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Epidemiology and Treatment of infections caused by extensively drug-resistant Pseudomonas aeruginosa. Clinical and microbiological impact of the new antipseudomonal agents. PseudoNOVA Study

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DOCTORAL THESIS

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Dedicado

A mi madre,

A mi abuela María Merlos y a mi abuelo Antonio “el Yiyo”,

A mis hermanas Elo y Mary,

A mis sobrinos,

A la tita Rita y a toda mi familia,

y a mi persona favorita, a mi esposo, …

a mi Carlos /Caalo/

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Abbreviations

aHR:	Adjusted Hazard Ratio
aOR:	Adjusted Odd Ratio
AUC:	Area under the ROC curve
BAT:	Best alternative therapy
BSI:	Bloodstream infection
CAUTI:	Catheter-associated urinary tract infection
CAZ-AV:	Ceftazdime-avibactam
CFU:	Colony-forming unit
CI:	Confidence interval
CR:	Carbapenem resistant
CRBSI:	Catheter-related bloodstream infection
Css:	Steady-state concentration
DTR:	Difficult to treat
ECDC:	European Centers for Disease Prevention and Control
EMA:	European Medicines Agency
EPINE:	Estudio de Prevalencia de las Infecciones Nosocomiales en España.
ESBL:	Extended-spectrum-β-lactamases
ESCMID:	European Society of Clinical Microbiology and Infectious Diseases
EUCAST:	European Committee on Antimicrobial Susceptibility Testing.
FDA:	The Food and Drug Administration
GNB:	Gram Negative Bacteria
HFIM:	Hollow fiber infection model
HR:	Hazard Ratio

IAI:	Intra-abdominal infection
ICU:	Intensive care unit
IDSA:	Infectious Diseases Society of America
IMI-REL:	Imipenem-relebactam
KPC:	*Klebsiella pneumoniae* carbapenemase
LPS:	Lipopolysaccharide
LRTI:	Lower respiratory tract infection
MBL:	Metallo-β-lactamase
MDR:	Multidrug-resistant
MER-VAB:	Meropenem/vaborbactam
Mex:	Membrane efflux
MIC:	Minimum inhibitory concentration
OM:	Osteomyelitis
OR:	Odd Ratio
OXA:	Oxacillinase
PBP:	Penicillin-binding proteins
PDR:	Pan-drug resistance
PJI:	Prosthetic joint infection
PK/PD:	Pharmacokinetics and pharmacodynamics
RCT:	Randomized controlled trial
RND:	Nodulation cell division family
SSTI:	Skin and soft tissue infection
ST:	Sequence type
TOL-TAZ:	Ceftolozane-tazobactam
URTI:	Upper respiratory tract infection
UTI:	Urinary tract infection
VAP:	Ventilator-associated pneumonia
XDR:	Extensively drug-resistant

Index

Abstract

The main objective of this thesis is to assess the clinical epidemiology and therapeutic options for the treatment of extensively drug-resistant (XDR) *Pseudomonas aeruginosa* strains.

The scientific production of this thesis consists of three studies:

In the first study, we assessed the influence of XDR phenotype on outcomes. We identified severity at presentation, having a high-risk source of bacteremia, and inappropriate definitive antibiotic therapy as risk factors for mortality. However, the XDR phenotype was not associated with poor prognosis.

The two following studies were focused on the antibiotic treatment of XDR *P. aeruginosa*. We studied two different sources of infections: 1) Urinary tract infection (UTI), as an example of a low-risk source, and 2) Respiratory infection, as an example of a high-risk source. In the UTI study, treatment with colistin or amikacin was not associated with worse outcomes in UTI caused by XDR strains. Finally, in the third study, focused on pneumonia, we observed through two models, one *in vivo*, from a real clinical case, and another *in vitro*, from a hollow fiber experiment, that subtherapeutic concentrations of ceftazidime/avibactam were associated with emergence of resistant mutants.

These findings are relevant in clinical practice given the limited therapeutic arsenal and the low evidence available for the treatment of XDR *P. aeruginosa* infections. Further studies are needed to reinforce these results.

Resumen

El objetivo principal de esta tesis es evaluar la epidemiología clínica y las diferentes opciones terapéuticas respecto al tratamiento de *Pseudomonas aeruginosa* extremadamente resistente (XDR).

Esta tesis, elaborada por compendio de publicaciones, consta de tres publicaciones:

En la primera de ellas se aborda el impacto del fenotipo XDR en las bacteriemias causadas por *P. aeruginosa*. Los peores desenlaces se asocian con el foco, la gravedad de la infección y con el antibiótico definitivo inapropiado, pero no con el fenotipo XDR.

En las dos siguientes publicaciones, la investigación se centra en el tratamiento de la *P. aeruginosa* XDR. Nos enfocamos en dos focos de infección: 1) Urinario, como ejemplo de foco de bajo riesgo y 2) Respiratorio, como foco de alto riesgo. En el artículo de la infección urinaria, el tratamiento con colistina o amikacina no se asocia con peores desenlaces. En el tercer estudio, enfocado en neumonía, observamos a través de un modelo *in vivo* (caso clínico real) y otro *in vitro* (experimento hollow fiber) que las concentraciones infraterapéuticas de ceftazidima/avibactam se asocian con el desarrollo de resistencias a este fármaco.

Estos hallazgos son relevantes en la práctica clínica habitual dado el escaso arsenal terapéutico disponible para el tratamiento de la *P. aeruginosa* XDR y la poca evidencia clínica actual. No obstante, son necesarios más estudios para confirmar estos resultados.

1

Introduction

1. INTRODUCTION

1.1. General characteristics

Pseudomonas aeruginosa was first isolated from green pus by Gessard in 1882. It is a Gram-negative nonfermenting bacillus that is ubiquitous in the environment and can be grown on a variety of media. It displays a predilection for infecting immunocompromised hosts, critically ill patients or those with chronic underlying diseases (1).

P. aeruginosa is a leading cause of hospital-acquired infections and has a great capacity for causing a wide range of infection (2). According to the EPINE (Estudio de Prevalencia de las Infecciones Nosocomiales en España) 2022, *P. aeruginosa* was one of the three most frequent pathogens isolated in ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infection (CAUTI) (2). Overall, it is the third most common cause of bloodstream infections based on the SENTRY antimicrobial surveillance program's data (3). Although community-acquired *P. aeruginosa* infections in immunocompetent patients are rarely seen, the pathogen can cause otitis externa and hot tub folliculitis (4).

1.2. Resistance mechanisms

P. aeruginosa shows resistance to many antibiotic families such as aminoglycosides, quinolones, and β-lactams. The main mechanisms of *P. aeruginosa* can be classified into intrinsic, acquired, and adaptive resistance. Overall, the intrinsic resistance of *P. aeruginosa* includes a reduced outer membrane permeability, expression of efflux systems that pump antibiotics out of the cell and the production of antibiotic-inactivating enzymes such as β-lactamases. *P. aeruginosa* can achieve the acquired resistance by either horizontal transfer of resistance genes or chromosomal gene mutations. The adaptive resistance of *P. aeruginosa* involves formation of biofilm and multidrug-tolerant persister cells (1,5,6).

The most import mechanisms of antibiotic resistance are showed in Figure 1 (6).

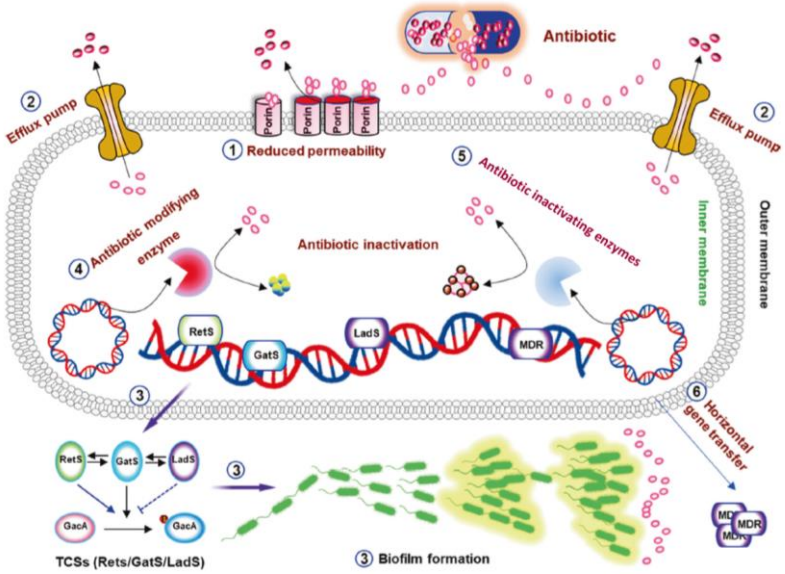


Figure 1. Mechanisms of antimicrobial resistance in *P. aeruginosa*. ① Outer membrane permeability, ② Efflux systems, ③ Biofilm-mediated resistance, ④ Antibiotic-modifying enzymes, ⑤ Antibiotic inactivating enzymes, and ⑥ Mutations and acquisition of resistance genes. Adapted from (6).

1.2.1. Instrisic resistance

Intrinsic resistance is encoded in the bacterium's chromosome and refers to its innate ability to decrease susceptibility to a specific antibiotic through inherent structural or functional characteristics. In the case of *P. aeruginosa*, the most frequent mechanisms are: 1) the expression of inducible AmpC cephalosporinase, 2) the presence of constitutive or inducible membrane efflux (Mex) pumps, particularly

MexAB-OprM (constitutive) and MexXY (inducible), and 3) the low permeability of its outer membrane (1,5).

- **Outer membrane permeability**

The outer membrane acts as a selective barrier to avoid antibiotic penetration. In case of *P. aeruginosa*, its permeability is even more restricted in contrast to other gram-negative bacteria. For instance, it is about 10- to 100-fold lower than *Escherichia coli* (1).

It a semi-permeable barrier composed of a phospholipid bilayer and lipopolysaccharide (LPS) that embeds proteins named porins. These porins configurate a water-filled channels which serves as the main conduit for diffusion of hydrophilic molecules such as β-lactam antibiotics. Among these porins, OprD is one of the most important due to it contains the binding sites for carbapenems. Thus, the absence of OprD in *P. aeruginosa* increases the resistance to this class of antibiotic (1,6).

- **Efflux systems**

Bacterial efflux pumps can drive multiple antibiotics out of the cell. Clinically relevant efflux pumps are part of the resistance nodulation cell division family (RND). They consist of three components: cytoplasmic membrane transporter (or pump), periplasmic linker proteins and outer membrane porin channel proteins (1,6,7). Figure 2 shows how this efflux pump works (7).

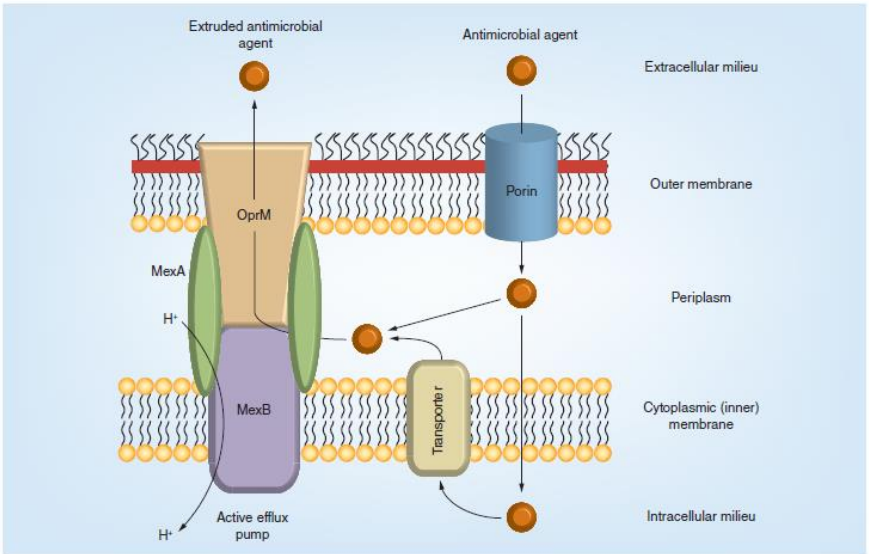


Figure 2. RND pumps remove antibiotic drugs from the periplasm either during entry into the bacterium or after removal from the cytoplasm by a transporter. This transporter is in the cytoplasmic membrane (MexB) and contacts the outer membrane channel protein (OprM) (7).

In case of *P. aeruginosa*, the most relevant RND pumps are MexAB-OprM and MexXY efflux pump, which are the responsible of low-level resistance to several antibiotic families. Whereas MexAB-OprM causes the efflux of quinolones and β-lactams (except for imipenem), MexXY affect to the aminoglycoside's family.

- **Antibiotic-inactivating enzymes**

The production of AmpC inducible β-lactamase plays a decisive role in the natural resistance of *P. aeruginosa* to aminopenicillins, some cephalosporins and imipenem. The β-lactamase, AmpC, is in the periplasm. Usually, it is present at low levels, but sub-inhibitory concentrations of certain β-lactams can be induced it. The resistance related to efflux pumps and AmpC goes hand in hand with the low outer-membrane permeability, because periplasm β-lactam concentrations depends on the success rate of their transportation through the porins of the outer membrane (8).

Two other intrinsic β-lactamases, oxacillinase (OXA-50) and an imipenemase, have also affected the basal β-lactam susceptibility levels (5,9). In addition, some *P. aeruginosa* isolates produce extended-spectrum-β-lactamases (ESBLs) which translate a high degree of resistance to most β-lactam antibiotics, such as penicillin, cephalosporin and aztreonam.

Regarding aminoglycoside resistance in *P. aeruginosa*, although it has been associated with multiple factors (*i.e.* lower cell membrane permeability, increased efflux, ribosomal changes), the enzymatic modification of amino and glycoside groups in the aminoglycoside molecular structure represents the main cause of aminoglycoside resistance (5).

1.2.2. Acquired antibiotic resistance through Chromosomal Gene Mutations

P. aeruginosa shows an exceptional capacity to acquire chromosomal alterations that increase its antimicrobial resistance to all currently used antibiotics. Among them, overproduction of chromosomal AmpC cephalosporinase is probably the most common mutation-driven β-lactam resistance mechanism. In a cohort of 190 *P. aeruginosa* isolates recovered from bloodstream infections in 10 Spanish centers, ampC overexpression was detected in over 24% of *P. aeruginosa* clinical isolates (10).

AmpC overexpression along with the mutational inactivation or downregulation of the carbapenem-specific OprD porin leads to imipenem resistance and reduces meropenem susceptibility. Both mechanisms in combination usually drives to resistance to all the classic antipseudomonal β-lactams (5). In case of ceftolozane-tazobactam and ceftazidime-avibactam, mutations in the structural modification of AmpC may also generate resistance (5).

On the other hand, mutational overexpression of relevant efflux pumps such as MexAB-OprM and MexXY is common in *P. aeruginosa* isolates (10% to 30%) (10). The combination of MexAB-OprM overexpression and OprD inactivation is one of the main causes of resistance to meropenem (5).

On the other hand, mutations in DNA gyrases and type IV topoisomerases confers resistance to fluoroquinolones. Regarding aminoglycoside, apart from MexXY overexpression and horizontally acquired mechanisms, mutations in fusA1 results in resistance to

this family. Finally, although the resistance to colistin is still low (<5%), mutations which leads to activation of the arnBCADTEF operon, overexpression of MexXY and downregulation of OprD are frequently linked to resistance in *P. aeruginosa* strains (5).

1.2.3. Horizontally acquired antibiotic resistance

P. aeruginosa can obtain antibiotic resistance genes through horizontal gene transfer from the same or different bacterial species or from the environment. Resistance to β-lactam, aminoglycoside, or quinolone resistance have been described due to this mechanism. Figure 3 shows the main mechanisms of horizontal gene transfer (8).

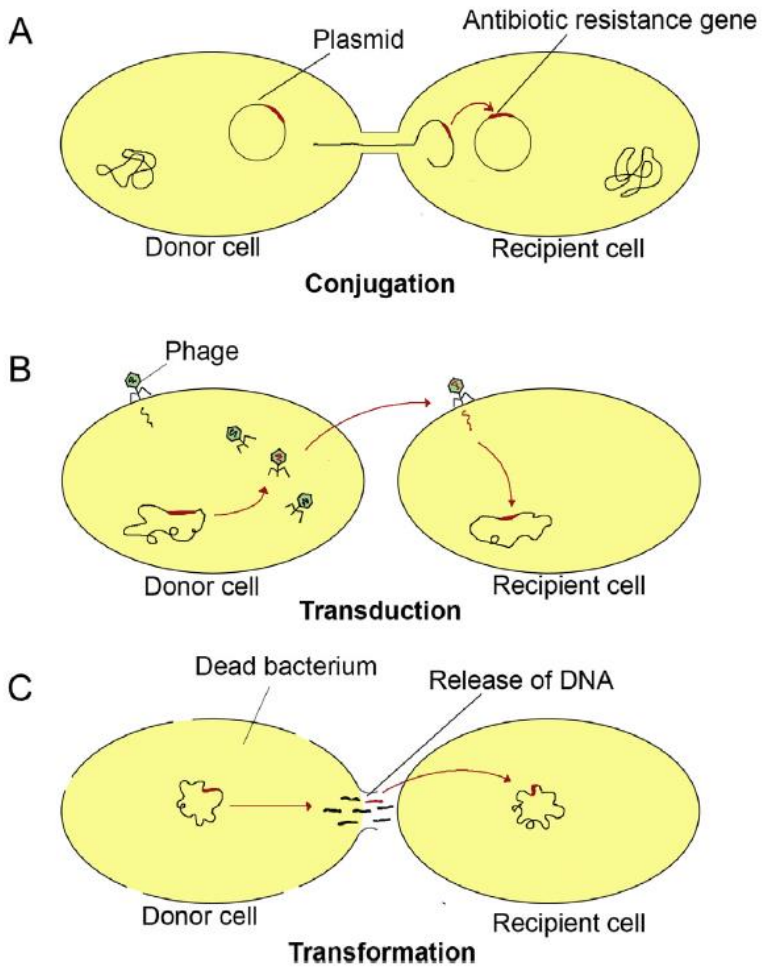


Figure 3. Mechanisms of horizontal gene transfer. (A) Conjugation: DNA is transferred through direct physical contact between cells. (B) Transduction: DNA is transferred from one bacterium to another by bacteriophages. (C) Transformation: bacteria take DNA released fragments of the environment and integrate it into their own genome. Figure from Z. Pang *et al*. (8).

In case of transferrable β-lactamases, ESBLs and carbapenemases currently are the most challenging. The main ESBLs reported in *P.*

aeruginosa include those in class D (such as OXA 2 or OXA-10 variants) and class A (PER, VEB, GES, BEL, and PME). Regarding the carbapenemases, metallo-β-lactamase (MBLs) are by far the most prevalent in *P. aeruginosa*, particularly VIM and IMP types. Furthermore, class A carbapenemases, such as GES and KPC, are arising in the last years (5).

1.2.4. Adaptative antibiotic resistance

Adaptive resistance refers to transient changes in gene and/or protein expression following an environmental stimulus to increase the bacteria resistance to the antibiotic treatment. In *P. aeruginosa*, biofilm formation and persistent cells or persisters are the most typical mechanisms of acquired adaptive antibiotic resistance (6).

Biofilm acts as a diffusion barrier to prevent antimicrobial agents from getting into the bacterial cells. On the other hand, multidrug-tolerant persister cells can survive to antibiotic action and are responsible for prolonged and recurrent infections (6).

1.3. Epidemiology of multidrug- or extensively drug-resistant *P. aeruginosa*

1.3.1. Definitions and prevalence

During the last years, multidrug- (MDR) or extensively drug-resistant (XDR) profiles has been defined according to Magiorakos *et al* (11): MRD is considered as nonsusceptibility to at least one agent in at least 3 antibiotic classes, XDR as nonsusceptibility to at least one agent in all but 1 or 2 antibiotic classes and pan-drug resistance (PDR) as nonsusceptibility to all agents in all classes. In recent years, a more practical definition has been proposed: difficult-to-treat (DTR) *P. aeruginosa*, including those strains non-susceptibility to all of the following: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin (12).

Setting aside theoretical definitions, the prevalence of MDR/XDR/DTR *P. aeruginosa* is high worldwide. The last surveillance report of the European Centers for Disease Prevention and Control (ECDC) stated that 18.7% of *P. aeruginosa* isolates had resistance for at least one antimicrobial group under surveillance (piperacillin-tazobactam, ceftazidime, imipenem or meropenem, ciprofloxacin or levofloxacin, and tobramycin) and 13% met the MDR criteria (13). Figures 4 and 5 shows the percentage of combined resistance and carbapenem resistance in Europe in 2021, respectively.

Figure 4: Percentage (%) of invasive *P. aeruginosa* isolates with combined resistance by country, 2021. Data from the ECDC Surveillance Atlas - Antimicrobial resistance (14)

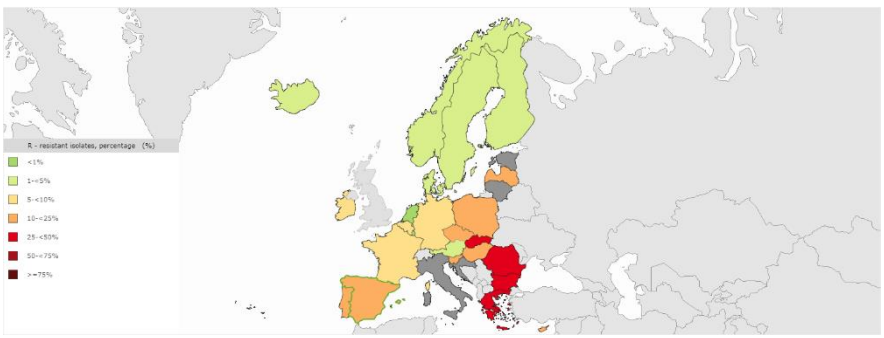
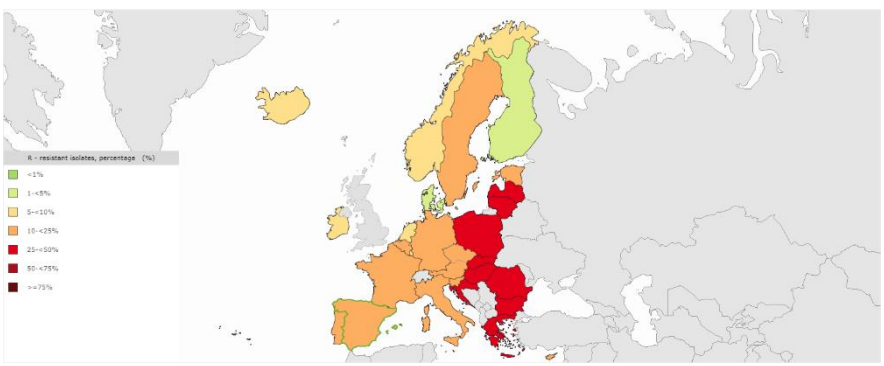


Figure 5: Percentage (%) of invasive *P. aeruginosa* isolates with carbapenem-resistence by country, 2021. Data from the ECDC Surveillance Atlas - Antimicrobial resistance (14)



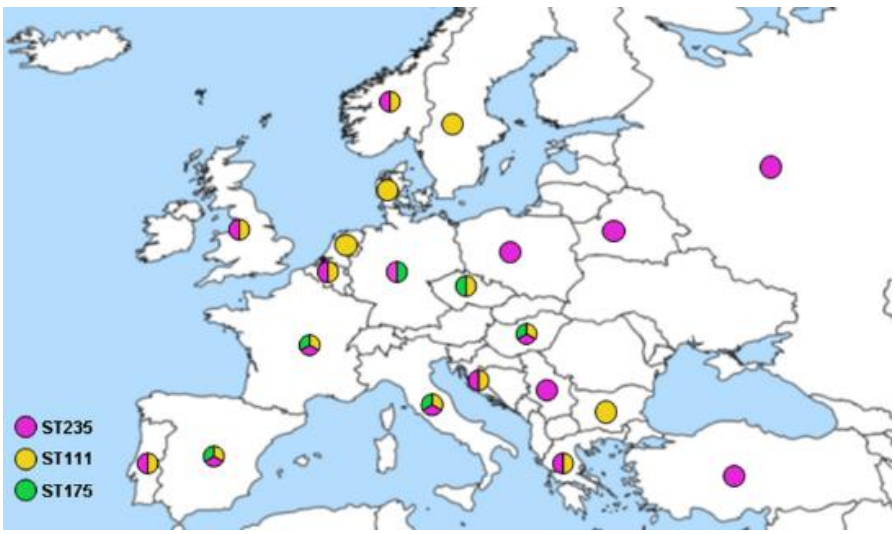
1.3.2. Epidemic High-Risk Clones

Molecular epidemiology surveys of *P. aeruginosa* isolates frequently reveal a remarkable clonal diversity. However, in case of MDR/XDR strains, this is much lower, especially among XDR isolates. Theses clones have been associated with multiple epidemic outbreaks and referred as “high-risk” clones.

They are widely disseminated in several hospital worldwide with geographical differences. The world-wide top 10 *P. aeruginosa* high-risk clones include sequence type 235 (ST235), ST111, ST233, ST244, ST357, ST308, ST175, ST277, ST654 and ST298 (15). ST235 is the most widespread high-risk clone. It is associated with the production multiple different acquired β-lactamases and appears to be especially virulent in cases of ExoU production.

In case of Europe, figure 6 shows the distribution of the more relevant high-risk clones.

Figure 6. European distribution of ST235, ST111 and ST175 high-risk clones. Adapted from (9)



ST175 is widely distributed in several European countries, including Spain (9). A Spanish nationwide survey showed that ST175 was detected in 40% of the 252 XDR isolates analyzed and in 29 of the 51 participating hospitals, being the most frequent high-risk clone (16). The ST175 high-risk clone combined multiple specific chromosomal mutations which were responsible for a typical resistance phenotype that includes all classical antipseudomonal agents apart from colistin and the new antipseudomonal drugs. Regarding ESBL/carbapenemases, they were only detected in 16.5% of XDR isolates from this clone. Finally, it seems the virulence of ST175 appears to be particularly low in comparison to other high-risk clones such as ST235.

1.3.3. The scenario of XDR *P. aeruginosa* in the Hospital del Mar

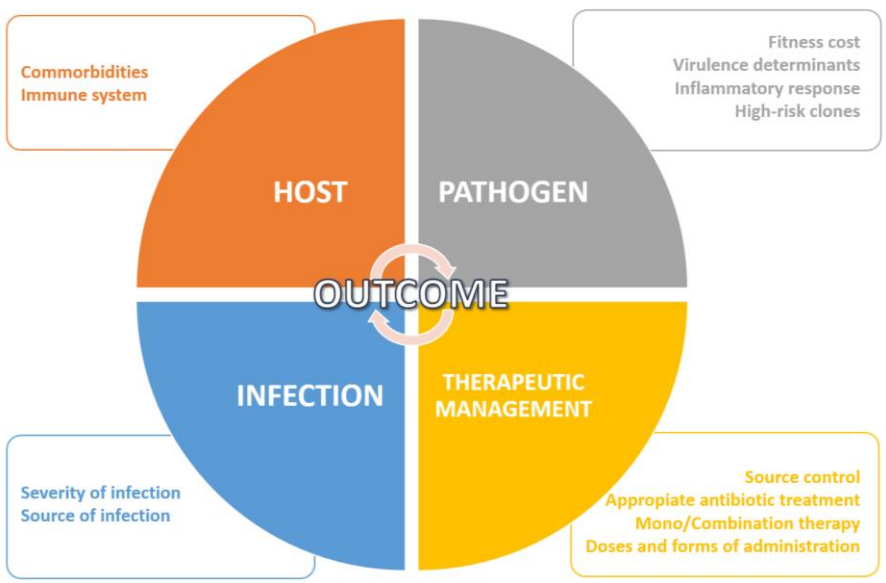
Previous studies showed that ST175 *P. aeruginosa* is by far the most frequent high-risk clone observed at our institution (16–19). In a Spanish multicenter study by our group (COLIMERO study) (19), 17/21 (81%) strains included from our center belonged to the ST175 high-risk. In another survey of *P. aeruginosa* molecular epidemiology and antimicrobial resistance in Spain, del Barrio-Tofiño *et al*. (16) found that all isolates recovered from our site also belonged to the ST175 high-risk clone.

More recent unpublished data derived from the PseudoNOVA study (see details in point 1.9) shows that ST175 is still the most frequent high-risk clone in our institution (50/54 (93%) patients). Most strains were non-susceptible to all classical antipseudomonal agents apart from colistin and amikacin. All were susceptible to ceftolozane-tazobactam whereas ceftazidime-avibactam showed a 91% of susceptibility. The underlying resistance mechanisms in the XDR phenotype were mainly a combination of chromosomal mutations such as hyperproduction of chromosomal AmpC β-lactamases, efflux pumps, OprD deficiency and/or quinolone resistance-determining region mutations.

1.4. Clinical impact of multidrug resistance in *P. aeruginosa*

It has been hypothesized that infections caused by MDR/XDR *P. aeruginosa* strains lead to worse outcomes than those caused by susceptible ones, although controversial findings have been reported over the years (20–24). This could partly be due to the difficulty of elucidating the influence of other factors on outcomes, such as underlying conditions, infectious syndrome severity, source of infection, therapeutic management, or bacterial virulence determinants (Figure 7).

Figure 7. The interplay between different factors and outcome in infections caused by MDR/XDR *P. aeruginosa.*



- **The host**

XDR *P. aeruinosa* colonization and infection usually happens in patients with multiple underlying diseases or immunocompromised hosts (4)(25). Vardakas *et al*. (26) suggested it may be difficult to determine whether outcomes in MDR infection are more influenced by patients' preexisting comorbidities than by multidrug resistance as such. Consequently, MDR/XDR phenotype seems to be the tip of the iceberg, warning of a more complex, vulnerable patient.

- **The pathogen**

In vitro studies have found that MDR/XDR strains have a lower growth rate and are poor in some virulence determinants such as bacterial motility or pigment production (27). It has been also hypothesized that the acquisition of resistance mechanisms may involve a fitness cost resulting in strains with lower virulence (28,29). On the other hand, not all resistance mutations lead to a biological cost such us the OprD deficiency (30). Indeed, some studies have identified that MDR strains can develop compensatory or suppressor mechanisms that allow them to recover their baseline fitness (31,32).

Other studies have found that some high-risk clones can be as virulent as susceptible strains suggesting that pathogenicity depends not only on the fitness cost of resistance, but also on the presence of certain virulence determinants such us exoU-positive genotype or O11 antigen serotype. The ST235 high-risk clone for example appears to be particularly virulent in cases of ExoU

production, whereas the virulence of ST175, the most prevalent high-risk clone observed at our institution, is especially low (33–35)

Finally, experimental *in vivo* animal models have shown that MDR/XDR *P. aeruginosa* strains produce a lower inflammatory response than susceptible strains (36).

- **The infection**

Apart from the severity of the baseline infection (sepsis or septic shock), previous studies have highlighted the importance of the source of infection. An increased risk of mortality among patients with respiratory tract infection have been showed in patients with *P. aeruginosa.* On the contrary, urinary tract or pancreaticobiliary tract infections have been associated with a reduced risk of mortality (37).

- **The therapeutic management**

Regarding antibiotic treatment, inappropriate initial antimicrobial therapy has been independently associated with increased mortality. Thus, the presence of resistant strains limits therapeutical options and enhances the risk of delay adequate therapy (37–39).

In addition, the onset time of the treatment, combination therapy, antibiotic dosing, or how antibiotic treatment is administered (extended- or continuous-infusion or intermittent-bolus) can affect the outcomes.

1.5. Current available antimicrobials for XDR P. aeruginosa treatment

1.5.1. "The old drugs"

Until quite recently, patients with infections caused by MDR/XDR *P. aeruginosa*, were basically treated with polymyxins or aminoglycosides in monotherapy or in combination with other antibiotics, with suboptimal clinical results, and with very high rates of renal toxicity (40,41). The susceptibility profile of MDR/XDR strains (close to 60% and 99% to amikacin and colistin (3), respectively) and the lack of therapeutic alternatives were behind this fact.

The effectiveness of aminoglycosides and/or polymyxins for treating XDR *P. aeruginosa* infections has already been assessed in previous studies. However, most of these included different sources of infection, used combination treatments, or had no control group, which makes interpretation difficult. *Pogue* et al. (42) compared ceftolozane-tazobactam vs. polymyxin or aminoglycoside-based therapy for the treatment of drug-resistant *P. aeruginosa* infections in a multicenter retrospective study. The authors reported statistical differences in clinical success rate (81% in the ceftolozane-tazobactam group vs. 61% in the comparative group), but not in mortality.

Focusing on colistin, the majority of published clinical studies are single-center retrospective studied with a small simple size. There are two studies accounting more than 100 patients (43,44). The most

frequent infectious source was low respiratory tract infection. Combination therapy was administered to 51 to 100% of patients with a clinical response and mortality rates ranging from 52% to 79% and 11% to 61%, respectively.

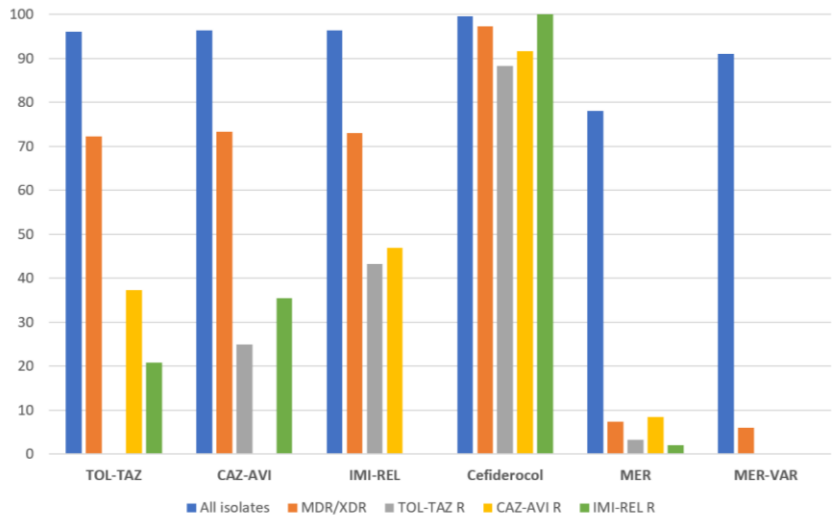
Regarding the effectiveness of aminoglycosides, they can be used in monotherapy in UTIs caused by drug-resistant *P. aeruginosa*, but this evidence has been frequently extrapolated from carbapenem-resistant Enterobacterales (45–47), with response rates ranging from 61% to 100%. In a systematic review (48), *Vidal* et al. demonstrated that aminoglycosides as single agents were as effective as beta-lactams or quinolones for achieving clinical improvement in patients with UTI, including those caused by *P. aeruginosa*.

1.5.2. The novel antipseudomonal agents

In recent years, the availability of new antipseudomonal agents, such as ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam, meropenem-varbobactam or cefiderocol, seem to provide an improvement on the treatment of infections caused by MDR/XDR *P. aeruginosa*, since they are supposed to have a higher clinical effectiveness with less side effects.

Their susceptibility profiles against *P. aeruginosa* are depicted in Figure 8.

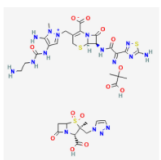
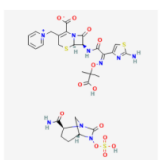
Figure 8 shows antimicrobial activity of ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam, cefiderocol, meropenem and meropenem-vaborbactam against *P. aeruginosa* based on SENTRY Antimicrobial Surveillance Program (49)(50).

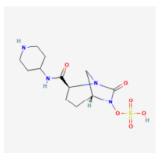
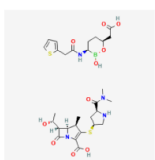
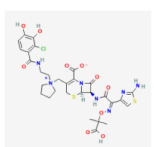


(*) Data for TOL-TAZ, CAZ-AVI, REL-IMI, Cefiderocol and MER are from (49) whereas data for MER-VAR are from (50).

The following tables summarize mechanism of action, spectrum of activity and mechanism of resistance (Table 1), clinical dosage, pivotal trials, indications approved by regulatory agencies and recommendations of guidelines (Table 2) and data from the main clinical studies of ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, imipenem-relebactam, and cefiderocol (Table 3).

Table 1. Mechanism of action, spectrum of activity and mechanism of resistance of ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, imipenem-relebactam, and cefiderocol (5,51,52).

Antibiotic	Mechanism of Action	Antimicrobial spectrum	Mechanism of Resistance
Ceftolozane-Tazobactam 	Combines a new antipseudomonal cephalosporin (ceftolozane) with a classic β-lactamase inhibitor (tazobactam). Ceftolozane inhibits cell-wall synthesis through binding of PBPs (strong affinity for PBP1b/c and PBP3). ↑stability against amp-C type β-lactamases and ↓affected by the changes in the porin permeability or efflux pumps. Tazobactam is a β-lactam sulfone that blocks class A and some class C β -lactamases.	Active against most of MDR/XDR *P. aeruginosa* and TEM, SHV, Amp-C and ESBL-producing Enterobacterales. No activity against carbapenemase producing bacteria.	-AmpC structural mutations. -Modification of PBPs. -Horizontally acquired β-lactamases that hydrolyse ceftolozane and are not inhibited by tazobactam (class D β-lactamases and OXAs). -Presence of MBL.
Ceftazidime-Avibactam 	Combination of a third-generation cephalosporin with a new β-lactamase inhibitor. Avibactam acts against some β-lactamases and protects ceftazidime from degradation.	Avibactam overcomes β-lactamases Ambler class type A (ESBL, KPC, GES), C (AmpC cephalosporinases) and some D (OXA-10, OXA-48). It does not retain activity against MBL.	-AmpC structural mutations. -Modification of PBPs. -Mutation in OXA-2 and OXA-10. -Presence of MBL. -OprD mutation and efflux pumps upregulation.

Imipenem-relebactam 	Combination of imipenem with a non-β-lactam bicyclic diazabicyclooctane β-lactamase inhibitor. Relebactam is structurally similar to avibactam with an additional piperidine ring.	Class A β-lactamases, including ESBLs and KPCs. Class C β-lactamases (AmpCs). Relebactam does not improve the spectrum of imipenem against OXA-48 and MBLs.	-MBL and GES carbapenemases. -Loss of the outer membrane entry porin OprD. -High-level expression of the chromosomally encoded AmpC enzyme.
Meropenem-vaborbactam 	Combination of meropenem with a cyclic boronic acid β-lactamase inhibitor with a high affinity for serine residues. It acts like a competitive inhibitor through the formation of a covalent bond with the β-lactamase without hydrolysis	Active against KPC and Ambler class tpe A and C β-lactamases. Not active against MBLs or OXA with carbapenemase activity.	-Porin mutations. -Efflux pump up.
Cefiderocol 	Siderophore cephalosporin. It uses active iron carriers to permeate the bacterial outer membrane.	Active against all β-lactamases, including MBLs, and AmpC. It is not affected by efflux pump extrusion or OprD porin channel loss.	-Mutations in major iron transport pathways. -Possible mutations in AmpC and β-lactamases.

4Abbreviations: MDR, multidrug resistant; XDR, extensively drug resistant; ESBL, extended spectrum β-lactamase; PBP, Penicillin-binding proteins; MBL, Metallo-β-Lactamase; KPC, Klebsiella pneumoniae carbapenemase.

Table 2. Clinical dosage, pivotal RTCs, indications approved by regulatory agencies and recommendations of guidelines of the new antipseudomonal agents.

Antibiotic	**Clinical dosage**	**Pivotal RCTs**	**Approved Indications**	**Place in guidelines** (53)(54)
TOL-TAZ	1.5 g (ceftolozane 1 g/tazobactam 0.5 g) every 8 h over 1 h 3 g (ceftolozane 2 g/tazobactam 1 g) iv every 8 h over 1 h for HAP/VAP	*ASPECT-cUTI (55): vs levofloxacin; cUTI (including acute pyelonephritis). *ASPECT-cIAI (56): (plus metronidazole) vs meropenem; cIAI. *ASPECT-NP (57): vs meropenem; HAP.	*FDA: cIAI, cUTI (lower dosage) HAP, VAP (higher dosage) *EMA: acute pyelonephritis, cUTI, cIAI, HAP and VAP (same dosage)	*IDSA: preferred treatment in DTR *P. aeruginosa* cystitis, cUTI, including pyelonephritis, and for infections outside of the urinary tract (high dose schedule outside uncomplicated UTI). *ESCMID: TOL-TAZ in monotherapy as a first line agent in severe infections due to CR *P. aeruginosa.*
CAZ-AVI	2.5 g (ceftazidime 2 g/avibactam 0.5 g) iv every 8 h over 2 h	*RECAPTURE (58): vs doripenem; cUTI (including acute pyelonephritis). *REPRISE (59): vs BAT; cUTI, cIAI *RECLAIM (60): (plus metronidazole) vs meropenem; cIAI. *REPROVE (61): vs meropenem; HAP, including VAP.	*FDA: cUTI, including acute pyelonephritis, cIAI, HAP, VAP. *EMA: cUTI, IAI, HAP, VAP, and for GNB with limited treatment options.	*IDSA: preferred treatment in DTR *P. aeruginosa* cystitis, cUTI, including pyelonephritis, and for infections outside of the urinary tract. *ESCMID: does not recommend CAZ-AVI in monotherapy as first line agent in severe infections due to CR *P. aeruginosa*, based on the lack of clinical evidence.
IMI-REL	1.25 g (imipenem 500 mg/cilastatin 500 mg/relebactam 250	*RESTORE IMI-1 (62): vs impipenem plus colistin; HAP/VAP, cIAI, or cUTI *RESTORE IMI-2 (63): (plus linezolid) vs	*FDA: cUTI, including acute pyelonephritis, cIAI, HAP, VAP.	*IDSA: preferred treatment in DTR *P. aeruginosa* cystitis, cUTI, including pyelonephritis, and for infections outside of the urinary tract.

	mg) iv every 6 h over 30 min	piperacillin/tazobactam plus linezolid; HAP/VAP.	*EMA: HAP, VAP, and for GNB with limited treatment options.	*ESCMID: does not recommend IMI-REL in monotherapy as first line agent in severe infections due to CR *P. aeruginosa*, based on the lack of clinical evidence.
MER-VAR	4 g (meropenem 2 g/vaborbactam 2 g) iv every 8 h over 3 h	*TANGO I (64): vs piperacillin/tazobactam; cUTI, including acute pyelonephritis *TANGO II (65): vs BAT; BSI, HAP/VAP, cIAI, cUTI. *None of them include *P. aeruginosa* strains.	*FDA: cUTI, including acute pyelonephritis. *EMA: acute pyelonephritis, cUTI, cIAI, HAP and VAP.	MER-VAR is not included in the options of the IDSA and ESCMID guidelines for the treatment of CR/MDR/XDR *P. aeruginosa* infections
Cefiderocol	2 g iv every 8 h over 3 h	*APEKS-cUTI (66): vs imipenem; cUTI, including acute pyelonephritis. *APEKS-NP (67): vs meropenem; HAP/VAP. *CREDIBLE (68): vs BAT; HAP, BSI or sepsis, cUTI.	*FDA: cUTI, including acute pyelonephritis, HAP, VAP. *EMA: cUTI, HAP, VAP, and for GNB with limited treatment options.	*IDSA: preferred treatment in DTR *P. aeruginosa* cystitis and cUTI, including pyelonephritis. For infections outside of the urinary tract caused by DTR *P. aeruginosa*, it is recommended as an alternative therapy if first-line agents are unavailable or not tolerated. *ESCMID: does not recommend cefiderocol in severe infections due to CR *P. aeruginosa*, based on the lack of clinical evidence.

Abbreviations: RCT, randomized controlled trial; TOL-TAZ, ceftolozane-tazobactam; CAZ-AV; ceftazdime-avibactam; IMI-REL, imipenem-relebactam; MER-VAB; meropenem/vaborbactam; CR, Carbapenem-resistant, MDR, multidrug resistant; XDR, extensively drug resistant; DTR, difficult to treat CR, GNB, Gram Negative Bacteria; URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection; VAP, ventilator-associated pneumonia, IAI, intra-abdominal infection; BSI, bloodstream infection; UTI, urinary tract infection; SSTI, skin and soft tissue infection; BAT; best alternative therapy; FDA, the Food and Drug Administration, EMA, European Medicines Agency, ESCMID, European Society of Clinical Microbiology and Infectious Diseases; IDSA, Infectious Diseases Society of America; iv, intravenous.

Table 3. Main clinical studies providing outcome information for infections due to MDR/XDR *P. aeruginosa* treated with new antipseudomonal agents.

Study reference	Study design (Country)	Patients (No., type of MDR phenotype and main source of infection)	Intervention/ Comparison	Outcome and Results
CEFTOLOZANE-TAZOBACTAM				
2016, Miller (69)	Post hoc analysis of RCT (ASPECT-cIAI)	TOL-TAZ 26 vs. Meropenem 29; MDR; IAI	TOL-TAZ vs meropenem	Clinical cure: TOL-TAZ 100% vs. meropenem 93.1%
2018, Gallagher (70)	Multicenter retrospective cohort study (USA)	205 patients. MDR 121 LRTI (58 VAP), 28 UTI, 26 Wound, 20 IAI, 16 BJI, 6 BSI	TOL-TAZ (No comparator)	-30-day and inpatient all-cause mortality: 19% -Clinical success 73.7% -Microbiological cure 70.7% TOL-TAZ in the first 4 days was independently associated with survival, clinical and microbiological success.
2018, Bassetti (71)	Multicenter retrospective real-world experience (Italy)	101 patients MDR 18%, XDR 51%, PDR 2% 32 LRTI, 21 SSTI, 14 cUTI, 13 cIAI, 9 OM, 6 primary BSI	TOL-TAZ (No comparator)	Clinical success: 83.2% *Lower rates were observed in patients with sepsis or undergoing continuous renal replacement therapy.
2019, Pogue (42)	Retrospective, multicenter, observational cohort (USA)	200 patients C-T: 64 LRTI, 16 UTI, 13 SSTI, 6 BSI, 7 others Comparator: 75 LRTI, 11 UTI, 6 SSTI, 6 BSI, 6 others	TOL-TAZ vs aminoglycosides or polymyxins	-Mortality: TOL-TAZ 20% vs. comparator 25% -Clinical cure: TOL-TAZ 81% vs. comparator 61% *Nephrotoxicity was less frequent in TOL-TAZ group (aOR 0.08, 95% CI 0.03-0.22).
2020, Sarah C. J (72)	Multicenter, retrospective study (USA)	226 patients MDR/XDR LRTI 149 (65.9%), IAI 11, primary BSI 4	TOL-TAZ (no comparator)	-Clinical failure: 37.6% -30-day mortality: 17.3% *25% received combination therapy (mainly aminoglycosides) *30% of LRTI received inhaled adjuvant therapy.

2021, Baladin (73)	Multicenter, retrospective, observational study (Spain)	95 patients XDR 48.4%, MDR 36.8%, Non-MDR 14.7% LRTI 54 (56.2%), IAI 10, URTI 8, UTI 6, IAI, CRBSI and SSTI 5, OM 2	TOL-TAZ (No comparator)	-Favorable clinical response 71.6% -Microbiological eradication 42.1% -Overall ICU mortality 36.5%. *TOL-TAZ monotherapy: 44.2%. *No outcome differences in the case of combination therapy
2022, Caffrey (74)	Retrospective, multicenter (USA)	212 patients MDR TOL-TAZ: 57; UTI 33, LRTI 30 Comparator: 155; UTI 97, LRTI 87	TOL-TAZ vs aminoglycosides or polymyxins	-Inhospital mortality: TOL-TAZ 15.8% vs comparator 27.7% (aOR 0.39, 95% CI 0.16 - 0.93) *UTI and meropenem as concomitant therapy ware more frequent in the aminoglycoside/polymyxin group (p <0.001 and 0.008, respectively).
2022, Holger (75)	Retrospective, observational cohort (USA)	206 patients MDR/XDR LRTI TOL-TAZ: 118 Comparator: 88	TOL-TAZ vs BAT	-Clinical failure: TOL-TAZ 23.7% vs comparator 48.9% (aOR 0.267, 95% CI 0.140–0.507). -No differences in 30-day mortality *More adverse drug reaction in comparator group (10% vs 30%; p<0.001)
2023, Almangou (76)	Retrospective, multicentre, observational cohort study (Saudi Arabia)	184 patients; MDR TOL-TAZ: 82; LRTI 23, VAP 16, Wound 14, UTI 7, IAI 7, other 14. Comparator: 102; LRTI 34, VAP 31, Wound 12, UTI 11, IAI 5, Other 5	TOL-TAZ vs colistin	*TOL-TAZ vs comparator: -Clinical cure: 77% vs 57% (aOR, 2.47; 95% CI 1.16-5.27). -Inpatient mortality: 39% vs 49% (p=0.175) *Acute renal injury was less frequent in TOL-TAZ group (15% vs. 41%; p<0.001).
CEFTAZIDIMA-AVIBACTAM				
2018, Stone (77)	Pooled analysis from 5 pivotal RTCs	91 patients MDR -CAV/AVI: 56; UTI 28; IAI 5, LRTI 23 -Comparator: 39; UTI 14, IAI 7, LRTI 18	CAZ-AVI vs Doripenem, meropenem or BAT	CAZ-AVI vs comparators: -Clinical cure: 85.4% and 87.9% -Favourable microbiological response: 57.1% and 53.8%

2019, Jorgensen (78)	Multicenter, retrospective, observational cohort (USA)	63 patients MDR LRTI 38, ITU 6, PJI and SSTI 6, IAI 3, CRBSI 2, Primary BSI 1	CAZ-AVI (no comparator)	-30-day mortality: 17.5% -Clinical response: 69.8% -30-day recurrence: 6.3%. *CAZ-AVI within 48 hours of infection onset was protective (aOR 0.409, 95% CI 0.180–0.930)
2020, Vena (79)	Multicenter, retrospective case series (Italy)	41 patients MDR−GNB other than CRE, *P. aeruginosa* (n = 33) LRTI 18, Primary bacteremia 5, Bone and other 3, IAI and SSTI 2	CAZ-AVI (no comparator)	-Clinical cure rate at the end of the follow-up period: 87.8% *The only risk factor for treatment failure at multivariate analysis was receiving continuous renal replacement therapy during CAZ-AVI.
2022, Corbella (80)	Retrospective cohort study (Spain)	61 patients, MDR/XDR LRTI 21, ITU and SSTI 14, IAI 7, CRBSI 3	CAZ-AVI (no comparator)	-Clinical cure rate by day 14: 54.1% -30-day all-cause mortality: 13.1% -90-day recurrence: 12.5%
2022, J. Chen (81)	Single-center retrospective observational study (China)	136 patients CR *P. aeruginosa* CAZ-AVI: 51; LRTI: 51, BSI 12, Other 13 Comparator: 85; LRTI: 84, BSI 37, IAI 4, Other 16	CAZ-AVI vs polymyxin B	CAZ-AVI vs comparator: -14-day mortality: 5.9% vs 27.1%, p=0.002 -30-day mortality:13.7% vs 47.1%, p<0.001 -In-hospital mortality: 29.4% vs 60.0%, p=0.001 -Bacterial clearance: 45.1% vs 12.9%, p<0.001 *CAZ-AVI: protector factor mortality (aHR 0.394; 95% CI 0.172–0.902), even after propensity-score matching adjustment (0.244, 95% CI 0.078–0.765).
Mularoni (82), Sempere (83) Davido (84)	Series of cases	3 XDR VIM: OM 1, URTI 1, abcess 1 1 XDR NMD1: LRTI	CAZ-AVI plus aztreonam (no comparator)	All cases presented clinical cure

IMIPENEM-RELEBACTAM				
2020, Motsh (62)	Randomized, controlled, double-blind, phase 3 trial (RESTORE-IMI 1) (Worldwide)	CR-GNB infections CR *P. aeruginosa* (71%) IMI-REL: 16; LRTI/VAP 8, UTI 7, IAI 1. Comparator: 8; HAP/VAP 3, ITU 3, IAI 2.	IMI-REL vs Colistin plus meropenem	-Overall response to treatment at 28 days: IMI-REL 13/16 (81%) vs comparator 5/8 (63%); adjusted difference of 3.1 (95% CI -19.8 to 38.2).
2021, Rebold (85)	Retrospective, observational case series (USA)	21 patients 16/21; 76% MDR *P. aeruginosa* LRTI 8, UTI 2, Device related infections 3, SSTI, IAI and OM 1	IMI-REL (no comparator)	-Clinical cure: 11/16 (69%) -Mortality: 3/16 (19%) -Microbiological recurrence: 5/16 (31%) *Resistance to IMI-REL developed in 1 *P. aeruginosa* strain.
2022, Shields (86)	Retrospective, observational case series	19 patients MDR	IMI-REL (no comparator)	*Resistance to IMI-REL developed in 5 (26%) *P. aeruginosa* strains.
MEROPENEM-VARBOBACTAM				
2021, Alosaimy (87)	Multicenter, retrospective cohort (USA)	126 patients, 11 *P. aeruginosa* Overall: LRTI (38%), IAI (19%)	MER/VAR (no comparator)	-30-day mortality: 18.3% -Recurrence: 11.9% *MER-VAR initiation within 48 hours was independently associated with negative outcomes (aOR 0.277; 95% CI, 0.081–0.941).
CEFIDEROCOL				
2020, Bassetti (68)	Randomised, open-label, multicentre, parallel-group, pathogen-focused, descriptive, phase 3 study (CREDIBLE-CR) (Worldwide)	151 patients with CR- GNB; 22 *P. aeruginosa*. -Cefiderocol: 12; LRTI 6, ITU 4, BSI 2 -Comparator: 10; LRTI 5, ITU 2, BSI 3	Cefiderol vs BAT	Cefiderocol vs comparator in *P. aeruginosa*: -All-cause mortality: 35% (6/17) vs. 17% (2/12). -Clinical cure: 58% (7/12 patients) and 50% (5/10 patients) *In overall cohort: - Cefiderocol had a greater all-cause mortality compared with BAT at day 14 (6.6% difference), day 28 (18.4% difference), and day 49 (20.4% difference) of treatment.

2020, Wunderink (67)	Randomised, double-blind, parallel-group, phase 3, non-inferiority trial (APEKS-NP) (USA)	148 patients, 48 with *P. aeruginosa* (4 ESBL and 4 carbapemase producers) Cefiderocol: 24 Comparator: 24 All nosocomial LRTI	Cefiderocol vs meropenem	*Cefiderocol vs comparator in non-fermenters: -Clinical cure at TOC: 66.7% vs 50% -Eradication at EOT: 33.3% vs 50% -All-cause mortality at day 28: 33% vs 50%
2021, Meschiari (88)	Prospective, observational study (Italy)	17 patients MDR LRTI 9 (VAP 7), IAI 3, Device related infections 2, SSTI and PJI 1, Primary BSI 1	Cefiderocol (no comparator)	-Clinical cure: 70.6% -Microbiological cure: 76.5%
2022, Timsit (89)	Post-hoc analysis of CREDIBLE-CR and APEKS-NP: Pathogen-focused, open-label analysis.	34 MBL-producing pathogens (19.5% in CREDIBLE-CR and 3.8% in APEKS-NP); 30% *P. aeruginosa*	Cefiderocol vs BAT or meropenem or imipenem	*Cefiderocol vs comparator in non-fermenters: -Clinical cure at TOC: 66.7% vs 60% -Eradication at EOT: 44.4% vs 40% -All-cause mortality at day 28: 11.1% vs 40%
2021, Bleibtreu (90)	National retrospective study (France)	13 XDR, 15% *P. aeruginosa*	Cefiderocol (no comparator)	-Overall mortality: 23% *Cefiderocol was used in combination as a salvage treatment. * 5 *P. aeruginosa* strains were not susceptible to cefiderocol.

Abbreviations: RCT, randomized controlled trial; TOL-TAZ, ceftolozane-tazobactam; CAZ-AV; ceftazdime-avibactam; IMI-REL, imipenem-relebactam; MER-VAB; meropenem/vaborbactam; MDR, multidrug resistant; XDR, extensively drug resistant; PDR, pandrug-resistant, CR, carbapenem resistant; CER; carbapenem-resistant Enterobacteriaceae; GNB, Gram Negative Bacteria; ESBL, extended spectrum β-lactamase; URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection; VAP, ventilator-associated pneumonia, IAI, intra-abdominal infection; BSI, bloodstream infection; ITU, urinary tract infection; SSTI, skin and soft tissue infection; PJI, prosthetic joint infection; OM, osteomyelitis; CRBSI catheter-related bloodstream infection, BSI, bloodstream infection; BAT; best alternative therapy; ICU, intensive care unit; aOR, adjusted Odd Ratio; aHR, adjusted Hazard Ratio; CI, confidence interval.

1.5.3. Combination treatment

The topic of whether combination therapy might improve patient outcomes is another major issue to be considered in the treatment of MDR/XDR *P. aeruginos*a infections.

Most of *in vivo* studies have been based on combinations with polymyxins and aminoglycosides. In a systematic review of polymyxins in monotherapy or in combination for the treatment of carbapenem-resistant (CR) gram-negative bacteria, *Zusman* et al. (91) suggested worse outcome in patients treated with colistin monotherapy, although most studies did not include *P. aeruginosa* infections. Other studies (92–94) also found benefits of using combination therapy with two active drugs in case of high-risk infection sources, mainly pneumonia. In case of bone and joint infections, a prospective clinical series showed a protective effect for patients treated with colistin in combination therapy, in contrast to β-lactam or colistin as monotherapy in infections caused by MDR/XDR *P. aeruginosa* strains (95). Taking everything into account, the international consensus guidelines for the optimal use of the polymyxins (96) favors “the use of polymyxins in combination with one or more additional agents to which the pathogen displays a susceptible MIC” in case of invasive infections due to CR *P. aeruginosa*. Regarding aminoglycosides, the use of monotherapy is restricted for urinary tract or a catheter-related bloodstream infections with complete source control (48,53). Finally, the ESCMID guidelines (54) consider “a good clinical practice to use the old antibiotics, chosen from among the *in vitro* active antibiotics on an individual basis and according to the source of infection”, in patients with non-severe or low-risk CR *P. aeruginosa* infections. However, when treating severe infections

caused by CR *P. aeruginosa* with polymyxins, aminoglycosides, or fosfomycin, combination treatment with *in vitro* active drugs is recommended.

Focused on the new antipseudomonal agents, the ESCMID guidelines (54) do not recommend nor discourage the combination therapy due the lack of information. Regarding the IDSA guidelines (53), “combination antibiotic therapy is not routinely recommended for infections caused by DTR-*P. aeruginosa* if in vitro susceptibility to a first-line antibiotic (ie, ceftolozane-tazobactam, ceftazidime-avibactam, or imipenem-relebactam) has been confirmed”.

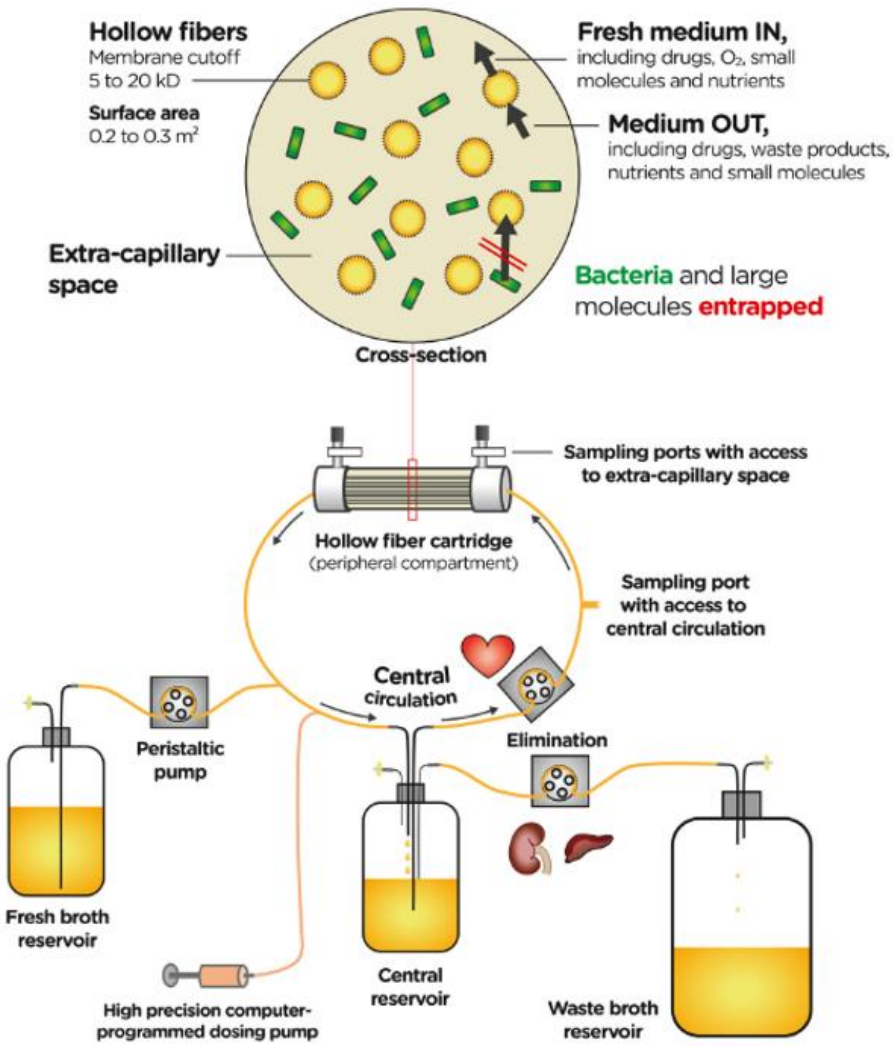
1.6. The Hollow-Fiber Infection Model

1.6.1. Introduction to the Hollow-Fiber infection model

The hollow fiber infection model (HFIM) is a dynamic two-compartment *in vitro* system that allows to culture bacteria continuously and “mimic” *in vivo* infections and drug concentration profiles (97).

It consists of a cartridge, a central reservoir, and a waste compartment. The HF cartridge holds thousands of small tubular fibers (filters) through which the medium is pumped from the central reservoir. These fibers have pores in their wall where bacteria are entrapped and serves as a peripheral infection site. Bacteria are provided with the optimal condition for its growth by the cartridge since they are continually exposed to fresh broth and oxygen and waste products are constantly removed (Figure 9).

Figure 9. Representation of the hollow fiber infection model. Figure from (98)



1.6.2. Application of Hollow-Fiber infection model

HFIM is the preferred *in vitro* model for evaluating pharmacokinetics and pharmacodynamics (PK/PD) indices and concentrations that best predict bacterial killing and resistance prevention (97–99).

There are distinct advantages to using this model, which include being able to analyze combination therapies, use extreme antimicrobial doses, work with multiple microorganisms, and quantify the resistance selection, all without the restrictions of animal models. This model provides a much deeper, dynamic analysis of the PK/PD behavior of antibiotics against bacterial strains (97–99).

The following table summarize the most relevant HF studies assessing the antibacterial activity of new antipseudomonal agents against XDR/MDR *P. aeruginosa* (Table 5).

Table 5. Main HF studies assessing the antibacterial activity of new antipseudomonal agents against XDR/MDR *P. aeruginosa*.

Study reference	Antibiotic	*P. aeruginosa* strains	Results
CETOLOZANO-TAZOBACTAM			
VanScoy, 2014 (100)	*Different doses (ranged from 62.5/31.25 to 2,000/1,000 mg) *Duration: 10 days	2 strains: a wild-type ATCC strain (MIC 0.5 mg/L) and a clinical isolate (MIC 4 mg/L)	Drug resistance selection was observed in the clinical isolate with intermediately intensive dosing regimens (125/62.5 through 1,000/500 mg).
Montero, 2018 (101)	*TOL-TAZ 2g/1g plus meropenem 2g every 8h. *Duration: 14 days	A single ST175 clone of XDR *P. aeruginosa:* MER MIC 8 mg/L and TOL-TAZ 2/4 mg/L.	TOL-TAZ+MER showed a >4 log10 CFU/ml bacterial density reduction and a suppression of regrowth up to day 14.
Montero, 2021 (102)	*3, 6 and > 9 g/4.5 g every 24 h in continuous infusion to simulate different Css (20, 45, 80 mg/dl) *Duration: 10 days	3 XDR *P. aeruginosa* type ST175 with different susceptibilities to C/T (MIC 2-16 mg/L)	Exposure to a Css of 20 mg/L led to the emergence of TOL-TAZ resistance in the susceptible isolate, whereas higher dosing regimens have a greater bactericidal effect, regardless of the TOL-TAZ MIC.
Montero, 2022 (103)	*Same dose 2g/1g; administered as: intermittent (1-h), extended (4-h), and continuous infusion. *Duration: 7 days	3 XDR *P. aeruginosa* type ST175 with different susceptibilities to C/T (MIC 2-16 mg/L)	Continuous infusion resulted in the greatest overall reduction in the number of bacterial colonies for both TOL-TAZ susceptible and resistant isolates. Only this regimen showed bactericidal activity against the three isolates.

CEFTAZIDIMA/AVIBACTAM			
Drusano, 2021 (104)	*4 experiments with different CAZ and AVI concentrations and forms of administration. *Duration: 10 days	One isolate with MICs of 1.0 mg/L (CAZ) and 4 mg/L (AVI).	CAZ-AVI had a bacterial cell kill driver of the time of AVI concentrations above 4.0 mg/liter (→ prevent from resistance due to classic porin downregulation, efflux pump overexpression) Low AVI AUC values were more common in continuous than in intermittent infusion (→ associated with amino acid deletion variants: large MIC changes and alteration the affinity for the active site of the β-lactamase).
IMIPENEM/RELEBACTAM			
Hirsch, 2012 (105)	*IMI: 30-minute infusions simulating either 500 (low dose) or 1,000 (high dose) mg doses every 6 h. *REL: 500 mg (given over 30 min) every 6 h. * Duration: 72 h	3 MDR *P. aeruginosa* strains (with OprD porin deletions and overexpression of AmpC).	*A ≥2 log reduction in bacterial population was shown at 24 hours. Failure with imipenem alone was seen against all isolates. *Sustained suppression of bacterial growth at 72 h was achieved with simulated doses of IMI/REL 500/500mg in one strain, and it was achieved in an additional strain when IMI dose was increased to 1,000 mg.
Jin Wu, 2018 (106)	*IMI at 500 mg plus REL at 125 or 250 mg administered intravenously every 6 h as a 30-min infusion *72 h	5 IMI-resistant strains with MIC ranged from 16 to 64 mg/L.	*For MIC 16 to 32 mg/L: Both doses of REL showed rapid and sustained bactericidal activity. *For MIC 64 mg/L: the lower dose of REL did not prove to be efficacious and the higher dose of REL took a longer time (>50 h) to reduce the number of CFU to below detectable limits.

Abbreviations: TOL-TAZ, ceftolozane-tazobactam; CAZ-AV; ceftazdime-avibactam; IMI-REL, imipenem-relebactam; MDR, multidrug resistant; XDR, extensively drug resistant; Css,steady-state concentrations; AUC; area under the ROC curve; MIC; minimum inhibitory concentration; CFU, Colony-forming unit, h, hours.

1.7. Summarize of the place of new antipseudomonal agents for the treatment of XDR *P. aeruginosa* infections considering *in vivo* and *in vitro* HFIM studies

- **Ceftolozane-tazobactam**

It represents a good option for the treatment of susceptible MDR/XDR *P. aeruginosa* infections. It is the only drug placed as the first choice for severe infections due to CR or DTR P. *aeurignosa* by both ESCMID and IDSA guidelines. Caution should be advised in the determination of optimal dosing and form of administration, mainly in the presence of renal impairment or in high-risk or high-inoculum infections to prevent form clinical failures and emergence of resistance. Administration of higher doses, in continuous infusion or in combination therapy could be helpful in these scenarios.

- **Ceftazidime-avibactam**

It is a good option for the treatment of MDR/XDR *P. aeruginosa* susceptible strains, including strains harboring carbapenemases, different from MBL. By IDSA guidelines, ceftazidime-avibactam as monotherapy is the preferred treatment option for the treatment of infections outside of the urinary tract caused by DTR *P. aeruginosa*. As well as ceftolozane-tazobactam dosage should be assessed with caution in the presence of renal impairment or in high-risk or high-inoculum infections. The combination with aztreonam could be of interest in infections caused by MBL-producers *P. aeruginosa*.

- **Imipenem-relebactam**

Considering its good *in vitro* activity against most MDR/XDR *P. aeruginosa* as well as the acceptable preliminary clinical data, imipenem-relebactam could be an option for the treatment of invasive MDR/XDR *P. aeruginosa* infections. Placed as a first treatment option for the treatment of infections outside of the urinary tract caused by DTR *P. aeruginosa* by IDSA guidelines.

- **Meropenem-varborbactam**

Vaborbactam is not expected to increase the coverage of meropenem on MDR *P. aeruginosa* strains. However, data from de SENTRY study (50) showed higher susceptibility rates of MDR strains in meropenem-vaborbactam than in meropenem. This fact may reflect a potential spread of KPC in MDR *P. aeruginosa* strains. Thus, it could be of interest in this setting.

- **Cefiderocol**

Cefiderocol has showed high susceptibility rates in MDR/XDR *P. aeruginosa* strains, even considering the newer β-lactam/β-lactamase inhibitors combinations. It could be a suitable option for the treatment of infections caused by MDR/XDR *P. aeruginosa*, particularly in the context of MBL producers. It is considered a treatment option in cUTIs caused by DTR *P. aeruginosa* by IDSA guidelines.

1.8. The PseudoNOVA Study

The PseudoNOVA study is a Spanish prospective, multicenter, observational cohort study, conducted between 2018 and 2022, of the clinical and microbiological impact of the new antipseudomonal agents ceftolozane-tazobactam and ceftazidime-avibactam on infections caused by high-risk clones of XDR *P. aeruginosa* in Spain. Results are compared with a retrospective cohort of patients treated with colistin before the arrival of the new antipseudomonal agents. Correlations with *in vitro* results are studied using a HF dynamic PK/PD model.

Patients admitted to participating hospitals during the study period with invasive infections caused by XDR *P. aeruginosa* and treated with ceftolozane-tazobactam or ceftazidime-avibactam were evaluated in terms of mortality, clinical cure, microbiological eradication, and selection of resistant mutants. Antibiotic regimen and dose selection were decided by the physician in charge without interference from the team of investigators. In this study, plasma levels of ceftolozane-tazobactam or ceftazidime-avibactam were performed blinded on days 3, 7, 14 and 21 of treatment (as appropriate). Clinical and microbiological results were compared with a retrospective cohort of patients treated with colistin. Strains that developed resistance during

antibiotic treatment were selected for study in a HFIM with a view to designing the most efficient strategies of antibiotic administration and to prevent the development of resistant mutants.

This thesis shows the background and first results of the PseudoNOVA study. It consists of three studies. The first study provides an historic picture of bloodstreams infections caused by XDR *P. aeruginosa* strains in the Hospital del Mar and set the basis of the PseudoNOVA study. The second study derives from a subgroup analysis of patients with UTI included in the colistin cohort of the PseudoNOVA study. Finally, the third study comes from a PseudoNOVA patient who developed *in vivo* resistance to ceftazidime-avibactam under the treatment with this drug. An *in vitro* HFIM was performed to correlate *in vivo* and *in vitro* results.

2

Hyphotesis

2. HYPOTHESIS

This thesis has four main hypotheses:

1. XDR phenotype is associated with worse outcome in infections caused by *P. aeruginosa* strains.

   It has been hypothesized that infections caused by antimicrobial-resistant strains lead to worse outcomes than those caused by susceptible ones, although controversial findings have been reported over the years. Although previous studies have assessed the impact on outcome of CR or MDR *P. aeruginosa*, little is known about the role of XDR strains, which have only few active drugs available and are more prone to be linked to high-risk clones.

2. The source of infection plays a major role in the choice of the antibiotic treatment.

   In case of *P. aeruginosa* infections, respiratory tract infections have been associated with an increased risk of mortality whereas urinary or pancreaticobiliary tract infections have been associated with lower mortality rates. UTI is therefore considered a low-risk source of infection. Thus, antibiotic monotherapy with aminoglycosides or colistin could be explored as an alternative therapeutic strategy to preserve the new antipseudomonal agents for more severe infections.

3. The new antipseudomonal agents ceftolozane-tazobactam and ceftazidime-avibactam will improve the prognosis of patients with severe infections caused by high-risk clones of XDR *P. aeruginosa*.

4. The HFIM will enable us to discover which doses, routes of administration and combinations of antibiotics would be the most effective and less likely to select resistant mutants during treatment for infections due to the XDR *P. aeruginosa* strains.

   Given the high risk of selection for and spread of mutants resistant to ceftolozane/tazobactam and ceftazidime/avibactam, it is of the utmost importance to monitor possible selection for resistance during treatment. Using the HFIM to identify the most efficient way to administer the antibiotic treatment could be an option to prevent the emergence of resistance.

3

Objetives

3. OBJETIVES

3.1. Primary objective

To assess the clinical epidemiology and different therapeutic options for the treatment of XDR *P. aeruginosa* infections.

3.2. Secondary Objectives

3.2.1. Secondary Objective number 1

To assess the clinical impact of XDR phenotype on patients with *P. aeruginosa* bacteremia.

3.2.2. Secondary Objective number 2

To evaluate the efficacy and safety of aminoglycosides or polymyxin monotherapy in comparison to other antibiotic regimens in complicated UTIs due to XDR *P. aeruginosa*.

3.2.3. Secondary Objective number 3

To evaluate the efficacy of three dosing regimens of ceftadizime-avibactam in an *in vitro* HFIM against an XDR *P. aeruginosa* strain and correlated these findings with the *in vivo* results.

4

Compendium of Publications

4. COMPENDIUM OF PUBLICATIONS

Listed below are the published articles that have been accepted by the Academic Committee of the Doctoral Program in Medicine:

4.1. Article 1

Risk factors for Mortality among Patients with *Pseudomonas aeruginosa* Bloodstream Infections: What is the influence of XDR phenotype on outcomes?.

Montero, Maria Milagro, López Montesinos Inmaculada, Knobel Hernando, Molas Ema, Sorlí Luisa, Siverio-Parés Ana, Prim Nuria, Segura Concepción, Duran-Jordà Xavier, Grau Santiago, Horcajada Juan Pablo.

J Clin Med. 2020 Feb 14;9(2):514. DOI: 10.3390/jcm9020514.

Journal of
Clinical Medicine

MDPI

Article

Risk Factors for Mortality among Patients with *Pseudomonas aeruginosa* Bloodstream Infections: What Is the Influence of XDR Phenotype on Outcomes?

María Milagro Montero [1], Inmaculada López Montesinos [1,*], Hernando Knobel [1], Ema Molas [1], Luisa Sorlí [1], Ana Siverio-Parés [2], Nuria Prim [2], Concepción Segura [2], Xavier Duran-Jordà [3], Santiago Grau [4] and Juan Pablo Horcajada [1]

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Abstract: This study aimed to assess the impact of extensively drug-resistant (XDR) phenotype on mortality in *Pseudomonas aeruginosa* bacteremia. A retrospective cohort study was performed in a tertiary hospital from January 2000 to December 2018. All consecutive prospectively recorded *P. aeruginosa* bacteremia in adult patients were assessed. In this study, 382 patients were included, of which 122 (31.9%) due to XDR *P. aeruginosa*. Independent factors associated with 14-day mortality were as follows: high-risk source of bacteremia (hazard ratio (HR) 3.07, 95% confidence interval (CI), 1.73–5.46), septic shock (HR 1.75, 95% CI, 1.12–2.75), and higher Pitt scores (one-point increments; HR 1.25, 95% CI, 1.12–1.38). Otherwise, the appropriateness of definitive antibiotic therapy was a protective factor (HR 0.39, 95% CI, 0.24–0.62). The same variables were also associated with 30-day mortality. XDR phenotype was not associated with 14- or 30-day mortality. In a subanalysis considering only high-risk source cases, combined antimicrobial therapy was independently associated with 14-day favorable outcome (HR 0.56, 95% CI, 0.33–0.93). In conclusion, XDR phenotype was not associated with poor prognosis in patients with *P. aeruginosa* bacteremia in our cohort. However, source of infection, clinical severity, and inappropriate definitive antibiotic therapy were risk factors for mortality. Combined antimicrobial therapy should be considered for high-risk sources.

Keywords: extensively drug-resistant Pseudomonas aeruginosa; multidrug resistance; high-risk clones; combined antimicrobial therapy; bacteremia; outcome

1. Introduction

Pseudomonas aeruginosa is one of the most difficult-to-treat microorganisms due to its intrinsic resistance profile and its extraordinary ability to develop additional resistance through selection of chromosomal mutations and acquisition of resistance genes [1,2]. In recent years, the emergence of high-risk clones that select multidrug- (MDR) or extensively drug-resistant (XDR) strains widely disseminated in hospitals throughout the world is also a factor of concern [2–4]. The increase in MDR/XDR strains seriously compromises antibiotic treatment options and consequently the probability of receiving appropriate early antimicrobial drugs, which may lead to poor outcomes, particularly in the presence of severe infections [5–7].

P. aeruginosa has an extraordinary capacity for causing a wide range of infections. Bloodstream infection (BSI) is considered one of its most serious and dreaded complications, with a reported mortality ranging from 18% to 61% [8]. It has been hypothesized that BSIs caused by antimicrobial-resistant strains lead to worse outcomes than those caused by susceptible ones, although controversial findings have been reported over the years [9–16]. These conflicting results could partly be due to the difficulty of elucidating the influence of other factors on outcomes, such as underlying conditions, infectious syndrome severity, source of infection, therapeutic management, or bacterial virulence determinants [9–17]. Those studies have some limitations: some results were obtained before Magiorakos et al. [5] standardized the terminology, or included different sources of infection, or were limited by insufficient sample sizes, and reliable conclusions could not be drawn. Furthermore, most were focused only on carbapenem-resistant or MDR *P. aeruginosa* strains, but not specifically on XDR isolates, which have only few active drugs available and are more prone to be linked to high-risk clones. In line with this, previous studies showed that almost all XDR isolates analyzed at our institution belonged to well-described *P. aeruginosa* high-risk clones, with sequence type 175 (ST175) being by far the most frequent high-risk clone observed [18–21]. Unpublished local data also showed that 85% of our XDR *P. aeruginosa* isolates were clonally related, showing an endemic situation at our center.

Hence, we present a retrospective analysis of a large cohort of *P. aeruginosa* bacteremia designed to assess the impact of XDR phenotype on mortality.

2. Methods

2.1. Hospital Setting, Study Design, and Participants

The study was conducted at the Hospital del Mar, a 420-bed tertiary-care university hospital in Barcelona, Spain. We performed a retrospective analysis of all patients aged ≥ 18 years old that had been prospectively recorded with positive blood cultures for *P. aeruginosa* from January 2000 to December 2018. Positive blood cultures were reported daily by the Microbiology Department. Patients were followed for up to 30 days from the onset of BSI to assess 14- and 30-day all-cause mortality. Only the first episode of bacteremia per each patient was considered. Polymicrobial BSIs were excluded.

The study was approved by the Clinical Research Ethics Committee of Parc de Salut (register no. 2019/8758I). The need for written informed consent was waived due to the observational nature of the study and retrospective analysis.

2.2. Study Objectives and Outcomes

We aimed to assess the impact of XDR phenotype on patients with *P. aeruginosa* bacteremia. The main outcomes were mortality at 14 and 30 days after the onset of bacteremia. An additional subanalysis was performed involving only the patients with high-risk sources of infection.

2.3. Variables and Data Source

Trained study investigators collected demographic, clinical, and microbiological data from the hospital or primary care electronic medical charts and the microbiology laboratory database, including the following: sex, age, underlying diseases, site of acquisition, source of infection, severity of BSI, antibiotic treatment, BSI microbiological resistance profile, and 14- and 30-day all-cause mortality. Follow-up information for up to 30 days after the onset of bacteremia was obtained by reviewing electronic medical charts.

2.4. Microbiological Studies

Bacterial growth in blood cultures was detected by the BacT/Alert® System (bioMérieux, Marcy l'Etoile, France). Bacterial identification was performed by standard biochemical tests and by MALDI-TOF since 2014. Antibiotic susceptibility testing was routinely performed by two dilution-based methods in two different periods due to changes in the laboratory workflow: broth microdilution panels MicroScan® using the WalkAway® system (Beckman Coulter, Brea, California, United States) from 2000 to 2008, and AST cards using the VITEK®2 System (bioMérieux, Marcy l'Etoile, France) from then on. Antimicrobials tested were the following: ciprofloxacin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, aztreonam, gentamicin, tobramycin, amikacin. and colistin. Antimicrobial susceptibility testing results were categorized according to the Clinical and Laboratory Standards Institute (CLSI) until 2013, and thenceforth according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Isolates categorized as intermediate were considered as resistant.

P. aeruginosa isolates were classified as XDR when they were non-susceptible to at least one agent in all but two or fewer antipseudomonal antimicrobial categories, and as MDR when they were non-susceptible to at least one agent in three or more antipseudomonal antimicrobial categories [5]. Those strains that did not meet the previous criteria were classified as non-MDR *P. aeruginosa*. Both non-MDR and MDR isolates were included in the non-XDR group.

Clonality and antibiotic resistance mechanisms were not investigated specifically for this study, but some strains had been analyzed by our group before [18,20,21]. As previously reported in detail, clonal relatedness of XDR *P. aeruginosa* isolates was analyzed using pulsed-field gel electrophoresis, multilocus sequence typing, and whole-genome sequencing. Regarding resistant mechanisms, overexpression of chromosomal β-lactamase AmpC or efflux pumps, OprD deficiency, or horizontal acquired enzymes were investigated, among others [18,20,21].

2.5. Definitions

P. aeruginosa bacteremia was defined as at least one positive blood culture obtained from a patient with signs and symptoms of infection.

Presence of comorbidities and severity of underlying diseases were assessed by the Charlson comorbidity index [22] and McCabe score [23]. Neutropenia was defined as an absolute neutrophil count of ≤500 cells/mm^3. Site of acquisition was defined according to the classification by Friedman et al. [24]. BSIs that did not meet the nosocomial or healthcare-associated criteria were considered as of community acquisition. BSI severity was evaluated by Pitt score [25], the need of intensive care unit admission, and the presence of septic shock, defined as the need for vasopressors to maintain a mean arterial pressure of at least 65 mmHg [26]. Source of infection was defined as the most likely origin of infection responsible for the BSI according to medical records. Catheter-related bloodstream infection, urinary tract infection, respiratory tract infection, skin and soft tissue infection, intraabdominal infection, and other sources (including endocarditis, otorhinolaryngologic, central nervous system, and surgical site sources) were included. When the origin of the infection was unclear, it was defined as BSI of unknown origin. For risk of mortality [10], sources of infection were divided into two groups: (a) low-risk sources or those involving either catheter-related bloodstream infections or urinary tract infections and (b) high-risk sources or those involving others.

Appropriate empiric antibiotic therapy was considered when at least 1 antipseudomonal antibiotic with in vitro activity was administered during the first 24 h after taking the blood cultures, whereas appropriate definite antibiotic therapy refers to the moment of knowing bacteria susceptibility results. When pneumonia was the source of infection, aminoglycoside monotherapy was considered an inadequate treatment [27]. Combination therapy was defined as two antipseudomonal drugs used for at least 24 h.

2.6. Statistical Analysis

Continuous quantitative variables were presented as median and interquartile range (IQR). For categorical variables, number of cases and percentages were used. The Student's *t*-test or Mann–Whitney U test were applied to compare continuous variables, and Fisher's exact test or Pearson's χ^2 test to contrast categorical variables, as appropriate. The Cox proportional hazards model was used to explore 14- and 30-day mortality events. Univariate and multivariate survival analyses were performed, and results were reported as the hazard ratio (HR) and 95% confidence interval (CI). The proportional hazard assumption checked by examining Schoenfeld residuals (for overall model and variable-by-variable) was not violated. The variables introduced into the multivariate analysis included those with a crude p value ≤ 0.1 in the univariate analysis or those considered clinically relevant for the outcome and the study hypothesis. The explained criteria are based on backward selection but controlling by clinical significance. All p-values were two-tailed and statistical significance was <0.05. Statistical analyses were performed using STATA 15.1.

3. Results

A total of 506 episodes of *P. aeruginosa* bacteremia were assessed during the study period. Of these, 124 were excluded: 114 had polymicrobial bacteremia, 9 patients presented more than one episode of *P. aeruginosa* BSI, and another was lost to follow-up. Therefore, 382 patients were finally recorded, and 122 (31.9%) of these episodes corresponded to XDR *P. aeruginosa* (Figure 1). In the non-XDR group, 35/260 (13.5%) were MDR and 225/260 (86.5%) non-MDR strains.

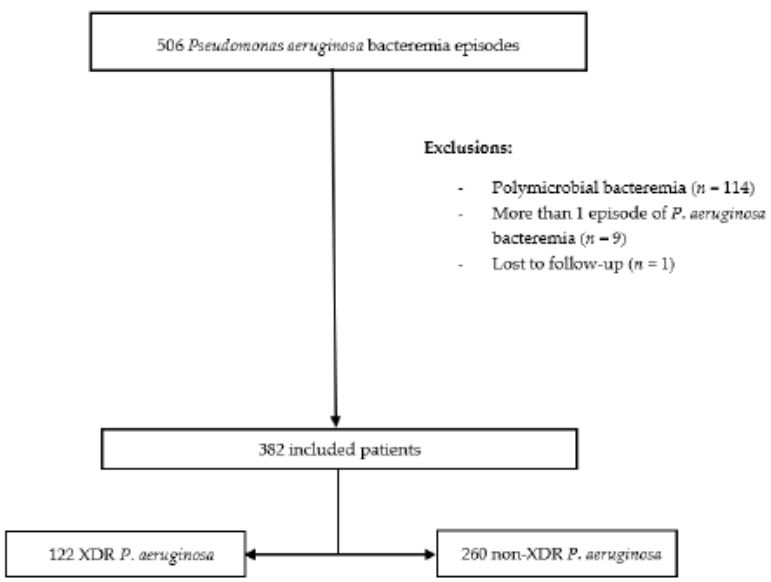

Figure 1. Flowchart of the patients included in the study.

The rate of antimicrobial susceptibility of the total cohort and according to XDR pattern are shown in Figure 2. With respect to the XDR antimicrobial susceptibility profile, the most common pattern observed was non-susceptibility to all antipseudomonal agents except colistin (98.4%, n = 120/122) and amikacin (89.3%, n = 109/122).

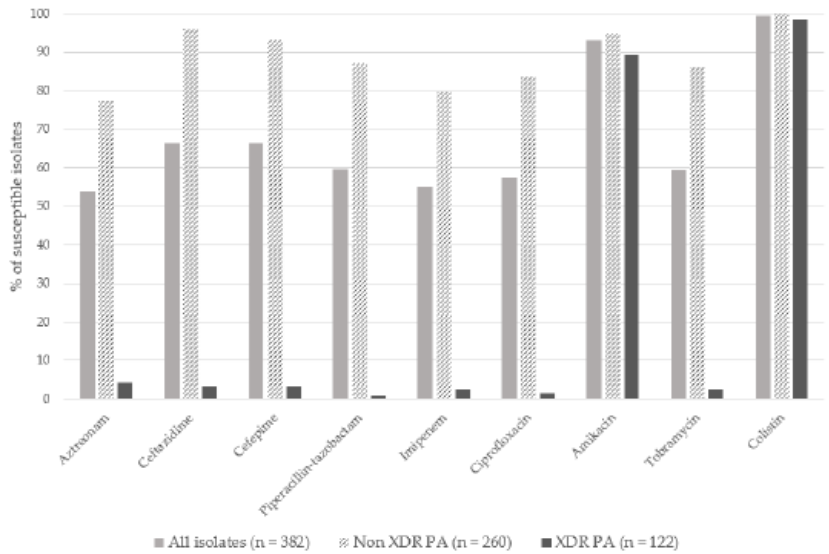

Figure 2. Susceptibility to different antimicrobial agents of the 382 isolates of *Pseudomonas aeruginosa*.

Demographics and clinical characteristics of the total cohort and according to XDR pattern are shown in Table 1. Compared to patients with non-XDR *P. aeruginosa* bacteremia, those with XDR *P. aeruginosa* were more likely to have a low-risk source of bacteremia (50.8% vs. 35.8%, p = 0.005), but a higher Pitt score (median points, 2 (IQR 1 to 4) vs. 2 (IQR 0 to 3); p = 0.017). Among patients with XDR bacteremia, the following significant differences were also observed when compared with non-XDR BSI episodes: history of hematologic malignancy (22.1% vs. 11.9%; p = 0.01), nosocomial acquisition (82.8% vs. 55%; $p < 0.001$), receiving inappropriate empirical antibiotic therapy (88.5% vs. 42.3%; $p < 0.001$), and use of combined antimicrobial therapy (51.6% vs. 33.5%; $p < 0.001$).

Table 1. Baseline characteristics of the patients. Data are presented as *n* (%), unless otherwise specified. Abbreviations: m (median), IQR (interquartile range), XDR PA (extensively drug-resistant *Pseudomonas aeruginosa*), ICU (intensive care unit).

Variable	All Episodes (*n* = 382)	Non-XDR PA (*n* = 260)	XDR PA (*n* = 122)	*p*-Value
Demographic information				
Age in years, m (IQR)	70.5 (60–78)	72 (60.25–78)	69 (59–76)	0.219
Male sex	276 (72.3)	190 (73.1)	86 (70.5)	0.599
Nosocomial acquisition	244 (63.9)	143 (55)	101 (82.8)	<0.001
Underlying condition				
Diabetes mellitus	94 (24.6)	63 (24.2)	31 (25.4)	0.803
Chronic obstructive pulmonary disease	82 (21.6)	53 (20.5)	29 (23.8)	0.475
Cirrhosis	28 (7.3)	18 (6.9)	10 (8.2)	0.656
Hemodialysis	25 (6.5)	18 (6.9)	7 (5.7)	0.662
Hematologic malignancy	58 (15.2)	31 (11.9)	27 (22.1)	0.010
Solid tumor malignancy	144 (37.7)	102 (39.2)	42 (34.4)	0.366
Neutropenia	70 (18.3)	51 (19.6)	19 (15.6)	0.341
Charlson comorbidity index, m (IQR)	4 (2–6)	4 (2–6)	4 (3–6)	0.911
McCabe score				
Non-fatal McCabe	107 (28)	73 (28.1)	34 (27.9)	0.966
Rapidly fatal McCabe	117 (30.6)	86 (33.1)	31 (25.4)	0.130
Ultimately fatal McCabe	158 (41.4)	101 (38.8)	57 (46.7)	0.145
Source of infection				
Catheter-related bloodstream infection	39 (10.2)	19 (7.3)	20 (16.4)	0.006
Urinary tract infection	116 (30.4)	74 (28.5)	42 (34.4)	0.237
Respiratory infection	95 (24.9)	67 (25.8)	28 (23)	0.552
Skin and soft tissue infection	19 (5)	15 (5.8)	4 (3.3)	0.297
Intraabdominal infection	45 (11.8)	36 (13.8)	9 (7.4)	0.067
Primary or Unknown	62 (16.2)	45 (17.3)	17 (13.9)	0.404
Other	6 (1.6)	4 (1.5)	2 (1.6)	1
High-risk source	227 (59.4)	167 (64.2)	60 (49.2)	0.005
Low-risk source	155 (40.6)	93 (35.8)	62 (50.8)	0.005
Baseline illness severity				
Pitt score, m (IQR)	2 (1–3)	2 (0–3)	2 (1–4)	0.017
Pitt score ≥ 2	206 (53.9)	133 (51.2)	73 (59.8)	0.112
Septic shock	103 (27)	65 (25)	38 (31.1)	0.207
ICU admission	96 (25.1)	61 (23.5)	35 (28.7)	0.272
Antibiotic management				
Appropriate empirical treatment	164 (42.9)	150 (57.7)	14 (11.5)	<0.001
Appropriate definitive treatment	332 (86.9)	227 (87.3)	105 (86.1)	0.737
Combined antimicrobial therapy	150 (39.3)	87 (33.5)	63 (51.6)	0.001
All-cause mortality				
Day 14	89 (23.3)	59 (22.7)	30 (24.6)	0.682
Day 30	118 (30.9)	77 (29.6)	41 (33.6)	0.431

3.1. Mortality and Risk Factors for Mortality

The 14-day all-cause mortality rate for all patients was 23.3% (89/382), whereas at day 30, it was 30.9% (118/382). No statistically significant differences in mortality rates were found between XDR and non-XDR *P. aeruginosa* groups at either day 14 or day 30.

Multivariate analysis using a Cox proportional hazards model, adjusted for sex and age, showed that the independent risk factors for 14-day all-cause mortality were as follows: a high-risk source of bacteremia (HR 3.07, 95% CI, 1.73 to 5.46; $p < 0.001$), presentation with septic shock (HR 1.75, 95% CI, 1.12 to 2.75; $p = 0.015$), and higher Pitt scores (one-point increments; HR 1.25, 95% CI, 1.12 to 1.38; $p < 0.001$). Receiving appropriate definitive antimicrobial therapy was a protective factor (HR 0.39, 95% CI, 0.24 to 0.62; $p < 0.001$). XDR *P. aeruginosa* BSI was not related to 14-day mortality (HR, 1.07; 95% CI, 0.68 to 1.67; $p = 0.777$) (Table 2).

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Table 2. Univariate and multivariate Cox model of 14-day all-cause mortality. Data are presented as *n* (%), unless otherwise specified. Abbreviation: HR (hazard ratio), CI (confidence interval), m (median), IQR (interquartile range), ICU (intensive care unit), XDR PA (extensively drug-resistant *Pseudomonas aeruginosa*), BSI (bloodstream infection).

Variables	Alive (*n* = 293)	Death (*n* = 89)	Crude HR (95% CI)	*p*-Value	HR Multivariate (95% CI)	*p*-Value
Demographic information						
Age in years, m (IQR)	70 (59.5–78)	71 (62.5–78)	1.01 (0.99–1.02)	0.252	1.01 (0.99–1.03)	0.119
Male sex	209 (71.3)	67 (75.3)	1.17 (0.72–1.89)	0.518	1.12 (0.69–1.82)	0.645
Nosocomial acquisition	186 (63.5)	58 (65.2)	1.05 (0.69–1.4)	0.796		
Underlying condition						
Diabetes mellitus	73 (24.9)	21 (23.6)	0.96 (0.59–1.56)	0.858		
Chronic obstructive pulmonary disease	57 (19.6)	25 (28.1)	1.49 (0.94–2.37)	0.09		
Cirrhosis	21 (7.2)	7 (7.9)	1.09 (0.51–2.37)	0.814		
Hemodialysis	22 (7.5)	3 (3.4)	0.47 (0.15–1.48)	0.196		
Hematology malignancy	42 (14.3)	16 (18)	1.22 (0.71–2.1)	0.465		
Solid tumor malignancy	114 (38.9)	30 (33.7)	0.81 (0.52–1.26)	0.354		
Neutropenia	51 (17.4)	19 (21.3)	1.22 (0.73–2.02)	0.445		
Charlson comorbidity index, m (IQR)	4 (2–6)	4 (2–6)	0.99 (0.93–1.07)	0.996		
McCabe score			1.16 (0.89–1.51)	0.249		
Non-fatal McCabe	83 (28.3)	24 (27)	0.98 (0.61–1.56)	0.932		
Rapidly fatal McCabe	96 (32.8)	21 (23.6)	0.67 (0.41–1.09)	0.104		
Ultimately fatal McCabe	114 (38.9)	44 (49.4)	1.41 (0.93–2.14)	0.105		
Origin of bacteremia						
Catheter-related bloodstream infection	37 (12.6)	2 (2.2)	0.18 (0.05–0.74)	0.017		
Urinary tract infection	103 (35.2)	13 (14.6)	0.36 (0.19–0.65)	0.001		
Respiratory infection	60 (20.5)	35 (39.3)	2.17 (1.42–3.32)	<0.001		
Skin and soft tissue infection	13 (4.4)	6 (6.7)	1.42 (0.62–3.25)	0.408		
Intraabdominal infection	35 (11.9)	10 (11.2)	0.94 (0.49–1.81)	0.852		
Primary or Unknown	40 (13.7)	22 (24.7)	1.84 (1.14–2.98)	0.013		
Other	5 (1.7)	1 (1.1)	0.69 (0.09–4.97)	0.715		
High-risk source	153 (52.2)	74 (83.1)	3.85 (2.17–6.67)	<0.001	3.07 (1.73–5.46)	<0.001
Low-risk source	140 (47.8)	15 (16.9)	0.26 (0.15–0.46))	<0.001		
Baseline illness severity						
Pitt score, m (IQR)	1 (0.5–2)	4 (1–4)	1.36 (1.24–1.49)	<0.001	1.25 (1.12–1.38)	<0.001
Pitt score ≥ 2	140 (47.8)	66 (74.2)	2.78 (1.73–4.48)	<0.001		
Septic shock	63 (21.5)	40 (44.9)	2.59 (1.71–3.95)	<0.001	1.75 (1.12–2.75)	0.015
ICU admission	56 (19.1)	40 (44.9)	2.92 (1.92–4.44)	<0.001		
Antibiotic management						
Appropriate empirical treatment	126 (43)	38 (42.7)	0.99 (0.65–1.51)	0.968		
Appropriate definitive treatment	266 (90.8)	66 (74.2)	0.35 (0.22–0.57)	<0.001	0.39 (0.24–0.62)	<0.001
Combined antimicrobial therapy	114 (38.9)	36 (40.4)	1.02 (0.67–1.88)	0.545		
XDR PA BSI	92 (31.4)	30 (33.7)	1.02 (0.68–1.56)	0.915	1.07 (0.68–1.67)	0.777

Regarding 30-day mortality, the independent risk factors for mortality observed in a Cox proportional hazards model, adjusted for sex and age, were as follows: a high-risk source of infection (HR 2.49, 95% CI, 1.56 to 3.99; $p < 0.001$), septic shock (HR 1.77, 95% CI, 1.18 to 2.65; $p = 0.006$), and Pitt scores (one-point increments; HR 1.25, 95% CI, 1.13 to 1.37; $p < 0.001$), whereas appropriate definitive therapy (HR 0.42, 95% CI, 0.27 to 0.66; $p < 0.001$) was identified as a protective factor. XDR *P. aeruginosa* BSI was not related to 30-day mortality (HR 1.14, 95% CI, 0.77 to 1.69; $p = 0.504$) (Table 3).

Table 3. Univariate and multivariate Cox model of 30-day all-cause mortality. Data are presented as *n* (%), unless otherwise specified. Abbreviation: HR (hazard ratio), CI (confidence interval), m (median), IQR (interquartile range), ICU (intensive care unit), XDR PA (extensively drug-resistant *Pseudomonas aeruginosa*), BSI (bloodstream infection).

Variables	Alive (*n* = 264)	Death (*n* = 118)	Crude HR (95% CI)	*p*-Value	HR Multivariate (95% CI)	*p*-Value
Demographic information						
Age in years, m (IQR)	70 (59–77.75)	72 (62.75–78)	1.01 (0.99–1.02)	0.159	1.01 (0.99–1.03)	0.052
Male sex	185 (70.1)	91 (77.1)	1.32 (0.85–2.04)	0.208	1.25 (0.8–1.95)	0.321
Nosocomial acquisition	162 (61.4)	82 (69.5)	1.31 (0.88–1.94)	0.178		
Underlying condition						
Diabetes mellitus	66 (25)	28 (23.7)	0.95 (0.62–1.46)	0.828		
Chronic obstructive pulmonary disease	50 (19)	32 (27.4)	1.47 (0.98–2.21)	0.062	1.1 (0.7–1.73)	0.675
Cirrhosis	19 (7.2)	9 (7.6)	1.06 (0.54–2.08)	0.876		
Hemodialysis	22 (8.3)	3 (2.5)	0.34 (0.11–1.06)	0.064	0.35 (1.01–1.15)	0.083
Hematology malignancy	38 (14.4)	20 (16.9)	1.15 (0.71–1.86)	0.575		
Solid tumor malignancy	102 (38.6)	42 (35.6)	0.88 (0.6–1.28)	0.505		
Neutropenia	48 (18.2)	22 (18.6)	1.03 (0.65–1.64)	0.884		
Charlson comorbidity index, m (IQR)	4 (2–6)	4 (2–6)	1.01 (0.95–1.07)	0.744		
McCabe score			1.2 (0.96–1.51)	0.116		
Non-fatal McCabe	77 (29.2)	30 (25.4)	0.89 (0.59–1.35)	0.596		
Rapidly fatal McCabe	87 (33)	30 (25.4)	0.72 (0.48–1.09)	0.124		
Ultimately fatal McCabe	100 (37.9)	58 (49.2)	1.43 (0.99–2.05)	0.005	1.29 (0.89–1.89)	0.178
Origin of bacteremia						
Catheter-related bloodstream infection	34 (12.9)	5 (4.2)	0.34 (0.14–0.83)	0.017		
Urinary tract infection	96 (36.4)	20 (16.9)	0.41 (0.25–0.66)	<0.001		
Respiratory infection	49 (18.6)	46 (39)	2.25 (1.55–3.26)	<0.001		
Skin and soft tissue infection	12 (4.5)	7 (5.9)	1.26 (0.58–2.69)	0.558		
Intraabdominal infection	29 (11)	16 (13.6)	1.18 (0.69–1.99)	0.537		
Primary or Unknown	39 (14.8)	23 (19.5)	1.38 (0.87–2.17)	0.165		
Other	5 (1.9)	1 (0.8)	0.51 (0.07–3.63)	0.500		
High-risk source	134 (50.8)	93 (78.8)	3.03 (1.96–4.76)	<0.001	2.49 (1.56–3.99)	<0.001
Low-risk source	130 (49.2)	25 (21.2)	0.33 (0.21–0.51)	<0.001		
Baseline illness severity						
Pitt score, m (IQR)	1 (0–2)	4 (1–4)	1.38 (1.28–1.49)	<0.001	1.25 (1.13–1.37)	<0.001
Pitt score ≥ 2	120 (45.5)	86 (72.9)	2.73 (1.82–4.1)	<0.001		
Septic shock	51 (19.3)	52 (44.1)	2.63 (1.83–3.79)	<0.001	1.77 (1.18–2.65)	0.006
ICU admission	44 (16.7)	52 (44.1)	2.99 (2.08–4.3)	<0.001		
Antibiotic management						
Appropriate empirical treatment	114 (43.2)	50 (42.4)	0.98 (0.68–1.41)	0.899		
Appropriate definitive treatment	240 (90.9)	92 (78)	0.42 (0.27–0.65)	<0.001	0.42 (0.27–0.66)	<0.001
Combined antimicrobial therapy	96 (36.4)	54 (45.8)	1.29 (0.9–1.87)	0.157		
XDR PA BSI	81 (30.7)	41 (34.7)	1.13 (0.77–1.65)	0.535	1.14 (0.77–1.69)	0.504

3.2. Subanalysis

Because the XDR group more frequently had a low-risk source of bacteremia, an additional analysis was performed that considered only high-risk sources of BSI (Table S1, Supplementary Materials). In multivariate analysis, factors independently associated with 14- and 30-day all-cause mortality were once again Pitt score, septic shock, and inappropriate definitive antibiotic therapy. In addition, at day 14, combined antimicrobial therapy was identified as a protective factor for mortality (HR 0.56, 95% CI 0.33 to 0.93; $p = 0.027$) (Tables S2 and S3, Supplementary Materials).

4. Discussion

Multiple reports have drawn attention to the worryingly high rates of XDR *P. aeruginosa* infections worldwide [1,2,28,29]. Although previous studies have assessed the impact on the outcome of carbapenem-resistant or MDR *P. aeruginosa* BSI [9–16], little is known about the role of XDR strains. In order to obtain a better understanding of this issue, we present here the results of the largest cohort of patients with monomicrobial XDR *P. aeruginosa* bacteremia published to date.

Our data show that the vast majority of XDR *P. aeruginosa* strains were non-susceptible to all antipseudomonal agents apart from colistin and amikacin. Although clonality and the resistance mechanisms of the isolates were not specifically investigated in this study, previous ones found that,

in XDR *P. aeruginosa* strains, the ST175 high-risk clone was by far the most frequent at our site [18–21]. In a Spanish multicenter study by our group (COLIMERO study) [18], 17/21 (81%) strains included from our center belonged to the ST175 high-risk clone. In another recent survey of *P. aeruginosa* molecular epidemiology and antimicrobial resistance in Spain, del Barrio-Tofiño et al. [19] found that all isolates recovered from our site also belonged to the ST175 high-risk clone. In these two previous reports [18,19], the underlying resistance mechanisms in the XDR phenotype were mainly a combination of chromosomal mutations such as hyperproduction of chromosomal AmpC β-lactamases, efflux pumps, OprD deficiency and/or quinolone resistance-determining region mutations, rather than horizontally acquired carbapenemases.

Our study showed that the all-cause mortality rate for patients with XDR *P. aeruginosa* bacteremia at day 30 was above 30%, which is similar to results reported in previous series of carbapenem-resistant or MDR isolates [9,10,13,16,17,30]. We observed that severity at presentation or a high-risk source of *P. aeruginosa* bacteremia were independent predictors of mortality, as has been previously reported [9,10,12,14–16]. On the other hand, although it is often assumed that antibiotic-resistant *P. aeruginosa* bacteremia results in worse outcomes, our study found that an XDR profile was not associated with higher mortality rates, even after considering only high-risk source BSI. Conflicting results have been reported for carbapenem-resistant or MDR *P. aeruginosa* infections [9–16]. In the case of XDR isolates, the evidence is even scarcer and there are only a few published studies. Recio et al. [31] also failed to identify the XDR phenotype as a predictor of mortality in a cohort of 70 patients with *P. aeruginosa* bacteremia in Spain that included 24 XDR strains (10 of them belonging to the ST175 high-risk clone and 11 to ST235). Another study carried out in Brazil [11] did not find an association in 120 patients with *P. aeruginosa* bacteremia, 31 of them XDR strains. On the other hand, a recent study of a cohort of 243 patients, including 87 XDR isolates (43 strains linked to ST175 high-risk clone and 33 to ST235) [16] found MDR phenotype as an independent predictor of 30-day crude mortality. In line with this, a recent Spanish study aimed at assessing the impact of virulence on the outcome of *P. aeruginosa* BSI [32] also found that XDR phenotype was statistically linked to 30-day mortality in a cohort of 593 bacteremia cases, 81 of them XDR isolates (61 ST175, 9 ST111, 2 ST235, and 2 ST244), although multivariate analysis data were not provided. Another Spanish study [33] focused on BSIs in solid organ transplant recipients identified a higher mortality rate for patients with bacteremia due to XDR *P. aeruginosa* (n = 31) compared to those with BSI caused by other microorganisms (n = 287), and it was found to be an independent risk factor for mortality (20/22 studied strains showed the ST175 pattern). Other studies [34,35] have addressed the impact of XDR strains on different sources of infection, not exclusively in bacteremia. For instance, a Thai study of 255 patients with *P. aeruginosa* infections, including 56 XDR strains, found that an XDR phenotype was an independent factor of mortality attributable to infection when compared to non-XDR isolates [34]. Samonis et al. found the same association with mortality in a cohort of 89 *P. aeruginosa* infections (52 of them with bacteremia and 22 XDR isolates) in cancer patients in Greece [35].

As was the case for carbapenem-resistant or MDR *P. aeruginosa* bacteremia, conflicting findings are also reported for XDR strains, suggesting that the prognosis of BSI depends on several factors apart from the resistance phenotype. In our study, no relevant differences were observed between the two groups either for preexisting comorbidities or severity of clinical presentation at the onset of bacteremia, although a low-risk source of bacteremia was more frequently observed in the XDR group. This finding could partially explain the lack of association between XDR phenotype and mortality, although the same results were obtained after adjusting for source of infection, which rules out this hypothesis. In addition, although patients with an XDR *P. aeruginosa* BSI have a higher probability of receiving inadequate empirical antimicrobial therapy, unexpectedly, this was not associated with worse outcomes. Some authors argue that in cases of *P. aeruginosa* bacteremia, a 48–72 h delay before receiving appropriate antibiotics may not be so crucial to patient outcome, since mortality is mainly due to other factors, such as severity of infection, having a high-risk source, or receiving inappropriate definitive antibiotic therapy [10,12,15,36,37], which is consistent with the results of the present study.

Another hypothesis to explain the lack of impact of XDR phenotype on mortality could be the influence of the acquisition of resistance mechanisms. It has been hypothesized that it may involve a fitness cost resulting in strains with lower virulence, and with a reduced inflammatory response in the host when compared to susceptible isolates [38–41]. On the other hand, it has also been reported that not all resistance mutations lead to a biological cost such as the OprD deficiency [42]. Indeed, some studies have identified that MDR strains can develop compensatory or suppressor mechanisms that allow them to recover their baseline fitness [12,43,44]. Other studies have found that some high-risk clones can be as virulent as susceptible strains, despite exhibiting many resistance mutations, suggesting that pathogenicity depends not only on the fitness cost of resistance, but also on the presence of certain virulence determinants such as exoU-positive genotype or O11 antigen serotype [16,17,31,32]. The ST235 high-risk clone, for example, appears to be particularly virulent in cases of ExoU production, whereas the virulence of ST175, the most prevalent high-risk clone observed at our institution, is especially low [16,17,31,32]. Thus, it may be inferred that the high prevalence of the less virulent ST175 high-risk clone at our institution may account in part for the lack of detrimental impact on mortality of the XDR phenotype.

One of the most interesting findings of our study is that combined antimicrobial therapy improves prognosis at day 14 in high-risk source bacteremia. Previous studies have addressed this issue [45–49], although they were not focused specifically on XDR *P. aeruginosa* bacteremia. Some of these [46–48] also found a protective effect for patients receiving two active drugs for high-risk sources, mainly pneumonia. In our study, lower respiratory tract infection was the most common high-risk source of infection, closely followed by primary bacteremia. Nevertheless, this finding should be interpreted with caution. As this issue was not one of the main purposes of our research, certain crucial variables, such as the duration of combined therapy, the onset time of this therapy, the antibiotic dosing, or how antibiotic treatment was administered (extended- or continuous-infusion or intermittent bolus) were not accounted for. Further studies are needed to clarify this question.

This study has several limitations. First, although cases were recorded prospectively, several data were obtained retrospectively and are therefore more vulnerable to potential bias. Second, the study was carried out in a single center and the results are not necessarily transferable to other settings with a different epidemiology. Third, although a sample of the included isolates was well characterized in previous studies, clonality and resistance mechanisms were not specifically investigated in all cases. Finally, some relevant variables such as the time until appropriate definitive antibiotic treatment or those related to combined antimicrobial therapy were not recorded, thus their impact on outcomes could have been potentially overlooked. On the other hand, this study has some highlighted strengths. It includes the largest published sample size of XDR *P. aeruginosa* bacteremia, eliminating the biases of many studies with small sample sizes, which reduce the statistical power to be able to draw reliable conclusions. Furthermore, a wide-ranging statistical analysis was performed to control for confounding variables, including a subanalysis in high-risk sources.

5. Conclusions

Our study identified severity at presentation, having a high-risk source of bacteremia, and inappropriate definitive antibiotic therapy as risk factors for mortality in patients with *P. aeruginosa* bacteremia. On the other hand, the XDR phenotype was not associated with poor prognosis. Decreased virulence in XDR strains, theoretical fitness costs, and a high prevalence of the less virulent ST175 high-risk clone at our institution may be among the reasons for these findings. Nevertheless, the mortality rate for *P. aeruginosa* bacteremia remains high in our cohort. Since antibiotic treatment is the only modifiable factor to try to improve outcome, strategies aimed at earlier identification of patients with risk factors for *P. aeruginosa* bacteremia should be implemented to avoid delays in the administration of effective antibiotic therapy, mainly in more severe patients and those with high-risk source bacteremia. Combined antimicrobial therapy should be considered in patients with bacteremia from high-risk sources.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0383/9/2/514/s1, Table S1: Baseline characteristics of patients with high-risk sources of infection, Table S2: Univariate and multivariate Cox model of 14-day all-cause mortality in patients with high-risk sources of infection, Table S3: Univariate and multivariate Cox model of 30-day all-cause mortality in patients with high-risk sources of infection.

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4.2. Article 2

Aminoglycoside or Polymyxin Monotherapy for Treating Complicated Urinary Tract Infections Caused by Extensively Drug-Resistant *Pseudomonas aeruginosa*: A Propensity Score-Adjusted and Matched Cohort Study.

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ORIGINAL RESEARCH

Aminoglycoside or Polymyxin Monotherapy for Treating Complicated Urinary Tract Infections Caused by Extensively Drug-Resistant *Pseudomonas aeruginosa*: A Propensity Score-Adjusted and Matched Cohort Study

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ABSTRACT

Introduction: Extensively drug-resistant (XDR) *Pseudomonas aeruginosa* (PA) infections are difficult to treat. We aimed to compare aminoglycosides or polymyxin monotherapy versus other antibiotic regimens (carbapenems, aztreonam, ceftazidime, cefepime, ceftolozane-tazobactam, or ceftazidime-avibactam) in complicated urinary tract infections (cUTI) caused by XDR-PA.

Methods: Study performed at a tertiary-care hospital from 2010 to 2019. All consecutive

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adult patients with XDR-PA urine cultures and diagnosed with cUTI were retrospectively reviewed. XDR phenotype was defined according to Magiorakos et al. A propensity score was used as a covariate in multivariate analyses and for matching. Primary outcome was early clinical failure and at end of treatment (EOT). Main secondary outcomes were 30- and 90-day mortality, microbiological clearance, and antibiotic-related side effects.

Results: Of the 465 episodes screened, 101 were included, 48% were treated with aminoglycoside or colistin monotherapy. Most XDR-PA were susceptible to colistin (100%) and amikacin (43%). Patients treated with antibiotic regimens other than aminoglycosides or polymyxin monotherapy were more likely to have hematologic malignancy ($p < 0.001$), higher SOFA score ($p = 0.048$), and bacteremia ($p = 0.003$). In multivariate models adjusted by propensity score, aminoglycoside or colistin monotherapy was not associated with worse outcomes. After propensity score matching, 28 episodes in each treatment group were matched. Adjusted ORs (95% CI) for early clinical failure and at EOT with aminoglycosides or polymyxin monotherapy were 0.53 (0.18–1.58) and 1.29 (0.34–4.83), respectively. Aminoglycoside or colistin monotherapy was not associated with higher 30-day (HR 0.93, 95% CI 0.17–5.08) or 90-day mortality (HR 0.68, 95% CI 0.20–2.31), nor with absence of microbiological clearance (OR 0.72, 95% CI 0.33–1.58). No statistically significant differences were found in terms of nephrotoxicity. *Clostridioides difficile* infection was observed only in the "other antibiotic regimens" group ($n = 6$, 11.3%).

Conclusions: Aminoglycosides or polymyxin monotherapy showed good efficacy and safety profile in treating cUTI caused by XDR-PA. These results may be useful for antibiotic stewardship activities.

Keywords: Extensively drug-resistant *Pseudomonas aeruginosa*; Urinary tract infections; Amikacin; Colistin; Antimicrobial stewardship

Key Summary Points

Aminoglycosides or polymyxin monotherapy might be an alternative for urinary tract infections (UTIs) caused by extensively drug-resistant (XDR) *P. aeruginosa*.

Aminoglycosides or polymyxin monotherapy was not associated with poor outcomes compared to other antibiotic regimens.

Patients treated with antibiotic regimens other than aminoglycosides or polymyxin monotherapy were more likely to have *Clostridioides difficile* infection

These results may be useful for antimicrobial stewardship activities.

INTRODUCTION

The increasing incidence of multidrug-resistant gram-negative bacteria (GNB) is a worldwide problem. *Pseudomonas aeruginosa* is particularly worrisome because of its extraordinary capacity to develop resistance [1]. The emergence of extensively drug-resistant (XDR) strains in recent years has increased the concern [2]. *P. aeruginosa* is frequently isolated in complicated urinary tract infection (UTI), mainly those of nosocomial or healthcare-related acquisition [3]. Aminoglycosides and colistin are usually active against GNB, including many XDR *P. aeruginosa* isolates [4]. Both agents have favorable pharmacokinetics characteristics, which theoretically makes them suitable molecules for the treatment of complicated UTIs. Aminoglycosides are excreted in high concentrations in the urine, exceeding plasma concentrations by up to 100-fold, and remain above therapeutic levels for 72 h or longer [5]. Approximately 60–70% of colistimethate sodium (CMS) is eliminated in the urine. Furthermore, the conversion of CMS into colistin

can occur in the renal tubular cells and in the bladder, suggesting that concentrations of formed colistin may be higher than those in plasma [6, 7]. However, as a result of concern about their nephrotoxicity [8, 9], clinical effectiveness [10, 11], and the risk of emergence of resistance in vivo [11, 12], combined antimicrobial therapy or broad-spectrum antibiotics such as carbapenems, ceftolozane-tazobactam, or ceftazidime-avibactam are frequently used to treat complicated UTIs caused by XDR *P. aeruginosa*.

On the other hand, previous studies [13, 14] have shown that in *P. aeruginosa* infections, UTI is associated with lower mortality rates and is therefore considered a low-risk source of infection. Thus, antibiotic monotherapy with aminoglycosides or colistin could be explored as an alternative therapeutic strategy, even in complicated UTIs. Furthermore, the prescription of broad-spectrum or combined antimicrobial therapy can also have deleterious effects, such us development and persistence of antimicrobial resistance [15], higher risk of *Clostridioides difficile* infection [16], and higher pharmacy costs [17].

We hypothesized that aminoglycosides or polymyxin monotherapy could be an alternative effective option for the treatment of complicated UTIs caused by XDR *P. aeruginosa*.

The aim of the present study was to evaluate the efficacy and safety of aminoglycosides or polymyxin monotherapy in comparison to other antibiotic regimens, including combined antimicrobial therapy, in complicated UTIs due to XDR *P. aeruginosa*.

METHODS

Hospital Setting, Study Design, and Participants

This study was conducted from January 2010 to June 2019 at the Hospital del Mar, a tertiary-care university hospital in Barcelona (Spain), within the framework of an antimicrobial stewardship (AMS) program.

All consecutive positive urinary cultures for XDR *P. aeruginosa* during the study period were retrospectively reviewed. XDR *P. aeruginosa* was defined as non-susceptible to one or more agent in all but no more than two antipseudomonal antimicrobial categories, according to Magiorakos et al. [18].

The inclusion criteria were patients aged at least 18 years old, diagnosed with acute pyelonephritis or complicated UTI and with a monomicrobial urine culture positive for XDR *P. aeruginosa*. Non-complicated UTIs and asymptomatic bacteriuria were excluded. All episodes were retrospectively reviewed by two authors (I.L.M. and S.G.-Z.). Patients treated with aminoglycosides or colistin in the form of CMS monotherapy were compared to those treated with other antibiotic regimens including carbapenems, aztreonam, ceftazidime, cefepime, ceftolozane-tazobactam, or ceftazidime-avibactam, alone or in combination (including also combinations with aminoglycosides or CMS). Dose selection was at the discretion of the responsible clinicians and was adjusted according to glomerular filtration rate (GFR).

Patients were followed for up to 90 days from the date of the urine culture. In cases of more than one episode of *P. aeruginosa* UTI, the second and following episodes were assessed if they occurred at least 90 days after the prior one. Patients who died within the first 48 h or did not complete follow-up were not included in the analysis.

Ethics

The study was approved by the Clinical Research Ethics Committee of the Parc de Salut Mar (register no. 2020/9321). The need for written informed consent was waived because of the observational nature of the study and retrospective analysis. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guideline and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Clinical Variables, Data Source, and Definitions

The main outcome variable was clinical failure assessed early (day 7) and at end of treatment (EOT). Secondary outcomes were crude 30- and 90-day mortality; recurrence, reinfection, microbiological clearance, and readmission rates within 90 days. The incidence of acute kidney injury (AKI), *C. difficile* infection, rash, hematological toxicity, hepatotoxicity, and neurological symptoms were also evaluated as secondary outcomes to study antibiotic-related side effects.

Demographic, clinical, and microbiological data were collected from hospital medical charts. Recorded data included the following: age and sex; comorbidities and severity of underlying diseases, assessed using the Charlson comorbidity index [19], and immunosuppression state, defined as neutropenia (absolute neutrophil count of 500 cells/mm^3 or less), chemotherapy or other immunosuppressant drugs, HIV infection, and/or congenital immunosuppression. Prior history of benign prostatic hypertrophy, urologic malignancy, obstructive nephropathy, recurrent UTI, and urological devices in the last 14 days were also recorded.

Severity of illness was calculated using the Sequential Organ Failure Assessment (SOFA) score [20], the need for intense care unit (ICU) admission, and the presence of septic shock [21]. The Pitt score [22] was applied in the case of bacteremia.

Acute pyelonephritis was considered if the patient had at least two of the following criteria: temperature above 37.7 °C, UTI symptoms (dysuria, urgency, suprapubic pain, and/or pollakiuria), local pain (lumbar back pain, costovertebral angle tenderness, and/or pelvic or perineal pain in men), and/or altered mental status in people up to 70 years. Those with the same criteria and a prior history of benign prostatic hyperplasia, intermittent or permanent indwelling urinary catheter (or withdrawal within 48–72 h before infection onset), or underlying urologic abnormalities such us nephrolithiasis, strictures, stents, history of renal transplant or urinary diversions or neurogenic bladder were classified as complicated UTI. The site of infection acquisition was defined according to Friedman et al. [23].

Appropriate empiric antibiotic therapy was considered when at least one antipseudomonal antibiotic with in vitro activity was administered during the first 24 h after urine cultures were taken. Appropriate definite antibiotic therapy was treatment based on the results of antibiotic susceptibility testing. Combination therapy was defined as two or more antipseudomonal drugs used for at least 48 h.

Adequate source control was defined as removal or insertion of indwelling urinary catheters, percutaneous drainage of the urinary tract (double-J stent, nephrostomy), or surgical intervention, as appropriate.

Clinical failure was considered if there was persistence or worsening signs and/or symptoms of UTI, the need to modify antibiotic therapy because of antibiotic side effects, the emergence of resistance to the study drug, and/or death.

Recurrence was defined as recurrent signs or symptoms of UTI and a urinary isolate of XDR *P. aeruginosa* with the same susceptibility profile as the index infection. Reinfection was defined as recurrent signs or symptoms of UTI with isolation of a *P. aeruginosa* strain with a different phenotypic profile from the prior one and/or a urinary isolate different from *P. aeruginosa*. Microbiological clearance was considered if there was no growth of *P. aeruginosa* in the final urine culture, if available. Episodes with missing urine samples during follow-up were classified as indeterminate. All microbiological assessments referred to up to 90 days following onset of the index UTI.

Antibiotic side effects (i.e., nephrotoxicity, *C. difficile* infection, rash) were also recorded. GFR, calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), was registered at baseline and at EOT. In case of AKI, the RIFLE score [24] was applied.

Microbiological Studies

Bacterial isolates were identified as *P. aeruginosa* following standard procedures. Antibiotic

susceptibility testing of isolates was performed by broth microdilution using MicroScan® panels [Beckman-Coulter] in the automated MicroScan® WalkAway system [Beckman-Coulter]. The following antimicrobials were tested: ciprofloxacin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, aztreonam, gentamicin, tobramycin, amikacin, and colistin. Ceftolozane-tazobactam was not in routine use for a large part of the study; it was tested by Etest® gradient diffusion (bioMérieux, Marcy-l'Etoile, France) from 2017 onwards. Antibiotic susceptibility testing results were categorized according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [25] in force at the time of urine culture.

Statistical Analysis

The required sample size (100 patients) was determined from the results of a previous study [26] to detect a 20% difference in early clinical failure between an aminoglycoside-based or colistin group vs. "other regimens" group for infections caused by drug-resistant *P. aeruginosa*; statistical power was set at 80%, alpha error at 0.05, and 0.2 estimated losses to follow-up.

Categorical variables were compared by the χ^2 test or Fisher exact test and continuous variables by Student's *t* test or Mann–Whitney *U* test, as appropriate. A logistic regression model examined associations between exposures and clinical failure and microbiological clearance whereas Cox proportional hazards regression was applied to assess mortality until day 30 and 90. Variables with a *p* value of at most 0.1 in univariate analysis and those clinically relevant were included in the multivariate models and selected manually using backward stepwise regression.

A propensity score for receiving monotherapy with aminoglycosides or colistin was calculated. Variables used for calculating propensity score were age, sex, Charlson comorbidity index, hematologic malignancy, positive blood cultures, SOFA score, and presentation with sepsis/septic shock. Its predictive ability was estimated by calculating the area under the receiver operating characteristic curve (AUC) with 95% confidence interval (CI). The variance inflation factor value was calculated for every variable included to control for the potential occurrence of collinearity between the propensity score and other potential confounders. We selected the best model according to the likelihood ratio test. The final model showed a *p* value of 0.71 for the Hosmer–Lemeshow goodness-of-fit test and an AUC of 0.8 (95% CI 0.71–0.88). The propensity score was used in two different ways, as a covariate of control for residual confounding in multivariate models, and to perform a matched cohort analysis in which patients receiving amikacin or CSM were matched 1:1 according to their propensity score with those receiving other antibiotic regimens. The caliper was set to a width equal to 0.2 of the standard deviation of the logit of the propensity score [27]. Clinical failure in the matched pairs was compared by conditional logistic regression whereas Cox regression was used to compared mortality. Sensitivity analyses for all the studied outcomes were performed excluding patients receiving amikacin or CMS as part of a combination therapy from the control group. All *p* values were two-tailed and those less than 0.05 indicated statistical significance. The STROBE recommendations were used to ensure the reporting of the study (Supplementary Material). Statistical analyses were performed using STATA 15.1.

RESULTS

Of the 465 cases with urine cultures positive for XDR *P. aeruginosa* screened, 101 episodes met the inclusion criteria and were included in the final analysis (Fig. 1). Only four patients had two episodes of UTI, the rest had a single episode. Most XDR *P. aeruginosa* were susceptible to colistin (100%) and amikacin (42.6%, *n* = 43/101). Complete antimicrobial susceptibility phenotypes are shown in the Supplementary Material.

In the aminoglycoside or CMS monotherapy group (*n* = 48), 27 episodes were treated with

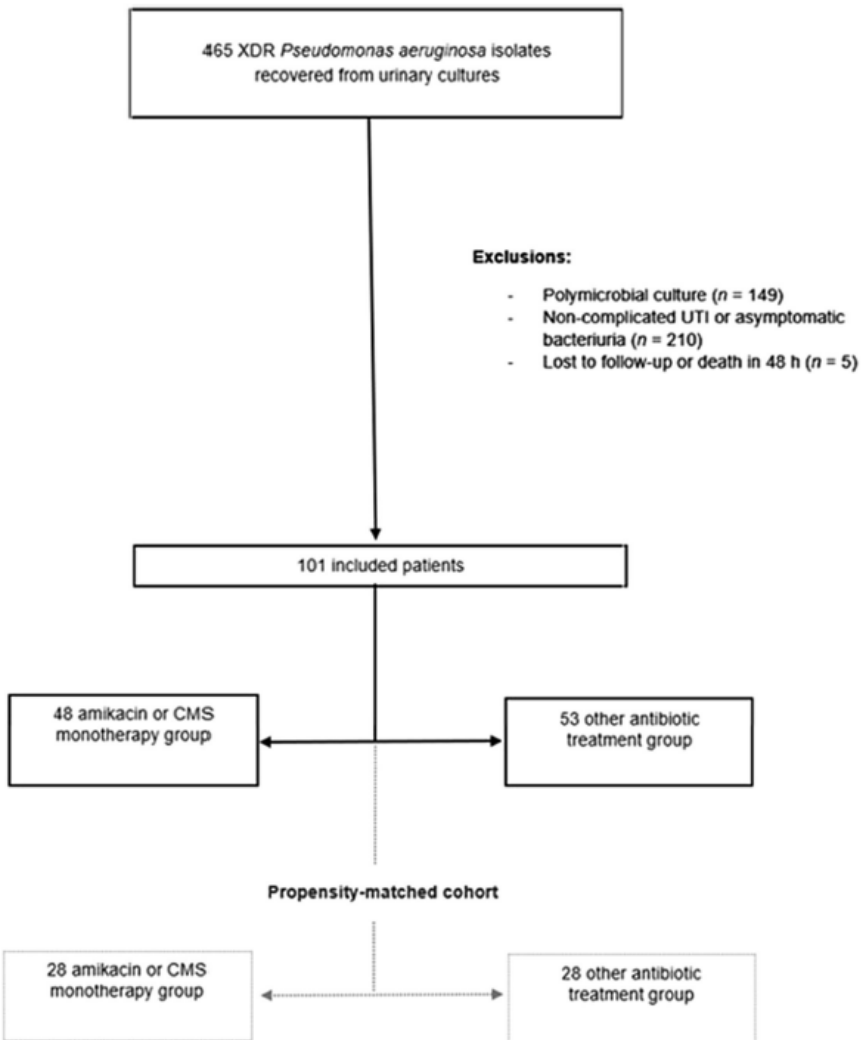

Fig. 1 Flowchart of the patients included in the study. XDR extensively drug-resistant, UTI urinary tract infection, CMS colistimethate sodium

CMS and 21 with aminoglycosides. Among those with other antibiotic therapies ($n = 53$), the most frequent antibiotic regimens were amikacin and/or CMS plus carbapenem ($n = 24$), CMS plus ceftazidime or cefepime ($n = 7$), and amikacin or CMS plus aztreonam

Table.1 Baseline characteristics of patients in overall and propensity-matched cohorts

Variable	Overall cohort (n = 101)			Propensity score matched cohort (n = 56)		
	Amikacin or CMS treatment (n = 48)	Other treatments (n = 53)	p value	Amikacin or CMS treatment (n = 28)	Other treatments (n = 28)	p value
Demographic information						
Age (years), m (IQR)	74.5 (67–84.5)	77 (67.5–82)	0.796	77 (69.5–87)	77 (66–82)	0.640
Male sex	40 (83.3)	40 (75.5)	0.331	23 (79.3)	21 (77.8)	0.899
Underlying condition						
Charlson comorbidity index, m (IQR)	4 (2–5.75)	4 (2–6)	0.961	3 (2–5)	4 (2–6)	0.337
Diabetes mellitus	13 (27.1)	17 (32.1)	0.583	8 (27.6)	12 (44.4)	0.188
COPD	15 (31.2)	16 (30.2)	0.908	5 (17.2)	8 (29.6)	0.273
Cirrhosis	2 (4.2)	2 (3.8)	1	–	1 (3.7)	0.482
Hematologic malignancy	1 (2.1)	16 (30.2)	< 0.001*	1 (3.4)	2 (7.4)	0.605
Solid tumor malignancy	24 (50)	25 (47.2)	0.776	15 (51.7)	11 (40.7)	0.410
Nephro-urological history						
Baseline GFR (ml/min), m (IQR)	58.1 (35–83)	50 (25.5–83.5)	0.835	43 (27–68.25)	48 (27–82)	0.476
Chronic kidney disease	10 (20.8)	15 (28.3)	0.385	8 (27.6)	9 (33.3)	0.640
Dialysis	1 (2.1)	5 (9.4)	0.208	1 (3.4)	2 (7.4)	0.605
Renal transplant	1 (2.1)	4 (7.6)	0.365	–	1 (3.7)	0.482
Benign prostatic hypertrophy	14 (29.2)	16 (30.2)	0.911	7 (24.1)	10 (37)	0.386
Obstructive urinary disease	6 (12.5)	6 (11.3)	1	3 (10.3)	1 (3.7)	0.612
Recurrent UTI	20 (41.7)	29 (54.7)	0.19	15 (51.7)	14 (51.9)	0.992
Indwelling urinary catheter in last 14 days	36 (75)	33 (62.3)	0.202	23 (79.3)	20 (74.1)	0.643
Other urological devices in last 14 days	6 (12.5)	12 (22.6)	0.205	2 (6.9)	1 (3.7)	1
Acquisition						
Healthcare-related	23 (51)	28 (52.8)	0.622	20 (69)	13 (48.1)	0.114
Nosocomial	25 (52.1)	25 (47.2)	0.622	9 (31)	14 (51.9)	0.114
HCA risk factors						

Table.1 continued

Variable	Overall cohort (n = 101)			Propensity score matched cohort (n = 56)		
	Amikacin or CMS treatment (n = 48)	Other treatments (n = 53)	p value	Amikacin or CMS treatment (n = 28)	Other treatments (n = 28)	p value
Hospital stay in last 3 months	24 (50)	33 (62.3)	0.234	13 (48.1)	17 (58.6)	0.432
Surgery in last 3 months	22 (45.8)	16 (30.2)	0.150	12 (41.4)	8 (29.6)	0.359
ICU admission in last 3 months	13 (27.1)	9 (17)	0.238	7 (24.1)	6 (22.2)	0.865
Residence in long-term care	8 (16.7)	6 (11.3)	0.567	8 (27.6)	1 (3.7)	0.026*
Antibiotic exposure in last 3 months	38 (79.2)	49 (92.4)	0.082	25 (86.2)	24 (88.9)	0.762
Baseline illness severity						
SOFA score, m (IQR)	1 (0–2.7)	2 (1–4)	0.048*	2 (0.5–3)	2 (0–4)	0.973
Sepsis or septic shock	11 (22.9)	21 (39.6)	0.072	10 (34.5)	6 (22.2)	0.310
ICU admission	5 (10.4)	7 (13.2)	0.764	5 (17.2)	2 (7.4)	0.424
Bacteremia	4 (8.3)	17 (32.1)	0.003*	4 (13.8)	4 (14.8)	0.913
Pitt score, m (IQR)	2 (0.5–2.7)	1 (0–1.5)	0.282	2 (0.5–2.7)	0.5 (0–1)	0.134
Management						
Appropriate empirical treatment	8 (16.7)	13 (24.5)	0.331	6 (20.7)	5 (18.5)	0.838
Appropriate definitive treatment	48 (100)	49 (92.5)	0.119	29 (100)	24 (88.4)	0.106
72 h delay to start appropriate antibiotic treatment	24 (50)	30 (56.6)	0.506	15 (51.7)	18 (66.7)	0.256
Adequate source control	44 (91.7)	46 (86.8)	0.432	27 (93.1)	24 (88.9)	0.580

Data are presented as n (%), unless otherwise specified

CMS colistimethate sodium, *COPD* chronic obstructive pulmonary disease, *GFR* glomerular filtration rate, *UTI* urinary tract infection, *HCA* healthcare acquired, *ICU* intensive care unit, *SOFA* Sequential Organ Failure Assessment, *m* median, *IQR* interquartile range

*Statistical significance at $p < 0.05$

(n = 6). Only 14 episodes were treated with amikacin- or colistin-free antibiotic regimens: ceftazidime (n = 5), ceftolozane-tazobactam (n = 5), aztreonam (n = 2), and carbapenems (n = 2). All patients treated with an aminoglycoside (n = 35; 21 in the monotherapy group vs. 14 in the "other therapies" group) received amikacin in a once-daily strategy, with the most frequent regimen being 1 g every 24 h [n = 22, 15/21 (71.4%) in the monotherapy

group vs. 7/14 (50%) in other regimens]. In the case of CMS ($n = 52$; 27 in the monotherapy group vs. 25 in the "other treatments group"), the most frequent doses were 2 million international units (IU) three times a day in 9 (33.3%), 3 million IU twice daily in 8 (29.6%), 1 million IU twice daily in 8 (29.6%), and 1 million IU once a day in 8 (29.6%) episodes.

Overall, 80% were men and the median age was 76 years. Most cases were considered complicated UTI ($n = 93$), whereas acute pyelonephritis was observed in only eight patients. The 20% of episodes were bacteremic UTI. Bloodstream infection was more frequently observed among patients treated with amikacin or CMS monotherapy than those who received other antibiotic regimens (32.1% vs 8.3%, $p = 0.003$).

After propensity score matching, 56 (55.4%) patients were matched, with 28 in each treatment group. Baseline epidemiological and clinical characteristics between treatment groups before and after propensity score analysis are shown in Table 1. No significant differences were observed in the baseline demographic or clinical characteristics after propensity score matching, apart from prior residence in long-term care facility ($p = 0.026$).

Primary Outcome: Clinical Failure

Early clinical failure rate was 28.7% (29/101): 18.7% (9/48) in the amikacin or CMS monotherapy group vs. 37.7% (20/53) in other antibiotic regimens ($p = 0.035$). Reasons for failure were persistence or worsening signs and/or symptom, 26 cases (7/29, 24.1% in amikacin or CMS monotherapy vs. 19/29, 65.5% in other antibiotic regimens); death, two patients (1/29, 3.5% in each group); and need to modify therapy because of antibiotic side effects, one patient (3.5%) in the amikacin or CMS monotherapy group.

The rate of clinical failure at EOT was 19.8% (20/101): 20.8% (10/48) in amikacin or CMS

Table.2 Crude and adjusted associations between different variables and clinical failure at day 7 and at end of treatment in overall and propensity-matched cohorts

	Overall cohort			Propensity-matched cohorts	
	Crude OR (95% CI)	aOR (95% CI)	*p* value	aOR (95% CI)	*p* value
Day 7					
Age (years), m (IQR)	1.01 (1–1.09)	1.05 (1.01–1.1)	0.041	1.01 (0.96–1.06)	0.725
Charlson comorbidity index, m (IQR)	1.06 (0.89–1.25)	1.09 (0.9–1.32)	0.356	1.05 (0.81–1.35)	0.717
SOFA score, m (IQR)	1.12 (0.9–1.38)	1.01 (0.82–1.31)	0.770	1.13 (0.82–1.55)	0.460
Amikacin or CMS treatment	0.38 (0.15–0.95)	0.5 (0.17–1.44)	0.198	0.53 (0.18–1.58)	0.251
Propensity score	0.16 (0.03–0.86)	0.34 (0.04–2.74)	0.311		
End of treatment					
Age (years), m (IQR)	1.03 (0.98–1.08)	1.05 (0.99–1.11)	0.101	1.04 (0.97–1.19)	0.301
Charlson comorbidity index, m (IQR)	1.18 (0.98–1.43)	1.24 (1.01–1.53)	0.047	1.39 (0.97–1.97)	0.071
SOFA score, m (IQR)	1.05 (0.82–1.34)	1 (0.76–1.31)	0.980	0.88 (0.57–1.36)	0.552
Amikacin or CMS treatment	1.13 (0.42–3.01)	1.58 (0.47–5.32)	0.462	1.29 (0.34–4.83)	0.707
Propensity score	0.48 (0.07–3.2)	0.35 (0.31–4)	0.401		

SOFA Sequential Organ Failure Assessment, *ICU* intensive care unit, *CMS* colistimethate sodium, *m* median, *IQR* interquartile range, *OR* odds ratio, *aOR* adjusted odds ratio, *CI* confidence interval

monotherapy vs. 18.9% (10/53) in other antibiotic regimens ($p = 0.805$). Reasons for failure were (amikacin or CMS monotherapy vs. other antibiotic regimens) persistence or worsening signs and/or symptoms, nine patients (4/20, 20% vs. 5/20, 25%); need to modify therapy because of antibiotic side effects, four cases (3/20, 15% vs. 1/20, 5%); death, four patients (1/20, 5% vs. 3/20, 15%); and emergence of resistance, three isolates (2/20, 10% and 1/20, 5%). In all cases, nephrotoxicity was the reason for switching antibiotic treatment because of antibiotic side effects.

Table 2 shows crude and adjusted analyses of variables involved in early clinical failure and at EOT. Monotherapy with amikacin or CMS was not associated with higher rates of clinical failure.

The estimations of the associations of CMS or amikacin in monotherapy with clinical failure at day 7 and at EOT in sensitivity analyses were consistent with the analysis in the whole cohort (Supplementary Material).

Secondary Outcomes: Mortality and Microbiological Clearance

The 30-day mortality rate was 8.3% (4/48 patients) among patients treated with CMS or amikacin in monotherapy and 11.3% (6/53 patients) among those who received other antibiotic regimens ($p = 0.744$). The 90-day mortality was 18.8% (9/48 patients) and 30.2% (16/53 patients), respectively ($p = 0.183$). In multivariate analysis, receipt of amikacin or CMS monotherapy was not associated with either crude 30- or 90-day mortality (Table 3). Sensitivity analyses for mortality did not show different trends (Supplementary Material).

Regarding the microbiological assessment, 51 patients had a follow-up urine culture within 90 days. No statistically significant differences

Table.3 Crude and adjusted associations between different variables and 30- and 90-day mortality in overall and propensity-matched cohorts

	Overall cohort			Propensity-matched cohorts	
	Crude HR (95% CI)	aHR (95% CI)	*p* value	aHR (95% CI)	*p* value
30-day mortality					
Age (years), m (IQR)	1.05 (0.99–1.12)	1.09 (1.01–1.19)	0.033*	1.12 (1.01–1.25)	0.046*
Charlson comorbidity index, m (IQR)	1.21 (0.99–1.49)	1.36 (1.07–1.73)	0.012*	1.73 (1.01–2.99)	0.049*
SOFA score, m (IQR)	1.36 (1.05–1.78)	1.37 (1.02–1.83)	0.036*	1.24 (0.75–2.06)	0.398
Amikacin or CMS treatment	0.73 (0.2–2.57)	1.25 (0.29–5.45)	0.763	0.93 (0.17–5.08)	0.937
Propensity score	0.16 (0.02–1.67)	0.27 (0.01–7)	0.438		
90-day mortality					
Age (years), m (IQR)	1.01 (0.98–1.05)	1.04 (0.99–1.09)	0.113	1.07 (0.99–1.15)	0.065
Charlson comorbidity index, m (IQR)	1.3 (1.14–1.49)	1.37 (1.17–1.59)	< 0.001*	1.59 (1.13–2.22)	0.007*
SOFA score, m (IQR)	1.22 (1.03–1.45)	1.22 (1.01–1.48)	0.037*	1.32 (0.88–1.98)	0.177
Amikacin or CMS treatment	0.59 (0.26–1.34)	0.96 (0.36–2.54)	0.933	0.68 (0.20–2.31)	0.534
Propensity score	0.2 (0.48–0.82)	0.34 (0.06–2.03)	0.236		

SOFA Sequential Organ Failure Assessment, *CMS* colistimethate sodium, *m* median, *IQR* interquartile range, *HR* hazard ratio, *aHR* adjusted hazard ratio, *CI* confidence interval

*Statistical significance at $p < 0.05$

were found between treatment groups after adjusting for confounders (Supplementary Material).

Adverse Events

Antibiotic-related side effects are shown in Supplementary Material. No statistically significant differences were found in terms of nephrotoxicity between groups. *C. difficile* was only observed in patients in the group treated with other antibiotic regimens (11.3%).

DISCUSSION

In the present study, we were unable to demonstrate that amikacin or CMS monotherapy was associated with worse outcomes in terms of mortality, clinical failure, or microbiological clearance than combination or other antibiotic therapies in cases of complicated UTI caused by XDR *P. aeruginosa* isolates, after controlling for confounders. Although these results cannot be interpreted as that amikacin or CMS monotherapy is equally effective as combination or other antibiotic therapies, they reinforce the message that alternative narrow-spectrum antibiotic use should be considered in some scenarios despite that we are facing a difficult-to-treat bacteria.

The challenge of treating XDR *P. aeruginosa* has been thoroughly discussed in the literature. Many clinicians favor combination treatment even though the clinical evidence of the superiority of combination therapy over monotherapy is scarce and of low quality [28, 29]. Although the use of combination therapy may be tempting in this type of infection, combination therapies increase antibiotic pressure in the hospital ecosystem and the selection of multidrug-resistant bacteria [15]. In this setting, the World Health Organization, not surprisingly, has urged the implementation of AMS programs to optimize antibiotic use and control increased multidrug resistance worldwide [30]. Further, although the new antipseudomonal agents ceftolozane-tazobactam and ceftazidime-avibactam have recently become available in daily clinical practice, the emergence of resistant mutants has been already reported [31, 32], suggesting that the "old drugs" still have a place.

The effectiveness of aminoglycosides and/or polymyxins for treating XDR *P. aeruginosa* infections has already been assessed in previous studies. However, most of these included different sources of infection, with few UTI episodes, or had no control group, which makes interpretation difficult. Pogue et al. [26] compared ceftolozane-tazobactam vs. polymyxin or aminoglycoside-based therapy for the treatment of drug-resistant *P. aeruginosa* infections in a multicenter retrospective study. A total of 200 patients were assessed, but only 27 of these had UTI. The authors reported statistical differences in clinical success rate (81% in the ceftolozane-tazobactam group vs. 61% in the comparative group), but not in mortality. Other authors have described their clinical experience of ceftolozane-tazobactam in the treatment of drug-resistant *P. aeruginosa* with large cohorts (more than 100 patients assessed) [33–35], with successful clinical outcome rates ranging from 63% to 83%. However, the limited number of UTIs included ($n < 30$) makes interpretation difficult.

In a systematic review of polymyxins in monotherapy or in combination for the treatment of carbapenem-resistant GNB, Zusman et al. [36] suggested a less than optimal outcome in patients who received colistin monotherapy, although most studies did not include *P. aeruginosa* infections, and UTI was not a frequent source of infection. Our group has previously assessed the performance of CMS in XDR *P. aeruginosa* infections [7, 37] and detected no differences between monotherapy and combination therapy or in clinical failure or mortality. One of those studies was specifically focused on UTIs [37]. In that prospective cohort of 33 patients, more than half of whom received CMS monotherapy, clinical cure was achieved in 89.5% of patients treated with CMS monotherapy.

Regarding the effectiveness of aminoglycosides, the evidence on monotherapy for treating UTIs caused by drug-resistant *P. aeruginosa* was extrapolated from carbapenem-resistant *Enterobacterales* [38–40], with response rates ranging from 61% to 100%. In a systematic review [11],

Vidal et al. demonstrated that aminoglycosides as single agents were as effective as beta-lactams or quinolones for achieving clinical improvement in patients with UTI, including those caused by *P. aeruginosa*. However, the impact of the new antipseudomonal agents was not assessed as a result of the date of publication.

Our data show that patients treated with other antibiotic regimens had more underlying comorbidities and severe disease compared to those in the amikacin or CMS group. It may be inferred that clinicians were reluctant to administer amikacin or CMS monotherapy in more complicated patients. To overcome this indication bias, a double propensity score analyses was performed and no differences between groups were found for the studied outcomes.

One of the main concerns in treatment with amikacin or CMS is nephrotoxicity. However, since many patients in the "other antibiotic regimens" group were also treated with combination therapies that included amikacin or CMS, this side effect was not properly assessed. In our study the rate of renal toxicity was in fact lower in the amikacin or CMS monotherapy group. There are several possible reasons for this, apart from the antibiotic treatment received: patients in the "other antibiotic regimens" group were more severely ill and some of the cases were probably sepsis-related; second, the kidney infection itself; third, the concomitant use of nephrotoxic drugs; and finally, a cautious attitude to using amikacin or CMS in patients with abnormal GFR baselines.

Another worrisome antibiotic-related side effect is the incidence of *C. difficile* infection. Aminoglycosides and polymyxins are not among the "high-risk" drugs for the development of *C. difficile* infection [16], in accordance with our findings. Reducing the risk of *C. difficile* infection could be another reason for using them in the treatment of XDR *P. aeruginosa* infections.

Perhaps the greatest challenge associated with XDR *P. aeruginosa* is achieving the appropriate balance between efficacy, security, and ecology. Strategies aimed at safeguarding broad-spectrum drugs should be approached with caution, particularly in less severe patients with a low-risk source of infection such us UTI, where the favorable pharmacokinetics characteristics of aminoglycosides and colistin could provide an excellent opportunity to use more ecological agents.

Our study has the inherent limitations of a retrospective design and a single-center study. As a result of imbalances in the baseline characteristics of the treatment groups, a double propensity-based approach was performed to reduce potential biases. Although the initial analysis included 101 patients, the matched cohort resulted in a smaller sample which reduces the statistical power of the study. It could have been of interest to study monotherapy with CMS or amikacin in more severe patients, but groups were too small for specific analyses to be performed. Another limitation is that many patients in the control group used aminoglycosides or colistin combined with other drugs. Although a sensitivity analysis was performed excluding those patients, as a result of the limited number of episodes treated with amikacin- or colistin-free antibiotic regimens ($n = 14$), results should be cautiously interpreted. In addition, not all patients had a urine control culture to assess microbiological clearance. Another potential limitation is that patient comorbidities were not determined using disease codes. Even though all clinical records were cautiously reviewed for two infectious diseases clinicians, there is a risk of misclassification or measurement error, particularly in a retrospective study. Finally, it would have been interesting to conduct genotypic studies. Prior studies have shown that the major XDR clone involved in our hospital is the less virulent ST-175 clone [4], which is widespread in our country and in Europe [1, 2]. Thus, our results might not be transferable to other settings with a different epidemiology. As strengths, a propensity score approach was used for controlling confounders at baseline. This is one of the recommended strategies to emulate the random assignment of clinical trials [41]. Finally, it explores more ecological agents in a difficult-to-treat bacteria, such as XDR *P. aeruginosa*, in a "real life" situation.

CONCLUSIONS

Our findings might reinforce that amikacin or CMS monotherapy does not have a detrimental impact on outcomes of complicated UTIs caused by XDR *P. aeruginosa* when compared with combination or other antibiotic therapies. These results may be useful for antibiotic stewardship activities given their clinical and ecological impact. However, further studies are needed to confirm these findings, particularly in more severely ill patients.

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Author Contributions. ILM and SG-Z designed the study, collected the data, and wrote the initial manuscript. DEE, SG, NP, and EP performed microbiological and PK/PD tests. ILM, SG-Z, ZRPB, and XDJ performed the statistical analysis. ZRPB, DEE, MPG, ES, LS, MM, RG, SG, and JPH reviewed and edited the final manuscript.

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Compliance with Ethics Guidelines. The study was approved by the Clinical Research Ethics Committee of the Parc de Salut Mar (register no. 2020/9321). The need for written informed consent was waived because of the observational nature of the study and retrospective analysis. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guideline and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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4.3. Article 3

Suboptimal Concentrations of Ceftazidime/Avibactam (CAZ-AVI) May Select for CAZ-AVI Resistance in Extensively Drug-Resistant *Pseudomonas aeruginosa*: In Vivo and In Vitro Evidence.

Lopez-Montesinos I, Montero M.M., Domene-Ochoa S., López-Causapé C., Echeverria D, Sorlí L., Campillo N, Luque S, Padilla E, Prim N, Grau S, Oliver A and Horcajada JP.

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Suboptimal Concentrations of Ceftazidime/Avibactam (CAZ-AVI) May Select for CAZ-AVI Resistance in Extensively Drug-Resistant *Pseudomonas aeruginosa*: In Vivo and In Vitro Evidence

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Abstract: This study correlates in vivo findings in a patient with an extensively drug-resistant (XDR) *P. aeruginosa* infection who developed resistance to ceftazidime-avibactam (CAZ-AVI) with in vitro results of a 7-day hollow-fiber infection model (HFIM) testing the same bacterial strain. The patient was critically ill with ventilator-associated pneumonia caused by XDR *P. aeruginosa* ST175 with CAZ-AVI MIC of 6 mg/L and was treated with CAZ-AVI in continuous infusion at doses adjusted for renal function. Plasma concentrations of CAZ-AVI were analyzed on days 3, 7, and 10. In the HIFM, the efficacy of different steady-state concentrations (Css) of CAZ-AVI (12, 18, 30 and 48 mg/L) was evaluated. In both models, a correlation was observed between the decreasing plasma levels of CAZ-AVI and the emergence of resistance. In the HIFM, a Css of 30 and 48 mg/L (corresponding to 5× and 8× MIC) had a bactericidal effect without selecting resistant mutants, whereas a Css of 12 and 18 mg/L (corresponding to 2× and 3× MIC) failed to prevent the emergence of resistance. CAZ/AVI resistance development was caused by the selection of a single ampC mutation in both patient and HFIM. Until further data are available, strategies to achieve plasma CAZ-AVI levels at least 4× MIC could be of interest, particularly in severe and high-inoculum infections caused by XDR *P. aeruginosa* with high CAZ-AVI MICs.

Keywords: ceftazidime/avibactam; *Pseudomonas aeruginosa*; multidrug-resistant; continuous infusion; PK/PD; hollow fiber

1. Introduction

The emergence and spread of extensively drug-resistant (XDR) *Pseudomonas aeruginosa* has become a matter of public health concern. The increase in XDR strains seriously compromises antibiotic treatment options [1]. Receiving ineffective antibiotic therapy is associated with worse outcomes and higher mortality rates among patients with *P. aeruginosa* infections [2,3].

In recent years, the availability of new drugs such as ceftazidime-avibactam (CAZ-AVI) has increased the therapeutic arsenal against these microorganisms [4]. However, the emergence of resistant mutants has already been reported [5] and strategies to monitor and prevent the selection of resistance during antibiotic treatment are urgently needed.

CAZ-AVI (Zavicefta®) [6] is a ceftazidime/β-lactamase inhibitor combination with activity against extended-spectrum beta-lactamase (ESBL) producers and carbapenem-resistant Enterobacterales (CRE), but is not active against metallo-β-lactamase (MBL) producers. The addition of avibactam to ceftazidime protects the cephalosporin from enzymatic degradation caused by *P. aeruginosa* strains (mainly due to Amp-C enzymes but also ESBLs and class A carbapenemases) and leads to decreased minimum inhibitory concentrations (MICs) of ceftazidime [7]. It has been approved by the U.S. Food and Drug Administration and the European Medicines Agency for the treatment of infections caused by *P. aeruginosa* strains from different sources (i.e., hospital-acquired bacterial pneumonia, complicated intra-abdominal infections, and urinary tract infections).

The current recommended dosage of CAZ-AVI for adult patients with normal renal function is a 2 h infusion of 2 g/0.5 g every 8 h. As a time-dependent antibiotic, the best pharmacokinetic parameter to achieve maximum bacterial killing is the percentage of free drug concentration that remains above the MIC (%fT > MIC) for 40–70% [8] of the dosing interval, and is maximized when concentrations in plasma are 4–5× MIC.

The recommended standard dosing regimen may be insufficient to treat infections caused by isolates with CAZ-AVI MIC values close to susceptibility breakpoints of 8 mg/L, due to the increased likelihood of not achieving effective concentrations [9]. In line with this, it has been suggested that the therapeutic target could be increased to 100% fT > 4–8 times MIC of the free drug [10,11] in complicated scenarios, such as critically ill patients or high inoculum infections such as pneumonia.

The use of prolonged infusion could offer advantages for attaining pharmacokinetic/pharmacodynamic (PK/PD) targets and optimizing antibiotic treatment [12]. In a randomized clinical trial including 60 patients with severe sepsis treated with b-lactam therapy, 82% and 29% of those receiving continuous infusion (CI) and intermittent dosing, respectively, achieved 100% $fT \geq$ MIC against target pathogens. In addition, the clinical cure rate was higher in the CI group than in the intermittent administration group (70% versus 43%; $p = 0.037$). In the specific case of CAZ-AVI, Goncette et al. [10] assessed its performance when administered by CI in a case series of 10 patients. The median daily dose of CAZ-AVI used was 10 g (interquartile range 5–10 g) with median reported steady-state concentrations (Css) level of 63.6 mg/L (interquartile range 47.6–80 mg/L). In this setting, CAZ-AVI in CI achieved clinical cure and microbiological eradication rates of 80% and 90%, respectively.

This study is based on an actual clinical experience in which a critically ill patient with pneumonia caused by an XDR *P. aeruginosa* strain with ceftazidime-avibactam MIC of 6 mg/L developed resistance to this agent. The patient was included in the PseudoNOVA observational study (see Methods section).

We hypothesized that the administration of higher doses of CAZ-AVI in CI could help optimize the PK/PD target for CAZ-AVI and prevent the emergence of resistant mutants in isolates with borderline MICs. To test this, we used an in vitro hollow-fiber infection model (HFIM) to evaluate the efficacy of three dosing regimens of CAZ-AVI given by CI against an XDR *P. aeruginosa* strain with MIC of 6 mg/L isolated from the mentioned patient and correlated these findings with the in vivo results. We also characterized emerging resistance mechanisms by whole genome sequencing (WGS).

2. Results

2.1. Clinical Study

We present the 2018 case of a 55-year-old female patient with a prior history of obesity (weight 90 kg, height 160 cm: body mass index 35 kg/m^2), type 2 diabetes mellitus and chronic kidney disease in need of a kidney transplant, who was receiving long-term

immunosuppressive treatment with everolimus 1 mg every 24 h, mycophenolate 360 mg every 8 h, and prednisone 5 mg every 24 h. She was included in the PseudoNOVA study due to ventilator-associated pneumonia caused by an XDR *P. aeruginosa* strain recovered from a bronchial aspirate (BAS) sample, which was susceptible to CAZ-AVI with MIC of 6 mg/L. A 15-day course of directed therapy with CAZ-AVI was prescribed, consisting of a loading dose of 2 g/0.5 g followed by 3 g/0.75 g given in CI every 24 h, adjusted for renal function (median Glomerular filtration rate (GFR), 51.5 mL/min). Plasma CAZ-AVI levels on days 3 and 7 were 81.4 mg/L and 77.4 mg/L, respectively. A control BAS confirmed no growth of *P. aeruginosa*. When the patient was assessed at the end of the treatment window, clinical cure and microbiological eradication were considered to have been achieved and she was extubated.

However, 20 days after the index episode, the patient developed acute tracheobronchitis. A new BAS was performed and XDR *P. aeruginosa* was again isolated. The CAZ-AVI MIC was 6 mg/L. In addition, CAZ-AVI-resistant subpopulations with MIC of 32 mg/L were detected in the same culture. CAZ-AVI was reinitiated at the same dosage, achieving plasma levels of CAZ-AVI of 54 mg/L, 58.1 mg/L and 27 mg/L on days 3, 7 and 10, respectively (median GFR of 91.5 mL/min). The patient showed a good clinical response and CAZ-AVI was stopped after 10 days of antibiotic treatment. Two further follow-up BAS were performed 1 day and 10 days after the end of antibiotic treatment. CAZ-AVI-resistant subpopulations of XDR *P. aeruginosa* were documented in both samples, with CAZ-AVI MICs of 24 to 48 mg/L, and 32 to 256 mg/L, respectively. They were interpreted as colonization. The patient was assessed as clinically cured, but with microbiological failure at the follow-up endpoint.

In both episodes (ventilator-associated pneumonia and acute tracheobronchitis), the patient received adjuvant treatment with nebulized colistimethate sodium (CMS) at doses of 2 million international units every 8 h.

2.2. HFIM

The initial *P. aeruginosa* strain was exposed to different concentrations of CAZ-AVI administered as CI in a 7-day in vitro HFIM. Total colony-forming unit (CFU)/mL reductions observed for the different regimens of CAZ-AVI during the HFIM are shown in Figure 1. The administration of CAZ-AVI in CI was bactericidal at concentrations of 5× and 8× MIC, corresponding to Css of 30 mg/L and 48 mg/L, but not at lower concentrations (Css of 12 mg/L and 18 mg/L). The mean bacterial density of the starting inoculum was 7.08 $\log_{10}$ CFU/mL. The behavior of CAZ-AVI in CI at Css of 12 mg/L and 18 mg/L was similar to the control regimen without antibiotic, with final bacterial densities of 8.65 and 8.18 $\log_{10}$ CFU/mL, respectively. CAZ-AVI in CI at higher concentrations (Css of 30 mg/L and 48 mg/L) achieved a continuous bacterial reduction of 4.16 and 4.48 $\log_{10}$ CFU/mL with bacterial densities of 2.92 and 2.60 $\log_{10}$ CFU/mL, respectively, at the end of the experiment.

2.3. Resistance Studies

In the 7-day HFIM study, CAZ-AVI-resistant mutants were not selected when CAZ-AVI was administered in CI at Css of 30 mg/L and 48 mg/L. Nevertheless, CAZ-AVI-resistant subpopulations emerged at lower concentrations of CAZ-AVI, corresponding to Css of 12 mg/L and 18 mg/L, administered in CI (Figure 2 and Supplementary Table S1).

2.4. In Vitro Susceptibility and Resistance Mechanisms

MLST analysis from WGS revealed that the XDR clinical isolates studied belonged to the widespread ST175 high-risk clone. The initial isolate was susceptible to ceftazidime-avibactam (MIC 6 mg/L) and resistant to the classical β-lactams (piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem and meropenem) due to an inactivating mutation in OprD (Q142X) and AmpC hyperproduction (G154R mutation in AmpR), as described previously for ST175 isolates. The subsequent CAZ-AVI-resistant clinical isolates

differed from the parent strain by a single SNP that resulted in the previously described Q146K mutation in AmpC [13]. The CAZ-AVI-resistant mutants obtained from the HFIM similarly differed from the parent strain by a single mutation in *ampC*, in this case a 21 bp deletion leading to the deletion of 7 amino acids (positions 236 to 242) in the Ω-loop (Table 1).

2.5. Drug Concentrations

The relation between observed and predicted CAZ-AVI concentrations over 7 days is shown in Supplementary Material. Agreement between observed and predicted results was evaluated with a Bland–Altman plot. For all Css of 12 and 18 mg/L, difference values lay within 1.96 standard deviations (SD) of the mean. For Css of 30 and 48 mg/L, on the other hand, two and three of the values deviated slightly from ±1.96 SD in 18 and 30 Css, respectively.

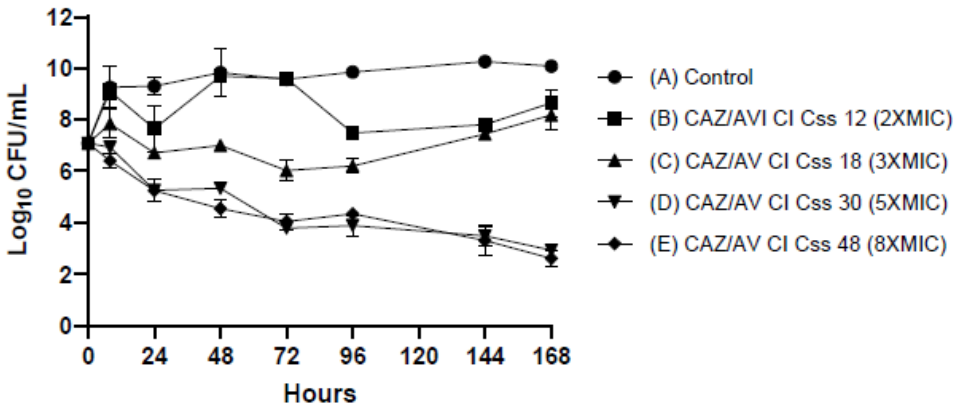


Figure 1. Mean reduction in bacterial density during 7-day HFIM assays (in duplicate) with the XDR *P. aeruginosa* index isolate treated with different Css of CAZ-AVI (12, 18, 30 and 48 mg/L) in CI. CFU, colony-forming unit; CI, continuous infusion; Css, steady-state concentration; MIC, minimum inhibitory concentrations. Errors bars are expressed as standard deviations (SD).

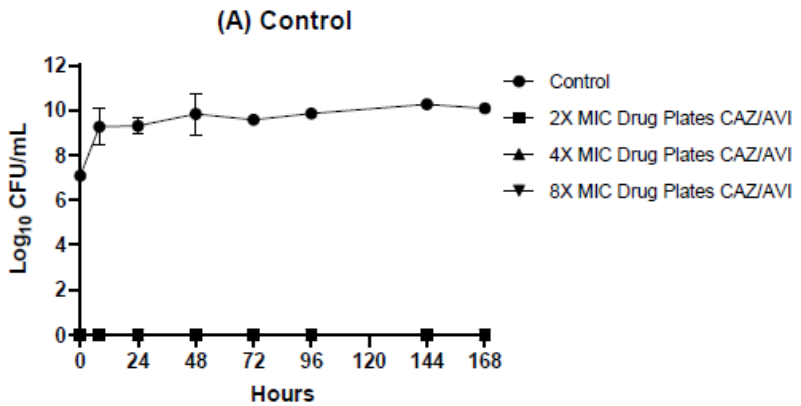
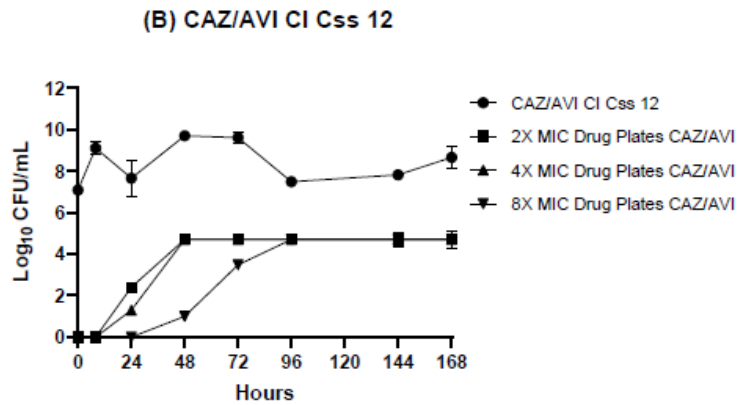
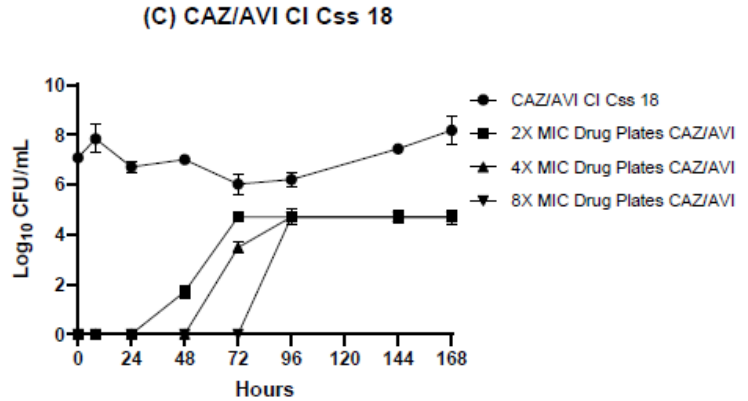


Figure 2. *Cont.*





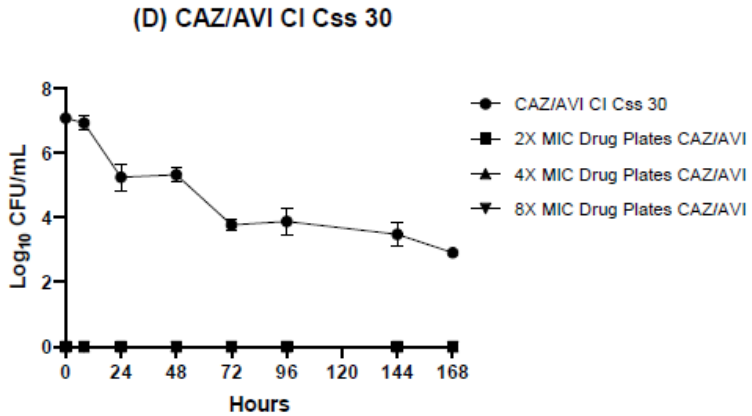


Figure 2. *Cont.*

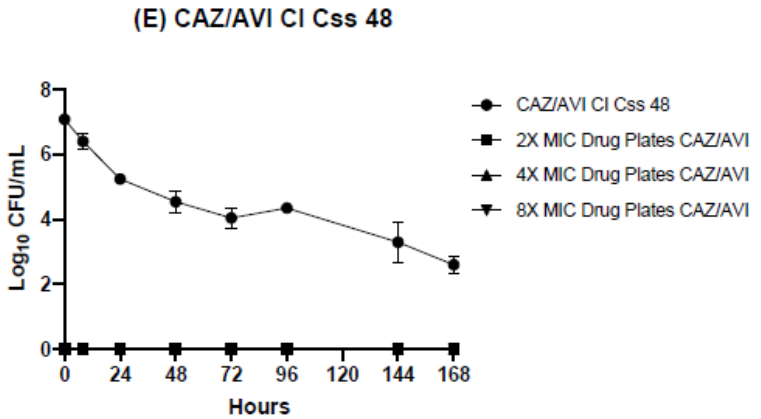


Figure 2. (**A**–**E**) Emergence of CAZ-AVI-resistance in the XDR *P. aeruginosa* isolate using Css of 12 (2× MIC), 18 (3× MIC), 30 (5× MIC) and 48 (8× MIC) mg/L in CI (performed in duplicate). CFU, colony-forming unit; CAZ/AVI, ceftazidime/avibactam; CI, continuous infusion; Css, steady-state concentrations; MIC, minimum inhibitory concentration. Errors bars are expressed as standard deviations (SD).

Table 1. Whole genome sequence resistome analysis of the studied *P. aeruginosa* clinical isolates and derived resistant mutants. PA, *P. aeruginosa*; CAZ/AVI, ceftazidime/avibactam; MIC, minimum inhibitory concentration; HFIM, hollow-fiber infection model.

PA Isolate	CAZ/AVI MIC	Resistome Summary
Episode 1 (Index isolate)	6 mg/L	*aadB*, *oprD* (Q142X), *mexZ* (G195D), *gyrA* (T83I, D87N), *ampR* (G154R), *parC* (L168Q, S87W), *armZ* (V266M)
Episode 2 (day 35)	6 mg/L	*aadB*, *oprD* (Q142X), *mexZ* (G195D), *gyrA* (T83I, D87N), *ampR* (G154R), *parC* (L168Q, S87W), *armZ* (V266M)
Follow up (day 46)	24 mg/L	*aadB*, *oprD* (Q142X), *mexZ* (G195D), *gyrA* (T83I, D87N), *ampR* (G154R), *parC* (L168Q, S87W), *armZ* (V266M), *ampC* (Q146K)
Follow up (day 55)	32 mg/L	*aadB*, *oprD* (Q142X), *mexZ* (G195D), *gyrA* (T83I, D87N), *ampR* (G154R), *parC* (L168Q, S87W), *armZ* (V266M), *ampC* (Q146K)
HFIM in vitro resistant mutants	>32 mg/L	*aadB*, *oprD* (Q142X), *mexZ* (G195D), *gyrA* (T83I, D87N), *ampR* (G154R), *parC* (L168Q, S87W), *armZ* (V266M), *ampC* (Δ236-242)

3. Discussion

In this translational study, the clinical experience of an actual patient was correlated with in vitro HFIM findings. We evaluated a critically ill patient with relevant comorbidities and ventilator-associated pneumonia and tracheobronchitis due to XDR *P. aeruginosa* ST175 with a borderline CAZ-AVI MIC at baseline, who developed resistance to CAZ-AVI during treatment. Using HFIM, we compared three concentrations of CAZ-AVI in CI against the index isolate to identify the most efficient way to administer the antibiotic and prevent the emergence of resistance in future cases.

In the clinical setting, we observed a correlation between decreasing levels of CAZ-AVI in plasma and the emergence of resistance to this drug. In the ventilator-associated pneumonia episode, CAZ-AVI plasma concentrations of T > 4–8 times the MIC (plasma levels of 81.4 mg/L and 77.4 mg/L on days 3 and 7) were achieved. The patient was cured, and a follow-up respiratory sample confirmed eradication of *P. aeruginosa*. With respect to the tracheobronchitis episode, selection of resistant mutants became apparent mainly as a

result of the sharp drop in CAZ-AVI in plasma during the study (plasma levels of 54 mg/L, 58.1 mg/L and 27 mg/L on days 3,7 and 10, respectively).

Several factors may have led to the reduction in CAZ-AVI concentrations. First, CAZ-AVI is mainly eliminated by the renal route [6]. In the present clinical case, an improvement in renal clearance was observed (median GFR rose from 51 mL/min to 91.5 mL/min in the pneumonia and tracheobronchitis episodes, respectively). In the second episode, however, CAZ-AVI doses were not adjusted for renal function. This most likely favored higher CAZ-AVI elimination rates and consequently lower plasma levels. It should be noted that increased renal clearance is frequently seen in critically ill patients with normal serum creatinine concentrations [14]. Furthermore, the PK of hydrophilic antimicrobials such as beta-lactams is affected by the presence of sepsis, leading to a potential increase in the volume of distribution [14]. Finally, obese patients may also have an increased volume of distribution and higher renal clearance, resulting in antibiotic exposure that is difficult to predict [11].

Of note, CAZ-AVI-resistant subpopulations with MIC of 32 mg/L were observed in the first respiratory sample from the tracheobronchitis episode, despite the fact that no microorganism growth had been documented in an earlier sample. Selection of CAZ-AVI-resistant subpopulations could be the consequence of lower CAZ-AVI concentrations at the site of infection. It has previously been reported that the ratio of epithelial lining fluid exposure to concentrations of ceftazidime and avibactam is about 30% of plasma exposure [15]. To prevent subtherapeutic antibiotic concentrations, previous authors [10] have suggested CAZ-AVI concentrations of ≥4–5 times the MIC at the site of the infection as the PK/PD target, rather than in plasma. This could be an interesting strategy, especially in complicated circumstances such as critically ill patients, isolates with elevated MICs and/or deep-seated infections. However, antibiotic-related side effects should be carefully monitored.

In the present clinical case, the patient had a favorable outcome in terms of clinical cure despite developing resistance to CAZ-AVI. This may be due in part to the fact that CAZ-AVI resistance was documented during the tracheobronchitis episode, which is considered a low-risk source of infection [2]. Indeed, the need for systemic antibiotics in this type of infection is controversial [16].

On the other hand, the emergence of CAZ-AVI resistance may further complicate antimicrobial therapy. In addition, borderline MICs may lead to the appearance of low-level resistance mechanisms that can ultimately compromise clinical outcome. In the case of ceftolozane-tazobactam, it was reported that higher MICs (>2 mg/L) predict 30-day mortality in patients with lower respiratory tract infections caused by MDR or XDR *P. aeruginosa* [17].

Although there are no clinical data to support a different therapeutic management that takes MIC values into account, it is obvious that a higher MIC will reduce the likelihood of achieving any PK/PD target, including $fT > MIC$. In this scenario, strategies such as the use of higher doses of CAZ-AVI, extended therapy, CI and/or combination therapy may be considered. In the latter circumstance, our group [18] demonstrated in vitro that combinations of CAZ-AVI plus colistin, amikacin or aztreonam were additive or synergistic in at least 85% of the XDR *P. aeruginosa* isolates studied, including CAZ-AVI-resistant *P. aeruginosa*. Clinical experience has also shown that administration of CAZ-AVI by prolonged infusion (≥3 h) reduces mortality by 46% [19].

In the HFIM, our in vitro findings showed similar trends to the in vivo results. A Css of 30 and 48 mg/L (corresponding to 5× and 8× MIC) had a bactericidal effect without selecting for resistant mutants, whereas a Css of 12 and 18 mg/L (corresponding to 2× and 3× MIC) clearly failed to prevent the emergence of resistance. WGS revealed that the development of CAZ-AVI resistance in XDR *P. aeruginosa* ST175 was caused by selection of a single *ampC* mutation, both in the patient and in the HFIM. Our results agree therefore with previous findings for CAZ-AVI and ceftolozane-tazobactam that point to AmpC mutations as the major resistance mechanism [20,21]. It should be noted that resistance emerged in our case, as in previous ones, in XDR strains that were already ceftazidime-resistant due to

a mutation leading to AmpC overexpression, which can be considered a prerequisite for subsequent CAZ-AVI and ceftolozane-tazobactam resistance development due to selection of AmpC structural mutations [22]. These results highlight the importance of optimizing antibiotic treatment, particularly in a rapidly adapting microorganism such as *P. aeruginosa*.

The main limitation of the study is that only a single isolate was studied, although it belongs to an XDR *P. aeruginosa* clone that is widespread in Spanish hospitals [15]. More in vivo and in vitro examples should be analyzed before drawing generalizable conclusions. Second, we evaluated plasma concentrations of ceftazidime but not avibactam. Considering the potential nonlinear synergetic effect between CAZ and AVI, using CAZ plasma concentration alone could be controversial. Conversely, CAZ and AVI displayed similar PK/PD profile in terms of lung penetration, volume of distribution, time-dependent activity, low plasma protein binding and renal clearance in previous reports [6,15,23]. It would also have been interesting to measure CAZ and AVI concentrations in epithelial lining fluid to corroborate hypothetical subtherapeutic concentrations at the site of infection. Another limitation is potential variation due to the method of MIC determination [24]. These variations must be taken into account to prevent potential under- or overdosing of patients. Finally, due to the observational nature of the study, the CAZ-AVI dose was decided by the physicians in charge and was not therefore accurately adjusted for renal clearance in accordance with current recommendations. Nevertheless, this reflects daily clinical practice and enabled us to test our hypothesis in vivo. As a strength, this is one of the few reports in the literature to correlate clinical studies with HFIM findings and represents an example of translational research to clinics.

4. Materials and Methods

4.1. The PseudoNOVA Project

The PseudoNOVA project is a Spanish prospective, multicenter, observational cohort study, conducted between 2018 and 2022, of the clinical and microbiological impact of the new antipseudomonal agents ceftolozane-tazobactam and CAZ-AVI on infections caused by high-risk clones of XDR *P. aeruginosa* in Spain. Correlations with in vitro results were studied using a hollow-fiber dynamic PK/PD model. Patients admitted to participating hospitals during the study period with invasive infections caused by XDR *P. aeruginosa* and treated with ceftolozane-tazobactam or CAZ-AVI were evaluated in terms of mortality, clinical cure, microbiological eradication, and selection of resistant mutants. Antibiotic regimen and dose selection were decided by the physician in charge without interference from the team of investigators. GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). In this study, plasma levels of ceftolozane-tazobactam or CAZ-AVI were performed blinded on days 3, 7, 14 and 21 of treatment (as appropriate). Strains that developed resistance during antibiotic treatment were selected for study in a HFIM with a view to designing the most efficient strategies of antibiotic administration and to prevent the development of resistant mutants.

4.2. Bacterial Isolates, Microbiological Studies, and Resistance Mechanisms

Local microbiology laboratories used standard microbiological techniques for the isolation, identification, and susceptibility testing of bacteria. *P. aeruginosa* isolates were considered XDR according to *Magiorakos* et al. [25]. CAZ-AVI MICs were determined by E-test and interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [26]. Strains of *P. aeruginosa* were frozen at −80 °C for subsequent study in the HFIM.

4.3. Antibiotics

CAZ-AVI (Zavicefta®) was provided by Pfizer (Ringaskiddy, County Cork, Ireland). CI dosing regimens of CAZ-AVI were simulated to achieve Css of 12, 18, 30 and 48 mg/L. The different Css were chosen to analyze concentrations above and below the therapeutic objective of 100% $fT \geq$ 4–5 times the MIC in plasma (2×, 3×, 5×, 8× MIC of the isolate).

4.4. HFIM

A 7-day, experimental HFIM was carried out in duplicate, as described previously [27]. The efficacy of different CI, Css of CAZ-AVI (12, 18, 30 and 48 mg/L) was evaluated. Polyethersulfone hemofilters were used as hollow-fiber cartridges with a volume of 50 mL (Aquamax HF03, Nikkiso, Belgium) [28]. Experiments were conducted in a humidified incubator set at 37 °C. Antibiotics were pumped directly into the central reservoir with a separate infusion pump to achieve the target Css. Treatment regimens were compared with a no-treatment control. Fresh drug-free growth medium (cation-adjusted Mueller–Hinton broth [CA-MHB]) was continuously infused into the central reservoir to dilute and simulate human drug clearance. An equal volume of drug-containing medium was removed from the central reservoir concurrently to maintain an isovolumetric system. Bacterial suspensions were inoculated into the extracapillary compartment of the hollow-fiber cartridge, where they were exposed to fluctuating drug concentrations. Bacterial samples were obtained from the cartridges at 0, 8, 24, 48, 72, 96, 144 and 168 h, then washed and suspended in solution in 1 mL Eppendorf tubes to minimize the carryover effect of the drug. Decimal serial dilutions were quantitatively cultured onto drug-free trypticase soy agar (BBL TSA II, Becton Dickinson) plates to determine bacterial densities ($\log_{10}$ CFU/mL). The lower limit of detection (LLOD) was 1.3 $\log_{10}$ CFU/mL. Bactericidal activity was defined as a reduction of 3 log10 CFU/mL from the initial bacterial density [29–31].

4.5. Antimicrobial Resistance Studies

A portion of the bacterial suspension was quantitatively cultured onto agar supplemented with CAZ-AVI at two, four, and eight times the reference MIC to evaluate amplification of resistant subpopulations. When growth was observed after 72 h, up to three colonies were selected to confirm reduced susceptibility to CAZ-AVI and be analyzed for changes in MIC values from baseline. Antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for broth microdilution using CA-MHB [32].

4.6. Characterization of Resistance Mechanisms by WGS

CAZ-AVI-susceptible and CAZ-AVI-resistant XDR *P. aeruginosa* isolates obtained from the patient, as well as CAZ-AVI-resistant isolates obtained from the HFIM were characterized by WGS, following previously established protocols [33]. Genomic DNA was obtained by using a commercially available extraction kit (High Pure PCR template preparation kit; Roche Diagnostics). Obtained paired-end reads were mapped to the *P. aeruginosa* PAO1 reference genome (GenBank accession: NC_002516.2) with Bowtie 2 v2.2.4 and pileup and raw files were obtained by using SAMtools v0.1.16 and PicardTools v1.140, using the Genome Analysis Toolkit (GATK) v3.4.46 for realignment around InDels. From the obtained raw files, SNPs were extracted if they met the following criteria: a quality score (Phred-scaled probability of the samples reads being homozygous reference) of at least 50, a root-mean-square (RMS) mapping quality of at least 25 and a coverage depth of at least 3 reads; excluding all ambiguous variants. As well, MicroInDels were extracted from the total pileup files when meeting the following criteria: a quality score of at least 500, an RMS mapping quality of at least 25 and support from at least one-fifth of the covering reads. After conversion of these filtered files to vcf, SNPs and InDels were annotated with SnpEff v4.2. Additionally, paired-end reads were de novo assembled using SPAdes v3.13.1 [34] for study the structural integrity of porin OprD, to determine the presence of horizontally acquired antimicrobial resistance determinants and to determine the Sequence Type [35]. Finally, sequence variants located within a set of 40 chromosomal genes (*gyrB, mexR, mexA, mexB, oprM, ampDh3, parS, parR, mexY, mexX, mexZ, galU, mexS, mexT, mexE, mexF, oprN, dacB, gyrA, nalD, nalC, dacC, pbpA, mpl, ampR, ampC, fusA1, ftsI, ampD, oprJ, mexD, mexC, nfxB, pmrA, pmrB, parC, parE, armZ, ampDh2*) were extracted and natural polymorphisms were filtered [33].

4.7. Data Availability

Genomic sequences have been deposited in the European Nucleotide Archive, under project number PRJEB55650.

4.8. Drug Concentrations in the HFIM

During the first 48 h (0, 3, 5, 7, 9, 23, 25 27, 29 and 47 h) of the study and once a day until the end of the study, antibiotic samples were collected from the peripheral compartment of the HFIM and immediately stored at −80 °C until analysis. Samples were taken for Css reporting and analyzed by HPLC [36]. The McWhinney BC et al. technique for beta-lactams was used [37]. The concentration–time profiles of the antibiotics were validated by means of a linear model using ADAPT II [38].

5. Conclusions

This case reflects "the perfect storm" leading to failure of antimicrobial drug therapy. Until further data are available, it seems reasonable to use more precise or even higher dosing regimens when using CAZ-AVI to treat severe and high-inoculum infections caused by XDR *P. aeruginosa* isolates with CAZ-AVI MICs close to the susceptibility breakpoint. Strategies aimed at achieving plasma levels at least 4 × MIC could be of interest to avoid subtherapeutic antibiotic exposure at the site of infection and prevent the emergence of resistant mutants, at least in this sub-group of patients. Clinical and microbiological studies are needed to assess the feasibility, effectiveness, and safety of this challenging question.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antibiotics11111456/s1, Figure S1: Bland-Altman plot of observed and predicted ceftazidime concentrations for the four regimens in the overall experiments: Css of 12 mg/L, Css of 18 mg/L, Css of 30 mg/L and Css of 48mg/L. Table S1: Mean bacterial density (log_{10} CFU/mL) during 7-day HFIM assays and the emergence of CAZ-AVI resistant subpopulation onto agar supplemented with CAZ-AVI at 2×, 4× and 8× baseline MIC.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the Hospital del Mar (protocol code 2017/7474/I and date of approval 27 July 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reason.

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Abbreviations

HIFM	Hollow-Fiber Infection Model
CAZ-AVI	Ceftazidime-avibactam
CI	Continuous infusion
Css	steady-state concentrations
XDR PA	extensively drug-resistant *Pseudomonas aeruginosa*
MIC	minimum inhibitory concentration
ICU	Intensive Care Unit
WGS	Whole genome sequencing
GFR	Glomerular filtration rate
CFU	Colony-forming unit

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5

Summary of Results

5. SUMMARY OF RESULTS

To study the influence of XDR phenotype on outcomes, we assessed a retrospective cohort of patients with *P. aeruginosa* bacteremia. 382 patients were included, 122 (31.9%) due to XDR *P. aeruginosa*. Independent factors associated with 14-day mortality were: high-risk source of bacteremia (Hazard ratio (HR) 3.07, 95% confidence interval (CI), 1.73-5.46), septic shock (HR 1.75, 95%CI, 1.12-2.75) and higher Pitt score (one-point increment; HR 1.25, 95%CI, 1.12-1.38). Otherwise, the appropriateness of definitive antibiotic therapy was a protective factor (HR 0.39, 95%CI, 0.24-0.62). The same variables were also associated with 30-day mortality. However, XDR phenotype was not associated with 14- or 30-day mortality. Therefore, we concluded that XDR phenotype was not associated with poor prognosis in patients with *P. aeruginosa* bacteremia in our cohort.

In the previous study we also showed that low-risk sources of infection, defined as those involving either catheter-related bloodstream infections or urinary tract infections were associated independently with lower mortality rates. Therefore, to assess the role of the source of infection in the choice of the antibiotic treatment, we performed another retrospective study including only patients diagnosed with complicated UTI caused by XDR *P. aeruginosa*. 101 patients were included, 48% were treated with aminoglycoside or colistin monotherapy. In multivariate models adjusted by propensity score, aminoglycoside or colistin monotherapy was not associated with worse outcomes. After propensity score matching, 28 episodes in each treatment group were matched. Although the sample size was small, aminoglycoside or colistin monotherapy was not associated with worse outcomes: adjusted Odd Ratio (OR) (95% CI) for early clinical failure and at EOT

with aminoglycosides or polymyxin monotherapy were 0.53 (0.18-1.58) and 1.29 (0.34-4.83), respectively; and adjusted HRs (95% CI) for 30-day and 90-day mortality were 0.93 (0.17-5.08) and 0.68 (0.20-2.31), respectively. No statistically significant differences were found in terms of nephrotoxicity. However, *Clostridioides difficile* infection was observed only in the 'other antibiotic regimens' group (n=6, 11.3%). We concluded that aminoglycosides or polymyxin monotherapy showed good efficacy and safety profile in treating complicated UTI caused by XDR *P. aeruginosa*. Thus, strategies aimed at safeguarding broad-spectrum drugs should be approached, particularly in less severe patients with a low-risk source of infection such us UTI, where the favorable pharmacokinetics characteristics of aminoglycosides and colistin could provide an excellent opportunity to use more ecological agents.

Finally, to assess other strategies aimed at preserving the new antipseudomonal agents ceftolozane-tazobactam and ceftazidime-avibactam, we used a HFIM to test an XDR *P. aeruginosa* which developed resistance *in vivo* and correlated the findings.

The patient was critically ill with ventilator-associated pneumonia caused by XDR *P. aeruginosa* ST175 with ceftazidime-avibactam MIC of 6 mg/L and was treated with ceftazidime-avibactam in continuous infusion. In both models, a correlation was observed between the decreasing plasma levels of ceftazidime-avibactam and the emergence of resistance. In the HIFM, a steady-state concentration (Css) of 30 and 48 mg/L (corresponding to 5× and 8× MIC) had a bactericidal effect without selecting resistant mutants, whereas a Css of 12 and 18 mg/L (corresponding to 2× and 3× MIC) failed to prevent the emergence of resistance. Ceftazidime-avibactam resistance development was

caused by the selection of a single ampC mutation in both patient and HFIM.

We concluded that to prevent from the emergence of resistance strategies aimed to achieve plasma ceftazidime-avibactam levels at least 4× MIC could be of interest, particularly in severe and high-risk infections such as pneumonia caused by XDR *P. aeruginosa* with high ceftazidime-avibactam MICs.

6

Summary of discussion

6. SUMMARY OF DISCUSSION

Although previous studies have assessed the impact on outcome of CR or MDR *P. aeruginosa* BSI, little is known about the role of XDR strains. To obtain a better understanding of this issue, in this thesis we assess the largest cohort of patients with monomicrobial XDR *P. aeruginosa* bacteremia published to date.

Our study identified severity at presentation, having a high-risk source of bacteremia, and inappropriate definitive antibiotic therapy as risk factors for mortality in patients with *P. aeruginosa* bacteremia. On the other hand, the XDR phenotype was not associated with poor prognosis. Decreased virulence in XDR strains, theoretical fitness costs, and a high prevalence of the less virulent ST175 high-risk clone at our institution may be among the reasons for these findings.

It is noteworthy than this study was carried out from January 2000 to December 2018, when most of new antipseudomonal agents were not available (only ceftolozane-tazobactam could be used from 2016 to 2018). In this scenario, the mortality rate for XDR *P. aeruginosa* bacteremia was high in our cohort (34% at day 30). When we broke down the data by source of infection, in high-risk sources it was 50% versus 18% in case of low-risk sources. Thus, it seemed clear that different treatment strategies should be addressed considering the source of infection.

Under this hypothesis, we developed our second study in which we evaluated the efficacy and safety of aminoglycosides or polymyxin

monotherapy in comparison to other antibiotic regimens in a low-risk source of infection such as UTIs due to XDR *P. aeruginosa.*

In this study, aminoglycoside or colistin monotherapy was not associated with worse outcomes neither in multivariate models adjusted by propensity score nor after propensity score matching. Although these results cannot be interpreted as that amikacin or colistin monotherapy is equally effective than as combination or other antibiotic therapies, they reinforce the message that alternative narrow-spectrum antibiotic use can be considered in some scenarios despite we are facing a difficult-to-treat bacteria. Once the new antipseudomonal agents have become commercially available, this fact is still more important in order to preserve them and avoid the emergence of resistant mutants.

Even though the clinical evidence of the ceftolozane-tazobactam and ceftazidime-avibactam over “the old drugs” is of low quality, currently, ceftolozane-tazobactam and ceftazidime-avibactam are considered firs-line agents to treat infections caused by XDR *P. aeruginosa* strains by the IDSA guidelines (a review about ceftolozane-tazobactam could be found in annexe I).

In line with this, the working hypothesis of the PseudoNOVA study was that use of ceftolozane-tazobactam and ceftazidime-avibactam to treat infections produced by high-risk clones of XDR *P. aeruginosa* would improve the prognosis of patients, with greater clinical efficacy and less toxicity than had so far been reported with “the old drugs”.

To the date, the clinical results of this study are in working process. However, in an exploratory study carried out in our institution prior to the PseudoNOVA study, we assessed the performance of ceftolozane-

tazobactam in the treatment of infections caused by XDR *P. aeruginosa* strains (see annexe 2). A total of 42 patients were assessed with different sources of infection, being UTI the most frequently observed (43%). The mortality rate at day 30 was lower (14.3%) than previously reported. However, these differences in mortality rates cannot be compared directly because the cohorts come from different time periods and have different clinical profiles.

Although it seems than the new antipseudomonal drugs have improved the prognosis of patients with XDR *P. aeruginosa*, or at least have increased the therapeutic arsenal, the emergence of resistant mutants has already been reported. In the PseudoNOVA study, 5/69 (7%) patients with XDR *P. aeruginosa* infections treated with ceftolozano-tazobactam or ceftazidime-avibactam developed resistance under the antibiotic treatment. The underlying resistance mechanisms were AmpC or OXAs mutations (unpublished data).

One of these patients was studied in a HFIM. The patient was critically ill with a high-risk source of infection such as VAP caused by XDR *P. aeruginosa* ST175 with a borderline ceftazidime-avibactam MIC at baseline. In both the *in vivo* and *in vitro* scenario, we observed a correlation between decreasing levels of ceftazidime-avibactam and the emergence of resistance to this drug.

The lower ceftazidime-avibactam concentrations at the site of infection in comparison to plasma could be among the reasons of selection ceftazidime-avibactam-resistant subpopulations. To prevent subtherapeutic antibiotic concentrations, previous authors (107) have suggested to get antibiotic concentrations of ≥ 4–5 times the MIC at the site of the infection as the PK/PD target, rather than in plasma. This could be an interesting strategy, especially in complicated

circumstances such as critically ill patients, isolates with elevated MICs and/or deep-seated infections. However, antibiotic-related side effects should be carefully monitored.

Perhaps the greatest challenge associated with XDR *P. aeruginosa* treatment is achieving the appropriate balance between efficacy, security, and ecology.

This research has many limitations. Regarding the two clinical studies, they had retrospective design and were single-center studies. Therefore, results were more prone to biases and not necessarily transferable to other settings with different epidemiology. In the study in which we assessed the performance of monotherapy with colistin and amikacin in complicated UTI, a double propensity-based approach was performed to reduce potential biases. Although the initial analysis included 101 patients, the matched cohort resulted in a smaller sample which reduces the statistical power of the study. Finally, although XDR *P. aeruginosa* isolates in our institution have been well characterized in previous studies (16–19), clonality and resistance mechanisms were not specifically investigated in none of them studies.

In the case of the HFIM study, only a single XDR isolate was studied. More *in vivo* and *in vitro* examples should be analyzed before drawing generalizable conclusions. Second, avibactam plasma concentrations were not assessed. Finally, variation due to the method of MIC determination (108) was another potential limitation. These variations must be considered to prevent potential under- or overdosing of patients.

As strengths, our research includes the largest published sample size of XDR *P. aeruginosa* bacteremia, eliminating the biases of many

studies with small sample sizes, which reduce the statistical power to be able to draw reliable conclusions. Furthermore, in one of our studies, we use of propensity score, one of the recommended strategies to emulate the random assignment of clinical trials, for controlling confounders. Finally, the HFIM study represents an example of translational research to clinics.

7

Conclusions

7. CONCLUSIONS

1. The XDR phenotype was not associated with poor prognosis in patients with *P. aeruginosa* bacteremia.
2. Severity at presentation, having a high-risk source of bacteremia, and inappropriate definitive antibiotic therapy were identified as risk factors for mortality in patients with XDR *P. aeruginosa* bacteremia.
3. In case of low-risk source of infection such us UTI caused by XDR *P. aeruginosa*, amikacin or CMS monotherapy do not have a detrimental impact on outcomes when compared with combination or other antibiotic therapies. This strategy may be useful for safeguarding the new antipseudomonal agents.
4. In case of high-risk source of infections such as pneumonia and/or infections with isolates with MICs close to the susceptibility breakpoint, administration strategies aimed at achieving plasma levels of antibiotic at least 4 x MIC could be of interest to avoid subtherapeutic antibiotic exposure at the site of infection and prevent the emergence of resistant mutants.

8

Future lines of Research

8. FUTURE LINES OF RESEARCH

As we have reviewed during this thesis, most treatment recommendations in the guidelines are based on very-low-certainty evidence or no evidence. Most studies are retrospective case series or observational cohorts with no comparator group or post-hoc analysis derived from RCTs with small sample sizes. In addition, outcomes are frequently defined in different ways, evaluated at different time points during the follow-up, and usually presented in aggregated form, which makes it difficult to compare results between publications. Furthermore, crucial variables about the use of antibiotic agents, such as mono- or combination therapy, dosing or how antibiotic treatment are administered (extended- or continuous-infusion or intermittent-bolus), are not routinely accounted for. Therefore, well-designed RTCs and specifically focus on MDR/XDR *P. aeruginosa* are urgently needed to overcome these biases and provide more robust evidence.

However, the development of RTCs is not easily feasible, mainly in XDR *P. aeruginosa* infections. In the meanwhile, observational studies should include larger patient populations and improve methods to reduce potential biases.

It is of special interest the role of combination therapy for serious or high-risk infections due to XDR *P. aeruginosa*, even when new antipseudomonal agents are susceptible.

More studies are needed to establish optimal dosing regimens (dosing, frequency or extended- or continuous-infusion or intermittent-bolus administration), and treatment durations especially when using β-

lactams agents. It is of special interest the impact of the administration of β-lactams by continuous or prolonged infusion, particularly in scenarios in which an aggressive PK/PD target is difficult to achieve, such as augmented renal clearance or deep-seated infection.

Finally, the use of HFIM to analyze the most effective doses and form of administration of new antipseudomonal agents, in monotherapy or in combination with other antibiotics, is another interesting line of research. The knowledge derived from it let us get a better understanding about how to use the new antipseudomonal drugs and prevent form the selection of resistant mutants.

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10. ANNEXES

10.1. Publication 1

Ceftolozane-tazobactam: When, how and why using it?. I López Montesinos, M Montero, L Sorlí, J P Horcajada. Rev Esp Quimioter. 2021 Sep;34 Suppl 1(Suppl1):35-37. doi: 10.37201/req/s01.10.2021. Epub 2021 Sep 30.

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Ceftolozane-tazobactam: When, how and why using it?

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ABSTRACT

Ceftolozane-tazobactam is currently the most active antipseudomonal agent, including multidrug-resistant extensively drug-resistant strains. Tazobactam provides additional activity against many extended-spectrum beta-lactamases *Enterobacterales*. Ceftolozane-tazobactam is formally approved for complicated urinary tract infection, complicated intra-abdominal infection, and hospital-acquired and ventilator-associated bacterial pneumonia. The clinical and microbiological success is over 70-80% in many series. However, resistant mutants to ceftolozane-tazobactam have been already described. Combination therapies with colistin or meropenem could be among the strategies to avoid the resistance emergence.

Key words: Ceftolozane-tazobactam, *Pseudomonas aeruginosa*, multidrug resistant, extensively drug resistant, extended spectrum β-lactamase.

INTRODUCTION

Ceftolozane-tazobactam (TOL-TAZ) combines a new antipseudomonal cephalosporin (ceftolozane) with enhanced antipseudomonal activity with a classic β-lactamase inhibitor (tazobactam). It exhibits bactericidal properties through inhibition of bacterial cell wall biosynthesis, which is mediated through penicillin-binding proteins (PBPs). Ceftolozane is a potent PBP3 inhibitor and has a higher affinity for PBP1b and PBP1c compared with other β-lactam agents. PBP1b and PBP1c are present in *Pseudomonas aeruginosa*. Moreover, ceftolozane has high stability against amp-C type betalactamases, which are frequently present in *P. aeruginosa*, and it is significantly less affected by the changes in the porin permeability or efflux pumps of the external membrane of gram negatives. Because of this ceftolozane has higher antipseudomonal activity than other antipseudomonals. Further, due to the combination with tazobactam, TOL-TAZ inhibits class A serine-betalactamases and extended-spectrum beta-lactamases (ESBL). TOL-TAZ also acts against non-ESBL class D oxacillinases, but it lacks activity against carbapenemases [1].

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SPECTRUM OF ACTIVITY

TOL-TAZ is an effective combination against several multidrug-resistant (MDR) Gram-negative bacilli, particularly MDR or extensively drug-resistant (XDR) *P. aeruginosa*. It is also active against AmpC and ESBLs producing *Enterobacterales*, but with a limited activity against ESBL-producing *Klebsiella pneumoniae*. Further, it remains activity against *Streptococcus* spp. (excluding *Enterococcus* spp.) and some anaerobes (*Bacteroides fragilis* and non-Bacteroides Gram-negatives) [2,3].

APPROVED INDICATIONS

TOL-TAZ was first approved for the treatment of adults with complicated intra-abdominal infection (cIAI) (in combination with metronidazole 500 mg every 8 hours) and complicated urinary tract infection (cUTI), including pyelonephritis. The dosage approved for these indications was 1.5 g 3 times a day. It was lately approved for adults with hospital-acquired and ventilator-associated bacterial pneumonia (HABP/VABP) at a dosage of 3 g every 8 h [2].

CLINICAL EXPERIENCE

The efficacy of TOL-TAZ in *P. aeruginosa* and ESBL *Enterobacterales* infections has been evaluated in several studies to the date (Table 1).

Regarding infections caused by *P. aeruginosa*, all these studies included patients treated with a dose of either 1.5 g every 8 h or 3 g every 8 h, with the high dose usually adminis-

Table 1 Clinical studies evaluating ceftolozane-tazobactam for *P. aeruginosa* and Enterobacterales infections. Adapted from [2]

Study reference	Design	No. and source of infection	Microorganism	Outcomes
Pseudomonas aeruginosa				
Miller 2016, Antimicrob Agents Chemother	Post hoc analysis of RCT: C-T vs. Meropenem	IAI (C/T: 26 vs. Meropenem 29)	MDR	Clinical cure: C-T 100% vs. meropenem 93.1%
Caston 2017, Antimicrob Agents Chemother	Case series with C-T	6 LRTI, 5 BSI, 3 IAI, 3 others	MDR	Mortality 25%, Clinical cure 75%, Microbiological cure 58.3%
Dinh 2017, Int J Antimicrob Agents	Case series with C-T	7 LRTI, 3 UTI, 2 IAI, 3 others	XDR	Mortality 27%, Clinical cure 67%, Microbiological cure 75%
Haidar 2017, Clin Infect Dis	Retrospective study	18 LRTI, 1 BSI, 1 ITU, 1 IAI	MDR/XDR	Mortality 10%, clinical cure 71,4%
Munita 2017, Clin Infect Dis	Retrospective study	18 LRTI, 6 BSI	CR	Mortality 22.3%, clinical cure 74%, Microbiological cure 100%
Díaz-Cañestro 2018, Clin Infect Dis	Prospective observational study	35 LRTI, 10 UTI, 4 IAI,3 BSI, 6 others	MDR/XDR	Mortality 27.6%, Clinical cure 63.8%, Microbiological cure 70%
Escola Verge 2018, Infection	Retrospective study	14 LRTI, 11 BSI, 6 UTI, 6 SSTI, 4 IAI, 8 others	XDR	Mortality 13.2%, Clinical cure 68.4%-86.6%, Microbiological cure 68.4%
Gallagher 2018, Open Forum Infect Dis	Retrospective study	121 LRTI, 28 UTI, 25 BSI, 20 IAI, 42 others	MDR	Mortality 19%, Clinical cure 73.7%, Microbiological cure 70.7%
Xipell 2018, J Glob Antimicrob Resist	Case series with C-T	8 LRTI, 7 UTI, 6 SSTI, 3 IAI	MDR/XDR/PDR	Mortality 22%, Clinical cure 88%, Microbiological cure 75%
Bassetti 2019, Int J Antimicrob Agents	Retrospective study	32 LRTI, 22 BSI, 21 SSTI, 14 UTI, 13 IAI, 6 others	Non-MDR/MDR/XDR/PDR	Mortality 5%, Clinical cure 83.2%
Pogue 2019, Clin Infect Dis	Retrospective study: C-T vs polymyxin or aminoglycoside	C-T: 64 LRTI, 16 UTI, 13 SSTI, 6 BSI, 7 others Comparator: 75 LRTI, 11 UTI, 6 SSTI, 6 BSI, 6 others	MDR/XDR	Mortality: C-T 20% vs. comparator 25% Clinical cure: C-T 81% vs. comparator 61%
Vena 2019, Clin Infect Dis	Case control study C-T vs polymyxin or aminoglycoside	C-T 16 vs comparator 32: 27 LRTI, 21 BSI	MDR/XDR	Mortality: C-T 18.8% vs. comparator 28.1% Clinical cure: C-T 81.3% vs. comparator 56.3%
Bosaeed 2020, Infect Dis	Retrospective study	LRTI 6, BSI 4, SSTI 3, UTI 2, IAI 3, bone 1	CR	Mortality 21%, Clinical cure 94.7%, Microbiological cure 73.7%
Coppola 2020, Microorganisms	Case series with C-T	SSTI 2, BSI 2, 1 other	MDR	Mortality 0%
Hart 2021, Open Forum Infect Dis	Retrospective study	UTI 45, SSTI 8, IAI 6, BSI 6, bone/joiont 4, brain 3.	MDR	Mortality 19%, clinical cure 68%
Enterobacterales				
Huntington 2016, J Antimicrob Chemother	Post hoc analysis of RCT: C-T vs. Levofloxacin	212 UTI, 7 BSI	186 Enterobacterales 85 ESBL	Clinical cure: C-T 90% vs. comparator 76.8% Microbiological cure: C-T 63% vs. comparator 43.8%
Popejoy 2017, J Antimicrob Chemother	Post hoc analysis of 2 RCT: C-T vs. Levofloxacin C-T vs. Meropenem	UTI: 54 C-T, 46 Levofloxacin IAI: 24 C-T, 26 Meropenem	ESBL	Clinical cure: C-T 97.4% vs. Levofloxacin 82.6% and vs Meropenem 88.5%. Microbiological cure: C-T 79.5% vs. Levofloxacin/Meropenem 62.5%
Arakawa 2019, J Infect Chemother	Nonrandomized open-label trial	90 UIT, 24 BSI	93 Enterobacterales 13 ESBL	For ESBL: Mortality 0%, Microbiological cure 38.5%
Mikamo 2019, J Infect Chemother	Nonrandomized open-label trial	130 IAI	58 Enterobacterales 5 ESBL	For ESBL: Mortality 0%, Clinical cure 100%, Microbiological cure 100%

Abbreviations: RCT, randomized controlled trial; C-T, ceftolozane-tazobactam; IAI, intra-abdominal infection; LRTI, lower respiratory tract infection; BSI, bloodstream infection; ITU, urinary tract infection; SSTI, skin and soft tissue infection; MDR, multidrug resistant; XDR, extensively drug resistant; CR, carbapenem resistant; PDR, pandrug resistant; ESBL, extended spectrum β-lactamase.

tered for high inoculum sources such as pneumonia, osteomyelitis, and abscesses. However, not only the source of infection should be considered to make the decision about the dosage but also the TOL-TAZ minimum inhibitory concentration (MIC). In a study aimed to evaluate the efficacy of different TOL-TAZ doses in patients with lower respiratory infection due to MDR- or XDR-*P. aeruginosa*, *Rodríguez Núñez* et al. found that mortality was significantly lower in patients with *P. aeruginosa* strains with MIC ≤2 mg/L and receiving high dose of TOL-TAZ compared with the group with higher MIC and standard dosage (16.2% vs 35.8%; P = .041). However, in the multivariate analysis only TOL-TAZ MIC >2 mg/L was identified as an independent predictor of mortality [4].

In case of third generation cephalosporin resistant *Enterobacterales*, the results of MERINO-3 (multicentre, parallel group open-label non-inferiority trial design comparing TOL-TAZ vs. meropenem in adult patients with bloodstream infection caused by ESBL or AmpC-producing *Enterobacterales*) will provide a better comprehension about the efficacy of TOL-TAZ in such infections [5].

RESISTANCE MECHANISMS

In vitro and *in vivo* data indicate that *P. aeruginosa* resistance to TOL-TAZ is due to several mechanisms. The most important seems to be a combination of mutations leading to hyperproduction and structural modified AmpC enzymes. It has been also suggested that specific PBP3 mutations may reduce its susceptibility. Finally, although to a minor extent, the overexpression of different efflux pumps could also affect to TOL-TAZ. With respect to acquired β-lactamases, TOL-TAZ shows no activity against metallo-beta-lactamases (MBL) -producing strains. Finally, extended-spectrum mutations in horizontally acquired OXA-type β-lactamases may lead to the emergence of resistance to TOL-TAZ [3].

Regarding *Enterobacterales*, tazobactam has no activity against serine carbapenemases or MBL, and has limited activity against AmpC and some ESBL [6].

COMBINATION THERAPY AGAINST MDR/XDR P. AERUGINOSA STRAINS

In order to avoid the selection of resistance, some studies have addressed the efficacy of combination antibiotic therapy with TOL-TAZ for treating MDR/XDR *P. aeruginosa* strains.

In an *in vitro* study aimed to evaluate the antibacterial activity of TOL-TAZ and colistin alone and in combination against a collection of 24 clinical XDR *P. aeruginosa*, *Montero* et al. demonstrated synergistic or additive effect for TOL-TAZ plus colistin (21/24), including TOL-TAZ-resistant strains [7]. The same group also evaluated the efficacy of TOL-TAZ in combination with meropenem against XDR strains in a hollow-fiber model. This approach showed that when TOL-TAZ was administered in combination with meropenem, there was a >4 log10 CFU/ml bacterial density reduction, without resistance emergence. This result suggests that a double beta-lactam strategy based on TOL-TAZ plus meropenem may be a useful combination for treating XDR *P. aeruginosa* [8].

CONFLICTS OF INTEREST

JPH has received honoraria as speaker or for advisory activities from Pfizer, MSD, Menarini, Angelini, Zambon. All other authors declare no conflicts of interest.

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10.2. Publication 2.

ORAL COMMUNICATION PRESENTED AT THE XXIII CONGRESS OF THE SPANISH SOCIETY OF INFECTIOUS DISEASES AND CLINICAL MICROBIOLOGY (SEIMC): Experiencia clínica de ceftolozano-tazobactam en infecciones invasivas por *Pseudomonas aeruginosa* extremadamente resistente. López Montesinos, L. Tena Caballero, M. Milagro Montero, L. Sorli Redó, N. Prim Bosch, S. Grau Cerrato y J.P. Horcajada Gallego. Enferm Infecc Microbiol Clin. 2019;37(Espec Cong 1):50

0074. EXPERIENCIA CLÍNICA DE CEFTOLOZANO-TAZOBACTAM EN INFECCIONES INVASIVAS POR *PSEUDOMONAS AERUGINOSA* EXTREMADAMENTE RESISTENTE

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Introducción: Ceftolozano-tazobactam (TOL/TAZ) ha demostrado actividad antibacteriana contra cepas de *Pseudomonas aeruginosa* extremadamente resistente (PAXDR). A continuación, describimos nuestra experiencia con TOL/TAZ en el tratamiento de infecciones invasivas por PAXDR en un hospital universitario entre febrero 2016 y agosto 2018.

Material y métodos: Se realizó un estudio observacional retrospectivo de infecciones por PAXDR, susceptibles a TOL/TAZ. Se estudiaron variables demográficas, clínicas y microbiológicas mediante un análisis multivariado.

Resultados: Se incluyeron 42 pacientes. Hombres 34 (85%), edad media de 69,4 años (desviación estándar, DE 13,8). Charlson 4,4 (2,7) y McCabe última o rápidamente fatal 37 (88,1%). Con respecto a los focos de infección: infección del tracto urinario (ITU) 18 (42,9%), neumonía o infección del tracto respiratorio (NITR): 12 (28,5%), infección de piel y partes blandas (IPPB) 6 (14,2%) e infección intraabdominal y otros focos 3 (7,1%), respectivamente; presentando bacteriemia secundaria 8 (19%) casos. En cuanto a la presentación clínica: sepsis grave o shock 9 (21,4%) y SAPS-2 35,4 (DE 11). Con respecto a la forma de emplear TOL/TAZ, en la mitad de los casos (22, 47,6%) fue terapia de rescate. En 21 (50%) pacientes se usó en monoterapia, principalmente en ITU 17 (80,9%). Con respecto al tratamiento combinado, TOL/TAZ se utilizó junto a meropenem en 12 (57%) y a colistina intravenosa en 4 (19%) pacientes, mayoritariamente en los siguientes focos: NITR 10 (47,6%), IPPB 5 (23,8%) e intraabdominal 3 (14,3%). Se utilizó triple terapia en 5 (23,8%) episodios. La curación clínica global fue del 72%: ITU 16 (88,9% del total de ITU), NITR 6 (50% del total de NITR), IPPB 5 (83,3% del total de IPPB) e infección intraabdominal 2 (66,7% del total de este tipo de infección). En el caso de infección de un foco de alto riesgo, es decir, diferente a ITU; la respuesta clínica observada fue menor: 53%. La mortalidad global a día 30 fue del 14,3% y el único factor asociado fue SAPS-2 (OR 1,11, IC95% 1,05-1,23). En el análisis multivariante, los focos de infección de alto riesgo se asociaron a mayor fracaso clínico (OR 8,9, IC95% 1,1-71,3). La no erradicación bacteriana (8 (19%)) fue más frecuente en NITR (5 (62,5%)), principalmente en EPOC (3 (60%)). Los efectos adversos fueron: infección por *C. difficile* y hepatotoxicidad, ambos 1 (2,3%) episodio.

Conclusiones: La monoterapia con TOL/TAZ se usó en ITU por PAXDR con un resultado excelente. Sin embargo, en pacientes con foco de infección de alto riesgo se prefirió la combinación de TOL/TAZ con meropenem o colistina con una media de respuesta clínica del 53%. Los eventos adversos fueron infrecuentes.