

## ORIGINAL ARTICLE

# IN VITRO ACTIVITY OF IMIPENEM AND CEFTAZIDIME AGAINST MUCOID AND NON-MUCOID STRAINS OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM PATIENTS IN IRAN

Mohammad Ahangarzadeh-Rezaee MSc\*, Qorban Behzadiyan-Nejad PhD\*, Parviz Owlia PhD\*\*, Shahin Najjar-Pirayeh PhD\*

\*Department of Microbiology, Faculty of Medical Sciences, Tarbiat Modarres University,

\*\*Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran

**Background** – Mucoid strains of *Pseudomonas aeruginosa* are surrounded by a capsule composed of  $\beta$ -1, 4 linked D-mannuronic acid and L-guluronic acid. The capsule provides a protective barrier against antimicrobial agents and the immune system, and is an important pathogen in immunocompromised hosts. In this study, we compared the activities of imipenem and ceftazidime against mucoid and non-mucoid clinical strains of *P. aeruginosa*.

**Methods** – From April to September 2000, one-hundred and thirty-three *P. aeruginosa* cultures were isolated from hospitalized patients in Tehran. In addition to determination of the minimum inhibitory concentrations (MICs) of two antipseudomonal antibiotics, ceftazidime and imipenem, against these isolates, mucoid strains were selected by capsule staining. Mucoid phenotype was confirmed by precipitation of alginate in cold ethanol. Data were analyzed by the Chi-square method.

**Results** – More than 70.6% of the strains were sensitive to imipenem, which inhibited most strains at an MIC of 4  $\mu$ g/mL. However, more than 72.9% of strains were resistant to ceftazidime, and only concentrations of 128  $\mu$ g/mL or more inhibited their growth. There was a statistically significant relationship between the presence of the alginate capsule and resistance against ceftazidime. Such a relation did not exist for imipenem.

**Conclusion** – Considering the extensive use of ceftazidime in Iran, performing susceptibility tests, as well as determining the mucoid phenotype in strains of *P. aeruginosa* is strongly recommended prior to antimicrobial therapy in immunocompromised patients.

**Keywords** • alginate • ceftazidime • imipenem • *Pseudomonas aeruginosa*

## Introduction

*Pseudomonas aeruginosa* is one of the most important opportunistic bacteria, causing a wide variety of infections especially in immunocompromised hosts such as burn patients, patients suffering from respiratory diseases like cystic fibrosis, and cancer chemotherapy patients.<sup>1-3</sup> This organism is intrinsically resistant to many antibiotics, or can

develop resistance during treatment.<sup>4</sup> Although *P. aeruginosa* has an outer membrane with low permeability, this characteristic alone does not adequately explain its intrinsic antimicrobial resistance.<sup>5</sup>

An additional mechanism that may interfere with access of antibiotics to their targets is production of an alginate capsule by mucoid strains of *P. aeruginosa*.<sup>1-3</sup> Alginate is a copolymer of O-acetylated  $\beta$ -1, 4 linked D-mannuronic acid and L-guluronic acid.<sup>6</sup> In some previous studies, it was shown that mucoid strains are more resistant to certain antibiotics than non-

• Correspondence: M. Ahangarzadeh-Rezaee MSc, Department of Microbiology, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran. P.O.Box:14115 -111, Fax: +98-21-28013030, E-mail: [ahanga\\_m@modares.ac.ir](mailto:ahanga_m@modares.ac.ir).

## In vitro Activity of Imipenem and Ceftazidime against *P. aeruginosa*

**Table 1.** Susceptibility distributions of two antibiotics against 133 clinical isolates of *P. aeruginosa*.

Antibiotic	MIC ( $\mu\text{g/mL}$ )											
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	> 128
Imipenem (No.)	0	0	0	0	0	94*	20	9	5	5	0	0
Ceftazidime (No.)	0	0	0	0	8*	10*	18*	5	6	13	8	65

\* Number of susceptible strains at minimum inhibitory concentration (MIC) breakpoints.

mucooid strains.<sup>7</sup>

In this study, we compared the activities of imipenem and ceftazidime against mucooid and non-mucooid clinical strains of *P. aeruginosa*. We selected ceftazidime which is extensively used for the treatment of pseudomonal infections in Iran and imipenem which is one of the newest antipseudomonal antibiotics with no data about its efficacy in Iran.

### Materials and Methods

#### Bacterial strains

A total of 133 *P. aeruginosa* isolates, cultured from patients treated between April and September 2000, were collected from specimens submitted to the clinical microbiology laboratories of selected hospitals in Tehran. The obtained specimens were from blood, wound swab, urine, sputum, stool and cerebrospinal fluid. The type of infection, source of each specimen, and patient characteristics were not important to the study design and are not presented here. All clinical isolates were identified and stored in skimmed milk at  $-70^{\circ}\text{C}$  until use. Strains were subcultured twice on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) prior to testing. *P. aeruginosa* ATCC 27853 was used as a quality control strain for the susceptibility test.<sup>8</sup>

#### Antimicrobial agents and MIC determination

Ceftazidime and imipenem-cilastatin were provided from Sigma-Tau, Italy. Agar dilution susceptibility tests used, were those outlined by the National Committee for Clinical Laboratory Standards (NCCLS).<sup>8</sup>

MICs were determined on plates of Mueller-Hinton agar containing serial two-fold dilutions of each antibiotic. Bacterial suspensions of  $10^4$  colony-forming units (CFU)/mL were inoculated onto the surface of the plates, and results were recorded after overnight incubation at  $35^{\circ}\text{C}$  in an aerobic atmosphere. The MIC was defined as the lowest antibiotic concentration with no visible growth.<sup>9-11</sup> For imipenem, NCCLS breakpoints of

$\leq 4$   $\mu\text{g/mL}$  (susceptible), 8  $\mu\text{g/mL}$  (intermediate) and  $\geq 16$   $\mu\text{g/mL}$  (resistant) were applied. For ceftazidime, NCCLS breakpoints of  $\leq 8$   $\mu\text{g/mL}$  (susceptible), 16  $\mu\text{g/mL}$  (intermediate), and  $\geq 32$   $\mu\text{g/mL}$  (resistance) were applied.

#### Differentiation of mucooid and non-mucooid strains

Using the Muir method, a thin film of bacterial suspension was prepared and dried in air. The film was covered with a piece of filter paper, cut to the size of the smear, and the slide was flooded with Ziehl-Neelsen carbolfuchsin. The slide was heated to steaming for 30 seconds using a low Bunsen flame. The slide was gently rinsed with 95% ethanol and then with distilled water. Mordant solution was added for 20 seconds followed by a wash with distilled water. Decolorization was performed by ethanol. For counter stain, 0.3% methylene blue was used for 30 seconds before examination under oil immersion lens. The cells stained red, and the capsule blue.<sup>12</sup> Precipitation of alginate was used for confirmation of capsule staining. For each isolate, a 48-hour culture was prepared in 100 mL nutrient broth (shaking at  $34-35^{\circ}\text{C}$ ) and alginate was precipitated by the addition of 50 mL cold ethanol. This mixture was kept at  $-20^{\circ}\text{C}$  for 30 min until formation of the alginate precipitate was complete.<sup>1,13</sup>

#### Statistical analysis

Antibiotic susceptibility results of mucooid and non-mucooid strains of *P. aeruginosa* were compared with Chi-square analysis using MS-TAT-C software (version 1.42, R.D. Freed, Michigan State University, USA).

### Results

Of 133 clinical isolates, 43 isolates had mucooid phenotype by Muir staining and precipitation of alginate. More than 70.6% of all strains were sensitive to imipenem with an MIC equal to 4  $\mu\text{g/mL}$ . However, more than 72.9% of all strains

**Table 2.** Comparative *in vitro* activity of imipenem and ceftazidime against mucoid and non-mucoid strains of *P. aeruginosa* according to minimum inhibitory concentration.

Antibiotic	Strain susceptibility, no. (%)					
	Susceptible		Intermediate		Resistant	
	M	NM	M	NM	M	NM
Imipenem	28 (65.12)	66 (73.33)	71 (16.27)	13 (14.45)	8 (18.61)	11 (12.22)
Ceftazidime	6 (13.95)	30 (33.33)	1 (2.32)	4 (4.44)	36 (83.73)	56 (62.23)

M = mucoid; NM = non-mucoid.

were resistant to ceftazidime, and only concentrations equal to 128 µg/mL or more, inhibited their growth.

Distribution of antimicrobial susceptibility of clinical isolates according to NCCLS susceptibility breakpoints are shown in Table 1.

Comparative *in vitro* activities of the two antibiotics (according to MIC determination) against mucoid and non-mucoid strains of *P. aeruginosa* are shown in Table 2.

While statistical analysis showed that mucoid strains were significantly more resistant than non-mucoid strains to ceftazidime ( $p < 0.01$ ), this was not the case for imipenem.

## Discussion

We designed the present study to examine the difference in susceptibility patterns of mucoid and non-mucoid clinical isolates of *P. aeruginosa* to imipenem and ceftazidime *in vitro*. Several investigators have shown that imipenem possesses an activity superior to that of β-lactams against *P. aeruginosa*, including strains resistant to third-generation cephalosporins.<sup>14,15</sup> The narrow range of the MICs of imipenem against our *P. aeruginosa* isolates is comparable to that reported by other investigators.<sup>10,16,17</sup> This property of imipenem is believed to be due to its stability against the β-lactamases and its rapid and lethal penetration of target sites and molecular structure.<sup>16,17</sup>

In earlier studies, we have shown that sub-inhibitory concentrations of gentamicin reduced alginate production in mucoid strains of *P. aeruginosa*.<sup>18–20</sup> In the present study, we observed that the alginate capsules of mucoid strains of *P. aeruginosa* could not act as a barrier against imipenem. This finding is comparable to the results of Slack and Nichol's studies, in which alginate impeded the penetration of all antibiotics except the β-lactams.<sup>20,21</sup> However, the alginate glycocalyx provides a barrier against penetration of ceftazidime, and this antibiotic was clearly inferior

to imipenem against our *P. aeruginosa* strains. In addition, this reduced susceptibility may be related to the more extensive use of ceftazidime in Iran. On the other hand, additional resistance mechanisms—especially production of extended-spectrum β-lactamases (ESBLs) and other enzymes—may contribute to ceftazidime resistance.<sup>4</sup>

## References

- 1 Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *P. aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev*. 1996; **60**: 539–74.
- 2 Hodges NA, Gordon CA. Protection of *P. aeruginosa* against ciprofloxacin and β-lactams by homologous alginate. *Antimicrob Agents Chemother*. 1991; **35**: 2450–2.
- 3 May TB, Shinabarger D, Maharaj R, et al. Alginate synthesis by *P. aeruginosa*: a key pathogenic factor in chronic pulmonary infections of cystic fibrosis patients. *Clin Microbiol Rev*. 1991; **4**: 191–206.
- 4 Bonfiglio G, Laksai Y, Franchino L, et al. Mechanisms of β-lactam resistance amongst *Pseudomonas aeruginosa* isolated in an Italian survey. *J Antimicrob Chemother*. 1998; **42**: 697–702.
- 5 Masuda N, Gotoh N, Ishii C, et al. Interplay between chromosomal β-lactamase and the MexAB-OprM efflux system in intrinsic resistance to β-lactams in *P. aeruginosa*. *Antimicrob Agents Chemother*. 1999; **43**: 400–2.
- 6 Yu J, Penaloza-Vazquez A, Chakrabarty AM, et al. Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of *P. syringae* pv. *syringae*. *Mol Microbiol*. 1999; **33**: 712–20.
- 7 Govan JR, Fyfe JA. Mucoid *P. aeruginosa* and cystic fibrosis: resistance of the mucoid form to carbenicillin, flucloxacillin and tobramycin and the isolation of mucoid variants *in vitro*. *J Antimicrob Chemother*. 1978; **4**: 233–40.
- 8 National Committee for Clinical Laboratory Standards. *Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria Grown Aerobically*. 4th ed. Approved Standard M7-A4. Wayne PA, USA: NCCLS; 1997.
- 9 Gerecke AA, Otuk G. Comparison of imipenem and five other antipseudomonal agents against gentamicin-susceptible and -resistant *Pseudomonas aeruginosa*. *Chemotherapy*. 1995; **41**: 334–6.
- 10 Garcia-Rodriguez JA, Blazquez AM, Fresnadillo MJ, et al. *In vitro* activity of meropenem against ciprofloxacin-resistant enterobacteriaceae and *P. aeruginosa*. *J*

## ***In vitro* Activity of Imipenem and Ceftazidime against *P. aeruginosa***

- Chemother.* 1996; **8**: 358 – 64.
- 11 Minami S, Akama M, Araki H, et al. Imipenem and cephem-resistant *P. aeruginosa* carrying plasmids coding for class B beta-lactamase. *J Antimicrob Chemother.* 1996; **37**: 433 – 44.
  - 12 Baron EJ, Finegold SM. *Diagnostic Microbiology.* 8th ed. Missouri, USA: Mosby Company; 1990: 37 – 8.
  - 13 Doig P, Smith NR, Todd T, et al. Characterization of the binding of *Pseudomonas aeruginosa* alginate to human epithelial cells. *Infect Immun.* 1987; **55**: 1517 – 22.
  - 14 Michael PR, Alford RH, McGee ZA. Superior activity of N-formimidoyl thienamycin against gentamicin-resistant *P. aeruginosa*. *Antimicrob Agents Chemother.* 1981; **20**: 702 – 41.
  - 15 Vurma-Rapp U, Kayser FH, Barberis-Maino L. Antibacterial properties of imipenem with special reference to the activity against methicillin-resistant Staphylococci, cefotaxime-resistant Enterobacteriaceae and *P. aeruginosa*. *J Antimicrob Chemother.* 1986; **18(suppl E)**: 27 – 33.
  - 16 Livermore DM. Clinical significance of beta-lactamase induction and stable derepression in gram-negative rods. *Eur J Clin Microbiol.* 1987; **6**: 439 – 45.
  - 17 Yoshimura F, Nikaido H. Diffusion of  $\beta$ -lactam antibiotics through the porin channels of *Escherichia coli* K-12. *Antimicrob Agents Chemother.* 1985; **27**: 84 – 92.
  - 18 Behzadiyan-Nejad Q, Souria E, Owlia P. *In vitro* effects of subinhibitory concentrations of gentamicin on *Pseudomonas aeruginosa* alginate production. *Pharm Pharmacol Commun.* 1998; **4**: 489 – 91.
  - 19 Owlia P, Behzadiyan-Nejad Q, Souria E. Microscopic study of the effects of subinhibitory concentrations of gentamicin on capsule production of *Pseudomonas aeruginosa*. *Arch Iranian Med.* 2001; **4**: 18 – 20.
  - 20 Ahangarzadeh-Rezaee M, Behzadiyan-Nejad Q, Najjar-Pirayeh S. Higher aminoglycoside resistance in mucoid *P. aeruginosa* than in non-mucoid strains. *Arch Iranian Med.* 2002; **5**: 108 – 10.
  - 21 Slack MP, Nichols WW. The penetration of antibiotics through sodium alginate and through the exopolysaccharide of a mucoid strain of *P. aeruginosa*. *Lancet.* 1981; **2**: 502 – 3.