation with a β-lactam associated with amikacin, ciprofloxacin or colistin (chosen based on local resistance rates) increases the probability of the appropriateness of the initial empirical schedule, that is, the *P. aeruginosa* strain is at least susceptible to one of the two antibiotics administered [91,93,94,98,99].

5) Value of antibiotic associations. Usually, the association of a β-lactam and an aminoglycoside shows *in vitro* synergistic activity. However, in clinical practice, the potential synergy of the association does not seem to turn into a tangible improvement of prognosis estimated as survival rate. Most studies carried out in patients with bacteremia [5,92,94,100-104] or VAP [91,105,106] by *P. aeruginosa*, as well as several meta-analyses [98,99,107], did not found significant differences in mortality rates between patients receiving β-lactam monotherapy and those receiving a β-lactam and aminoglycoside association. Nevertheless, there are several aspects raising doubts with respect to the strength of these results. Most studies were retrospective analyses, treatments were not randomized, the most severe patients tended to be treated with antibiotic associations [107] and analyses were not adjusted by possible confounding factors. In a significant number of patients, the origin of bacteremia was an urinary tract infection or venous catheter removal, thus, non-severe infections and low bacterial load. In addition, in the aminoglycoside arm nephrotoxicity masking the benefits of the association could not be ruled out since renal failure is an important prognostic factor in critically ill patients. On the other hand, in other studies, a favorable effect of the association versus monotherapy has been reported in the treatment of bacteremia caused by *P. aeruginosa* [2,108], particularly in neutropenic patients [109-111], in cystic fibrosis exacerbations [112] and in a meta-analysis of studies on bacteremia by Gram-negative bacilli [113]. However, these results are neither conclusive because in the monotherapy arm patients treated with aminoglycosides were often included [110,113]. The efficacy of aminoglycosides is lower than that of β-lactams [92,111] except in urinary tract infections [114].

The results of all these so far published studies on *P. aeruginosa* infections comparing monotherapy of a β-lactam with combinations of β-lactams and aminoglycosides, are at least questionable since the aminoglycoside concentration in serum was never optimized in the first 24-48 hours. This could be a critical issue explaining the apparent lack of *in vivo* synergy and other possible favorable effects of the combination, particularly in the case of *P. aeruginosa* infection for two reasons: the first one in relation to the mechanism of synergy and the second one related to the adaptive resistance phenomenon. At low or intermediate tobramycin concentrations (<4 mg/L) the main mechanism of bacterial lysis is the block of protein synthesis at the ribosome, while at more elevated concentrations (≥8 mg/L), the main lytic mechanism is the aminoglycoside interaction with divalent cations stabilizing lipopolysaccharide molecules of the outer membrane. Since aminoglycosides molecules are bigger than Ca^{2+} and Mg^{2+} ions, their substitution by the aminoglycoside causes the disruption of the external membrane, with the subsequent increase in permeability [115]. In Gram-negative bacilli, and specially in *P. aeruginosa,* the outer membrane constitutes the main barrier for penetration of many antibiotics. The achievement of a high aminoglycoside concentration in the infectious foci is, probably, an important target if synergistic activity is to be obtained.

The result of *P. aeruginosa* exposure to aminoglycosides is an early and rapid concentration-dependant bacterial lysis followed by a refractory phase characterized by a low and concentration-independant bacterial destruction known as adaptive resistance [24]. This phenotype of partial and transitory resistance is due to the fact that the aminoglycoside, even at subinhibitory concentrations, induces the expression of genes codifying the MexXY efflux pump [116]. A similar phenomenon is observed in anaerobic or hyperosmolar media, at acidic pH and in the presence of elevated concentrations of divalent ions (Ca^{2+} or Mg^{2+}) [117]. This effect is more pronounced against *P. aeruginosa*. Several of these conditions are present in urine and bronchial secretion. Adaptative resistance justifies, among

others, the administration of aminoglycosides as single daily doses. If after the first aminoglycoside dose, a Cmax approximately 10 times the MIC is not reached, the intrinsic bactericidal activity of the antibiotic is lower than optimal, not surpassing the MPC and reduces the possibility and/or the degree of β-lactam synergy. This decrease in efficacy precisely occurs during the first 24-48 hours of treatment, when there is a need for a rapid elimination of the high bacterial load and for countering selection of resistant mutants, this justifying the β-lactam and aminoglycoside association. Clinical experience supports the importance of optimizing the aminoglycoside PK/PD parameters from the beginning. A published study [118] analyzed outcome in 78 patients with pneumonia treated with antibiotic regimens including aminoglycosides with the aim of determining if optimization of PK/PD parameters result in more rapid therapeutic responses (defined as days until fever and leukocytosis resolution). The logistic regression analysis predicted 90% probability of fever and leukocytosis resolution after 7 days if during the first 48h treatment with the aminoglycoside a Cmax/MIC >10 ratio was reached [118]. In another study including 38 patients with bacteremia by *P. aeruginosa*, the probability of clinical cure was ≥90% when the Cmax/MIC ratio was at least 8 [119].

Until mid 90's, aminoglycosides (gentamicin, netilmicin and tobramycin) were used at doses of 3 to 5 mg/kg/day with bid or tid schedules. These regimens reached a Cmax of approximately 5 mg/L from day 2-3 on [106,120,121]. The potential effects on outcome when aminoglycosides are administered at suboptimal doses are hardly valorable in infections by *P. aeruginosa* (tobramycin MIC are usually 2 mg/L). From 1990's on, schedules progressively changed to single daily doses of 5-7 mg/kg/day (gentamicin and tobramycin) and of 15-20 mg/kg/day for amikacin [122]. Nevertheless, even with these doses, often Cmax continues to be suboptimal (especially for the treatment of *P. aeruginosa* infections) due to the elevated V_d and/or increase of renal clearance normally present in patients with severe sepsis or septic shock, mechanical ventilation, neutropenia, polytraumatism, severely burn, cystic fibrosis or morbid obesity (if doses are calculated for the lean body mass) [123-126]. In an ICU study, septic patients were treated with a mean gentamicin dose of 6.6 ± 2.3 mg/kg and only 1 out of 24 patients (4%) reached the desired Cmax ≥30 mg/L [127]. In another study carried out in patients with severe sepsis or septic shock treated with an amikacin initial dose of 25 mg/kg, the desired Cmax of at least 60 mg/L was not reached in up to 30% of cases [128]. Other authors have reported similar results [129-133]. In a 2013-14 French study, two years after the implementation of a guideline for aminoglycoside administration [77], 37% prescriptions were not in line with the recommendations [134]. With the aminoglycoside once daily administration the risk of renal toxicity is reduced through the reduction in the time that the proximal tubule is exposed to the antibiotic. Treatment duration for the aminoglycoside in the combined therapy with a β-lactam should be limited to the first 3-5 days [135].

In VAP patients, a low aminoglycoside Cmax might be primarily unfavorable due to its limited diffusion to the alveolar space and specially to the bronchial secretion [136-141] and the potential loss of activity in both sites. A reduction in the activity of tobramycin has been observed in the presence of pulmonary surfactant, particularly at low concentrations (0.25-1 x MIC) [142], probably due to its linkage to surfactant phospholipid proteins. In bronchial secretion, aminoglycosides are partially inactivated, mainly if the sputum is purulent, due to the electrostatic binding to mucin polysaccharides and to the DNA, to the presence of divalent cations and to pH ≤ 7 [143]. Concentrations up to 25 times higher the MIC of tobramycin are required to achieve bactericidal activity in sputum [144,145].

Even though clinical experience does not permit to firmly rule out the existence of a favorable result when associating a β-lactam and an aminoglycoside, if a benefit exists, it does not imply a significant improvement in the prognosis and it does not justify the risk of the aminoglycoside nephrotoxicity. In most clinical situations, the treatment of choice for a β-lactam susceptible *P. aeruginosa* infection is β-lactam monotherapy except in the following cases: 1) during the first 72 hours if the infection presents criteria of severe sepsis or septic shock, 2) in the neutropenic patient, and 3) in nervous central system (meningitis, abscess) or endovascular (endocarditis) infections. Use of associations including a β-lactam should be considered even for the treatment of infections caused by β-lactam resistant pathogens, especially if the resistance level is moderate (MIC 2-4 times higher than the breakpoint value). In this situation, the potential synergy with the second antibiotic could revert β-lactam non-susceptibility, if succeed in lowering the MIC below the resistance level.

6) Clinical efficacy of different antibiotics as monotherapy. Clinical experience evidences that monotherapy with β-lactams shows higher efficacy and/or lower toxicity than monotherapy with aminoglycosides [92,111,114] or colistin [146-148] and similar to monotherapy with a fluoroquinolone (ciprofloxacin) [149-151] in the treatment of gramnegative infections, including those by *P. aeruginosa*. However, in some infection sites, as in external malignant otitis, prostatitis, or cystic fibrosis bronchial infections, the use of ciprofloxacin may have advantages over a β-lactam, based on the possibility of oral administration, better penetration in the infectious foci and the probable greater activity in biofilms.

7) Measures to increase antibiotic concentrations in the infectious foci. As mentioned in points 1 and 2, to optimize the PK/PD index and to avoid selection/amplification of resistant subpopulations, high (aminoglycosides, fluoroquinolones) and maintained (β-lactams) antibiotic concentrations are required in the infectious foci. Nevertheless, in certain infection sites (as in pneumonia in the intubated patient, ventriculitis, meningitis), even with the maximum tolerated dose, MPCs are not exceeded or the associated toxicity is unacceptably high. In these cases, the possibility of directly introducing the antibiotic into the infectious foci using

Table 3 **Recommendations for antibiotic treatment of acute invasive infection produced by *P. aeruginosa***

1. Consider surgical control of the foci (drainage, debridement) and removal of any infected foreign body (catheter u others).
2. Include a β-lactam with activity against *P. aeruginosa*.
3. Choose the β-lactam having: a) the highest probability to achieve the optimal value of the adequate pharmacokinetic/pharmacodynamic index, and b) the lowest risk of selection/amplification of the resistant subpopulation.
4. For empirical treatment schedules, consider possible antibiotics associations during the first 48-72 h, in order to: rapidly decrease the bacterial population, avoid selection of resistant mutants (or resistant subpopulations in heteroresistant strains) and to increase the probability of the strain to be susceptible at least to one of the two antibiotics.
5. For directed treatment schedules, consider possible antibiotics associations if the infection presents criteria for severe sepsis or septic shock, in central nervous system infections, in endocarditis, in case of neutropenia (< 500/cells/mm^3) and when *P. aeruginosa* is resistant to β-lactams.
6. Whatever antibiotic is chosen, it is essential to optimize the dose, route and way of administration. Consider the use of the inhalatory route in case of a severe respiratory tract infection or caused by a multidrug resistant strain.

the inhalatory, intrathecal or other routes (depending on the infection site) should be considered. Antibiotic administration by the inhalatory route allows concentrations in bronchial mucous and the epithelial lining fluid around 100 times higher than those obtained with the same dose by iv route. This result in a higher probability of bacteriological eradication, even for microorganisms considered as resistant in *in vitro* susceptibility tests together with a reduction in the risk of selection and growth of the resistant population.

The review of clinical experience on the treatment of *P. aeruginosa* respiratory infections using inhaled antibiotics surpasses the extension limit of the present document. In chronic respiratory infections by *P. aeruginosa* in cystic fibrosis patients, inhaled tobramycin, colistin or aztreonam are considered treatments of choice, from the first exacerbation by *P. aeruginosa,* even in case of strains susceptible to β-lactams [19,152]. Studies performed in VAP patients [153-164], and several meta-analyses on VAP [165-167] or bronchiectasis infections [168], indicate that the addition of inhaled antibiotics improves clinical success and bacteriological eradication, especially when causal microorganisms harbour resistance mechanisms. In a study on patients with VAP by *P. aeruginosa,* the administration of inhaled antibiotics was compared with the administration of the same compounds by iv route, randomly assigning patients to receive ceftazidime and amikacin as treatments [169]. In the inhaled treatment arm, several patients were infected by strains exhibiting intermediate resistance to the antibiotics used, while in the iv treatment arm, in case of intermediate resistance to amikacin, this drug was changed to ciprofloxacin. No statistically significant differences in clinical outcome were observed. Resistances only emerged in the iv treatment arm [169]. In the respiratory infection by *P. aeruginosa*, if the infection presents severity criteria, the radiologic image is extensive or shows cavitations, or the isolated strain is multidrug resistant, inhaled treatment administration of tobramycin, colistin or aztreonam through a vibrating-membrane nebulizer should be considered. Presence of severe hypoxia (PaO_2/FiO_2 < 200) might contraindicate the use of inhalatory route.

Table 3 shows main recommendations in relation to antibiotic treatment for acute invasive infections by *P. aeruginosa*.

ANTIBIOTICS ACTIVE AGAINST *P. aeruginosa*

β-lactams. Nowadays, in most Spanish hospitals resistance rates in *P. aeruginosa* to piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem or meropenem are ≥20% (table 2). Ceftolozane-tazobactam is active against nearly 95% isolates and the ceftazidime-avibactam association restores ceftazidime susceptibility in nearly 80% resistant strains. With the exception of imipenem, poorly stable at room temperature, all other β-lactams active against *P. aeruginosa* should be administered at high doses and using extended or continuous infusion after an initial loading dose. This recommendation is based on: their time-dependant bactericidal activity, the possible inoculum effect of a high bacterial load (present at treatment initiation), the need for optimization of the PK/PD parameter for the high MIC against *P. aeruginosa,* the increase in V_d and/or renal clearance [170] and the need to exceed the MPC. In relation to the latter, table 1 shows MPC values for different β-lactams against a *P. aeruginosa* strains not harboring additional resistance determinants. Several studies have reported for ceftazidime and meropenem values similar to those shown in table 1 [171-173]. With a MPC value >32 mg/L of ceftazidime, cefepime, aztreonam, piperacillin-tazobactam and imipenem, the probability that concentrations of these antibiotics in serum are within the mutant selection window is very high, even when administered at maximum doses by extended/continuous infusion. The risk is especially high if the infection involves a bacterial load equal to or higher than the spontaneous mutation rate (10^{-6}-10^{-8} CFU). The risk is moderate for meropenem (MPC of 8 mg/L) administered at 6 g daily dose by extended infusion, and very low for ceftolozane-tazobactam (MPC of 2 mg/L) at 1.5-3 g dose by 3-4 hours infusion every 8 hours. In an *in vitro* study using one wild-type and one hypermutant *P. aeruginosa* strains exposed to ceftazidime, meropenem and ceftolozane-tazobactam, high-level resistance first to ceftazidime and after to meropenem was rapidly developed in both strains [31]. None of the selected mutants showed cross-resistance with ceftolozane-tazobactam. Development of resistance to ceftolozane-tazobactam was slower and only was of high-level in the hypermutant strain [31]. Other studies have confirmed the

greater ability of ceftazidime versus meropenem in selecting *P. aeruginosa* resistant mutants both from wild-type and hypermutant strains [174].

In clinical practice, most isolates of *P. aeruginosa* harbors one or more resistance mechanisms and MPC values are higher than those for fully susceptible strains. In these cases, failure and/or resistance development may occur with meropenem and, eventually, with ceftolozane-tazobactam monotherapies, even at high doses.

The main side effect with the use of a β-lactam high dose is neurotoxicity produced by inhibition of GABA- $GABA_A$ receptors binding, characterized by a slow and progressive appearance of somnolence, confusion, disorientation, agitation, myoclonus, asterixis, seizures, non-convulsive epileptic status and coma. The electroencephalogram shows a diffuse slow wave activity with triphasic waves, suggestive of toxic encephalopathy. Neurotoxicity is more frequent with cefepime, followed by ceftazidime, cefazoline and the remaining β-lactams. Patients with pathologies involving the central nervous system, with renal impairment and advanced age are especially vulnerable [175,176]. Some authors consider that steady state concentrations should not exceed the 100 mg/L threshold to avoid neurological toxicity with piperacillin, aztreonam or ceftazidime [177,178].

Piperacillin-tazobactam has been identified as a factor responsible for the delay in renal function restoration in the critically ill patient [179].

The review of the resistance mechanisms to different β-lactams in table 1 shows that ceftazidime and piperacillin share the same primary resistance mechanism, as well as occurs for cefepime and aztreonam and for meropenem and imipenem. Resistance to any of these antibiotics makes probable (but not certain) the resistance to its couple [180].

High number of *in vitro* studies on the association of two β-lactams or one β-lactam with other antibiotics, mainly aminoglycosides and fluoroquinolones, has been published. Chromosomal cephalosporinases (AmpC) of the species *Enterobacter, Citrobacter, Serratia* and *Pseudomonas* hydrolyze aztreonam, but the half-life of this reaction is long enough to maintain the enzyme inactivated along several generations of bacterial growth. In this way aztreonam can protect ceftazidime, and specially cefepime, from hydrolysis by AmpC in *P. aeruginosa* strains resistant by derepressed production of the enzyme [181-185]. The benefit is higher in the case of cefepime due to its more rapid cross of the external bacterial membrane. However, clinical experience is limited to a study including 13 patients with infection by *P. aeruginosa* resistant to all β-lactams treated with the association of cefepime plus aztreonam. Outcome was favorable for 69% of cases. Nevertheless, 11 out of 13 patients additionally received an aminoglycoside and 5 inhaled colistin. Aztreonam is resistant to hydrolysis by MBLs. It could be associated with ceftazidime-avibactam for the treatment of infections caused by *P. aeruginosa* strains producing a MBL plus derepressed AmpC. In a *Galleria mellonella* larvae model of *P. aeruginosa* infection, several β-lactam associations (not including aztreonam) showed *in vivo* synergism, which was bad correlated with *in vitro* interaction results [186]. Another possible synergistic mechanism for β-lactam combinations is the complementarity of PBPs inhibition profiles. In a recently published study [187], the association of cefepime, piperacillin or meropenem with zidebactam, a non- β-lactam PBP2 specific inhibitor, was synergistic against MDR and MBL-producing *P. aeruginosa* strains. However, there are not clinical experience, not even in infection animal models, supporting the potential advantage of the combination of a potent PBP2 inhibitor (carbapenem) with a potent PBP3 inhibitor (ceftazidime, cefepime or aztreonam).

Aminoglycosides. Tobramycin is the aminoglycoside showing the highest intrinsic activity against *P. aeruginosa*, being two-times more active than gentamicin and from 3 to 4 times than amikacin. Nevertheless, amikacin is susceptible to inactivation by a lower number of enzymes, thus being active against a higher percentage of *P. aeruginosa* isolates (90-95%) compared to tobramycin (80%).

The concentration-dependent bactericidal activity of aminoglycosides reaches its optimal efficacy in the treatment of *P. aeruginosa* infection when a Cmax/CMI ≥10 ratio is obtained in the first 24-48 hours of treatment initiation [118,119]. Aminoglycosides, due its hydrophilic nature, are distributed in the interstitial space and renally eliminated. The increase in V_d and in renal clearance, observed in critically ill patients with an important systemic inflammatory response, significantly reduces the aminoglycoside concentration in serum after the first dose. The recommended dose in the first 48-72 h of treatment, in patients with normal renal function and severe *P. aeruginosa* infection, is up to 8 mg/kg for gentamicin or tobramycin and of 20-30 mg/kg for amikacin [77].

The combination of an aminoglycoside and a β-lactam might be *in vitro* synergistic against gramnegative bacilli by means of the increase in the permeability of the external membrane, as previously commented. Another mechanism that could contribute, at least in part, to the synergy is the one observed in AmpC-producing *P. aeruginosa* resistant to cefepime. The addition of tobramycin at 7 mg/kg/day doses suppress protein synthesis, and with that, β-lactamase expression, facilitating the cephalosporin activity [188].

Fluoroquinolones. The current resistance rate to ciprofloxacin and levofloxacin in *P. aeruginosa*, in most Spanish hospitals, exceeds 30% (table 2). Ciprofloxacin is intrinsically more active than levofloxacin (MIC 2-4 dilutions lower).

The concentration-dependent bactericidal activity of fluoroquinolones reaches an optimal efficacy with Cmax/MIC >8. Nevertheless, the bactericidal effect of fluoroquinolones is slower than that of aminoglycosides and lysis of resistant mutants requires longer exposures. Bacteriological eradication without resistance development has been related with AUC_{24h}/MIC >100 [189,190]. The combination of both indexes minimizes resistance emergence [90]. MPC of ciprofloxacin and levofloxacin is 2 and 8 mg/L, respectively [191]. Diffusion of fluoroquinolones (especially levofloxacin) to cerebrospinal flu-

id, lung parenchyma, bronchial secretion and prostate is superior to that of β-lactams, aminoglycosides and colistin.

In *in vitro* studies carried out with *P. aeruginosa*, the association of levofloxacin and imipenem precluded emergence of resistance, even when strains exhibiting intermediate resistance to one or both antibiotics due to loss of OprD or efflux pumps overexpression were used [192,193]. In several studies, the association of levofloxacin with meropenem had more rapid bactericidal effect and resulted in resistance suppression [194] or meropenem MPC decrease [195], even when the strain was resistant to levofloxacin [196]. Levofloxacin and meropenem are eliminated by MexAB and the overexpression of this pump should affect both. The authors suggest that the β-lactam access to the pump through the periplasmic space could saturate its ability to extract levofloxacin from the cytoplasm [194]. The association of ceftazidime or cefepime with a fluoroquinolone (ciprofloxacin, levofloxacin or moxifloxacin) at 0.5 x MIC concentrations was synergistic for more than 50% *P. aeruginosa* strains [197]. However, in another study, the association of ceftazidime with ciprofloxacin led to emergence of resistance due to overexpression of MexAB [198].

Clinical experience indicates that ciprofloxacin is similar to [151] or better than imipenem [149] in the treatment of severe nosocomial pneumonia. Ciprofloxacin associated to metronidazole was similar to imipenem in intraabdominal infections [199] and equivalent to the association of ceftazidime and amikacin in febrile episodes in neutropenic patients [150]. In a study including 740 patients with VAP, treatment with meropenem monotherapy (1 g every 8 hours) was compared with meropenem associated with ciprofloxacin (400 mg/12 hours), in both cases by iv route. Treatment allocation was randomized. No differences in mortality, days in the ICU or hospital, clinical or microbiological response or emergence of resistance were observed. Nevertheless, in the analysis of the subgroup of 56 patients who had infection due to *P. aeruginosa*, *Acinetobacter* spp., and multidrug-resistant Gram-negative bacilli, the combined initial treatment was appropriate in 84% patients (versus 18.8%; p< 0.001) and the response was favorable for the association in microbiological eradication (64% versus 29.4%; p = 0.05) and favorable but non-significant in 28-days clinical resolution rates, days in the ICU and days with mechanical ventilation [200]. In the analysis of a series of 235 episodes of bacteremia by *P. aeruginosa,* definitive treatments with associations including ciprofloxacin showed a significantly lower 30-days mortality if the strain was susceptible. On the contrary, the association with tobramycin did not modify the prognosis [2]. A similar result was reported in another study on patients with bacteremia by gramnegative bacilli and Pittsburgh score <4 [201].

The use of high fluoroquinolone doses, exceptionally might produce confusion, orofacial dyskinesias, myoclonus, psychosis and non-convulsive epileptic status [175] by $GABA_A$ inhibition or NMDA receptor activation.

Colistin. Around 98% *P. aeruginosa* strains are colistin susceptible with MICs of 0.5-1 mg/L (table 2). Colistin Cmax after standard doses does not exceed 2-3 mg/L. Although its bactericidal activity is concentration-dependent, the therapeutic margin is very narrow and the increase in serum concentrations is not possible due to the risk of renal toxicity. The activity decreases in the presence of high inocula [202,203]. In *P. aeruginosa* rat models of pneumonia, a $fAUC_{0-24}$ /MIC of 40 predicted a bacterial reduction ≥ 2 $\log_{10}$ [204]. Some *P. aeruginosa* strains, apparently susceptible to colistin, presented heteroresistance [205] with the MIC of the resistant subpopulation far above the achievable maximum concentration in serum. Colistin should not be used as monotherapy, especially if the MIC is > 1 mg/L, the bacterial load is high or in the case of low accessible foci (lung, CNS). The association with a β-lactam (cefazidime or meropenem), a fluoroquinolone (ciprofloxacin or levofloxacin) or rifampicin can exert synergistic effects [206-211]. It is recommended to start treatment with a 6-9 MU iv loading dose to avoid the delay of 48-72 hours needed to reach the stationary state [212,213], followed by iv 4.5 MU/12 hours. Nevertheless, in a recent study [214] no relation between 28-days mortality and administration of a loading dose followed by high doses (9 MU/days) was observed when compared with the use of lower doses (4-6 MU/day) without loading dose. On the contrary, renal toxicity and appearance of seizures were significantly more frequent with the use of high doses. The most frequently isolated microorganisms were *Acinetobacter baumannii* and *Klebsiella pneumoniae*, and against both, colistin MIC was low (MIC_{90} 0.5 mg/L). Thus, probably, an optimal exposure was achieved with both doses [215]. Until more experience in the treatment of infections caused by microorganisms exhibiting MIC ≥ 2 mg/L is available, the administration of a loading dose followed by high doses should be considered.

Diffusion of colistin in the alveolar space and the bronchial secretion is limited [216], and its activity significantly decreases in the presence of mucus [217]. As well, concentration in cerebrospinal fluid is only 5% of the serum concentration [218].

Fosfomycin. Against nearly 33% *P. aeruginosa* strains, MIC of fosfomycin is ≤64 mg/L. Its time-dependent bacteriostatic activity is highly influenced by the inoculum size [219]. Heteroresistance is frequent among susceptible strains, and for this reason monotherapy is not recommended. The association with tobramycin [220,221], amikacin [222,223], ciprofloxacin [224,225] and different β-lactams [226-229] is frequently synergistic and decreases emergence of resistance [220-222]. Clinical experience is limited to the treatment of MDR *P. aeruginosa* exacerbations of cystic fibrosis. In the largest published study, 30 exacerbations in 15 patients treated with iv 5 g/8h fosfomycin associated with tobramycin, colistin or a β-lactam were analyzed [230]. The authors considered that treatment outcome was favorable. In a literature review analyzing 6 studies, including 33 patients treated with fosfomycin (associated with other antibiotic in 25 cases), 91% patients had a favorable outcome [231]. Optimal efficacy against *P. aeruginosa* is obtained with 16-24 g/day continuous infusion [226]. The disodium salt for iv administration contains 13.5 mEq of sodium per gram; caution is needed when

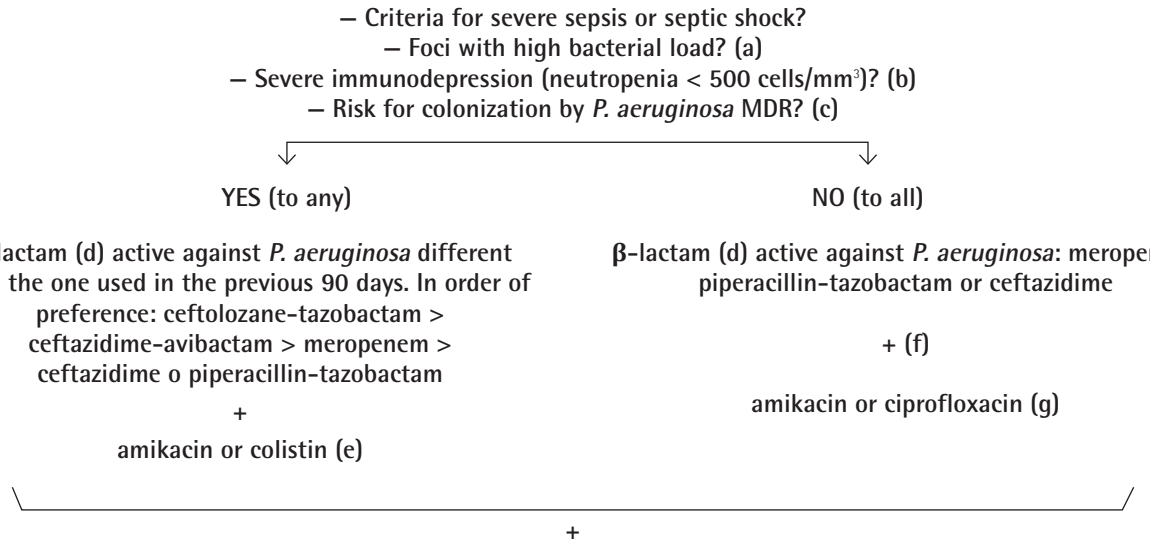

Figure 1 Election of empirical antibiotic treatment active against *P. aeruginosa*

a) High bacterial load not surgically correctable (extensive pneumonia or pneumonia with necrosis/cavitation)

b) Includes neutropenia < 500 cells/mm^3 and treatment with corticoid doses >20 mg/kg during >3 weeks

c)Treatment within the last 30-90 days with a β-lactam active against *P. aeruginosa*, admission during > 3-5 days in an hospitalization unit with a prevalence of MDR *P. aeruginosa* >10-20% or previous history of colonization/infection by MDR *P. aeruginosa*

d) Initial loading dose followed by high doses administered as continuous (or extended) infusion during the first 48-72 h

e) According to local epidemiology and susceptibility of possible previous isolates

f) Monotherapy in case of urinary tract infection or venous catheter infection. Association with amikacin or fluoroquinolone (levofloxacin or ciprofloxacin) in situations with high bacterial load (pneumonia)

g) Ciprofloxacin as treatment of choice for malignant external otitis, prostatitis and bronchial infection in patients with cystic fibrosis

administered to patients with heart insufficiency or under hemodialysis. Rapid high doses administration may produce hypopotassemia.

ANTIBIOTICS OF CHOICE FOR THE TREATMENT OF INFECTIONS CAUSED BY *P. aeruginosa*

Empirical treatment (figure 1). Empirical selection of the most appropriate antibiotic treatment for a possible infection by *P. aeruginosa* is based on: a) presence of severity criteria and b) presence of risk factors for infection by a strains harboring resistance mechanisms. Severity criteria include criteria of severe sepsis or septic shock, severe immunodepression (especially neutropenia <500 cells/mm^3), and infections involving high bacterial load, being not surgically controllable, as extensive pneumonia or pneumonia with cavitations/necrosis. The possibility of infection by a MDR strain should be considered in patients treated with a β-lactam active against *P. aeruginosa* within the previous 30-90 days, and in hospitalized patients admitted to units with a prevalence of MDR/XDR *P. aeruginosa* ≥10-20% for >3-5 days or which have history of previous colonization/infection by MDR/XDR *P. aeruginosa*. Among risk factors for selection of MDR/XDR strains we do not include exposure to β-lactams not active against *P. aeruginosa*, fluoroquinolones or aminoglycosides, since, under these conditions, the probability of colonization by strains resistant to antipseudomonal β-lactams is lower.

If the patient fulfills any of the above criteria, treatment with a β-lactam different from the one received within the previous 90 days should be used. By order, preference should be given to 1.5-3 g/8 h iv ceftolozane-tazobactam, 2 g/8 h iv meropenem and 2 g/8 h iv ceftazidima or 4.5 g/6 h iv piperacillin-tazobactam. They should be administered as extended infusion (ceftolozane-tazobactam, meropenem) or continuous infusion with a loading dose (ceftazidime, piperacillin-tazobactam), together with a second antibiotic as 25 mg/kg/día iv ami-

kacin as single daily dose or colistin (loading dose of 6-9 MU iv followed by 4.5 MU/12 h iv). For the election of the second antibiotic, it should be taken into account the epidemiology of the unit or hospital, and in the case of previous colonization/infection by *P. aeruginosa*, the susceptibility of the isolate.

If the patient does not fulfill severity criteria and has not risk factors for infection by a MDR/XDR *P. aeruginosa* strain, treatment could be initiated with a β-lactam (meropenem, ceftazidime or piperacillin-tazobactam) alone (urinary tract infection or venous catheter infection) or associated with amikacin or a fluoroquinolone (levofloxacin or ciprofloxacin) when bacterial load is higher (pneumonia).

In any of the previous situations, adequate surgical control of the infectious foci (drainage, de-obstruction, debridement) and/or removal of the infected foreign body (catheter or others) is critical.

Once culture results and antibiogram are available, treatment should be adjusted to the susceptibility of the isolated microorganism. If *P. aeruginosa* infection is confirmed and clinical evolution is favorable, from the 3rd day on treatment can be continued as monotherapy with a β-lactam chosen in accordance with the antibiogram. If all cultures are negative and clinical evolution is favorable, from the 3rd day on treatment can be continued as monotherapy with the initial β-lactam. If a rectal swab is available, and the patient is not colonized by *P. aeruginosa*, treatment continuation with a β-lactam not active against this microorganism can be considered.

Directed treatment. Election of antibiotic treatment when the susceptibility profile of the isolated *P. aeruginosa* strain is known, can be made according to the following recommendations:

a) Strain resistant to meropenem, ceftazidime and piperacillin-tazobactam, but susceptible to ceftolozane-tazobactam and ceftazidime-avibactam.

Against these strains, MIC of ceftolozane is often 2-4 mg/L. A possible treatment is 3 g/8 h iv ceftolozane-tazobactam. ESBL- or class A carbapenemase (GES o KPC)- producing *P. aeruginosa* strains can be resistant to ceftolozane-tazobactam, maintaining susceptibility to ceftazidime-avibactam that can be used at 2.5 g/8 h iv doses. If the strain produces a MBL-type carbapenemase, therapeutic options are limited to the use of associations of aztreonam with ceftazidime-avibactam with or without colistin.

b) Strain resistant to one of the β-lactams active against P. aeruginosa.

In case of resistance to ceftazidime and/or piperacillin-tazobactam, treatment can be ceftolozane-tazobactam, ceftazidime-avibactam or meropenem. The election depends on the risk of emergence of resistance, which in turn is related with the expected size of the bacterial load in the infectious foci. If the infection involves a high bacterial load (pneumonia), it is adviced to give priority to the antibiotic having the greatest probability to surpass the MPC, in this case, ceftolozane-tazobactam. Meropenem can be used for urinary tract infections, venous catheter infections or other infections with low bacterial load. In case of resistance to meropenem, treatment can be ceftolozane-tazobactam, ceftazidime or piperacillin-tazobactam. Again, the decision should be taken based on the bacterial inoculum size.

Table 4 **Initial posology of antibiotics with activity against *P. aeruginosa* for the treatment of severe infections**

Antibiotic	Posology
Ceftazidime	1-2 g loading dose + 6 g/24 h CI
Ceftazidime-avibactam	2/0.5 g/8 h EI
Piperacillin-tazobactam	2/0.25 g loading dose + 16/2 g/24 h CI
Ceftolozane-tazobactam	1/0.5 or 2/1 g/8 h EI
Aztreonam	1-2 g loading dose + 6 g/24 h CI
Meropenem	1-2 g loading dose + 2 g/8 h EI
Fosfomycin	2-4 g loading dose + 16-24 g/24 h CI
Colistin	6-9 MU loading dose + 4.5 MU/12 h
Ciprofloxacin	400 mg/8 h in 30-60 minutes
Levofloxacin	500 mg/12 h in 30-60 minutes
Tobramycin	8 mg/kg/24 h in 60 minutes
Amikacin	25 mg/kg/24 h in 60 minutes

CI: continuous infusion; EI: extended infusion (3-4 h); MU: million units

c) Strain susceptible to all β-lactams.

In this case, treatment options can be meropenem, ceftazidime or piperacillin-tazobactam. However, in VAP, severe pneumonia in COPD patients or in patients with bronchiectasis, and pneumonia with cavitation/necrosis, treatment with ceftolozane-tazobactam at 3 g/8 hours should be considered due to the high risk of resistance emergence.

In any of the three previous situations, in case of septic shock and in neutropenic patients, along the first 48-72 hours of directed treatment, an additional antibiotic (chosen according to the strain susceptibility) can be added: 400 mg/8 h ciprofloxacin or 8 mg/kg/day iv tobramycin (25-30 mg/kg/day amikacin in case of resistance to tobramycin). Occasionally, the resistance pattern makes necessary associations with colistin 4.5 MU/12 h or fosfomycin at 16-24 g/day iv dose administered as continuous infusion. Inhaled antibiotics (tobramycin, colistin or aztreonam) are reserved for cases of severe pneumonia or pneumonia caused by MDR *P. aeruginosa* strains. Nevertheless, their use should also be considered for infections caused by strains not harboring resistance mechanisms when the patient is intubated or suffers a relevant chronic bronchial pathology (GOLD-4 COPD, cystic fibrosis, bronchiectasis, bronchiolitis), circumstances in which the high bacterial load together with the limited antibiotic diffusion to bronchial secretions drives to an important risk for treatment failure and/or resistance emergence.

The treatment of CNS infections by *P. aeruginosa* poses two additional problems: antibiotic diffusion through the

meninges and the risk of encephalopathy (seizures) associated with elevated doses of β-lactams (cefepime, ceftazidime or imipenem) and to lesser extent with fluoroquinolones. Treatment can be 2 g/8 h iv meropenem or ceftazidime [232], associated or not (according to the strain susceptibility) with 400 mg/8 h iv ciprofloxacin. Among other potentially efficacious antibiotics, if the strain is susceptible, they should be considered 16-24 g/day fosfomycin and intratecal or intraventricular administration [233,234] of 5-20 mg/day tobramycin, 30 mg/day amikacin or 10-20 mg/day colistin as colistimethate (1 mg of colistimethate = 12,500 UI) [235]. Up to now, no experience with the use of ceftolozane-tazobactam is available.

Table 4 shows initial doses of antibiotics active against *P. aeruginosa* for severe infections.

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