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Antimicrobial Susceptibility Pattern in the clinical isolates of Pseudomonas aeruginosa at NIMS Medical College & Hospital, Jaipur, India

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Abstract
Pseudomonas aeruginosa has become an important nosocomial and opportunistic pathogen in the second half of last century. The aim of the study was to isolate and asses the antimicrobial susceptibility of Pseudomonas aeruginosa at NIMS Medical College & Hospital, Jaipur. Total 300 samples were tested and 40 Pseudomonas aeruginosa were isolated. The Pseudomonas aeruginosa were isolated using a standard protocol which included tests for motility, oxidase production, catalase production, biochemical reactions, oxidation- fermentation test, amino acids activity, nitrate reduction, malonate test and gelatin hydrolysis. Antimicrobial susceptibility were performed by Kirby-Baeur disc diffusion method and result interpreted according to CLSI guidelines. The resistance pattern of Pseudomonas aeruginosa among various antimicrobials are Meropenem (43%), Imepenem (0%), Piperacillin+tazobactam (20%), Polymyxin-b (53%), colistin (30%), Tigecycline (83%), amikacin (53%), Ampicillin+salbactum (85%), ciprofloxacin (30%), Cefipime (23%), Ceftazidime (33%), penicillin-g (100%). The highest resistance seen in penicillin-g (100%) and the lowest resistance in Imepenem (0%).

Keywords: Pseudomonas aeruginosa, penicillin-g, imepenem.

1. Introduction
Pseudomonas aeruginosa is a pathogen associated with wide range of nosocomial infections and is an important pathogen isolated from various clinical samples. It plays a very significant role in Hospital Associated Infections (HAI), predominant ones being pneumonia, urinary tract infection (UTI) along with skin and soft tissue infections [1]. Its role as an important opportunistic pathogen has been widely recognized. This bacteria can cause complication in treatment of AIDS, Cancer and Cystic fibrosis patients, viewed as a massive threat to human beings in coming future [2, 3]. Pseudomonas aeruginosa has been observed as a common isolate amongst the non-fermenters routinely isolated from various clinical samples. Genus Pseudomonas an aerobic, motile, gram negative bacilli, oxidase and catalase positive, non-lactose fermenter is a free living Saprophyte of soil, water, sewage or wherever decomposing organic matter is found. It is also found in human gut and skin [4]. It has been recognized as a major human pathogen since early 1940. The ability of Pseudomonas aeruginosa to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community as well as hospital settings. Pseudomonas aeruginosa has innate resistance to various antibiotics. In addition to this intrinsic resistance, Pseudomonas aeruginosa easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants [5]. Development of multidrug resistance by Pseudomonas aeruginosa isolates requires several different genetic events, including acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes. The ongoing emergence of resistant strains that cause nosocomial infections contributes substantially to the morbidity and mortality of hospitalised patients.

Materials & Methods
This study was carried out in the department of microbiology NIMS Medical College and Hospital, Jaipur from January 2015 to June 2015. Various clinical samples such as Urine, Sputum, Pus, Blood, Ear swabs, Pleural fluid & Tips were included in the study and analysed. Samples were inoculated on nutrient agar, blood agar and MacConkey agar and were incubated overnight at 37^0C and observed next morning. Pseudomonas aeruginosa was initially identified on the basis of Gram stain from the colony, colony morphology and oxidase reaction. They were further identified on the basis of pigment production, biochemical reactions which include indole, methyl red, voges proskeur, citrate, triple sugar iron, and urease. They were further identified by Oxidation / fermentation (Hugh and Leifson's media) for glucose and lactose, Lysine, ornithine decarboxylase, arginine dihydrolase activity, Nitrate reduction test, growth at 42 ̊C, Malonate test and gelatin hydrolysis. All the isolates confirmed as Pseudomonas aeruginosa were tested for antibiotic sensitivity by disc diffusion (Modified Kirby-Bauer disc diffusion method) according to CLSI guidelines [6].

Result
A total of 300 clinical samples were tested out of which 40 Pseudomonas aeruginosa were isolated.

Table 1: Type and number of clinical specimens collected during the study showing Pseudomonas aeruginosa

Specimens	Total No.	Percentage
Ear swab	12	30 %
Urine	9	22 %
Pus	8	20 %
Sputum	4	10 %
Blood	2	5 %
ET Tip	2	5 %
ET Secretion	1	3 %
Foleys tip	1	2 %
Catheter tip	1	3 %
Total	40	100 %

Table 2: Sex wise distribution of cases

Sex	Total No.	Percentage
Male	26	65 %
Female	14	35 %
Total	40	100 %

Table 3: Distribution of cases according to age

Age (in years)	No. of cases	Percentage
0-9	2	5%
10-19	4	10%
20-29	6	15%
30-39	4	10%
40-49	9	23%
50-59	7	18%
60-69	5	13%
70-79	2	5%
80-89	1	3%
Total	40	100%

Table 4: Distribution of Clinical Samples among patients of OPD / IPD / ICU

Specimens	OPD	IPD	ICU	OPD %	IPD %	ICU%
Ear Swab	4	8	0	40	44	0
Urine	2	5	2	20	28	17
Sputum	2	1	1	20	6	8
Pus	2	3	3	20	17	25
Blood	0	1	1	0	6	8
ET Tip	0	0	2	0	0	17
ET Secretion	0	0	1	0	0	8
Catheter tip	0	0	1	0	0	8
Foleys tip	0	0	1	0	0	8
Total samples	**10**	**18**	**12**	**100%**	**100%**	**100%**

Table 5: Resistance and Susceptibility pattern of Pseudomonas aeruginosa to various Antimicrobials used in the study.

Antimicrobials	Resistance	Sensitive
Meropenem	17 (43%)	23 (58%)
Imepenem	0 (0%)	40 (100%)
Piperacillin+tazobactam	8 (20%)	32 (80%)
Polymyxin- B	21 (53%)	19 (48%)
Colistin	12 (30%)	28 (70%)
Tigecyclline	33 (83%)	7 (18%)
Amikacin	21 (53%)	19 (48%)
Ampicillin+salbactum	34 (85%)	28 (15%)
Ciprofloxacin	12 (30%)	7 (70%)
Cefipime	9 (23%)	31 (78%)
Ceftazidime	13 (33%)	27 (68%)
Penicillin- G	40 (100%)	0 (0%)

Discussion

Pseudomonas aeruginosa is being increasingly implicated in human diseases. It is an important opportunistic and nosocomial pathogen which is becoming a great threat in treating infections. Despite advances in surgical interventions, sanitation facilities and the introduction of wide variety of antimicrobial agents with antipseudomonal agents, life threatening infections caused by Pseudomonas aeruginosa continue to be in hospital infections. The present study was conducted to determine the antibiotic sensitivity pattern of Pseudomonas aeruginosa isolated from various clinical samples. In the present study rate of isolation of Pseudomonas aeruginosa was compared with other studies. In the present study maximum isolates of Pseudomonas aeruginosa were isolated from Ear swabs (30%) followed by pus (20%) which is comparable with the studies done by Ayesha ansari *et al*.[7] and Rajat rakesh *et al*.[8]. In the present study it was found that infections caused by Pseudomonas aeruginosa can occur at all ages. The youngest in our study was 6 years and the eldest was 88 years. The mean age was found to be 42.22 years. The highest incidence was seen in the age group of 40-49 years which is comparable to the study done by T Rakhee *et al*.[9] and Yahuda Carmeli *et al*.[10]. In the present study Pseudomonas aeruginosa was isolated from 65% Males and 35% Females which correlates with the study of DE Premalatha *et al*. [11] and H Ravichandra Prakash *et al*. [12]. The present study shows 100% resistance to Penicillin-G followed by Ampicillin+salbactum (85%), Tigecyclline (83%), Amikacin, Polymyxin-B (53% each), Meropenem (43%), Ceftazidime (33%), Colistin, Ciprofloxacin (30% each), Cefipime (23%), Piperacillin Tazobactam (20%) and Imepenem (0%) which was compared with the studies done by Ayesha ansari *et al*, Zeynab Golshai *et al*.[13], Rajat rakesh *et al* and Mohansoundaram KM[14]. This study shows that Pseudomonas aeruginosa is becoming resistant to commonly used antibiotics due to the excessive consumption of antibiotics exerting selective pressure on bacteria, frequently used invasive devices and severe underlying diseases. The empirical antibiotic treatment should be avoided and treatment should be carried out using antibiotic susceptibility tests and efforts should be made to prevent spread of resistant bacteria.

Conclusion

The ability of Pseudomonas aeruginosa to survive, multiply and to disseminate in hospital environment is a threat to the hospital management for development of hospital infections among patients admitted in the hospital. Non judicial use of antibiotics further enhances the chances of development of antibiotic resistance. It is a challenge to the clinician and management authorities of the hospital to prevent the spread of Pseudomonas aeruginosa in the hospital and to prevent the spread of antimicrobial resistance. It is critically important that the antibiotic susceptibility pattern should be regularly and continuously monitored. To prevent the spread of resistance among bacteria it is the need of the hour that strict antibiotic policies, surveillance programmes for its isolation, drug resistance and infection control procedures need to be implemented. It can also be planned by continuous interaction and efforts of microbiologists, clinician, management authorities and community to promote greater understanding of this problem. Hand hygiene to prevent spread of the organism, rational use of antibiotic, should be encouraged and monitored. Better surgical and medical care including optimum cleaning and disinfection practices should be provided to the patient during hospital stay.

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