REPORT

PREVALENCE AND RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA AGAINST VARIOUS ANTIBIOTICS

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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, immunospressed 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).
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), gentamicin (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 10⁸ cfu/ml. It was then further diluted to give 10⁴ 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 microorganisms, among these 314 (6.67%) demonstrated the growth of *Pseudomonas aeruginosa*. The present study
shows 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 *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-amoxiclav 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 quinoline out of the cell (John Smith et al., 2000). The resistance pattern against gentamicin 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 meropenem 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 consistence 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 cefoxacile was 1 to > 2048 µg/ml and maximum isolates of *Pseudomonas aeruginosa* (20%) were inhibited at 256 µg/ml. Ampicillin/sublactam showed MIC range 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 amoxicillin 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 ceftazidime were found to be the most effective drug while ampicillin, ampicillin/sublactam, cefoxacile, 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.

**REFERENCES**


