

ORIGINAL ARTICLE

HIGHER AMINOGLYCOSIDE RESISTANCE IN MUCOID *PSEUDOMONAS AERUGINOSA* THAN IN NON-MUCOID STRAINS

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

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

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

Background- *Pseudomonas aeruginosa* is an important opportunistic pathogen. The mucoid strains of this organism produce a hyperviscous glycocalyx (alginate) that provides a barrier against antimicrobial agents and the immune system, thereby increasing its virulence and antibiotic resistance.

Methods- The in vitro activities of gentamicin, tobramycin and amikacin against 133 clinical isolates of *Pseudomonas aeruginosa* were tested by a disk diffusion method. The Muir method was used for capsule staining and identifying mucoid and non-mucoid strains.

Results- The mucoid strains ($n = 43$) were statistically significantly more resistant to amikacin, gentamicin and tobramycin than the non-mucoid strains ($n = 90$) ($p = 0.001$, 0.0002 and 0.009 , respectively).

Conclusion- Mucoid strains of *Pseudomonas aeruginosa* were more resistant to gentamicin, amikacin and tobramycin than non-mucoid strains. Therefore, these antimicrobial agents should be avoided in the treatment of mucoid *Pseudomonas aeruginosa* infections.

Keywords • alginate • amikacin • gentamicin • *Pseudomonas aeruginosa* • tobramycin

Introduction

Pseudomonas aeruginosa is nearly ubiquitous in nature and is quite innocuous in most environments. However, *P. aeruginosa* can cause severe and life-threatening infections in immunosuppressed hosts such as patients with burns; patients suffering from respiratory diseases, chemotherapy cancer patients, and children and young adults with cystic fibrosis.¹⁻⁴ This opportunistic pathogen produces a number of unique virulence factors. Extracellular toxins, proteases, hemolysins, and exopolysaccharides are some of the types of virulence factors that have been implicated in the pathogenicity of *P. aeruginosa*.^{1,5-7}

owlia@shahed.ac.ir.

The extracellular glycocalyx (alginate) is produced in copious amounts by mucoid strains of *P. aeruginosa*. Alginate is a linear copolymer composed of D-mannuronic acid and L-gluronic acid.^{2,8} There is also evidence that alginate provides a barrier against penetration by aminoglycosides.² The aim of this study was to compare the levels of resistance of mucoid and non-mucoid strains of *P. aeruginosa* to gentamicin, amikacin and tobramycin.

Materials and Methods

Bacteria

One hundred and thirty-three clinical isolates of *P. aeruginosa* were obtained as specimens submitted to the clinical microbiology laboratories of selected hospitals in Tehran. *P. aeruginosa* strain ATCC (American Type Culture Collection)

• Correspondence: P. Owlia PhD, Department of Microbiology, Faculty of Medicine, Shahed University, No. 29, Keshavarz Blvd., Dehkadeh St., Tehran, Iran. Fax: +98-21-8966310, E-mail:

27853 and *Escherichia coli* strain ATCC 25922

Table. Comparative in vitro resistance of mucoid and non-mucoid isolates of *Pseudomonas aeruginosa* to antibiotics.

Antibiotic	No. of strains (%)					
	Susceptible		Intermediate		Resistant	
	M	NM	M	NM	M	NM
Gentamicin	4 (9.3)	25 (27.8)	1 (2.33)	0 (0)	38 (88.37)	65 (72.2)
Amikacin	8 (18.6)	36 (40)	6 (14)	3 (3.3)	29 (67.4)	51 (56.7)
Tobramycin	6 (14)	26 (28.9)	0 (0)	0 (0)	37 (86)	64 (71.1)

M = mucoid; NM = non-mucoid.

were included as controls to verify the accuracy of the antibiotic susceptibility test procedure.⁹

Antibiotic susceptibility testing

Gentamicin, amikacin and tobramycin were selected because these antibiotics are commonly used in Iran. The susceptibility of various *P. aeruginosa* strains was determined by the disk diffusion method.⁹

Bacterial suspensions of 10⁴ CFU/mL were spread onto the surface of Müller-Hinton agar plates by swab. Fifteen minutes after inoculating the plates with bacteria, three antimicrobial disks were applied using sterile forceps. The plates were inverted and incubated overnight at 35°C for 18 hours, after which the diameter of each zone of inhibition was measured.⁹

Differentiation of mucoid and non-mucoid strains

Mucoid strains were identified using the Muir method,¹⁰ in which a thin film of suspension was prepared and air-dried, the film was covered with a piece of filter paper the size of the smear, and the slide was flooded with Ziehl-Neelsen carbol fuchsin and heated to steaming for 30 seconds by a low Bunsen flame.

The slide was gently rinsed with 95% ethanol and then with distilled water. Mordant solution was added for 20 seconds and then washed with distilled water. Decolorization was performed using ethanol. For counterstaining, 0.3% methylene blue was used for 30 seconds prior to examination of the preparations under the oil immersion lens. The cells were stained red, and the capsules blue.¹⁰

Statistical analysis

The susceptibility of mucoid and non-mucoid strains of *P. aeruginosa* to the antibiotics were determined using SAS, version 6. A *p* value of

less than 0.05 was considered significant.

Results

From the 133 clinically isolated *P. aeruginosa* strains, 43 were identified to be mucoid by the Muir staining method.

The in vitro activities of antibiotics against mucoid and non-mucoid strains of *P. aeruginosa* are shown in the Table. Overall, there was more to gentamicin (*n* = 103) and tobramycin (*n* = 101) than to amikacin (*n* = 80) (Figure).

Statistical analysis showed that mucoid strains were significantly more resistant than non-mucoid strains to gentamicin, amikacin and tobramycin (*p* = 0.001, 0.0002 and 0.009, respectively).

Discussion

It appears that alginate, an extracellular glycocalyx, probably acts as a barrier against aminoglycosides.² In earlier studies, we showed that subinhibitory concentrations of gentamicin reduced alginate production in mucoid strains of *P. aeruginosa*.^{11,12} Govan and Fyfe reported that the mucoid forms of *P. aeruginosa* are more resistant to carbencillin, flucloxacillin and tobramycin than the non-mucoid isolates.¹³ In contrast, Demko and Thomassen observed increased sensitivity of

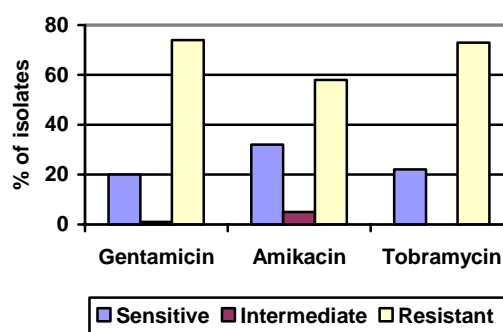


Figure. In vitro activities of gentamicin, amikacin and tobramycin against 133 clinical isolates of *P. aeruginosa*.

alginate-producing strains to carbencillin, tobramycin and ticarcillin.⁵ Nevertheless, there is evidence that alginate provides an ionic barrier against penetration of aminoglycoside antibiotics.² Slack and Nichols used antibiotic diffusion through agar as a criterion for direct measurement of the permeability of the alginate layer to antibiotics.⁷ They found that, with the exception of β -lactams, alginate did in fact impede the penetration of antibiotics such as aminoglycosides. However, Gordon et al observed that the alginate-to-antibiotic ratio could greatly influence the perceived permeability barrier. When this ratio is high, aminoglycosides (but not β -lactams) are retained in the alginate layer. However, low alginate-to-antibiotic ratios quickly result in disruption of the gel structure and faster penetration of aminoglycosides.⁸ They suggested that high levels of antibiotic saturate the negative charge of alginate and result in a breakdown in the permeability layer. In a study by Rastegar Lari of 2,122 patients admitted to Tohid Burn Center in Tehran from 1995–1997, 3,365 bacterial strains including *P. aeruginosa* (73.39%), *Staphylococcus aureus* (9.1%) and other organisms (17%) were isolated.¹⁴ The frequency of *P. aeruginosa* resistance to gentamicin, carbencillin, cotrimoxazole, ceftizoxime and tetracycline was over 95% in their study.

Ciofu et al characterized 42 paired mucoid and non-mucoid *P. aeruginosa* isolates collected from Danish cystic fibrosis patients in 1997 by RiboPrinting, antibiotic susceptibility (ciprofloxacin, tobramycin, meropenem, colistin, ceftazidime, piperacillin, azteronam, meropenem) and β -lactamase activity.¹⁵ They determined that the non-mucoid/mucoid minimum inhibitory concentration (MIC) ratio for tobramycin was 1.3, and that mucoid isolates were generally more susceptible to antibiotics. This result is in direct contrast to our findings and may be due to the differences in the methods used: they determined susceptibility by MIC, whereas we used the disk diffusion method.

In the current investigation, mucoid strains of *P. aeruginosa* were more resistant to gentamicin, amikacin and tobramycin as compared to non-mucoid strains. Overall, there was more resistance to gentamicin, followed by tobramycin and amikacin.

The results of this study suggest that the capsule may act as a barrier against aminoglycosides. Results indicate that familiarity with the type of *P. aeruginosa* (mucoid or non-mucoid) can aid in the selection of appropriate antibiotic therapy. Nevertheless, antibiograms must be performed before antibiotic therapy is begun.

References

- 1 Abi-Hanna P, Quinn J. Meropenem: a comprehensive review. *Pharm Therapeu.* 1998; **23**: 632-8.
- 2 Govan JRW, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev.* 1996; **60**: 539-74.
- 3 Hodges NA, Gordon CA. Protection of *Pseudomonas aeruginosa* against ciprofloxacin and β -lactams by homologous alginate. *Antimicrob Agents Chemother.* 1991; **35**: 2450-2.
- 4 Masuda N, Sakagawa E, Ohya S. Outer membrane proteins responsible for multiple drug resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 1995; **39**: 645-9.
- 5 Demko CA, Thomassen MG. Effect of mucoid property on antibiotic susceptibility of *Pseudomonas aeruginosa*. *Curr Microbiol.* 1980; **4**: 69-73.
- 6 Masuda N, Gotoh N, Ishii C, et al. Interplay between chromosomal beta-lactamase and MexAB-OprM efflux system in intrinsic resistance to beta-lactams in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 1999; **43**: 400-2.
- 7 Slack MP, Nichols WW. The penetration of antibiotics through sodium alginate and through the exopolysaccharide of a mucoid strain of *Pseudomonas aeruginosa*. *Lancet.* 1981; **2**: 502-3.
- 8 Gordon CA, Hedges NA, Marriott C. Antibiotic interaction and diffusion through alginate and exopolysaccharide of cystic fibrosis-derived *Pseudomonas aeruginosa*. *J Antimicrob Chemother.* 1988; **22**: 667-74.
- 9 Anhalt JP, Washington HA. Antimicrobial susceptibility tests of aerobic and facultatively anaerobic. In: Washington JA, ed. *Laboratory Procedures in Clinical Microbiology*. 2nd ed. New York: Springer-Verlag; 1985: 285-303.
- 10 Baron EJ, Tenenbaum SM. *Diagnostic Microbiology*. 8th ed. New York: Mosby; 1990: 37-8.
- 11 Behzadiyan-Nejad Q, Sourji E, Owlia P. In vitro effects of sub-inhibitory concentrations of gentamicin on *Pseudomonas aeruginosa* alginate production. *Pharm Pharmacol Commun.* 1988; **4**: 489-91.
- 12 Owlia P, Behzadiyan-Nejad Q, Sourji E, Sadri H. Microscopic study of the effects of sub-inhibitory concentrations of gentamicin on capsule production of *Pseudomonas aeruginosa*. *Arch Iran Med.* 2001; **4**: 18-20.
- 13 Govan JRW, Fyfe JAM. Mucoid *Pseudomonas aeruginosa* and cystic fibrosis: resistance of the mucoid form to carbencillin, flucloxacillin and tobramycin and the isolation of mucoid variants in vitro. *J Antimicrob Chemother.* 1978; **4**: 233-40.
- 14 Rastegar Lari A, Bahrami Honar H, Alaghebandan R. *Pseudomonas* infections in Tohid Burn Center, Iran. *Burns.* 1998; **24**: 637-41.

- 15 Ciofu O, Fussing V, Bagge N, et al. Characterization of paired mucoid/non-mucoid *Pseudomonas aeruginosa* isolates from Danish cystic fibrosis patients: antibiotic resistance, beta-lactamase activity and RiboPrinting. *J Antimicrob Chemother.* 2001; **48**: 391-6.