

Short Communication

EMERGENCE OF CIPROFLOXACIN RESISTANCE AMONG *PSEUDOMONAS AERUGINOSA* ISOLATED FROM BURN PATIENTS

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ABSTRACT

Background: Increasing resistance of *Pseudomonas aeruginosa* to ciprofloxacin in ICU/burn units has created a problem in the treatment of infections caused by this microorganism.

Methods: Fifty *P. aeruginosa* strains were isolated from burn patients hospitalized in the Kerman Hospital during May 1999-April 2000 and were tested for *in-vitro* sensitivity to different antibiotics by disc diffusion breakpoint assay. The isolates were subjected to minimum inhibitory concentration (MIC) test by agar dilution method. Existence of the plasmids was also investigated in the isolates.

Results: Thirty-four patients infected with ciprofloxacin strains showed MIC of 8 µg/ml [$p < 0.001$]. Sixteen patients were infected with sensitive strains exhibiting MIC range of $0.0125-0.125 \pm 0.033$ µg/ml. The isolates were also also resistant to other antibiotics [$p < 0.001$]. Plasmid isolation and agarose gel electrophoresis (0.7%) revealed three plasmid bands in strains 8 and 16, and one band in strain 35.

Conclusion: The emergence of ciprofloxacin resistance of *P. aeruginosa* in burn patients is alarming since this antibiotic has only recently been introduced onto the market in Iran. One important observation was that some isolates exhibited cross resistance to other antibiotics. Furthermore, some strains were carriers of plasmids which might have acted as the potential source of acquired resistance in the hospital setting.

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Key Words • *Pseudomonas* • ciprofloxacin • plasmids

Introduction

The *P. aeruginosa* is one of the leading causes of nosocomial infection, ranking second among the gram-negative pathogens reported to the National Nosocomial Infection

Surveillance System.^{1,2} Due to its ubiquitous presence, *P. aeruginosa* can be found in clinical samples as a contaminant without any relation to disease, however, this organism is responsible for some of the most serious and lethal infections in immunocompromised patients.^{3,4} There are a limited number of antimicrobial agents with reliable activity against *P. aeruginosa*, including penicillins, cephalosporins, carbapenems and fluoroquinolons particularly ciprofloxacin.^{5,6} The changing and easy acquisition of

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Table 1: Distribution of number and percentage of *Pseudomonas aeruginosa* samples obtained from patients in burn unit of the hospital.

Burn(%)	Age groups(Yrs) *					Total
	1-10	11-20	21-30	31-40	41-49	
1-30	2 (15.4)	4 (28.6)	1 (8.3)	-	-	7 (14.0)
31-40	6 (46.1)	4 (28.6)	4 (33.3)	4 (57.1)	3 (75.0)	21 (42.0)
41-50	5 (38.5)	3 (21.4)	5 (41.7)	-	1 (25.0)	14 (28.0)
<50	-	3 (21.4)	2 (16.7)	3 (42.0)	-	8 (16.0)
Total	13 (100.0)	14 (100.0)	12 (100.0)	7 (100.0)	4 (100.0)	50 (100.0)

* Mean \pm SD = 21 \pm 14.3

resistance to *P. aeruginosa* requires rapid surveillance procedures to represent the whole reality of the situation at any given time. In this study we report on the emergence of ciprofloxacin resistance among *P. aeruginosa* isolated from burn patients in Kerman, Iran.

Materials and Methods

Bacterial source:

Fifty strains of the *P. aeruginosa* were isolated from burn patients at the Kerman Hospital, Iran, during May 1999 to April 2000. The genus and species of the isolates were confirmed by standard bacteriological tests.⁷

Antibiotics:

All antibiotic discs were provided by Darupaksh, Iran, including tetracycline (Te), carbenicillin (CB), chloramphenicol (C), ciprofloxacin (Cp), kanamycin (K), tobramycin (Tob), amikacin (AN), and cefotaxime (CTX). Ciprofloxacin powder (99.8% purity) was obtained from Razak Company Ltd, Karaj, Iran. Chemicals for plasmid isolation were purchased from Sigma (USA). Restriction enzymes [AvaII, ECOR1, BamHI and SmaI] were received from Sina-Gene Company, Tehran, Iran.

Antibiotic susceptibility tests:

The susceptibility of the above mentioned isolates to antibiotics was determined by disc diffusion break point assay. MIC was measured by agar dilution test in 19.9 ml sterile Muller-Hinton agar as recommended by the National Committee for Clinical Laboratory Standards [NCCLS].⁸ The MIC was defined as the lowest concentration of antibiotics that would inhibit the growth after 18-24 h of incubation at 37 °C. The bacterial inoculum was 10⁵-10⁶ cells/ml. On the basis of multi-modal distributions, the strains were categorized into sensitive (S), and resistant (R) according to the indicated break point.

Plasmid DNA extraction:

Plasmid DNA from each isolate was extracted by the method of Holmes and Quigely and sized related to phage λ -DNA digested with Hind III.⁹ Electrophoresis was carried out in horizontal bed apparatus using 1mM Tris-Borate buffer (pH:7.2) either at 60 V for 4 hours or 90 V for 2 hours.

Statistical analysis:

All data were analyzed using Epi Info software version 6.4. Chi-square test was used for determination of the significance of association.

Table 2: Number of antibiotic resistant *Pseudomonas aeruginosa* with respect to ciprofloxacin and other antibiotics.

Antibiotics	Sensitive	Resistant	P-value
Ciprofloxacin	16	34	<0.001
Kanamycin	4	46	<0.001
Tetracycline	0	50	<0.001
Chloramphenicol	0	50	<0.001
Carbenicillin	0	50	<0.001
Cefotaxime	7	43	<0.001
Amikacin	6	44	<0.001
Ceftriaxone	4	46	<0.001
Tobramycin	4	46	<0.001

Results

Out of 250 specimens studied in the burn unit of the Kerman Hospital over a period of one year, 50 were infected with *P. aeruginosa*. Twenty-two isolates were collected from females and 28 from males. The mean \pm standard deviation (SD) of their age was 21.3 ± 14.3 years. The distribution of the resistance of the isolates to different antibiotics is shown in Table 1. On the basis of criteria established by NCCLS, the overall susceptibility pattern of the isolates with respect to ciprofloxacin and other antibiotics are shown in Table 2. Out of 50 strains examined, sixteen were sensitive to Cp, 4 to K, 6 to AN, 4 to CFT, and 4 to TOB [$P < 0.001$]. The MICs of ciprofloxacin for the above isolates are shown in Table 3. The

correlation between MIC and zone diameter were also consistent with that expected. Plasmid isolation and agarose gel electrophoresis (0.7%) revealed three plasmid bands in strains 8 and 16 with one plasmid band in strain 35 (Fig. 1).

Discussion

There is growing concern that the control of infectious diseases is threatened by the upward trend in the number of bacteria that are resistant to multiple antibiotics. Resistant infections are associated with increased morbidity, prolonged hospital stays, greater direct and indirect costs, and greater opportunity for the spread of infection to other individuals.¹⁰

In this study, we have shown that the

Table 3: Minimum inhibitory concentration (MIC) of ciprofloxacin for *Pseudomonas* isolates.

No. of sensitive or resistant strains	R or S	MIC 50 ($\mu\text{g/ml}$)	Total MIC ($\mu\text{g/ml}$)	Range (95% CI *)
5	S	0.0125	0.025	0.001-0.041
5	S	0.031	0.068	0.034-0.066
6	S	0.0625	0.125	0.067-0.133
34	R	4	8	4.283-8.517

*Confidence interval, S= sensitive, R= resistant

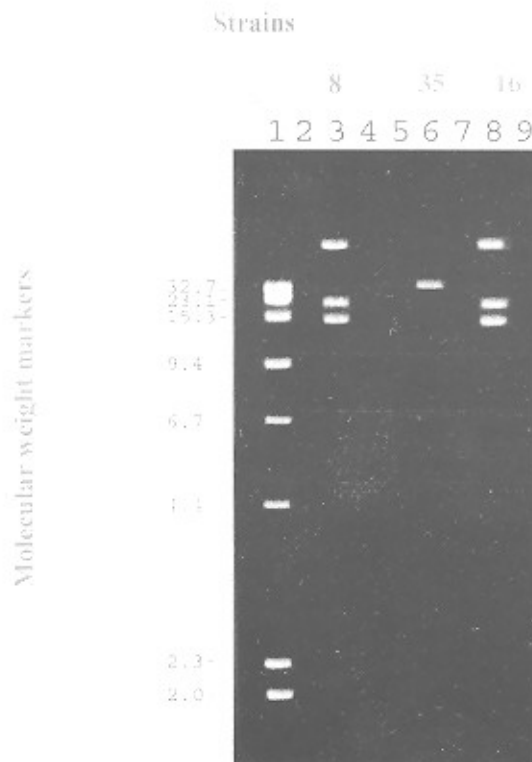


Figure 1: Agarose gel electrophoresis of the plasmids from *P. aeruginosa* strains 8, 16 and 35.

ciprofloxacin resistant strains of *P. aeruginosa* are emerging in the burn unit of the Kerman Hospital and some strains also exhibited cross resistance with other clinically useful antibiotics. The high level of ciprofloxacin resistance in many of these isolates with MIC of 8 $\mu\text{g/ml}$ indicates the presence of multiple mutations in the genome. Continued exposure to ciprofloxacin would explain the occurrence of strains with high levels of resistance to this antibiotic in a hospital setting. To our surprise, a high percentage of *P. aeruginosa* isolates were obtained from patients with prolonged hospitalization. Furthermore, some strains carried plasmids which could be a potential source for acquired resistance (Fig. 1).

There is little information available regarding the emergence of ciprofloxacin resistance pathogens in third world

countries.^{11,13} Qadri et al. studied antimicrobial resistance patterns of bacteria, isolated in 1992 from two tertiary care centers in Riyadh.¹¹ Outstanding number of *P. aeruginosa* exhibited MIC of 4-8 $\mu\text{g/ml}$. Similarly, the level of resistance of bacteria to ciprofloxacin in third world countries was compared by Green and Tillotson. Their data confirmed that ciprofloxacin is a safe drug in children in developing countries although resistant strains are emerging.¹² Sharma et al. reported ciprofloxacin resistance among multi-drug resistance strains of *Salmonella senftenberg* in India.¹³ The level of resistance is rapidly increasing in this organism. In Iran, few reports exist on ciprofloxacin resistance in bacteria and this study was an attempt to address this issue.¹⁴

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