REPORT

PREVALENCE AND RESISTANCE PATTERN OF *PSEUDOMONAS AERUGINOSA* AGAINST VARIOUS ANTIBIOTICS

JAMSHAID ALI KHAN, ZAFAR IQBAL, SAEED UR RAHMAN*, KALSOOM FARZANA AND ABBAS KHAN**

Department of Pharmacy University of Peshawar, Pakistan
**Postgraduate Medical Institute Hayatabad Medical Complex, Peshawar, Pakistan*
***Faculty of Pharmacy, Bahauddin Zakariya University, Multan*

ABSTRACT

A prospective study on various clinical isolates from patients admitted from various parts of NWFP and Afghanistan at Post Graduate Medical Institute (PGMI) Hayatabad Medical Complex, Peshawar was conducted from January 2000 to December 2004 to ascertain the prevalence and antimicrobial susceptibility of *Pseudomonas aeruginosa* infections. Among 4709 positive isolates, 314 (6.67%) were *Pseudomonas aeruginosa*. The highest rate of infection due to *Pseudomonas aeruginosa* was observed in orthopedic ward (24.61%) and OPD (20%), in other wards the infection was between 13% to 1.5%. Gender-wise prevalence showed 61.78% male and 38.22% females were infected by *Pseudomonas aeruginosa.*

The highest percentage of *Pseudomonas aeruginosa* isolates were observed in pus (57.64 %) and urine (24.2 %) samples. Maximum *Pseudomonas aeruginosa* isolates were found between March to August and the highest percentage 13.846% was observed in June. Using the disc diffusion method, the resistance patterns of 314 isolates against 14 antimicrobial agents were determined. The highest resistance was observed against ampicillin (≥ 98.4%), ampicillin/ sulbactam (85.3%), co-amoxiclave (83.8%) and ofloxacin (68.4%) and least resistance was observed against amikacin (24%). Similarly the MIC for ampicillin (4 to >2048 µg/ml), ampicillin/sulbactam (1 to 2048 µg/ml) and co-amoxiclave (1 to >2048 µg/ml) against clinical isolates of *Pseudomonas aeruginosa* was also high. High resistance of *Pseudomonas aeruginosa* against various commonly used antibiotics showed the alarming situation. The control of drug resistant *Pseudomonas aeruginosa* required rational prescribing and proper use of antibiotics.

Keywords: *Pseudomonas aeruginosa*, Prevalence, nosocomial infections, MIC, antibiotics.

INTRODUCTION

Despite advance in medical and surgical care and introduction of wide variety of antimicrobial agents against anti-pseudomonal activities, life threatening infection caused by *Pseudomonas aeruginosa* continue to cause complications in hospital acquired infections. It contributes substantially to wound related morbidity and mortality world wide (Mayhall, 1996).

Pseudomonas aeruginosa is primarily an opportunistic pathogen that causes infections in hospitalized patients particularly in burns patients where the skin host defenses is destroyed, orthopedic related infection, respiratory diseases, immunosppressed and catheterized patients. It may be the cause of the chronic debilitating pulmonary infection, which is one major cause of death in-patients with cystic fibrosis (Govan, 1992). Generally it contributes substantially to wound related morbidity and mortality world wide (Mayhall, 1996).

The organism enters into the blood, causing sepsis that may spread to the skin and lead to ecthyme gangrenosum, the black necrotic lesion. Several external otitis and skin lesion occurs in swimming pools and hot tubs users, particularly where chlorination is inadequate. *Pseudomonas aeruginosa* is the most common cause of osteochondritis of the foot, corneal infections caused by contact lens user (Warren, 2000), corneal ulceration (Dark, 1988), endocarditis (Fang *et al.*, 1993) and in neurosurgery unite associated with contaminated sinks/water (Bert *et al.*, 1998).

Infections due to *Pseudomonas aeruginosa* are seldom encounter in healthy adults but in last two decades the organism has become increasingly recognized as the etiological agent in a variety of serious infection in hospitalized patients with impaired immune defense (John Smith *et al.*, 2000) including HIV infections (Donnel, 1993; Flores *et al.*, 1993).

**Corresponding author:* Tel: +92-91-9216750, e-mail: zafar_iqbal@upesh.edu.pk

MATERIAL AND METHODS

Study location

The antimicrobial susceptibility studies were performed at PGMI Hayatabad Medical Complex, Peshawar. This hospital provides the health facilities not only to the local's population but also to the other parts of the province and immigrants from Afghanistan.

Bacterial isolates

Pseudomonas aeruginosa isolated from various samples collected from different wards of hospitals and OPD department using standard laboratory procedures (Gilardi, 1991) for the isolation and identification of pathogen. The Muller-Hinton agar (Oxoid, U.K.) medium was used for the growth of the bacteria. The susceptibility of various antibiotics against clinical isolates of *Pseudomonas aeruginosa* was determined using appropriate antimicrobial susceptibility antibiotics discs (Oxoid, U.K.) using disc diffusion method recommended by National Committee for Clinical Laboratories Standards (NCCLS, 1993). The *Pseudomonas aeruginosa* (ATCC 27853) was used as control organism. The results were interpreted as susceptible, intermediate susceptible or resistant by measuring the diameter of zone of inhibition, according to the criteria designed by NCCLS 1993.

Determination of minimum inhibitory concentrations (MIC)

Stock solutions of selected antibiotics were prepared according to their labeled potencies and stored immediately at -70°C.

Agar dilution method was used to determine the minimum inhibitory concentrations (MIC). Serial two-fold dilution of amikacin (AK), imipenem (IMP), meropenem (MEM), ceftazidime (CAZ), enoxacin (ENO), piperacillin/ tazobactam (TZP), sparfloxacin (SPF), azteronam (ATM), tobramycin (TOB), gentamycin (GM), ofloxacin (OFX), co-amoxiclave (AUG), ampicillin/sulbactam (SAM) and ampicillin (AMP) were tested against *Pseudomonas aeruginosa* (Monica, 1991)

Four to five well isolated colonies of various *Pseudomonas aeruginosa* isolates from a blood agar plates were inoculated in tube containing 5 mL of tryptone soya broth (Oxid, U.K.) and incubated at 35°C until it achieved or exceeded the turbidity of 0.5 McFarland standard, then adjusted using sterile saline to give the density equivalent approximately 10^8cfu/ml. It was then further diluted to give 10^4 cfu/mL and 10 µL of this inoculum was transferred on Mueller-Hinton agar plates containing 4% sodium chloride and different concentrations of antibiotics. The plates were incubated at 37°C for 18-24 hours (Monica, 1991; Jennifer, 2001).

RESULTS AND DISCUSSION

Pseudomonas aeruginosa emerged as an important pathogen and responsible for the nosocomial infections that is one of the important causes of morbidity and mortality among hospital patients. The objective of the present study was to evaluate the epidemiological data of *Pseudomonas aeruginosa* strains in patients treated at PGMI, Hayatabad Medical complex, Peshawar and to evaluate the antimicrobial susceptibility patterns of bacteria against various antibiotics. During 2000 to 2004, total 4709 samples exhibited the growth of micro-organisms, among these 314 (6.67%) demonstrated the growth of *Pseudomonas aeruginosa*. The present study

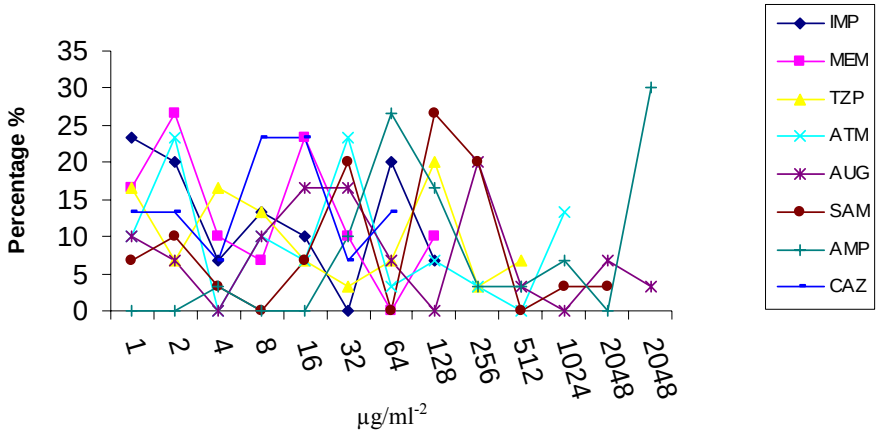

Fig. 1: MIC of different ß-Lactam antibiotics against clinical isolates of Pseudomonas aeruginosa.

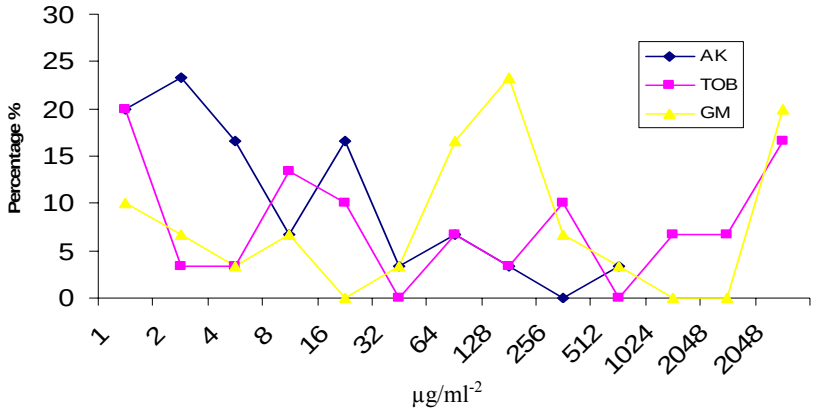

Fig. 2: MIC of amino glycosides against clinical isolates of *Pseudomonas aeruginosa.*

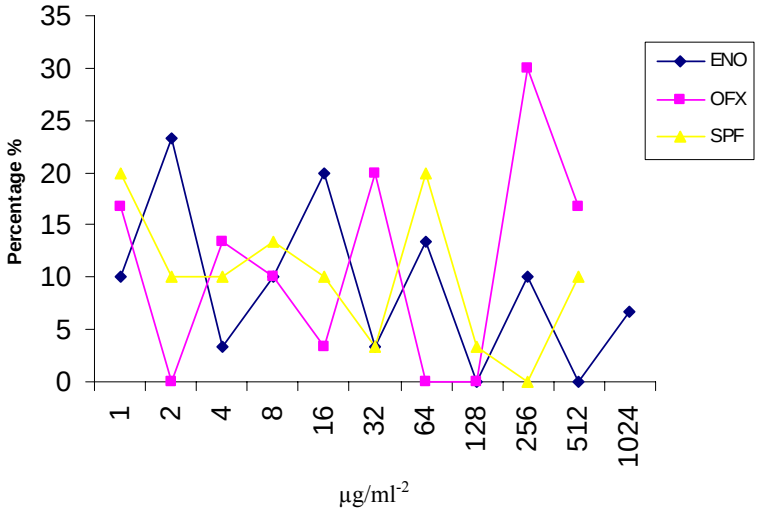

Fig. 3: MIC of quinolones against clinical isolates of *Pseudomonas aeruginosa.*

shows that that the prevalence of *Pseudomonas aeruginosa* is also a serious problem in other countries (Agarwal *et al.*, 2006; Ako *et al.*, 2006; Balkhy *et al.*, 2006; Lizioli *et al.*, 2003).

In present studies, the highest percentage (24.61%) of *Pseudomonas aeruginosa* infections were observed in orthopedic ward, 20% in OPD, 13.0% in general medical ward, 7.69% in gynecology and obstetrics, 6.15% in ICU, and 6.15% in surgical, 3.08% in ENT, 1.54% in plastic surgery. The gender-wise prevalence of clinical isolates showed that infections caused by of *Pseudomonas aeruginosa* are very common in male (61.78%) compared with female (38.22%).

In present studies, maximum clinical isolates of *Pseudomonas aeruginosa* were isolated from the pus samples (57.64%), followed by urine (24.2%), CSF 5.4%, HVS 2.3%, blood 3.18% and miscellaneous 7.32%. These results are in line with other finding, where prevalence of *Pseudomonas aeruginosa* was high in clinical samples of pus and urine (Arshi *et al.*, 2007; Murase *et al.*, 1995)

Pseudomonas aeruginosa isolated from various clinical samples has lost susceptibility and showed increasing resistance to β-lactamase inhibitor antibiotics; ampicillin/ sulbactam 85.3%, co-amoxiclave 83.8% while ampicillin showed ≥ 98.4%. Increasing resistance of *Pseudomonas aeruginosa* against β-lactamase inhibitor antibiotics may be due to excessive β-lactamase production and/or active efflux mechanism may also contribute to the full expression of β-lactam resistance in *Pseudomonas aeruginosa*. Multi drug efflux pumps in the inner and outer membrane of *Pseudomonas aeruginosa* may protect the bacterium from to β-lactam agents (Srikumar *et al.*, 1997).

In present studies the resistance against ofloxacin and sparfloxacin was observed 68.4% and 39%, respectively. The quinolone resistant *Pseudomonas aeruginosa* showed

the presence of new outer membrane protein in the range of 51-54 KDa. These proteins apparently actively transport quinolone out of the cell (John Smith *et al.*, 2000). The resistance pattern against gentamycin 67.8%, tobramycin 44%, aztreonam 37%, piperacillin/tazobactam 35.1%, enoxacin 33%, ceftazidime 30.2%, meropenem 28%, imipenem 26.7%, and amikacin 24% was also observed in this study. The amikacin, imipenem and meropenam showed high susceptibility against *Pseudomonas aeruginosa* in this study. These finding are in good agreement with the other similar studies (Van, 2003)

The MIC of 14 antibiotics was determined against 30 clinical isolates of *Pseudomonas aeruginosa*. The susceptibility of each bacterial isolates to these antibiotics tested varied greatly, ranging from 1 to > 2048 µg/ml. Amikacin (Fig.2) exhibited the MIC range of 1 to 512 µg/ml. Maximum clinical isolates of *Pseudomonas aeruginosa* (23.33%) were inhibited at 2 µg/ml, 20% at 1 µg/ml and 16.67% at 4 µg/ml and 16 µg/ml each. Imipenem (fig.1) showed MIC range of 1 to 128 µg/ml. 23.33 % clinical isolates of *Pseudomonas aeruginosa* inhibited at 1 µg/ml and 20% fell in the category of 2 µg/ml and 64 µg/ml each. Meropenem exhibited the range of 1 to 128 µg/ml. Maximum clinical isolates of *Pseudomonas aeruginosa* (26.67%) were inhibited at 2 µg/ml, it was followed by 23.33% at 16 µg/ml. Ceftazidime exhibited the range of 1 to 64 µg/ml. Maximum isolates (23.33%) inhibited at 8 and 16 µg/ml. Enoxacin (fig.3) showed MIC range of 1 to 1024 µg/ml. 23.33% isolates inhibited at 2 µg/ml and 20% inhibited at 16 µg/ml. Piperacillin/tazobactam exhibited MIC range of 1 to 512 µg/ml. Maximum isolates (20%) fell in the category of 128 µg/ml. These results are consistance with other findings (Elhag, 1999; Al-Lawati, 2000). Similarly sparfloxacin showed MIC range of 1 to 512 µg/ml. Maximum isolates (20%) exhibited MIC at 1 µg/ml and 64 µg/ml. Azteronam showed MIC range of 1 to 1024 µg/ml and maximum isolates (23.33%) inhibited at 2µg/ml and 32µg/ml each. Tobramycin exhibited broadest range 1 to >2048 µg/ml. 20% isolates showed MIC level at 1µg/ml, followed by 16.67% at 2048 µg/ml. Gentamycin exhibited MIC range 1 to > 2048 µg/ml. Maximum isolates (23.33%) inhibited at 128 µg/ml and 20% at >2048 µg/ml. Ofloxacin showed MIC range of 1 to 512 µg/ml. Maximum clinical isolates of *Pseudomonas aeruginosa* inhibited at 256 µg/ml. MIC range of co-amoxiclave was 1 to > 2048 µg/ml and maximum isolates of *Pseudomonas aeruginosa* (20%) were inhibited at 256 µg/ml. Ampicillin/sulbactam showed MIC rang of 1 to 2048 µg/ml and maximum isolates (26.67%) fell in the category of 128 µg/ml. *Pseudomonas aeruginosa* demonstrated poor susceptibility toward β-lactam/β-lactamase inhibitors combination drugs for treatment, is a continuing problem (Carmeli *et al*, 1999). Ampicillin exhibited the broadest rang 4 to >2048 µg/ml. Maximum isolates inhibited (30%) at >2048 µg/ml followed by 26.67% at 64 µg/ml. The comparable MIC of ampicillin and gentamycin with the present findings were also observed against clinical isolates of *Pseudomonas aeruginosa*, *E. coli* and *S. aureus* (Odelola *et al.*, 1989).

CONCLUSION

Pseudomonas aeruginosa is one of the most important bacterial pathogen seriously contributing the problem of hospital infection, particularly in orthopedic related infection, burn patient, immunosuppressant and catheterized patients. Amikacin, imipenem and ceftazi-dime were found to be the most effective drug while ampicillin, ampicillin/sulbactam, coamoxiclave, ofloxacin and gentamycin were showed maximum resistance to *Pseudomonas aeruginosa.* Drug resistance to *Pseudomonas aeruginosa* is rapidly increasing. The antimicrobial agents are losing their efficacy because of the spread of resistant organism due to indiscriminate use of antibiotic, lack of awareness, patient non compliance, and unhygienic condition.

The solution can be planned by continuous efforts of clinician, microbiologist, pharmacist and community to promote greater understanding of this problem, better hygiene, post operative care and management.

REFERNCES

Agarwal R, Dheeraj Gupta and Pallab Ray (2006). Epidemiology, risk factors and outcome of nosocomial infections in a Respiratory Intensive Care Unit in North India. *Journal of infection,* **53**(2): 98-105.

Ako-Nai A K, Ikem IC, Akinloye OO, Aboderin AO, Ikem RT and Kassim OO (2006). Characterization of bacterial isolates from diabetic foot infections in Ile-Ife, South western Nigeria. *The Foot*, **16**(3): 158-164.

Al-Lawati AM, Crouch ND and Elhag KM (2000). Antibiotic consumption and development of resistance among Gram negative bacilli in intensive care units in Oman. *Ann. Saudi Med*., **20**: 324-327.

Arshi S, Manzoor T, Syed S and Assadullah S (2007). *In vitro* sensitivity pattern of *Pseudomonas aeruginosa* strain isolated from patient skims-role of antimicrobial in the emergence of multiple resistance strains. *J. K. Practitioner*, **14**(1): 31-34.

Balkhy HH, Gwen Cunningham and Fong Khew Chew (2006). Hospital- and community-acquired infections: a point prevalence and risk factors survey in a tertiary care center in Saudi Arabia. *International Journal of Infectious Diseases*, **10**(4): 326-333.

Bert F, Maubec and Bruneau B (1998). Multi-resistance *Pseudomonas aeruginosa* out break associated with contaminated tape water in a neurosurgery intensive-care unit. *Journal of Hospital Infection*, **39**: 53-62.

Carmeli Y, Troillet N, Eliopoulos GM and Samore MH (1999). Emergence of antibiotic- resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antiseudomonal agents. *Antimicrob. Agents Chemother*., **43**:1379-1382.

Dark JKG (1988). Pathogenesis and therapy of *Pseudomonas aeruginosa*. *Eye*, **2**: 46-55.

Donnel DGO (1993). Sinusitis due to *Pseudomonas aeruginosa* in patients with human immunodeficiency virus infection. *Clin. Infect. Dis*., **16**: 404-406.

Elhag KM, Reed M and Al-Lawaty HM (1999). The prevalence of antibiotic resistance among Gram negative bacilli from intensive care units in Oman. *Saudi Medical Journal*, **20**: 373-377.

Fang G, Keys TF and Gentry LO (1993). Prosthetic value endocarditis resulting from nosocomial bacteremia. A prospective, multicenter study. *Ann. Intern. Med*., **119**: 560-567.

Flores G, Stavola JJ and Noel GJ (1993). Bacteremia due to *Pseudomonas aeruginosa* in children with AIDS. *Clin. Infect. Dis*., **16**: 706-708.

Gilardi GL (1991). Pseudomonas and related genera. *Manual of clinical microbiology*, 5th edn. Washington DC: American Society for Microbiology, pp.429-441.

Govan JRW (1992). Microbiology of lung infection in cystic fibrosis. *British Medical Bulletin*, **48**: 912-930.

Jennifer M Andrews (2001). Determination of minimum inhibitory concentration. *Journal of Antimicrobial Chemotherapy*; **48**(Suppl-SI): 5-16.

John Smith MB, John Payne E, Thomas Berne V (2000). *The surgeons Guide to antimicrobial Chemotherapy*, pp.38-74.

Lizioli A, Privitera G and Alliata E (2003). Prevalence of nosocomial infections in Italy: result from the Lombardy survey in (2000). *Journal of Hospital Infection*, **54**(2): 141-148.

Mayhall CG (1996). Nosocomial burn wound infection. Mayhall GC Ed. *Hospital epidemiology and infection control.* William and Wilkins Co., Baltimore, MD, USA, pp.225-236.

Monica Cheesbrough (1991). *Medical Laboratory Manual for Tropical Countries*, Volume-II. University Press Cambridge (UK), pp.286-287.

Murase M, Miyamoto H, Handa T, Sahaki S and Takenchi N (1995). Activities of antipseudomonal agent against clinical isolates of *Pseudomonas aeruginosa. Jpn. J. Antibiot.*, **48**(10):1581-1589.

National Committee for Clinical Laboratory Standards (1993). Performance standards for antimicrobial disc susceptibility tests. 5th ed. Approval standard M2-A5.*National Committee for Clinical Laboratory Standards*. Villanova. Pa.

Odelola HA, Jaiyebo O, Anukamand PA and Akannia AO (1989). Comparative in vitro antibacterial activity of two brands of antibiotics against clinical isolates of some bacterial genera. *Afr. J. Med. Sci*., **18**: 307-310.

Srikumar R, Li XZ and Poole K (1997). Inner membrane efflux components are responsible for β-lactam specificity of multi drug efflux pumps in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **179**: 7875-7881.

Van Elder J (2003). Multicentre surveillance of *Pseudomonas aeruginosa* susceptibility pattern in nosocomial infection. *J. Antimicrobial Chemotherapy*, **51**: 347-352.

Warren Levinson, (2000). Ernest Jawetz. Medical Microbiology and Immunology, 6th edn. McGraw-Hill, New York, p.123.