

ISOLATION AND IDENTIFICATION OF PSEUDOMONAS AERUGINOSA FROM EAR SAMPLES AND ITS ANTIBIOGRAM ANALYSIS

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ABSTRACT

Objectives: The aim of this study was to determine the frequency of *Pseudomonas aeruginosa* in middle and outer ear and to check the antibiotic susceptibility pattern of commonly used antibiotics.

Material and Methods: In this study a total of 42 samples were found as positive for *P. aeruginosa* out of 100 and tested for six antibiotics (cefixime, ciprofloxacin, amikacin, imepenem, gentamicin, aztreonam).

Results: 42 samples out of 100 were found positive for *P. aeruginosa*. Gender wise distributions were higher for female as compared to males. 22 samples (52%) were positive for females and for male 20 samples (48%) were found positive. Antibiotic sensitivity pattern was checked. Pathogen was highly sensitive to Gentamicin 30 (71.4%), followed by Aztreonam 28(66.6%), Cefixime 22 (52.3%), Imepenem 18(42.8%), amikacin 15(35.5%) and Ciprofloxacin 12(28.5%).

Conclusion: Females were more susceptible to *Pseudomonas* infections. Isolates of *P. aeruginosa* from samples showed highest sensitivity (71.4%) to Gentamicin while maximum resistance was showed to Ciprofloxacin (47.6%).

Key Words: *Pseudomonas aeruginosa*, ear infections, antibiotics susceptibility.

INTRODUCTION

Pseudomonas aeruginosa is a rod shaped Gram negative bacterium and is an opportunistic pathogen that causes many diseases in humans and animals¹. Necrotizing otitis externa is an extension of external otitis into the mastoid or temporal bone that may prove life-threatening. Osteomyelitis that occurs most often in elderly patients with diabetes mellitus may be caused by *P. aeruginosa*. However, all immunocompromised patients are at risk especially those with human immunodeficiency virus (HIV) infection^{4 6 8 11}.

Otitis media is an infection of middle ear mostly caused by bacteria, virus and fungi resulting in inflammation of mucosal lining. Otitis media if occur recurrently may cause damage of ossicles, cochlea and facial nerve, thus may result in hearing loss. It can be acute or chronic. The acute form is usually associated with the upper respiratory tract infection whereas persistent form is known as chronic supportive otitis media (CSOM)². The chronic form is a major problem in developing countries like Pakistan. This infection is more common in lower socioeconomic group and children are mostly affected. Most common etiological agents of CSOM are *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*

ae, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Aspergillus* species and *Candida* species but these organisms vary in various geographical areas².

The pathogen has evolved resistance to many antibiotics but the emergence of isolates of *P. aeruginosa*, with Extended Spectrum β -Lactamases (ESBLs) has become a serious problem¹⁶.

MATERIALS AND METHODS

This study was conducted at the Microbiology Department of Abasyn University, Peshawar from May to September 2012. All clinical samples were collected from patients at Hayat Abad Medical Complex, Peshawar.

Isolation, characterization and identification:

Pus samples were collected using standard microbiological techniques. Pus samples were collected from ears using sterile swabs and were brought to Microbiology laboratory of Abasyn University for further processing, within 1 hour of collection. Cysteine Lactose Electrolyte Deficient (CLEED) agar, Blood agar and MacConkey agar were used as growth media for the culturing of samples and were incubated at 37°C for 24 hours to get the growth⁵. Identification of microorganisms was done with the help of gram staining and biochemical tests including catalase¹⁷, oxidase¹⁸, Triple Sugar Iron (TSI) agar¹⁹ and Indole¹⁷. These were carried out following standard routine techniques⁷.

Antibiotic susceptibility Testing

The Antibiotic susceptibility pattern for *P. aeru-*

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ginosa was confirmed by standard Kirby-Bauer disc diffusion method⁹. Muller Hinton agar was prepared. It was sterilized by autoclaving for 15 min at 121°C. 25 ml of media was poured in sterile Petri dishes and incubated at 37°C overnight to check sterility. The positive isolates of *Pseudomonas aeruginosa* were checked for their sensitivity against antibiotics such as: cefixime, ciprofloxacin, amikacin, imipenem, gentamicin, aztreonam of standard strengths. The plates were incubated at 37°C for 24h. After incubation, the diameter of zones of inhibitions was then measured. The susceptibility (sensitive, intermediate or resistance) were determined according to national committee for control laboratory standards¹³ and Clinical and Laboratory Standard Institute (CLSI)²⁰.

RESULTS

A total of 100 pus samples were processed for isolation of *Pseudomonas aeruginosa*. Out of 100 samples 42 were found positive for *Pseudomonas aeruginosa* (Fig 1). Out of 42 samples, gender wise distributions were higher for female as compared to males. 22 samples (52%) were positive for females and for male 20 samples (48%) were found positive (Fig 2). Different biochemical tests were conducted for identification and Characterization of *P. aeruginosa*. Culture sensitivity testing of these samples was conducted against 6 antibiotics for Pseudomonas infections by means of Disc diffusion method¹³.

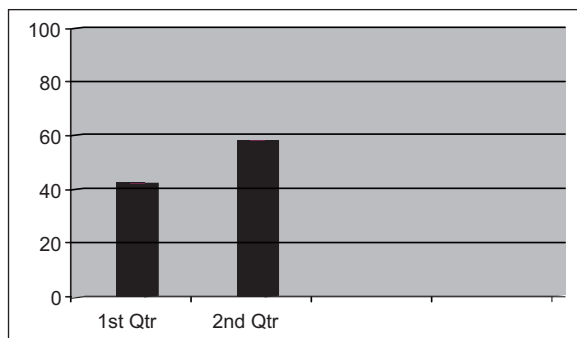


Figure 1: 1st Qtr showing Positive Samples, 2nd Qtr showing Negative samples for *P.aeruginosa*

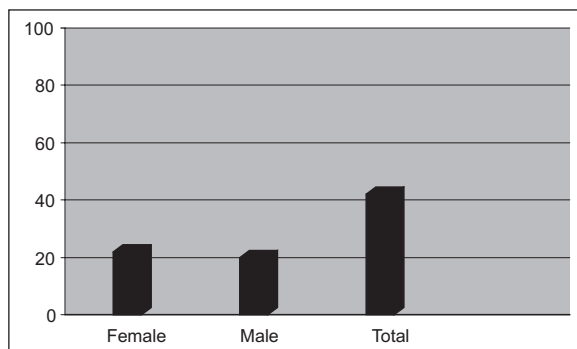


Figure 2: Gender wise distribution of *P.aeruginosa* positive samples

S. No.	Antibiotics	Sensi-tive	Inter-mediate	Resis-tant
1.	Gentamicin	30	5	7
2.	Aztreonem	28	5	9
3.	Cefixime	22	9	11
4.	Imepenem	18	11	13
5.	Amikacin	15	10	17
6.	Ciprofloxacin	12	10	20

The Antibiogram for *Paeruginosa* shown in Table 1, revealed that Gentamicin was highly effective as 30 (71.4%) samples showed sensitivity for it, followed by Aztreonem 28(66.6%) > Cefixime 22(52.3%) > Imepenem 18(42.2%) > Amikacin 15 (35.7%) > Ciprofloxacin 12(28.5%). The resistance was high against Ciprofloxacin 20 (47.6%) followed by Amikacin 17(40.47%) > Imepenem 13 (30.9%)> Cefixime 11 (26.19%) > Aztreonem 9 (21.4%)> Gentamicin 7 (16.6%). The Intermediate patterns observed were in order of Imepenem 11 (26.19%) > Amikacin 10(23.8%) and Ciprofloxacin 10 (23.8%) > Cefixime 9 (21.4%) >Gentamicin 5(11.9%) and Aztreonem 5(11.9%).

DISCUSSION

In the current study 42% isolates were positive for *Pseudomonas aeruginosa*, which showed nearly same results as were obtained by Alo et al¹ and Motoya¹². Both of them isolated 48.7% *Pseudomonas aeruginosa* from ear infections.

Gender wise distribution of *Paeruginosa* was lower in male 48% as compared to female 52%. The current result is in consistence with Ihsan et al⁹ who isolated 54.2 % from female and 45.8% from male while with in the study of Rashid et al¹⁴, 60% isolates were positive for male and 40% for Female. This difference may be due to difference in geographical distribution.

The antibiogram profile showed that *Pseudomonas* was highly sensitive to Gentamicin 30 (71.4%) followed by Aztreonam 28(66.6%), Cefixime 22(52.3%), Imepenem 18(42.2%), Amikacin 15 (35.7%) and Ciprofloxacin 12 (28.5%). These results are in accordance with results showed by Motayo et al¹², in which *Pseudomonas aeruginosa* showed the highest sensitivity to Gentamicin and were highly resistant towards Ciprofloxacin.

The study of Rashid et al¹⁴, showed that *Pseudomonas aeruginosa* was highly resistant to Cefixime 93.3%, Gentamicin 77.3%, Ciprofloxacin 75.5%. These results are different from the present study results in which *Pseudomonas aeruginosa* was highly resistant to Ciprofloxacin 20 (47.6%) followed by Amikacin 17 (40.47%), Imepenem 13 (30.9%), Cefixime 11 (26.19%), Aztreonem 9 (21.4%) and Gentamicin 7 (16.6%). This difference may be due to the differences in geographical distribution or may be due to the frequency of exposure

of the pathogen towards particular antibiotic in different regions.

CONCLUSION

This study revealed that among these 6 commonly used antibiotics. Gentamicin was the effective one as 71.4% samples showed sensitivity to this, whereas the pathogen has evolved high resistance towards Ciprofloxacin as 47.6% samples were resistant to it.

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