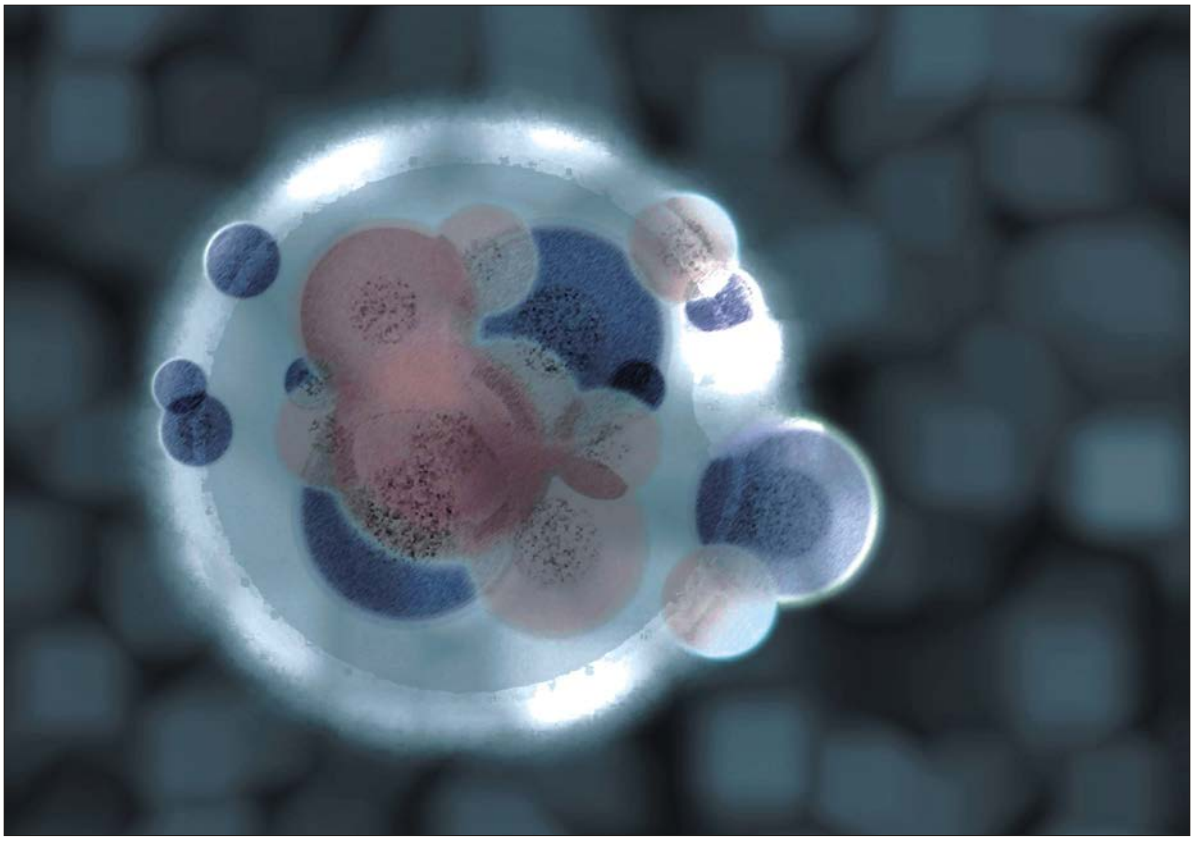

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Evaluation of the Sensitivity of *Pseudomonas aeruginosa* Clinical isolates to Ciprofloxacin

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Abstract: In this study, the sensitivity of 100 *P. aeruginosa* isolates to ciprofloxacin has been investigated by determining minimum bactericidal concentration. The susceptibilities of isolates to other antibiotics were tested using agar disk diffusion method. The isolates contains 67 isolate from urine, 19 isolate from wound, 10 isolate from sputum, two isolate from blood, one isolate from stool and one isolate from ear. Thirty-five percent of isolates showed resistance to 10 antibiotics and also to ciprofloxacin and were sensitive only to imipenem. Thirty-five antibiotypes were recognized for all the isolates. The rates of resistances were determined to antibiotics as follows: gentamicin 49%, ticarcillin 100%, ceftizoxime 78%, co-trimoxazole 97%, amikacin 35%, carbenicillin 65%, ceftriaxone 65%, piperacillin 53%, imipenem 2%, kanamycin 65% and ofloxacin 71%. Fifty-five percent of isolates were resistant to ciprofloxacin. In conclusion, ciprofloxacin-resistant *P. aeruginosa* has been frequent in our clinical isolates, this data remind the worldwide emerging resistance against ciprofloxacin and this is a serious problem in therapeutic management of *P. aeuroginosa* infections and has a local and worldwide concern.

Key words: Minimum bactericidal concentration, antibiotic, resistance, clinical isolates, disk diffusion method

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic gram negative bacilli and one of the most important causes of nosocomial infections especially in patients with burns, cystic fibrosis and neutropenia. *P. aeruginosa* shows high resistance to different classes of antimicrobial agents (Algun *et al*., 2004).

P. aeruginosa is an opportunistic pathogen found along with other *Pseudomonas* sp. as part of the normal flora of the human skin (Larson *et al*., 2002). When the host is immunocompromised, this opportunistic bacterium can quickly colonize and infect the burn and wound sites. Since *P. aeruginosa* can rapidly disseminate from the wounds into other organs via the bloodstream and can produce a number of virulence factors, the clinical outcome in these patients can lead to sepsis which is often fatal. In case studies of burn patients who developed *P. aeruginosa* septicemia, the mortality rate was more than 75% (Holder, 1985; Wurtz *et al*., 1995). Antibiotics, with the exceptions of the fluoroquinolones, are generally ineffective against most serious infections by *P. aeruginosa*. The introduction of fluoroquinolones offers a promising effective therapy of these types of infections caused by *P. aeruginosa* but unfortunately, the numbers of isolates of *P. aeruginosa* which have developed drug resistance to fluoroquinolones have increased rapidly in recent years (Dale *et al*., 2004).

Ciprofloxacin is a commonly used antibiotic in clinical practice (Chaudhry *et al*., 1999). It is a broad-spectrum fluoroquinolone with coverage against most gram-positive and gram-negative orgamisms, including *Pseudomonas aeruginosa* (Chaudhry *et al*., 1998; Snyder and Katz, 1992; Knauf *et al*., 1996; Kunimoto *et al*., 1999). This study was conducted to examine the susceptibility of clinical isolates of *P. aeruginosa* to ciprofloxacin.

MATERIALS AND METHODS

Bacterial strains and culture media: A total of 100 isolates were collected from Clinical specimens (wound, urine, sputum, blood, stool and ear) submitted to hospital diagnostic laboratories in Urmia/Iran from June to September 2005. The isolates were further processed by the standard methods to identify as *P. aeruginosa*. Isolated bacteria were maintained for long storage on skimmed milk medium (BBL) by adding 10% glycerol in -60° C, cultures were maintained for daily use on Nutrient agar slants on 4°C.

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Preparation of ciprofloxacin powder: Ciprofloxacin powder was kindly provided by Exir pharmaceutical company, Tehran, Iran. The pure content of active ciprofloxacin was 96% in the provided powder.

Determination of antimicrobial activity of ciprofloxacin: For determining of the bacterial isolates sensitivity to ciprofloxacin, classic broth dilution susceptibility test were used (Sahm and Weissfeld, 2002). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of isolates to ciprofloxacin were determined. The initial concentration of antibiotic in the first tube was 52 μg mL^{-1}, this solution was diluted serially in 8 steps. 1.5×10^{8} inoculums of the isolates were added to each concentration of ciprofloxacin in Muller Hinton Broth (MHB). A tube containing growth medium without ciprofloxacin and an un-inoculated tube were used as a positive and negative growth control respectively. Antibacterial activity was measured by determining MBC by culturing on MHA medium in a sterile Petri dish. Five microlitre of each tube streaked on MHA plates, the highest dilution that inhibits bacterial growth on MHA after overnight incubation was taken as MBC (Sahm and Weissfeld, 2002). *In vitro* resistance was defined as MBC of 4 or more μg mL^{-1} for isolates of *P. aeruginosa* (Chaudhry *et al*., 1999).

Determination of the isolates sensitivity to antibiotics: The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method (Bauer *et al*., 1966) with NCCLS breakpoints. *P. aeruginosa* ATCC27853 was used as reference strain. To represents the different classes of antimicrobial agents commonly used for the treatment of *P. aeruginosa* infections, we used Piperacillin (100 mcg), gentamicin (10 mcg), ofloxacin (5 mcg), ticarcillin (75 mcg), kanamycin (30 mcg), imipenem (10 mcg), amikacin (30 mcg), co-trimoxazole (1.25/23.75 mcg), ceftizoxime (30 mcg), ceftriaxone (30 mcg), carbenicillin (100 mcg) (Hi-media, Mombay, India).

RESULTS

A total of 100 *P. aeruginosa* isolates were collected from clinical specimens submitted to the hospital clinical microbiology laboratories of selected hospitals in Urmia, Iran. The frequency of isolates from each kind of clinical specimen has been shown in Table 1.

Sensitivity of bacterial isolates to ciprofloxacin: As shown 50 isolates (50% of all isolates) were resistant and the other isolates were sensitive to ciprofloxacin (Table 2).

Table 1: Types of specimens obtained from patients with *P. aeruginosa* infections

	Source of isolates					
	Urine	Wound	Sputum	Blood	Stool	Ear
No. of isolates	67	19	10	2	1	1
% of isolates	67	19	10	2	1	1

Table 2: The average of Minimum bactericidal concentrations (MBC)* of ciprofloxacin for 100 isolates of *P. aeruginosa*

	Minimum bactericidal concentrations (MBC) (μg mL^{-1})							
	0.39	0.78	1.56	3.12	6.25	12.5	25	50
No. of isolates	9S	17S	10S	14S	15R	19R	4R	12R
% of isolates	9	17	10	14	15	19	4	12

*: MIC amount for each isolate was equivalent to MBC, R: Resistant, S: Sensitive

Table 3: The rates of resistance to different antibiotics for 100 clinical isolates of *P. aeruginosa*

	Antibiotics										
	Ti	Co	Ck	Of	Cb	K	Cf	Pip	G	Ak	I
Resistance (%)	100	97	78	71	65	65	65	53	49	35	2

Ti: Ticarcillin, Ck: Ceftizoxime, Co: Co-trimoxazole, Ak: Amikacin, Cb: Carbenicillin, Cf: Ceftriaxone, Pip: piperacillin, I: Imipenem, K: Kanamycin, Of: Ofloxacin

Sensitivity of bacterial isolates to antibiotics: Thirty-five percent of isolates showed resistance to 10 antibiotics in adding to ciprofloxacin and were sensitive only to imipenem. Thirty five antibiotypes were recognized for all the isolates. The rates of resistances were showed in Table 3.

DISCUSSION

The data obtained from this research indicate that the prevalence of resistance of *P. aeruginosa* isolates to tested antibiotics was relatively high. Increasing resistance to the various anti-*pseudomonas* agents has been reported worldwide and this poses a serious problem in therapeutic management of *P. aeuroginosa* infections (Carmeli *et al*., 1999; Obritsch *et al*., 2004). Approximately 65% of *P. aeruginosa* isolates in this study were multi-drug resistant strains, however the prevalence of multi-drug resistance isolates of *P. aeruginosa* in the other studies are much lower than our study (Ohara *et al*., 2007).

Ciprofloxacin is a bactericidal, rapidly acting antimicrobial agent with a wide spectrum and is very effective against many gram negative bacterial pathogens, its effect against gram negative bacilli, including *P. aeruginosa*, is one of the most important features of this antibiotic (Algun *et al*., 2004).

In the present study, 50% of this isolates were resistant to ciprofloxacin (Table 2).

In another study has been done on 2067 clinical isolates of *P. aeruginosa* in United kingdom the resistance amount of isolates to ciprofloxacin were 7.3% (Lambert, 2002). In another survey has been done on *Pseudomonas aeruginosa* isolated from burn patients at two hospitals of Tehran, Iran in 2003, the resistance amount of isolates to ciprofloxacin were 86.7% (Shahcheraghi *et al*., 2003), however the higher resistance of burn isolates must be considered.

Chaudhry *et al*. (1999) reviewed *in vitro* sensitivities of ocular isolates of *P. aeruginosa* between July 1991 and September 1998. They found that only nine of 423 (0.021%) ocular isolates of *P. aeruginosa* were resistant to ciprofloxacin.

Reports from the United States have shown dramatic increases in antimicrobial resistance to ciprofloxacin (from 15 to 32%) however the overall incidence of ciprofloxacin resistance among *P. aeruginosa* isolates has been reported to range between 30 and 40% (Jones *et al*., 2002; Van Eldere, 2003).

In conclusion the present study is important from a practical point of view. Ciprofloxacin-resistant *P. aeruginosa* has been frequent in our clinical isolates, however there is not any burn isolates in our research (Table 1). This data and the previously reported by others (Kunimoto *et al*., 1999) remind the worldwide emerging resistance against ciprofloxacin. If the present trend continues, clinicians may encounter more frequent ciprofloxacin resistance among common *P. aeruginosa* isolates and this has a local and worldwide concern.

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