Lack of beta–lactamases in *Pseudomonas aeruginosa* of animal origin and water

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Abstract

Cicuta, M.E.; Roibón, W.R.; Barceló, M.C.; Arzú, O.R.; Amable,V.I.: ***Lack of beta–lactamases in Pseudomonas aeruginosa of animal origin and water.*** *Rev. vet. 22: 1, 3–7, 2011.*
In order to know the susceptibility to carbapenems (imipenem and meropenem), ceftazidime and aztreonam as phenotypical indicator substrates of β–lactamases, 30 strains of *Pseudomonas aeruginosa* (18 from animal clinical samples and 12 from non–chlorinated water) were analysed. There were not synergism indicating their production, and the results differ from those of human where prevalence of resistance is a great problem when instauring treatment.

Key words: *Pseudomonas aeruginosa*, antibiotic resistance, β–lactamases.

Resumen

Cicuta, M.E.; Roibón, W.R.; Barceló, M.C.; Arzú, O.R.; Amable,V.I.: ***Ausencia de beta–lactamasas en Pseudomonas aeruginosa de origen animal y agua.*** *Rev. vet. 22: 1, 3–7, 2011.*
Con el fin de conocer la sensibilidad a carbapenems (imipenem y meropenem), ceftazidime y aztreonam como indicadores fenotípicos de sustratos de β–lactamasas, se analizaron 30 cepas de *Pseudomonas aeruginosa* (18 de muestras clínicas animales y 12 de aguas no clorinadas). No se halló sinergismo indicador de su producción. Estas estirpes se diferencian de las aisladas de seres humanos, donde alcanzan un alto grado de resistencia que dificulta considerablemente el tratamiento.

Palabras clave: *Pseudomonas aeruginosa*, resistencia antibiótica, β–lactamasas.

INTRODUCTION

The various bacterial antibiotic resistance mechanisms include alteration / modification of the target site, degradation of the antibiotic molecule and reduction of effective intracellular antibiotic concentration as a result of decreased permeability and energy–dependent (or active) efflux [10]. Resistance genes are either carried on the chromosomes of wild–type bacteria or on elements of extachromosomal, sometimes extraneous origins, such a resistance plasmids and transposons [8].

The most common mechanism by which bacteria are resistant to antibiotics is by producing enzymes that inactivate the drugs. The β–lactam antibiotics (penicillins and cephalosporins) can be inactivated by enzymes known as β–lactamases. Hundreds of β–lactamases have been described; they can be both plasmid or chromosomally encoded, and have varying degrees of activity against the different β–lactam antibiotics. Many bacteria produce multiple β–lactamases. In response to the proliferation and spread of β–lactamases, the pharmaceutical industry has developed some β–lactam antibiotics that are more resistant to hydrolysis by these enzymes. In addition, some combination drugs have been produced which contain both a β–lactam antibiotic and a β–lactamase inhibitor; the inhibitor has high affinity for the β–lactamase enzyme, irreversibly binds to it, and thereby preserves the activity of the β–lactam antibiotic [7]. About one–tenth of isolates of Gram–negative pathogens seem to produce extended–spectrum beta–lactamases (ESBL) [1].

Gram–negative bacteria pursue various molecular strategies for development of resistance to β–lactam antibiotics: (a) generation of extended–spectrum β–lactamases (ESBL) due to extension of the spectrum of already widely disseminated plasmid–encoded β–lactamases by amino acid substitution; (b) acquisition of genes encoding ESBL from environmental bacteria as, for instance the CTX–M–type β–lactamases from *Kluyvera* spp.; (c) high–level expression of chromosome–encoded β–lactamase (*bla*) genes as bla_{OXA} or bla_{ampC} genes due to modifications in regulatory genes, mutations of the β–lactamase promoter sequence as well as integration of insertion sequences containing

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an efficient promoter for intrinsic *bla* genes; (d) mobilization of *bla* genes by incorporation in integrons and horizontal transfer into other Gram–negative species such as the transfer of the *ampC* gene from *Citrobacter freundii* to *Klebsiella* spp.; (e) dissemination of plasmid–mediated carbapenemases as KPC and metallo–β–lactamases, e.g. VIM and IMP; (f) non–expression of porin genes and/or efflux pump–based antibiotic resistance [17]. *Pseudomonas aeruginosa* exhibits resistance to a variety of penicillins and cephalosporins, mediated by an alteration in porin proteins that form channels in the cell membrane [7].

The best–studied members of these pumps are the MexAB–OprM system of *Pseudomonas aeruginosa* that are known to efflux antibiotics, heavy metals, dyes, detergents, solvents, plus many other substrates [18]. Drug efflux systems pump out a broad range of chemically and structurally unrelated compounds from bacteria in an energy–dependent manner, without drug alteration or degradation [16]. Although drug efflux pumps are found in Gram–negative and Gram–positive bacteria, efflux mediated resistance in Gram–negative bacteria is a more complex problem due to the molecular architecture of the cell envelope. As a consequence, drug resistance in many cases is attributable to synergy between reduced drug intake (mainly due to low outer membrane permeability) and active drug export (via efflux pumps) [12].

The MexAB–OprM system of *P. aeruginosa* contributes to the antimicrobial resistance of wild–type strains and has the broadest substrate range of all characterized *P. aeruginosa* efflux pumps. Substrates of this pump include β–lactams, β–lactamase inhibitors, quinolones, macrolides, tetracyclines, chloramphenicol, novobiocin, sulfonamides, trimethoprim and thiolactomycin [9].

P. aeruginosa is a leading cause of nosocomial infections in public health. Carbapenem are potent agents for the treatment of this pathogen. However, the prevalence of carbapenem resistance among these bacteria has been increasing worldwide, particularly those mediated by acquired metallo–ß–lactamase (MBLs) [14]. MBLs eclipse the clinical efficacy of several broad–spectrum ß–lactam antibiotics and the existing ß–lactamase inhibitors. Moreover, the genetic elements usually associated with MBLs genes, facilitate their fast and widespread dissemination. Additionally, infections attributable to MBL–producing bacteria have been associated with an increased morbility and mortality [15].

P. aeruginosa is frequently isolated from canine otitis in this region. From 882 canine otitis swabs 222 strains were isolated. This indicates that it was the responsible ethiologic agent in 25.2% of cases of this pathology. The resistance to aminoglucosides was always over 50% with a maximun of 82.6% to amikacin and 81.4% to gentamicin. Low resistence to piperacillin (5.6%), fluoroquinolons (11.3%) and the polipeptide colistin (23%), resting the ultimate antibiotics to threat the infection. Similar susceptibility we obtained from *P. aeruginosa* isolated from another animal clinical samples such as nasal, bronchial, conjuntival, vaginal and piodermitis swabs. We did not know the resistant pattern of this bacteria from non–chlorinated water, obtained by perforation, of this region. So we analysed carbapenems, ceftazidime and aztreonam as phenotypical indicator substrates of β–lactamases [2] in the strains of both origin.

MATERIAL AND METHODS

Thirty strains of *P. aeruginosa* (18 from pathological clinical samples: 10 canine otitis, 3 ant–bear fecal swabs, 2 diarrhoeic suckling–pig faeces, 2 rabbit abscess, 1 ovine purulent exudate and 12 from non–chlorinated water) were studied for their susceptibility to antibiotics such as polimyxin (300 U), colistin (10 µg), piperacillin (100 µg), gentamicin (10 µg) and ciprofloxacin (5 µg) using antibiotic–diffusion in agar Mueller–Hinton by Kirby–Bauer antibiogram [11].

The strains were also analysed to determine phenotipically the presence of MBL: monodiscs of ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg),

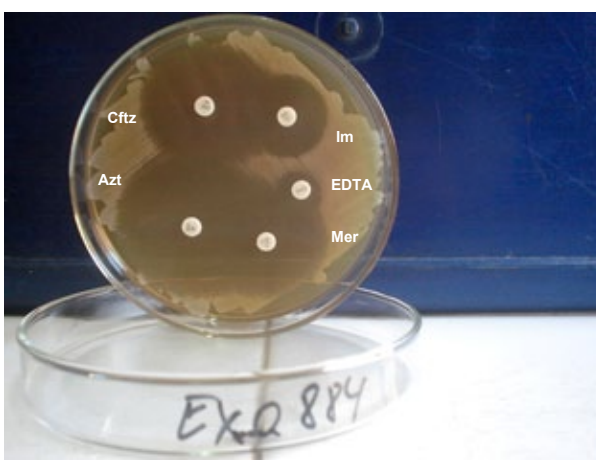

Figure 1. Antibiogram of *P. aeruginosa* of canine otitis (ExO884) susceptible to all antibiotics.

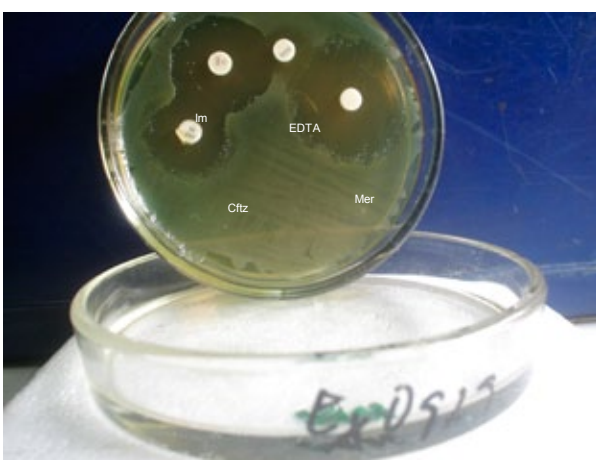

Figure 2. Antibiogram of *P. aeruginosa* of canine otitis (ExO929) resistant to imipenem (Im = 21 mm) and sensitive to other antibiotics.

Table 1. Percentage of sensibility of 30 strains of *P. aeruginosa*.

n	origin	Im	Mer	Azt	Pip	Cftz	Coli	Poli	G	Cipro
18	clinical samples	83.3	100	100	100	100	77.7	–	66.6	66.6
12	non–cholin. water	83.3	100	91.6	100	91.6	–	100	83.3	66.6

Im: Imipenem, Mer: Meropenem, Azt: Aztreonam, Pip: Piperacillin, Cftz: Ceftazidime, Coli: Colistin, Poli: Polimyxin, G: Gentamicin, Cipro: Ciprofloxacin.

meropenem (10 µg) and one of ethilendiaminotetraacetic (EDTA, 1 µmol) as MBL inhibitor, were used according the agar diffusion method [3, 11]. The test is positive when there is synergism between EDTA and carbapenem discs [2].

RESULTS

Of 18 strains from animal clinical samples (Table 1) 100% were susceptible to meropenem, aztreonam, piperacillin and ceftazidime, 15/18 (83.3%) to imipenem, 14/18 (77.7%) to colistin and 12/18 (66.6%) to gentamicin and ciprofloxacin.

Of 12 strains from non–chlorinated water (Table 1), 100% were susceptible to meropenem, piperacillin, and polimyxin, 11/12 (91.6%) to aztreonam and ceftazidime, 10/12 (83.3%) to imipenem and gentamicin and 8/12 (66.6%) to ciprofloxacin. There was not synergism between EDTA and carbapenem discs (Figures 1, 2, 3 and 4). The detail of seven strains found resistant to one ß–lactam antibiotic and the diameter average of the inhibition halos are in Tables 2 and 3 respectively.

Table 2. Detail of resistance of 7 strains of *P. aeruginosa* to ß–lactam antibiotics.

ref.	origin	Im	Mer	Azt	Cftz
Ex O 929	canine otitis	R (21)	S (30)	S (25)	S (20)
Ex O 934	canine otitis	R (21)	S (35)	S (25)	S (21)
As 153	ant–bear rectal	R (11)	S (30)	S (24)	S (20)
A 8	non chol.water	S (23)	S (36)	S (25)	R (17)
A 10	non chol.water	S (22)	S (30)	R (10)	S (25)
A 12	non chol.water	R (19)	S (30)	S (24)	S (25)
A 13	non chol.water	R (21)	S (35)	S (25)	S (22)

Im: Imipenem, Mer: Meropenem, Azt: Aztreonam, Cftz: Ceftazidime, S: sensible, R: resistant. In brakets: diameter of inhibition halos (mm).

Table 3. Diameter average of inhibition halos of *P. aeruginosa* from clinical samples and water (mm).

n	origin	Im	Mer	Azt	Cftz
18	clin.samples	25.2	34.3	29.1	24.9
	ranks	11–40	27–50	21–45	18–40
12	non–ch.water	23.0	33.3	23.9	23.3
	ranks	19–27	28–40	10–28	17–25

Im: Imipenem, Mer: Meropenem, Azt: Aztreonam, Cftz: Ceftazidime.

DISCUSSION

As the phenotypic screening was negative for detecting MBLs producing isolates, the resistance to imipenem observed in four of them, is more likely due to a decrease in the expression of OprD, an outer membrane protein channel that acts as the passage for imipenem entry in *P. aeruginosa* [13]. Owing that MBLs do not efficiently hydrolyse aztreonam, its sensibility would be a good predictor of the enzyme presence in resistant bacteria to imipenem and meropenem [3]. This was not the case in this work, where all the strains susceptible to aztreonam were also sensitive to meropenem.

Reduced of outer membrane permeability results in reduced antibiotic uptake, leading to low–level drug resistance. In the presence of drug efflux pumps, the

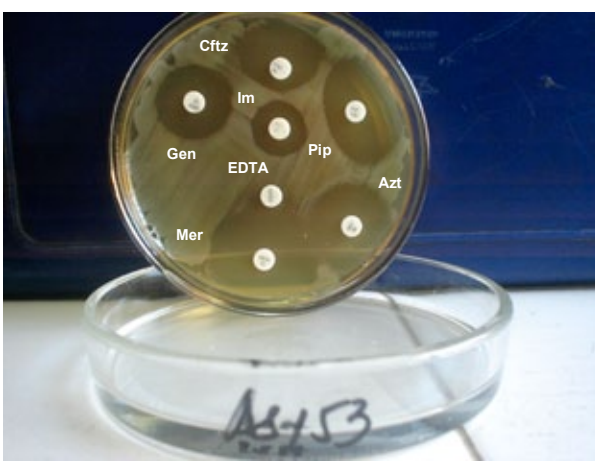

Figure 3. Antibiogram of *P. aeruginosa* of ant–bear rectal swab (AsI53) resistant to imipenem (Im= 11 mm) and sensitive to other antibiotics.

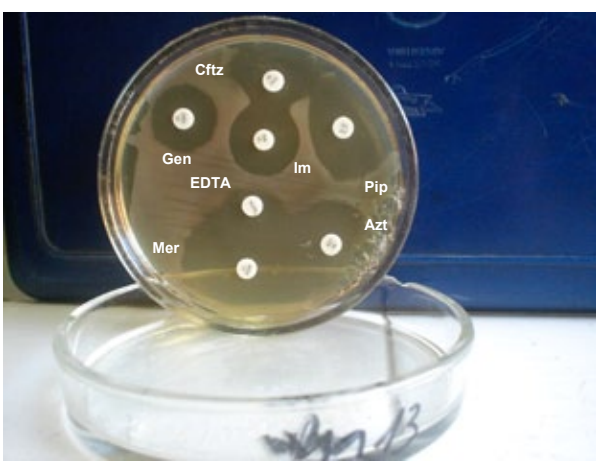

Figure 4. Antibiogram of *P. aeruginosa* of non– chlorinated water (AI3) resistant to imipenem (Im= 21 mm) and sensitive to other antibiotics.

resistance is amplified multiplicatively by synergism between reduced uptake and active efflux. This effect has been shown in 12 isolates of *P. aeruginosa*, of animal origin [4]. The synergism of reduced uptake and efflux is most evident in the multiplicative action of the outer membrane permeability barrier and active efflux, which results in high–level intrinsic and/or acquired resistance in many clinically important Gram–negative bacteria [8]. This mechanism may be the responsible of the resistance found in the strains of this work.

In contrast with public health where approximately 40% of *P. aeruginosa* isolates are resistant to ceftazidime, imipenem or levofloxacin [1], direct selective pressure is the most probable culprit of increased resistance. The chances for horizontal transfer of resistance and even virulence genes are increased by merely putting together bacteria that do not often interact [19]. Carbapenem resistance in human clinical isolates of *P. aeruginosa* has risen notably in recent years. This bacteria has different mechanisms of carbapenem resistance such as decreased levels of OprD or overexpression of the MexAB–OprM efflux system. Also the emergence of MBL–producing bacteria is becoming a severe therapeutic problem [20].

The introduction of carbapenems in antimicrobial chemotherapy in humans resulted in emergence of carbapenem hydrolyzing β–lactamases (carbapenemases); first in *P. aeruginosa* and *Acinetobacter* spp., later in *Enterobacteriaceae* [5]. At present, various β–lactamases are widespread in nearly every Gram–negative pathogenic species. Often, these enzymes are responsible for therapy failure because of mediating multidrug–resistance. A rigorous surveillance network to track the evolution and spread of resistance is also needed and would probably result in significant savings in healthcare [21].

P. aeruginosa, recognized as human opportunistic pathogen is increasingly responsible for lethal nosocomial infections. Treatment options are dramatically declining worldwide. This species has emerged as a relevant animal pathogen too. This is a consequence of massive antibiotic use and the large versatile genome of this organism. The strains we have studied have demonstrated important resistance to aminoglucosides other than β–lactamases. We agree with some authors [6] that antimicrobial profiling of *P. aeruginosa* animal isolates may contribute to a better understanding of the development and the epidemiology of *P. aeruginosa* multi–drug resistance in our densely populated biosphere with increasing human–animal interactions.

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