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The Resistance of *Pseudomonas aeruginosa* Strains Isolated from Cancer Patients to Various Groups of Antibiotics

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Abstract.- *Pseudomonas aeruginosa* causes a wide variety of infections in immunocompromised hosts such as cancer chemotherapy patients. Antimicrobial resistance among clinical isolates of *P. aeruginosa* is of great concern in these immunocompromised patients who have been hospitalized for extended periods of time and have received broad-spectrum antimicrobial therapy or cancer chemotherapy. Antibiotic resistance may complicate the treatment of infections and can adversely affect clinical outcomes and patient treatment costs. Surveillance of antimicrobial agents with activity against *P. aeruginosa* is thus very important. In the present study the in vitro activity of fluoroquinolones was compared with that of cephalosporins and aminoglycosides against 50 blood culture isolates of *P. aeruginosa* from hospitalized cancer patients. Susceptibility testing was performed by broth dilution method according to NCCLS guidelines. The overall respective MICs at which 50% and 90% of isolates inhibited (MIC_{50} and MIC_{90}) were as follows: ciprofloxacin, 4 and 8 µg/mL; ofloxacin, 16 and 64 µg/mL; pefloxacin, 16 and 128 µg/mL; ceftazidime, 16 and 64 µg/mL; amikacin, 32 and 128 µg/m; and tobramycin, 4 and 64 µg/mL. For the quinolones, the order of activity against *P. aeruginosa* strains was ciprofloxacin > ofloxacin > pefloxacin, norfloxacin. Among cephalosporins 90% of isolates of *P. aeruginosa* were resistant against ceftazidime, whereas resistance to amikacin and tobramycin was 43% and 50%, respectively.

Key words: Antibiotic resistance, MIC determination, Antibiotic sensitivity, *Pseudomonas aeruginosa*.

INTRODUCTION

Pseudomonas aeruginosa is one of the most important opportunistic bacteria, causing a wide variety of infections especially in immuno-compromised hosts such as burn patients, patients suffering from respiratory diseases like cystic fibrosis, and cancer chemotherapy patients (May *et al*., 1991; Hodges and Gordn, 1991; Govan and Deretic, 1996). Infections with *P. aeruginosa* is of greatest concern in critically ill and immuno-compromised patients who have been hospitalized for extended periods of time and have received broad-spectrum antimicrobial therapy or cancer chemotherapy (Kiska and Gilligan, 1999; Pollack, 2000). The spectrum of human infections caused by *P. aeruginosa* ranges from superficial skin infections to fulminant sepsis and is the leading cause of nosocomial respiratory infections (Kiska and Gilligan, 1999; Pollack, 2000)

P. aeruginosa is uually esistant to antimicrobials from several different structural classes. The development of resistance in *P. aeruginosa* is either intrinsically or through acquisition of genetic determinants for resistance over time. Most isolates of *P. aeruginosa* are resistant to ampicillin, amoxicillin-clavulanate, anti staphylococcal penicillins, narrow- and extended-spectrum cephalosporins tetracyclines, macrolides, rifampin, and chloramphenicol. *P. aeruginosa* is also resistant to ampicillin-sulbactam and trimethoprim-sulfamethoxazole. Antimicrobial resistance in *P. aeruginosa* may arise because of outer membrane impermeability, increased activity of multidrug efflux pumps, target site alterations, or enzymatic degradation (*e.g.*, aminoglycoside-modifying enzymes and ß-lactamases). Resistance to non-carbapenem ß-lactams in *P. aeruginosa* is most commonly associated with overproduction of a naturally produced cephalosporinase (AmpC) (Kiska and Gilligan, 1999; Pollack, 2000)

Surveillance of antimicrobial agents with activity against *P. aeruginosa* is very important among clinical isolates of *P. aeruginosa* because it may complicate the treatment of infections and can adversely affect clinical outcomes and patient

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treatment costs (Carmeli *et al*., 1999; Harris *et al.*, 1999). The present study was therefore investigated to determine the in vitro activities of cephalosorins, aminoglycosides and flouroquinolones antimicrobial agents against *P. aeruginosa* isolated from cancer patients. In this study, we compared the in vitro activities of different antimicrobial agents that are being used in our centre for the treatment of pseudomonal infections and studied their susceptibility and resistance pattern against these antimicrobial agents.

MATERIALS AND METHODS

The study was carried out at the Clinical Pathology Labs, Institute of Nuclear Medicine and Oncology over a period between January 2004 to June 2005.

Patients

All hospitalized cancer patients undergoing anticancer therapy with suspected blood stream infections were studied. No discrimination was made on the basis of age or gender. Patients already on antimicrobial therapy and those having fever due to non-infectious causes, such as blood transfusion, drug infusion etc. were excluded from the study.

Bacterial strains and culture conditions

A total of 50 *P. aeruginosa* isolates, isolated from blood cultures of patients treated between January and June 2004, were studied. Isolation was made by adding Five ml blood obtained from peripheral veins of the patients to brain heart infusion (BHI) broth (Oxoid, Hampshire, UK). The blood culture bottles were incubated at 37°C and regular subcultures were done. Identification of the *P. aeruginosa* isolates was done by Gram staining and standard biochemical tests according to the manual of clinical microbiology (Cheesbrough, 1984). Biochemical characterization of *P. aeruginosa* (Table I) was performed by oxidase, indole, urease and sugar fermentation tests according to Cheesbrough (1984). Identified *P. aeruginosa* stains were stored as glycerol stocks at – 70°C until use. *P. aeruginosa* ATCC 27853 was used as a quality control strain for the susceptibility test (NCCLS, 1997). Only one isolate/patient was used for sensitivity testing.

Table I.- Biochemical identification of *Pseudomonas aeruginosa* strains isolated from cancer patients.

Name of test	Results
Gram staining	-
Oxidase test	+
Indole test	-
Urease test	-
Citrate test	+
Sugar fermentation	
Glucose	-
Lactose	-
Kliger iron agar medium	
Slope	Red
Butt	Red
Gas	-
H_2S	-

Antimicrobial agents and MIC determination

Ciprofloxacin, pefloxacin, ofloxacin and norfloxacin was obtained from local commercial suppliers. MIC was determined in Mueller-Hinton broth (Oxoid, UK) containing serial two-fold dilutions of each antibiotic with inoculated bacterial suspensions of $5x10^5$ CFU/ml as outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 1997). The results were recorded after overnight incubation at 37°C. The MIC was defined as the lowest antibiotic concentration with no visible growth. The MIC_{50} and MIC_{90} was defined as the minimum concentration of antimicrobial that inhibited 50% or 90% of the isolates respectively. For ciprofloxacin, NCCLS breakpoints of $\leq$ 1 µg/mL (susceptible) and $\geq$ 4 µg/mL (resistant) were applied. For ofloxacin, NCCLS breakpoints of $\leq$ 2 µg/mL (susceptible), and $\geq$ 8 µg/mL (resistance) were applied. For pefloxacin, NCCLS breakpoints of $\leq$ 4 µg/mL (susceptible), and $\geq$ 16 µg/mL (resistant) were applied. For ceftazidime, NCCLS breakpoints of $\leq$ 8 µg/mL (susceptible), and $\geq$ 32 µg/mL (resistance) were applied. For amikacin, NCCLS breakpoints of $\leq$16 µg/mL (susceptible), $\geq$ 64 µg/mL (resistance) were applied. For Tobramycin, NCCLS breakpoints of $\leq$ 4 µg/mL (susceptible), $\geq$ 16 µg/mL (resistance) were applied (NCCLS 1997, 2002).

Table II.- ***In vitro*** **activities of different antimicrobial agents against** ***Pseudomonas eruginosa*** **isolates**

Antimicrobial agents	NCCLS resistance break point (µg/mL)	NCCLS susceptibility break point (µg/mL)	Range	Resistance (%)	Sensitivity (%)
Amikacin	64	16	0.5-256	43	47
Ciprofloxacin	4	1	0.125-64	80	20
Ceftazidime	32	8	0.5-256	90	10
Norfloxacin	16	4	0.5-256	100	0
Ofloxacin	8	2	0.5-256	95	5
Pefloxacin	16	4	0.5-256	100%	0%
Tobramycin	16	4	0.5-256	50%	50%

RESULTS

Characterization of P. aeruginosa *strains*

P. aeruginosa are Gram negative, motile bacteria. These are oxidase positive, catalase positive, indole negative, urease negative and citrate positive bacteria. (Table I). It produces a characteristic pink-red slope and butt. Fifty *P. aeruginosa* strains characterized above were then used for determination of MIC against different antimicrobial agents.

MIC determination

The overall susceptibility result was shown in Table II. Our results indicate that ciprofloxacin showed high activity against *P. aeuginosa* strains. The MIC_{50} of ciprofloxacin was 4 µg/mL and ranged from 0.125-64 µg/mL. Twenty percent of isolates of *P. areuginosa* were inhibited at concentration of 1 µg/mL. However it inhibited all the isolates of *P. areuginosa* at concentration of 32 µg/mL. Ofloxacin have moderate activity against *P. areuginosa* with MIC ranging from 0.5-256 µg/mL with more than 90% of isolates being susceptible at a concentration of 64 µg/mL and 95% resistant at 16 µg/mL (resistant break point). *P. areuginosa* isolates were found less susceptible to other fluoroquinolones, pefloxacin and norfloxacin. The activity of pefloxacin and norfloxacin was least active against *P. areuginosa* isolates, each demonstrating MIC_{90} of 128 µg/mL respectively. A high rate of resistance was observed among the tested strains with 80%, 95%, 100% resistance for ciprofloxacin, ofloxacin, pefloxacin and norfloxacin respectively. The order of activity of quinolones among *P. areuginosa* was ciprofloxacin > ofloxacin > pefloxacin = norfloxacin.

For 3rd generation cephalosporins (ceftazidime) 50% of *P. areuginosa* isolates have MIC 16 µg/mL ranging from 0.5-256 µg/mL and only 10% of isolates were susceptible at 8 µg/mL. In case of aminoglycosides (amikacin and tobramycin) good activity was seen against *P. aeruginosa* strains with MIC ranging from 0.5-256 µg/mL. For amikacin 50% isolates have MIC 32 µg/mL and 90% have MIC128 µg/mL. About 47% strains were susceptible at 16 µg/mL whereas 50% of isolates were susceptible at concentration 4 µg/mL in case of tobramycin.

Therefore order of activity of these drugs against our *P. aeruginosa* isolates was Amikacin > Tobramycin > Ciprofloxacin > Ceftazidime >. Ofloxacin > Pefloxacin = Norfloxacin.

DISCUSSION

The potential for antimicrobial resistance is an important concern for clinicians treating patients with confirmed or suspected *P. aeruginosa* infections as they are often resistant to a broad range of antimicrobial agents. The results of the present study indicated the *in vitro* activities of fluoroquinolones, cephalosporins and aminoglycosides against blood culture isolates of *P. aeruginosa*. It is clear that among fluoroquinolones, ciprofloxacin have fivefold greater in vitro activities against *P. aeruginosa* than either pefloxacin or ofloxacin. Though ciprofloxacin was found active against isolates of *P. areuginosa* but only 20% of these being susceptible at 1 µg/mL susceptibility

break point whereas 80% strains were resistant. The MIC_{50} and MIC_{90} of *P. areuginosa* isolates against ciprofloxacin were 4 and 8 µg/mL respectively. The MIC_{90} for Ofloxacin against bacterial isolates was 64 µg/mL which is eight fold higher than Ciprofloxacin. In case of Pefloxacin 90% of isolates have MIC128 µg/mL with none of the isolate being susceptible at NCCLS susceptibility break point (16 µg/mL). Lowest invitro activities of pefloxacin, norfloxacin was observed among floroquinolones. *In vitro* activity against *P. areuginosa* showed that MIC values of ciprofloxacin are generally lower than other floroquinolones but higher than recommended by NCCLS. Among cephalosporins, ceftazidime is the most commonly used antipseudomonal agent had 90% resistance. However, good activity was observed for aminoglycosides against *P. areuginosa* with 47% and 50% of isolates sensitive to amikacin and tobramycin respectively.

In the present study, *P. aeruginosa* demonstrated high resistance to fluoroquinolones and cephalosporin group of antibiotics whereas better activity against aminoglycoside group of antibiotics was observed.

Bonfiglio *et al.* (1998) studied the current resistance level of widely used antipseudomonal antibiotics in more than one thousand clinical isolates of *P. aeruginosa* and found susceptibility level for ceftazidime as 13.4%; amikacin, 10.6%; and ciprofloxacin 31.9%.

A survey of bloodstream infections due to gram-negative bacilli and frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY Antimicrobial Surveillance Programme also showed high resistance to third-generation cephalosporins and ciprofloxacin (Diekema *et al*., 1999).

A four-year evaluation of frequency of occurrence and antimicrobial susceptibility patterns of bacteria from bloodstream infections in Latin American medical centers was studied (Sader *et al*., 2002) where resistance rates among Gram-negative bacilli were much higher. *P. aeruginosa* resistance rates to amikacin, and ciprofloxacin showed a significant increase during the 4-year period evaluated. Resistance rates to most of antimicrobial agents for a number Gram-negative rods involved implicated in bacteremia, has reached worrisome levels and continues to increase.

A decreased susceptibility to fluoroquinolone was observed among gram negative isolates in Taiwan after wide use of these antimicrobial agents in different study periods was reported (Sheng *et al*., 2002). Similarly significant resistance to fluoroquinolones was also reported by Madhusudhan *et al*. (2003).

In Europe antimicrobial susceptibility of isolates from 3136 bacteraemic versus17261non-bacteraemic patients was reported in MYSTIC surveillance programme. Ceftazidime, gentamicin and ciprofloxacin generally exhibited the lowest activities against the most commonly isolated organisms (Unal *et al*., 2004).

Antimicrobial susceptibility of the pathogens of bacteraemia in the UK and Ireland between 2001-2002 and resistance rates of *P. aeruginosa* to ciprofloxacin, ceftazidime was between 4% and 7% (Reynolds *et al*., 2004).

Bouza *et al.* (1999) reported the resistance rates of 1,014 isolates from different public hospitals of Spain and reported ≤15% of resistance among all isolates to amikacin, ceftazidime and tobramycin. Resistance to quinolones was higher 24.5% than other antimicrobial agents. However, resistance level was higher among isolates from intensive care units of hospitalized patients.

The current study was important as changing and easy acquisition of resistance in *P. aeruginosa* require rapid surveillance procedures to represent the whole reality of a situation at a given time in single institution as opposed to other studies that involved contributing centers and large number of isolates. The present study provided important information on the current resistance pattern among our isolates against flouroquinolones and cephalosporins as compared to other antimicrobial agents. Resistance against fluoroquinolone and cephalosporins appears to be increasing more rapidly than to other agents. It can also be argued that according to the accepted selective pressure theory hypothesis a causal relationship exists between antimicrobial use and development of

resistance. An extensive use of ceftazidime and ciprofloxacin in our wards either for therapy or antibacterial prophylaxis might have contributed to such high resistance rates. On the other hand, additional resistance mechanisms, especially production of extended-spectrum β-lactamases (ESBLs) and other enzymes in ceftazidime resistance or gyrase gene mutation in fluoroquinolone resistance might have contributed to higher resistance levels that could be investigated in further studies.

In conclusion, antimicrobial resistance rates are increasing and require vigilance with respect to both the appropriate use of antimicrobial agents and continued surveillance for changes in rates of resistance among *P. aeruginosa* infections. A careful monitoring of antimicrobial use in hospitals is required to identify situations in which prescription patterns are contributing to the development of resistance. There is a need of constant monitoring at national, regional level as these surveillance efforts are imperative to provide clinicians with information for choosing empirical treatment regimens.

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