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Antibiotic susceptibility Pattrens of *Pseudomonas aeruginosa* in tertiary care hospital

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Abstract
Pseudomonas aeruginosa is a leading cause of nosocomial infections. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* varied markedly with the antibiotic tested. In this study a total of 40 clinical specimens were investigated, 10 were from females and 30 for males. Most of them belonged to the age group 10-60 years. Urine and blood were the more noticeable sources of specimens of *Pseudomonas aeruginosa*. Most isolates of *Pseudomonas aeruginosa* showed maximum resistance to Azithromycin (90%) and Oxacillin (75%), they showed less resistance to Cefoperazone+ Sulbactam (10%) and Imipenam (25%). All isolates were sensitive to the Amikacin, Ofloxacin and Tobramycin. Present study shows the occurrence of resistance and sensitivity to various anti-pseudomonal agents. Cefoperazone + Sulbactam were the only anti-pseudomonal drug against the *Pseudomonas aeruginosa* was fully sensitive. We recommend a more limited and a more rational use of this drug in this hospital setup. Amikacin and Imipenam are the choice drugs for optimal management of infections caused by *Pseudomonas aeruginosa*.

Keywords: Antibiotic, *pseudomonas aeruginosa*, tertiary care hospital

1. Introduction
The name *Pseudomonas* is the combination of two words, i.e the Greek and Latin words which means "false unit"(pseudo = false, Greek; Monas = single unit, Latin). For unicellular organisms "Monas" had been used in the initial history of microbiology. In 1786 Otto Friedrich Muller, from Copenhagen, who had described the *pseudomonads*, this had found into the group of vibriones (which was defined as group of shaking bacteria) after many years later studied it was found that Pseudomonas were involved in motility. Therefore, they assigned the name of "Pseudo", because due to shaking in nature and also it show motility. Due to presence in water *pseudomonas* identified as important microorganism. *Pseudomonas aeruginosa* is a gram-negative, rod-shaped bacterium which belongs to the family Pseudomonadaceae. It is 1-5 µm long and 0.5-1.0 µm wide. *Pseudomonas aeruginosa* is an obligate bacterium, mean they can survive in the presence and absence of oxygen. *Pseudomonas aeruginosa* has a great role in degradation it degrades organic compounds such as benzoate. They can survive in some harsh environment like in disinfectant solutions and also in nutrient deficient conditions [1]. They use oligotrophic which contain more dissolved oxygen as compared to plant, *Pseudomonas aeruginosa* have plasmid that play role in degradation of wastes and organic matters [2]. They can cause many infections such as critical illness and hospitalized infection as seen in many hospitalized individuals [3]. Their entry route may be common and cause urinary tract infection and surgical site infection. *Pseudomonas aeruginosa* is an opportunistic human pathogen means it easily disseminates in the human mostly in the patients with cystic fibrosis, cancer, or AIDS [4]. *Pseudomonas aeruginosa* is such a potent pathogen that causes more invasive diseases. It is a leading Gram-negative opportunistic pathogen at most medical center carrying a 40-60% mortality rate. It complicates 90% of cystic fibrosis deaths; and lastly, it is always listed as one of the top three most frequent Gram-negative pathogens and is linked to the worst visual diseases [5]. The aim of the research work was to find out the antibiotic susceptibility patterns of *Pseudomonas aeruginosa* in a tertiary care hospital.

2. Materials and Methods
This investigation was carried out in the microbiology lab of Pakistan Institute of Medical sciences (PIMS) Islamabad, one month of period in 2016.

Media i.e Mac-conky agar, blood agar, cysteine lactose electrolyte deficient medium (CLED) and Mueller Hinton medium (MH) were used and the reagents i.e oxidase reagents, catalase reagents and gram staining reagents the equipment incubator, autoclave, Inoculating loops and wooden sticks, Antibiotic disc, Watt man paper were used too for the investigation of antibiotic susceptibility.

2.1 Specimens Collection
Specimens were collected from patients who were hospitalized for more than week durations. A total of 40 clinical specimens was investigated for bacterial culture and identification. Only one isolate from each patient was considered in the study. Specimens were taken from different sources like blood, urine, tracheal secretions and were inoculated on routine culture media like Mac-conky agar, blood agar and CLED agar. Specimens were processed for bacterial species identification by standard microbiological procedures. Several tests were performed that included gram's staining, colony morphology, sugar fermentation tests and biochemical tests such as oxidase test, urease test for the confirmation of the isolates as *pseudomonas aeruginosa*.

2.2 Inoculation on Mac-Conkey and Blood Agar
After inoculation of the specimen on Mac-Conky and Blood agar two types of colonies were formed (a) yellowish and (b) Grayish. Yellowish colonies were indicated Lactose fermenter (LF) such as *E.coli* while grayish colonies indicated the Non-lactose fermenter (NLF's) such as *Pseudomonas* species. In case of appearance of Grayish colonies confirmation test were performed such as oxidase test, catalase test.

2.3 Procedure of Oxidase test
Watt man filter papers were soaked with the oxidase reagent (tetramethyl-p-phenylienediamine dihydrochloride). Then the paper was moistened with sterile distilled water and colonies were picked with wooden loop sticks and streaked on the filter paper and the inoculated area was observed for a color change to deep blue or purple within 10-35 seconds. In case of positive oxidase test (blue or purple color) sensitivity test was performed on Mueller Hinton media in which antibiotic discs were used and incubated at 37ºc for 24 hours. Green pigmentation appeared which indicated the presence of *Pseudomonas aeruginosa* while the absence of green color indicated the presence of other *pseudomonas* species.

2.4 Urine samples
Urine samples were cultured on cystein lactose electrolyte deficient (CLED) media.

2.5 Susceptibility tests
Antimicrobial susceptibility tests were done by the Kirby-Baur disk diffusion method as per recommendation of national (NCCLS). A panel of anti-pseudomonal antimicrobial is as follows: amikkacin, azithromycin, cefoperazone- sulbactum, ofloxacin, oxacillin, imipenem, and tobramycin.

3. Result
Pseudomonas aeruginosa were isolated and identified by standard microbiological procedures, a total of 40 clinical specimens was investigated. 10 were from females and 30 for males. Most of them belonged to the age group 10-60 years as shown in Table 1. Urine and blood were the more noticeable sources of specimens of *pseudomonas aeruginosa*. Source of clinical isolates is shown in Table 2.

Table 1: Age and gender wise investigation of *pseudomonas aeruginosa*

Age (year)	Male	Female	Total no. (%)
<15	4	2	6 (15%)
16-40	21	6	27 (67.5%)
41-60	5	2	7 (17.5%)
Total	30	10	40 (100%)

Table 2: Specimens distribution of *Pseudomonas aeruginosa* clinical isolates

Sources of specimen	No	Percentage
Blood	20	50%
Urine	15	37.5%
Tracheal secretion	5	12.5%
Total	40	100%

3.1 Antibiotic susceptibility patterns
Antibiotic susceptibility patterns of *pseudomonas aeruginosa* varied markedly with the antibiotic tested. Most isolates of *pseudomonas aeruginosa* showed maximum resistance to Azithromycin (90%) and oxacillin (75%), they showed less resistance to cefoperazone + sulbactam (10%) and imipenam (25%). All isolates were sensitive to the amikacin, ofloxacin and tobramycin. The resistance pattern of *pseudomonas aeruginosa* to various antibiotic tested was in order: azithromycin (90%), oxacillin (75%), ofloxacin (37.5%), tobramycin (30%), cefoperazone - sulbactum (10%), amikacin (30%), imipenem (25%) as shown in Table 3.

Table 3: Antimicrobial susceptibility of *pseudomonas aeruginosa*

Antibiotic	Sensitive no (%)	Resistance no (%)
Amikacin	28 (70%)	12 (30%)
Azithromycin	4 (10%)	36 (90%)
Cefoperazone +Sulbactam	36 (90%)	4 (10%)
Imipenam	30 (75%)	10 (25%)
Ofloxacin	25 (62.5%)	15 (37.5%)
Oxacillin	10 (25%)	30 (75%)
Tobramycin	28 (70%)	12 (30%)

4. Discussion
In this study, a total 40 samples of *pseudomonas aeruginosa* were isolated and identified from various clinical sources, from the hospitalized patients and their antimicrobial susceptibility was determined. Most of them belonged to age group of 16-40 years (67.5%) and elderly age > 50 years (7%). This could be due to prolonged hospitalization and other associated co-morbidities in these age groups. The distribution of *pseudomonas aeruginosa* specimens may vary with each hospital as each hospital and each health facility has a different environment associated with it. More than 80% of the *pseudomonas aeruginosa* isolates were obtained from blood and urine samples. Similar results had been obtained in various studies in India reported by [6, 7] respectively. Increasing resistance to different anti-pseudomonal drugs have been reported worldwide [8, 9] and this is a serious therapeutic problem in the management of disease due to one of the main features in this study was the sensitivity of *pseudomonas aeruginosa* to Cefoperazone +Sulbactam and Imipenem. These hospitals associated organisms. The resistance profile of *pseudomonas aeruginosa* was tested against seven anti-microbial agents. Many studies have shown varying degrees of resistance to imipenem [10-13]. Cefoperazone+ Sulbactam (90%), followed by Amikacin (70% sensitive) proved to be the most effective drugs for *pseudomonas aeruginosa*. An earlier study reported from Kathmandu, Nepal [14] shown amikacin (75.4% sensitive) and

Cefoperazone+ Sulbactam (92.3% sensitive) for *pseudomonas aeruginosa*. High resistance to Azithromycin (92%) had been reported in studies done in India [15-17]. Similarly, higher rates of resistance to oxacillin (80.5%) had been reported in a study done in North Kerala, India [18]. In this study, whereas beta-lectamase inhibitor drug cefoperazone- sulbactum showed a lower resistance of 4% only, indicating beta-lactamase inhibitor markedly expands the spectrum of activity of beta lactams [19] which makes the combination drug the preferred choice against *pseudomonas aeruginosa* infections. Thus, it is concluded that preferred should be given towards use of combined antibiotics in the treatment of *pseudomonas* infections [20].

5. Conclusion

The results of the present study clearly showed the occurrence of resistance to various anti-pseudomonal agents. Cefoperazone +Sulbactam were the only anti-pseudomonal drug against the *P. aeruginosa* was fully sensitive. We recommend a more limited and a more rational use of this drug in this hospital setup. Amikacin, and Imipenam are the choice drugs for optimal management of infections caused by *pseudomonas aeruginosa*.

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