FREQUENCY AND SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA IN PUS SAMPLES

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ABSTRACT

Objectives: The aim of this study was to isolate *Pseudomonas aeruginosa* as causative agent of pus and to determine the drug sensitivity of the identified organisms.

Material and Methods: In this study a total of 20 *P. aeruginosa*-positive pus cultures were obtained out of 272 and tested for ten antibiotics (Gentamicin, Imipenem, Amikacin, Norfloxacin, Tobramycin, Ciprofloxacin, Aztreonam, Cephalosporin, Cefotaxime, Sulzone.

Results: The highest rates of resistance were found for Cephradine (90%), Cefotaxime (75%), Aztreonam (55%), Gentamicin (40%), Sulzone (35%), Tobramycin (30%), Amikacin and Ciprofloxacin (25% each), while low resistance was seen in case of Imipenem (10%), Norfloxacin (5%) as compare to other antibiotics.

Conclusion: Isolates of *P. aeruginosa* from pus showed highest sensitivity (90%) to imepenem while maximum resistance was showed to Cephradine (90%) and Cefotaxime (75%).

Key Words: *Pseudomonas aeruginosa*, opportunistic infections, pus cultures, antibiotics.

INTRODUCTION

Pseudomonas aeruginosa is one of the most common gram-negative microorganisms identified in the clinical specimens of hospital admitted patients. It is a commensal of human microflora in healthy people and is frequently isolated as an opportunistic pathogen in recurrent infections of hospitalized patients[1]. It can infect almost any external site or organ, and therefore, can be isolated from various body fluids such as sputum, urine, wounds, eye or ear swabs and from blood[2]. This organism is often hard to treat because of both the intrinsic resistance and acquire resistance i.e. mutations in chromosomal genes, to multiple groups of antimicrobial agents, including β-lactams, aminoglycosides and fluoroquinolones[3,4]. An increased resistance of *P. aeruginosa* to β-lactam drugs is because of producing metallo-beta-lactamases i.e. enzymes that efficiently hydrolyze all β-lactams[5]. The implication of these emerging resistance in the successful treatment of infections caused by *P. aeruginosa* cannot be overemphasized[6]. It causes infections in hospitalized patients particularly in burns, orthopedic related infection, respiratory diseases, immunosuppressed and catheterized patients. Inherently resistant to many antimicrobial agents, it also contributes substantially to wound related morbidity and mortality worldwide[7]. Keeping in view the occurrence of Pseudomonas spp in different habitat, its pathology and resistance to antibiotics, this study was aimed to isolate *P. aeruginosa* from pus sample and to determine its antibiotic susceptibility profile.

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MATERIALS AND METHODS

This study was conducted at the Microbiology Department of Abasyn University Peshawar from June to August 2011.

Isolation, characterization and identification:

Pus samples were collected from Hayatabad Medical Complex, Peshawar and brought to Microbiology laboratory of Abasyn University for further processing. Blood agar, MacConkey agar and Cysteine Lactose Electrolyte Defient (CLED) agar were used as growth media for the culturing of samples[8,9]. Each sample was inoculated on Blood, MacConkey and CLED agar with the help of wire loop. The plates were then incubated at 37°C for 24 hours to get the growth/colonies. Positive samples were then processed further for identification using standard operating procedures. Gram staining was used to differentiate and identify gram positive and gram negative bacteria. For the confirmation of *P. aeruginosa*, biochemical tests including Indole test, Triple Sugar Iron (TSI) test, Urease test, Simmon's Citrate test, Oxidase test and Motility test were performed[10].

Antibiotic susceptibility Testing

Antibiotic susceptibility pattern of *P. aeruginosa* to different antibiotics was confirmed by standard Kirby-Bauer disc diffusion method[11]. Muller Hinton agar was

prepared and sterilized by autoclaving at 121° for 15 min. 25 ml of media was poured in 90 mm sterile Petri dishes and incubated at 37°C overnight to check sterility. All the clinical isolates of *Pseudomonas aeruginosa* were tested for their sensitivity against antimicrobials including: imipenem, norfloxacin, amikacin, cefotaxime, tobramycine, cephradine, aztreonam, gentamicin, ciprofloxacin and sulzone of standard strengths. The plates were incubated at 37°C for 18 h and after incubation, plates were examined for zones of inhibition and reported the organism sensitive, intermediate, resistant according to national committee for control laboratory standards[12].

RESULTS

A total of 272 pus samples were processed for isolation of *Pseudomonas aeruginosa*. Out of 272 samples, 20 (7.35%) were found positive for *Pseudomonas aeruginosa* (Fig. 1). Out of 20 positive samples for *P. aeroginosa* 12 (60%) were isolated from males while 8 (40%) were from females (Fig. 2). Different biochemical tests were conducted for identification and Characterization of *P. aeruginosa*. Culture sensitivity testing of these samples was conducted against 10 most commonly used antibiotics for Pseudomonal infections by means of Disc diffusion method[11].

The antibiogram (Table 1) of *P. aeruginosa* showed that most of the isolates 18 (90%) were highly sensitive to Imepenem. The sensitivity of isolates against Imepenem was then followed by Norfloxacin 17 (85%) > Tobramycin 10 (50%) > Amikacin 8 (40%) > Gentamicin and Ciprofloxacin 6 (30% each) > Aztreonam 3 (15%) > Cefotoxime and Sulzone 2 (10% each) and Cephradine 1 (5%). Most of the isolates showed resistance to Cephradine i.e. 18 (90%) while Norfloxacin 1 (5%) was found least resistant in the sensitivity profile. Some of the antibiotics used to determine their efficiency against *P. aeruginosa* isolates from pus samples showed an intermediate zone of inhibition i.e. Sulzone showed an intermediate response to about 11 (55%) isolates followed by

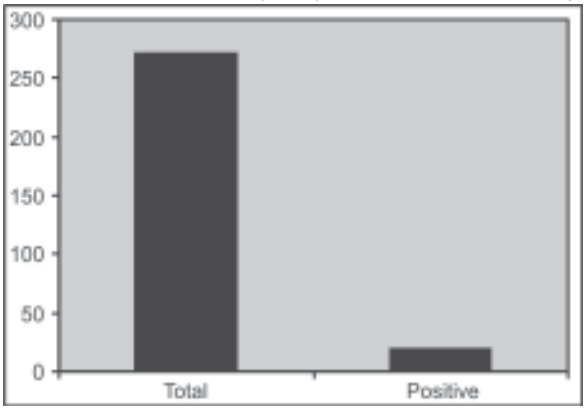

Fig. 1: Frequency of *Pseudomonas aeruginosa* in pus samples

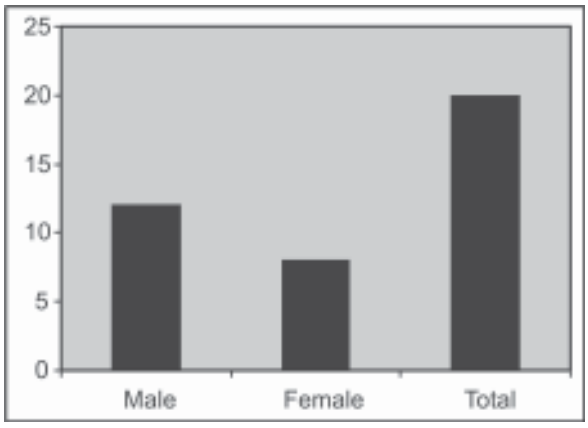

Fig. 2: Distribution of *Pseudomonas aeruginosa* among males and females

Table 1: Sensitivity profile of selected antibiotics against *P. aeroginosa* isolated from pus samples

S. No.	Antibiotics	Resistant	Intermediate	Susceptible
1	Imepenem	2	0	18
2	Norfloxacin	1	2	17
3	Amikacin	5	7	8
4	Sulzone	7	11	2
5	Cefotoxime	15	3	2
6	Tobramycin	6	4	10
7	Cephradine	18	1	1
8	Aztreonam	11	6	3
9	Gentamicin	8	6	6
10	Ciprofloxacin	5	9	6

Ciprofloxacin 9 (45%) > Amikacin 5 (35%) > Aztreonam and Gentamicin 6 (30% each) > Tobramycin 4 (27%) > Cefotoxime 3 (20%) > Cephradine and Norfloxacin 1 (5% each).

DISCUSSION

P. aeruginosa is involved in the etiology of several diseases mostly found in pus infections. Our result showed that among 272 patients, *P.aeruginosa* account for 7.35% pus infections. Akhter et al.[13] did similar study and reported 18% positive pus samples for *P. aeruginosa* while Amadi et al. 14 reported 64% pus samples infected with *P. aeruginosa*. The rapid and inappropriate use of antibiotics is responsible for the development of resistance in Pseudomonas species.

The antibiogram studies showed that *P. aeruginosa* was highly susceptible to Imipenem followed by Norfloxacin, Tobramycin, Amikicin, Gentamicin, Ciprofloxacin, Aztreonem, Cefotoxime, Sulzone and Cephradine. A similar antibiogram was obtained by Anjum and Mir[2] which indicates that *P. aeruginosa* was highly sensitive to Imipenem followed by other antibiotics. It might be expected that difference in response to different antibiotics is due to some drugs sold in developing countries do not contain the concentration of active substances stated on their labels even at the time of manufacture.

In our study, high resistance was faced by Cefradine (90%) against *P. aeruginosa* which was followed by Cefotaxime (75%) > Aztreonam (55%) > Gentamycin (40%) > Sulzone (35%) > Tobramycin (30%) > Amikicin and Ciprofloxacin (25% each) > Imipenem (10%) and Norfloxacin (5%). In this regard, antimicrobial resistance profile of *P. aeruginosa* isolated from surgical wounds was reported by Nwachukwu et al.[14]. According to them, Out of 16 isolates of *Pseudomonas aeruginosa*, 16(100%), 10(62.50%), 8(50.00%), 7(43.70%), 6(37.50%), and 2(22.20%) were resistant to Ampicillin, Ceftriaxone, Gentamycin, Streptomycin, Ciprofloxacin, and Nalidixic acid respectively. In isolates of *P. aeruginosa* from swimming pools and hot tubs, Lutz and Lee[15] reported that these were resistance to amikacin (9%), aztreonam (22%), ceftriaxone (4%), gentamicin (9%), imipenem (26%) and tobramycin (9%). A similar study conducted by Ullah et al.[16] showed inconsistent pattern of antimicrobial resistance in *Pseudomonas aeruginosa* to different classes of antibiotics except imipenem to which resistance was very low. Keeping in view the resistance pattern of pathogens to commonly used antibiotics, it is also necessary to carry out a large scale study with newer antimicrobials. This will hopefully reduce the resistant pattern and thus the treatment cost, and initiate quality patient care.

CONCLUSION

The drug of choice against the isolates of *P. aeruginosa* from pus was imepenem to which clinical isolates showed highest sensitivity (90%) while maximum resistance was showed to Cephradine (90%) and Cefotaxime (75%).

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