Patricia Ruiz-Garbajosa
Rafael Cantón

Epidemiology of antibiotic resistance in *Pseudomonas aeruginosa*. Implications for empiric and definitive therapy

Servicio de Microbiología. Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria. Madrid. Spain

ABSTRACT

Pseudomonas aeruginosa is one of the major pathogens causing hospital-acquired infections. It can easily develop antibiotic resistance through chromosomal mutations or by horizontal acquisition of resistant determinants. The increasing prevalence of multi-drug-resistant (MDR) or extensively-drug-resistant (XDR) *P. aeruginosa* isolates is associated with the dissemination of the so-called high-risk-clones, such as ST175. Infections caused by MDR/XDR are a cause of concern as they compromise the selection of appropriate empiric and definitive antimicrobial treatments. Introduction of new antibiotics with potent activity against MDR/XDR *P. aeruginosa* opens new horizons in the treatment of these infections.

Key words: *P. aeruginosa*, multidrug-resistance, ceftolozane-tazobactam

Epidemiología actual de la resistencia en *Pseudomonas aeruginosa*. Implicaciones en la terapia empírica y dirigida

RESUMEN

Pseudomonas aeruginosa es uno de los principales patógenos nosocomiales. Presenta una gran capacidad para desarrollar resistencias, bien por mutaciones cromosómicas o por adquisición de genes localizados en elementos transferibles. La emergencia de *P. aeruginosa* multirresistente (MR) y extremadamente resistente (XR) se ha asociado con la diseminación de los denominados clones de alto riesgo, como el ST175. Las infecciones causadas por estos clones comprometen la adecuación del tratamiento antimicrobiano empírico y definitivo. La introducción de nuevos antibióticos con potente actividad frente a *P. aeruginosa* MR/XR abre nuevos horizontes en el tratamiento de estas infecciones.

Palabras clave: *P. aeruginosa*, multirresistencia, ceftolozano-tazobactam

Correspondencia:
Patricia Ruiz-Garbajosa
Servicio de Microbiología. Hospital Universitario Ramón y Cajal. 28034-Madrid.
E-mail: pruizg@salud.madrid.org

INTRODUCTION

Pseudomonas aeruginosa is a non-fermentative gram-negative bacteria with an extraordinary ability to colonize a large variety of ecological niches, particularly moist environments. Currently, *P. aeruginosa* is one of the major pathogens causing hospital-acquired infections, in particular affecting patients with impairment of immune defences or admitted in the Intensive Care Unit (ICU)[1,2]. This organism is not only intrinsically resistant to a wide range of antimicrobials, but also has an extraordinary capacity for developing resistance to commonly used antimicrobials through the selection of mutations in chromosomal genes or by horizontal acquisition of resistant determinants. The increasing prevalence of multidrug-resistant (MDR) strains is a cause of concern as it compromises the selection of appropriate empirical and definitive antimicrobial treatments. This situation is associated with worse outcomes and higher mortality, particularly in patients with severe *P. aeruginosa* infections, including bacteraemia and ventilator associated pneumonia[3].

EPIDEMIOLOGY OF *P. aeruginosa* IN THE HOSPITAL SETTING

The European Centre for Disease Prevention and Control 2011-2012 Point-Prevalence Survey for health-care associated infections (HCAIs) found that almost 9% of all infections were caused by *P. aeruginosa*, and that it was the fourth most common pathogen in European hospitals[1]. Similar data was reported in a survey conducted by the Centers for Disease Control and Prevention in 2011, which found that 7.1% of HCAIs were caused by *P. aeruginosa* in the United States[2]. In

Spain, the 2016 EPINE survey found that *P. aeruginosa* was the second cause of hospital-acquired infections, and that it represented 10.5% of all these infections[4]. This prevalence is higher in the ICU setting, for instance the 2016 ENVIN-HELICS survey conducted by the Spanish Society of Intensive Care Medicine reported a 13% prevalence of *P. aeruginosa* infections[5].

Depending on the infection site, *P. aeruginosa* is one of the leading causes of ventilator-associated pneumonia (VAP), followed by bloodstream and urinary tract infections[1,2,4]. In ICUs in Spain, *P. aeruginosa* is the first cause of VAP, accounting for almost 21% of episodes[5].

EPIDEMIOLOGY OF ANTIBIOTIC RESISTANCE MECHANISMS IN *P. aeruginosa*

P. aeruginosa is intrinsically resistant to a wide range of antimicrobials mainly due to low outer membrane permeability, the expression of efflux pumps and the production of an inducible AmpC cephalosporinase. Moreover, it can also easily develop resistance to antimicrobials commonly used in the treatment of *P. aeruginosa* infections such as piperacillin/tazobactam, ceftazidime, carbapenems, fluoroquinolones or aminoglycosides. According to the data reported by The European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2015, the mean resistance percentages among *P. aeruginosa* invasive isolates for piperacillin/tazobactam, carbapenems and fluoroquinolones were close to 20%, while for ceftazidime and aminoglycosides they were 13%[6]. An increasing trend for piperacillin/tazobactam resistance was observed in Europe between 2011 and 2015, while carbapenem and ceftazidime resistance remained stable during this period[6]. Nevertheless, important variations in resistance rates were described in the different European countries, with higher resistance rates in the southern and eastern countries compared with the northern countries[6]. A multicentre study including *P. aeruginosa* isolates recovered from bloodstream infections from Spanish hospitals reported higher resistance rates for piperacillin/tazobactam, ceftazidime, fluoroquinolones and aminoglycosides (with the exception of amikacin) than those reported by EARS-Net[6,7]. However, carbapenem resistance was similar to that described by EARS-Net[6,7]. Sader et al in the SENTRY surveillance program found a moderate *in vitro* activity of piperacillin/tazobactam, ceftazidime and carbapenems against *P. aeruginosa* respiratory isolates collected from hospitalized patients with pneumonia US and European hospitals[8]. Moreover, resistance rates were higher in European than in US hospitals[8]. Amikacin and colistin were the most active antibiotics against blood and respiratory *P. aeruginosa* isolates[7,8] (table 1).

On the other hand, the prevalence of MDR *P. aeruginosa* has increased in the last decade reaching values of 30% in some areas, such as in eastern European countries[9]. A considerable proportion of MDR strains meets the criteria of XDR, which is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories[6]. A multicentre study on

Table 1 **Antimicrobial susceptibility of *P. aeruginosa* isolates recovered from patients with bloodstream infections and pneumonia**

	Blood isolates[a] (%R)[c]	Respiratory isolates[b] (%R)[c]	
Antimicrobial agent	Spanish hospitals (n=190)	EU hospitals (n=1,250)	USA hospitals (n=1,439)
Piperacillin/tazobactam	27.9	36.1	27.1
Ceftazidime	23.7	31.3	20.4
Cefepime	38.4	27.9	19.6
Imipenem	22.6	-[d]	-[d]
Meropenem	15.2	14.4	9
Ciprofloxacin	28.4	-[d]	-[d]
Levofloxacin	31.6	36.6	29.5
Gentamicin	21.1	24.8	13
Tobramycin	18.4	23.1	8.3
Amikacin	1.6	11.2	3.8
Colistin	1.1	1	1.1

[a]Data adapted from Cabot G et al.[7]

[b]Data adapted from Sader HS et al.[8]

[c]Percentage of resistant isolates according to EUCAST criteria

[d]Antimicrobial not tested

P. aeruginosa bloodstream infections in Spain found that 15% of the isolates were XDR[9]. Moreover, the EARS-Net reported a significant increase in Spain of invasive isolates with combined resistance to three or more antimicrobial groups (piperacillin–tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems), with rates ranging from 4% in 2005 to 14% in 2015[6]. Among XDR strains the polymyxins and amikacin were the antimicrobials that retained higher activity[6,9].

The mutational-mediated mechanisms, especially the hyperproduction of the chromosomally encoded AmpC betalactamase, the repression or inactivation of the carbapenem porin OprD, or the upregulation of efflux pumps are the main mechanisms involved in the development of antibiotic resistance in *P. aeruginosa*. Thus, the emergence of XDR or MDR strains is usually a consequence of the accumulation of several of these chromosomally mediated resistance mechanisms in the bacteria[7,9]. In addition, the acquisition of plasmid-mediated resistance genes coding for carbapenemase enzymes is an increasing problem in *P. aeruginosa*[7,9]. The metallo-betalactamases (MBLs) are the most commonly detected carbapenemases in *P. aeruginosa*, with VIM and IMP types being the most widely distributed[9]. Class A carbapenemases (mainly KPC type) are less frequent, but have been documented to be widespread in certain geographical areas, particularly in South America[9].

Data on the current prevalence of *P. aeruginosa* producing carbapenemase are scarce due to superimposed resistance phenotypes with other resistance mechanisms. Antibiotic resistance surveillance studies in Spain showed that the prevalence of carbapenemase producing isolates has increased from 0.08% in 2003 to 2.7% in 2009, with a predominance of VIM enzymes[10,11]. *P. aeruginosa* producing carbapenemase isolates are also associated with MDR or XDR phenotypes. Thus, the detection of carbapenemase production in *P. aeruginosa* is important for not only for the adequate selection of antimicrobial therapy but also for hospital epidemiology surveillance and infection control.

POPULATION STRUCTURE OF MDR/XRD *P. aeruginosa*

Molecular epidemiology studies of antibiotic susceptible *P. aeruginosa* isolates from hospital origin have described a highly polyclonal population[9]. However, the emergence of MDR/XDR *P. aeruginosa* revealed the existence of interhospital-disseminated MDR/XDR clones, denominated as high-risk clones (HRCs). The ST111, ST175, and ST235 clones have been described as the most successful *P. aeruginosa* HRCs, grouping the majority of MDR/XDR strains[9]. The ST111 and ST235 HRCs show a worldwide distribution, while ST175 clone is confined to European countries[9]. A wide dispersion of XDR *P. aeruginosa* belonging to ST175 clone has been found in Spanish hospitals[12,13]. In the majority of these strains the mutational mechanisms were responsible for the XDR phenotype, although hospital outbreaks of ST175 *P. aeruginosa* producing VIM-2 or VIM-20 have also been reported[9,12,13].

NEW ALTERNATIVES FOR THE ANTIBIOTIC EMPIRICAL AND DEFINITIVE TREATMENT OF MDR/XRD *P. aeruginosa*

The inappropriate empirical antibiotic therapy of MDR/XRD *P. aeruginosa* infections has been associated with increased mortality, length of hospital stay and increased hospital costs[2]. Antibiotic combinations are frequently used for the treatment of these infections, although the value of combination therapy compared to that of monotherapy remains controversial[2]. Moreover, amikacin and colistin are among the antipseudomonal antibiotics with greatest coverage against MDR/XRD *P. aeruginosa,* but both of them are associated with side effects and toxicity. In this scenario, new antibiotics with activity against MDR/XRD *P. aeruginosa* have been developed, and they represent an accurate alternative option for the treatment of infections produced by this organism.

Ceftolozane/tazobactam is an antibacterial consisting of ceftolozane, a novel antipseudomonal cephalosporin, with tazobactam, a well-established betalactamase inhibitor, that has been recently approved for the treatment of complicated intra-abdominal infections (plus metronidazole) and complicated urinary tract infections. The addition of tazobactam did not produce significant enhancement of the *in vitro* activity of ceftolozane against *P. aeruginosa* isolates, but enhanced the coverage of Enterobacteriaceae isolates producing extended-spectrum betalactamases. Ceftolozane has demonstrated potent *in vitro* activity against *P. aeruginosa* ($MIC_{50/90}$, 0.5/4 mg/L) and in different studies has shown higher activity compared with piperacillin/tazobactam, ceftazidime or meropenem[14,15]. More than 90% of clinical *P. aeruginosa* isolates show an MIC ≤ 8 mg/L[15]. Ceftolozane/tazobactam remains active against the majority of MRD/XDR isolates ($MIC_{50/90}$, 4/>64 mg/L), since it is not affected by some of the main resistance mechanisms in *P. aeruginosa* (AmpC hyperproduction, efflux pumps and/or loss of OprD) (figure 1)[14,15]. *In vitro* studies have demonstrated that the development of ceftolozane/tazobactam resistance is much slower than that of resistance to other antipseudomonal agents (ej. ceftazidime)[16]. In spite of the good antipseudomonal activity of ceftolozane/tazobactam, this is hydrolysed by carbapenemases such as metallo-betalactamases (MBLs). However, in Spain, since accumulation chromosomal mutations are the main mechanism responsible for MDR/XRD phenotypes, ceftolozane/tazobactam is a suitable therapeutic option in the current epidemiological scenario, not only for definitive therapy but also for empiric therapy.

Ceftazidime/avibactam, another new antibiotic with antipseudomonal activity, has also been approved for the treatment of complicated intra-abdominal infections (plus metronidazole) and complicated urinary tract infections. It is the combination of a third-generation antipseudomonal cephalosporin with the novel non-betalactam betalactamase inhibitor avibactam. Avibactam inhibits class A (ESBL and KPC), class C (AmpC) and some class D (such as OXA-48)

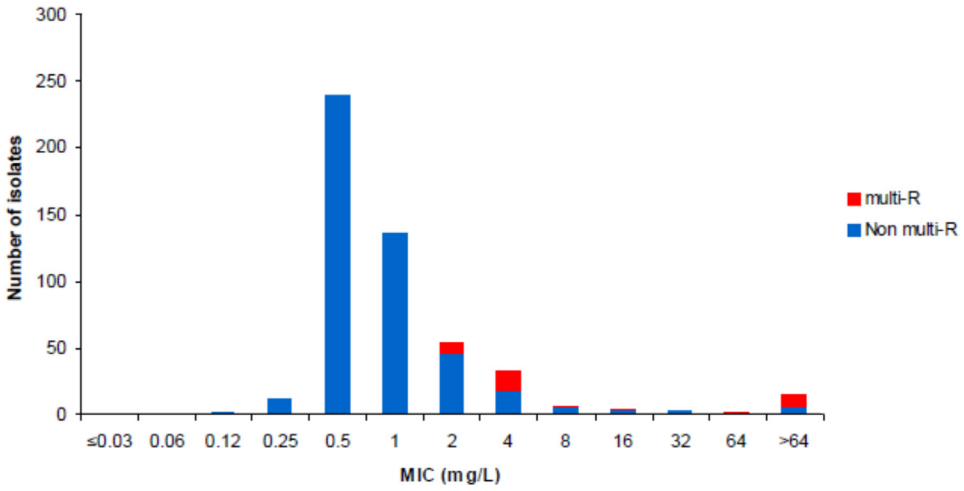

Figure 1 | **Activity of ceftolozane/tazobactam remains in *P. aeruginosa* with and without multidrug resistance phenotype recovered in Spain (data obtained from reference 14)**

betalactamases. Unfortunately, avibactam dose not inhibit MBLs. Furthermore, the addition of avibactam to ceftazidime increases the antipseudomonal spectrum of the latter by approximately 10%[17]. Ceftazidime/avibactam inhibited 82% and 76% of MDR and XDR strains at CMI ≤8 mg/L, respectively[17]. As with ceftolozane, ceftazidime/avibactam is not active against *P. aeruginosa* producing MBLs.

In summary, ceftolozane/tazobactam and ceftazidime/avibactam are new alternatives with potential to improve outcomes of patients with MDR/XDR *P. aeruginosa* infections. Prevalence of different resistance mechanisms in *P. aeruginosa* influences the positioning of ceftolozane/tazobactam or ceftazidime/avibactam for empiric use in infections due to this organism. Moreover, since carbapenemase production in *P. aeruginosa* is being increasingly reported, the screening of this resistance mechanism in MDR/XRD strains would be indicated or mandatory before starting definitive therapy with these new antibiotics.

CONFLICT OF INTEREST

RC has participate in educational programs organized by AstraZeneca and MSD and had a research project founded by Cubist.

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