

## Frequency and Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* in Ear Swabs

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**Abstract:** *Pseudomonas aeruginosa* is a Gram negative, an obligate aerobe bacteria that is found in soil, water and also on plants. *P. aeruginosa* accounts for a significant proportion of nosocomial infection and are responsible for about 13 % of eye, ear, nose and throat infections. Total of 237 samples of ear swabs were received at Dr. Ziauddin Hospital North Nazimabad (Campus) Karachi. Samples of pus from external auditory canal were taken with sterile cotton swab and are cultured on blood, chocolate and MacConkey agars media and were incubated for 24 to 48 hours. Antibiotic sensitivity was tested and interpreted by method according to CLSI criteria. *P. aeruginosa* was isolated from 37 samples and rests of 70 samples were positive for different microorganisms. Majority of organisms were sensitive to Meropenem (100%), Ceftazidime (100%), Polymyxin-B (100%) and Colistin (100%). From the current study it was concluded that *P. aeruginosa* is a potential cause of ear associated infection; we should consider it in every patient with a history of acute and prolong ear discharge. Antibiotic sensitivity should be carried out for such bacteria instead of empirical antibiotic use to reduce treatment failure and resistance.

**Key words:** *P. aeruginosa* • Meropenem • Ceftazidime • Polymyxin-B • Colistin

### INTRODUCTION

*Pseudomonas aeruginosa* is a Gram-negative bacillus, oxidase positive and an obligate aerobe. It derives its energy only by oxidation of sugars and it does not ferment glucose [1]. *P. aeruginosa* is often opportunistic; hospital acquired affecting those already in poor state of health and immune-suppressed, *P. aeruginosa* infections are especially prevalent among patients with burns, wound, cystic fibrosis, acute leukemia, organ transplants and intravenous drug user [2].

Characteristic bluish pus exudates due to the production of pyocyanins with grapes like odor are from infection site [3]. *Pseudomonas* nosocomial infection is usually due to patients undergoing ventilation, antibiotic treatment, chemotherapy or surgery. The patients in ICUs brought serious infection from community [4, 5]. Data

from medical ICUs during surveillance period 1992-1997 indicated that *P. aeruginosa* caused about 3 % blood infection; 21% of pneumonias; 10% urinary tract infections; 13 % of eye, ear, nose and throat infections and 5 % of cardiovascular infections [6].

Otitis is a general term for inflammation or an infection in human ear. It is divided as otitis externa, which involves outer ear and ear canals. Otitis media involves middle ear. Otitis interna involves inner ear [7]. Otitis media is an infection or inflammation of the middle ear. This inflammation often begins when infections that cause sore throats, colds, or other respiratory or breathing problems spread to the middle ear. These can be viral or bacterial infections [8]. Middle ear infection or suppurative otitis media can be acute or chronic [9]. Acute otitis media is a bacterial infection of the mucosal line ear containing spaces of the temporal bone with a

rapid onset of signs and symptoms, such as pain, fever, irritability, anorexia, or vomiting. Otitis media with effusion is characterized by the presence of an asymptomatic middle-ear effusion, although it can be associated with a “plugged ear” feeling [10].

Chronic suppurative otitis media is the condition when the ear drum has been perforated. In an acute attack of otitis media the infection remains patent and becomes chronic upon secondary invaders. They are *S. aureus* and *P. aeruginosa* as the cause of persistent chronic infection [11]. Otitis externa is the chronic inflammation of the external auditory meatus. It may be caused by bacteria and fungi, particularly *P. aeruginosa*, *S. aureus*, *Candida albicans* and *Aspergillus* species [12].

The most commonly encountered infection is otitis externa (swimmer’s ear) of which *P. aeruginosa* causes 35-70% [13]. This can be treated satisfactorily with aural toilet without resorting to antibiotics. However in malignant otitis media and otitis externa can occur and can be life threatening in extreme cases [14]. The aim of this study is to analyze the frequency of *P. aeruginosa* in different ear swab samples.

## MATERIALS AND METHODS

This study was carried out from January 2007 to December 2008 at Dr. Ziauddin Hospital North Nazimabad Campus Karachi. Questionnaires for getting information including patients, age, sex, address, code number and laboratory results report forms were used to collect data. The study subjects were adults, children of both sexes coming to health unit complaining of fever due to ear pain and ear discharge.

**Collection of Specimen:** All samples were collected by standard microbiological technique.

**Procedure:** Ear swab was collected by inserting and rotating a sterile swab as deep as possible in the ear canal without hurting the patient. The swab was then placed in transport medium (Carry-Blair Medium Oxoid).

### Processing of Ear Swabs for Culture and Sensitivity:

**Processing:** Inoculation was done on sheep blood agar, Chocolate agar and MacConkey agar plates with the swab by rotating it. Then with the help of sterile wire loop streaking the sample in four quadrants of the plate.

Sheep blood agar and Chocolate agar plates were incubated in 5% CO<sub>2</sub> at 37°C while MacConkey agar plates were incubated at 37°C in ambient air aerobically. The plates were then examined after 24 and 48 hours.

**Identification:** After overnight incubation suspected colonies of *P. aeruginosa* were initially identified on the basis of colonial morphology on blood and chocolate agar which appear as large colonies with metallic sheen, mucoid, rough or pigmented and often give beta-hemolysis. On MacConkey agar these colonies appear as non-lactose fermenting with green pigment or metallic sheen. Gram stain from colonies was also performed which showed Gram negative bacilli [16]. Analytical Profile Index (API) 20 NE were performed for Biochemical identification of *P. aeruginosa*:



API 20 NE

**Antibiotic Susceptibility Testing:** Antibiotic susceptibility testing was performed on Muller Hinton agar (MHA) according to CLSI [17] guidelines.

The following antibiotics were tested for antimicrobial susceptibility: amikacin (30ug), aztreonam (30ug), cefipime (30ug), ceftazidime (30ug), gentamicin (10ug), ciprofloxacin (5µg), polymyxin (300units), colistin (10ug), piperacillin/tazobactam (100ug/10ug) and cefoprazon/sulbactam (105ug).

The bacterial suspension was made and compared with McFarland standard 0.5 that is equivalent to 1.5x10<sup>8</sup> cfu. The MHA plates were then inoculated by streaking evenly with the sterile swabs. In the similar way also control plates were streaked. A 90 mm plate was used five discs were applied on each plate. Then MHA plates were incubated at 37°C for 18-24 hours.

Zone of inhibition around each antibiotic disc was measured with caliper. The zone edges were taken as points of complete inhibition of growth as determined by naked eye. The zones of inhibition were measured with scale from back side of agar plates in millimeters and were interpreted by referring to recommendation of CLSI guideline [17].

**Data collection and Entry:** A Performa was used to collect relevant details from the patients.

**Statistical Analysis:** For statistical analysis Statistix 9 software was used. ONE-Way ANOVA Test were used for significance relationship amongst the categorical parameters. P-value <0.005 are considered as significant.

**RESULTS**

A total 237 samples (Table 1), (Figure 1) of ear swabs were received, out of these 107 (45%) samples were positive for various pathogens, *P. aeruginosa* were present on 37 isolates (34.6%) of those 107 positive samples.

Seventy samples (65.4%) were positive for other organisms (Table 2) that include *S. aureus* 22 isolates (31%), *Staphylococcus lugdunensis* 18 isolates (26%), *Proteus* species 10 isolates (14%), *Enterobacter* species eight isolates (11%) *Acinetobacter* species four (6%), *Haemophilus influenzae* four isolates (6%), *E. coli* two isolates (3%) while Beta hemolytic group A streptococci and fungus are 1% each.

Figure 2 shows antimicrobial susceptibility and resistance pattern of *P.aeruginosa*. The organism was susceptible to imipenem, meropenem, polymyxin B, colistin, piperacillin/tazobactam, aztreonam, cefipime,

Total samples	237	P-value
Positive samples	107 (45%)	0.1035 non significant
Negative samples	130 (55%)	

Name of Microorganisms	No of Isolates and (positive%)	P-value
<i>Staphylococcus aureus</i>	22 (31)	0.4631 non significant
<i>Staphylococcus lugdunensis</i>	18 (26)	
<i>Proteus</i> species	10 (14)	
<i>Enterobacter</i> species	8 (11)	
<i>Acinetobacter</i> species	4 (06)	
<i>Haemophilus influenzae</i>	4 (06)	
<i>Escherichia coli</i>	2 (3)	
Beta Hemolytic group A streptococci	1 (1)	
Fungus	1 (1)	

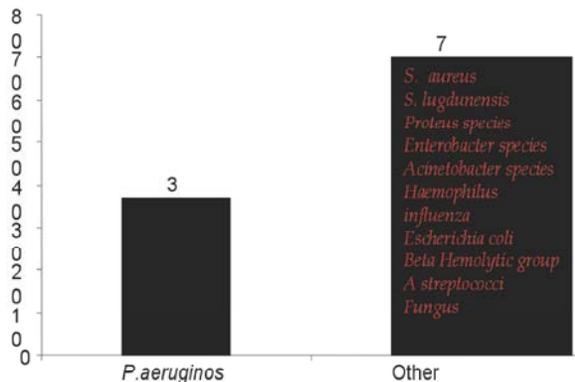


Fig 1: Frequency of *P. aeruginosa* and other microorganisms

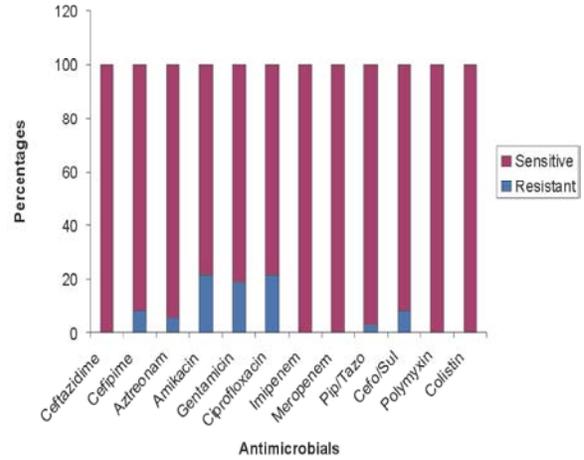


Fig 2: Antimicrobial Susceptibility Pattern of *P.aeruginosa*

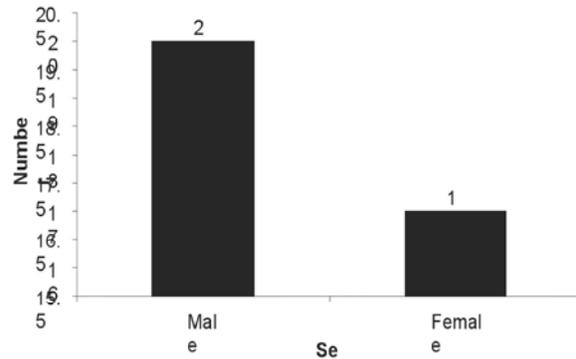


Fig 3: Male to Female Ratio in Total *P.aeruginosa* Positive Samples (n=37)

cefoperazone/sulbactam, gentamicin, ciprofloxacin, amikacin with 100, 100, 100, 100, 97, 94, 92, 92, 81, 78 and 78% respectively.

Figure 3 shows Male to Female ratio in total *P.aeruginosa* positive samples. In total 37 positive samples male were 54 % and female were 46 % which was not very remarkable difference.

**DISCUSSION**

The Gram negative bacteria *P. aeruginosa* is an obligate aerobe that is present in soil, water and on plants. *P. aeruginosa* can be frequently isolated from tap water in patient’s rooms in Hospital [18]. *P. aeruginosa* accounts for a significant proportion of nosocomial infection and the isolated strains shows multidrug resistance (MDR) profile with special reference to intensive care unit (ICU) [19, 20].

*P. aeruginosa* caused about 13 % eye, ear and nose and throat infections[21]. Acute otitis externa affects annually 0.4% persons in United States, while the chronic form is commonly of fungal or allergic origin; it affects 3 to 5 % percent of the population [22-25]. Research at United States Armed Forces Research Unit showed that 50% of bacterial cases involve *P. aeruginosa* followed by *S. aureus* and then other aerobic and anaerobic bacteria [19, 26-28].

In our study, *P. aeruginosa* represented 34.6% of total ear swab isolates and 65.4% were other isolates. Iqbal *et al* examined 200 ear swabs the bacterial isolates were *P.aeruginosa* 41.5% *staphylococcus* 19%, *Proteus mirabilis* 18%, *Klebsiella pneumonia* 10.5%, *E.coli* 4%, Beta-hemolytic *streptococcus* 5% and *Serratia* species 2% [29]. In another study, Gul *et al* found that the frequency of *P.aeruginosa* in chronic suppurative Otitis media was 52.2%, *S. aureus* 15%, *Proteus* species 6.5% and *Klebsiella* species 2.6% [30]. These results support our study with slight difference. On the other hand a study cited by Gad *et al* in Egypt showed 20 % *P. aeruginosa* in 80 positive samples out of 445 [31], which is low as compared to our study.

The sensitivity pattern of *P. aeruginosa* antibiotics appeared as 100% sensitivity imipenem, meropenem, Ceftazidime, Polymyxin-B and colistin. One earlier study cited by Gales *et al* showed that the meropenem was the most effective antibiotic against *P. aeruginosa* [32]. However more recent studies demonstrated resistant strains of *P. aeruginosa* to Meropenem [33, 34]. Local study showed 100% and other study shows 98% sensitive to imipenem, some study shows 84% sensitive to ceftazidime, gentamicin 70%, amikacin 92%, cefipime 84%, aztreonam 83% and ciprofloxacin 95% [30]. According to study cited by Gales *et al* [32] in Egypt among cephalosporin, Cefipime was the most effective against *P. aeruginosa*. Many studies findings support ours [32, 35, 36]. In our study ceftazidime is 100%, amikacin 78.3%, gentamicin 81%, cefipime 91.89%, aztreonam 94.5% and ciprofloxacin 78.3 % sensitive, both studies shows close similarity in sensitivity pattern.

In this study 54% male and 46% females were found infected by *P. aeruginosa* which is not quite great difference as compared to another local study in which 61.78% male and 38.22% female were infected [37].

## CONCLUSION

*P. aeruginosa* is a major cause of ear associated infections. We should consider in every patient with history of acute and prolonged ear discharge. Empirical antibiotic coverage needed to cover this pathogen as it develops resistance very rapidly. Antibiotic therapy should be revised after result of culture & sensitivity so that proper antibiotics given to eradicate the pathogen otherwise it will be life threatening.

Regarding the susceptibility pattern of isolates the resistance to amikacin (22%), ciprofloxacin (22%) is increasing day by day. Continuous monitoring of patients is required for preparing antibiograms. These susceptibility results help the physician for deciding the empirical regime for the patients.

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