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Resistance of Antimicrobial in *Pseudomonas aeruginosa*

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A B S T R A C T

Increasing number of reports had documented the continued emergency of resistance among Pseudomonas aeruginosa strains to common antibiotics drug, world-wide. This study investigated the antimicrobial resistance patterns of *P. aeruginosa* clinical isolates from hospitalized patients. Ongoing surveillance of *P. aeruginosa* resistant against antimicrobial is fundamental to monitor trends in susceptibility patterns and appropriately guide clinicians in choosing empirical or directed therapy. This study was conducted from August 2015 to December 2015 in Department of Microbiology at NIMS Medical College & Hospital Jaipur, Rajasthan, India. One hundred twenty six isolates of *P. aeruginosa* were isolated from different clinical specimens and fully characterized by standard bacteriological procedures. Antimicrobial susceptibility pattern of each isolates was carried out by the Kirby-Bauer disk diffusion method as per CLSI guidelines. Majority of *P. aeruginosa* were isolated from Pus, Sputum, Urine specimens. The isolate pathogen shows resistance to Amikacin (18.45%), ciprofloxacin (31.74%) and Cefoperazone – sulbactam (36.50%). All the isolates were (100%) susceptible to Meropenem and Imipenem. The result confirmed the occurrence of drug resistance strains of *P.aeruginosa*. Meropenem, Imipenem, Amikacin, ciprofloxacin were found to be the most effective antimicrobial drugs. It therefore calls for a very judicious, rational treatment regimens prescription by the physicians to limit the further spread of antimicrobial resistance *P. aeruginosa*

Introduction

Antimicrobial agents have been the only easily and widely used therapeutic option available to counter the infections caused by infectious microbial agents. However, microbial populations have developed various strategies to overcome these microbial agents – a major contributing factor in the development anti-microbial resistance world-wide.

Pseudomonas aeruginosa is an aerobic, motile, nutritionally versatile, gram negative bacteria. *Pseudomonas aeruginosa* is ubiquitous, human opportunistic pathogen and has implications on morbidity, mortality and healthcare costs both in hospitals and in the community (Franco BE *et al.*, 2009). Infections caused by *Pseudomonas aeruginosa* is frequently life threatening and

difficult to treat as it exhibits intrinsically high resistance to many antimicrobials and the development of increased, particularly multi drug resistance in health care settings (Poole K 2011). Ongoing surveillance of *Pseudomonas aeruginosa* resistance against antimicrobial agents is fundamental to monitor trends in susceptibility pattern and to appropriately guide the clinicians in choosing empirical or directed therapy, especially when new antimicrobial agents may not be readily available in the near future (Gales AC *et al.*, 2001). Ongoing studies on current antimicrobial resistance profile of P. aeruginos are essential to find out the susceptibilities of this pathogen against commonly prescribed antibiotics in any health care facility. This would help the physicians to optimize the current therapeutics treatment options. Thus, in our study we assessed the in vitro activity level of antimicrobial drugs against clinical isolates of *Pseudomonas aeruginosa* obtained from the NIMS Medical College & Hospital, Jaipur.

Materials and Methods

This investigation was carried out in the Department of Microbiology, NIMS Medical College & Hospital, Jaipur, Rajasthan, India during August 2015 to December 2015. Specimens were collected from patients who were hospitalized for more than one week duration. A total 126 consecutive clinical isolates of *P. aeruginosa* were collected for bacterial culture and identification. Only one isolate from each patient was considered in this study.

Sample Processing

The specimens were collected from the hospitalized patients admitted from different wards of hospital. These were processed for bacterial species identification by standard microbiological procedures. Specimens were taken from various sources like pus/wound, sputum, urine, broncho-alveolar lavage (BAL) fluid, tracheal aspirate and were inoculated on routine culture media like Blood agar, MacConkey agar. MacConkey agar showed lactose non-fermenting pale colonies with oxidase positive.

Conformation of *Pseudomonas* spp

After obtaining the pure strains, the strains subjected the grams staining and biochemical identification tests to identify Pseudomonas spp. For this purpose the samples are inoculated with Peptone water, Urease media, Citrate, TSI (Triple Sugar Iron) media and kept an incubator at 37^0C for 18 hrs. Next day the result will noted on Citrate media, Urease media, TSI media. Part of growth on peptone water was subjected to indole test with Kovac, s reagent and part for motility testing by Hanging drop method. A strain of Pseudomonas showed Indole negative, Urease test negative, TSI medium showed alkaline slant and no reaction in butt and Citrate test positive. Nitrate reduction test was positive in Pseudomonas (Konemen, 2006).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of all the *Pseudomonas aeruginosa* isolates was performed by Kirby-Bauer disk diffusion method and the result were interpreted by the Clinical Laboratory Standard Institute (CLSI) guidelines 2014. All the clinical isolates of *P. aeruginosa* were tested for their sensitivity against a panel of anti-pseudomonal antimicrobials of standard strength as follow: Amikacin 30mcg, Piperacillin 100mcg, Ceftriaxone 30mcg,

Cefoperazone-Sulbactum 75–10 mcg, Ciprofloxacin 5mcg, Co-Trimoxazole 25mcg, Imipenem 10mcg and Meropenem 10mcg (Hi Media Laboratories Pvt. Ltd., Mumbai, India). *P. aeruginosa* ATCC 27853 was used as quality control strain.

Results and Discussion

A total 126 strains of *Pseudomonas aeruginosa* were isolated and identified by standard microbiological procedures, out of a total 654 clinical specimens were investigated. The rate of isolation of *P. aeruginosa* was 126 (19.26%). Of these 126 strains of P. aeruginosa, 78 (61.90%) were from males and 48(38.09%) from females patients shown in Table 1. Most of them belonging from the age group 21-40 (45.23%) years followed by patients of >60 years (24.60%) of age as shown in Table 2. Wound/Pus, Sputum, Urine and Tracheal Aspirate were the predominant source of specimens of *P. aeruginosa* clinical isolates as shown in Table 3.

In our study, a total of 126 isolates of *Pseudomonas aeruginosa* were isolated and identified from various clinical specimens from the hospitalized patients and their antimicrobial sensitivity determined. Most of them are belong to older age group of 21-40 years (45.23%) and elderly age group >60 years (24.60%). This could be explained as due to decreased immunity, prolonged hospitalization and other associated co-morbidities in these age groups.

A study done in Ahmadabad in Gujarat state of India shown (29%) of patients were aged between 31-45 years (Rajat RM *et al.*, 2012). Similarly, a high prevalence of *P. aeruginosa* infection was found in the 35-50 years age group (Mohanasoundaram KM 2011). The distribution of specimens of *P. aeruginosa* may vary with each hospital as each hospital facility has a different environment associated with it. More than 80% of the *P. aeruginosa* isolates were obtained from Pus/Wound, Urine and Tracheal Aspirates. Increasing resistance to different anti-pseudomonal drugs particularly among hospital strains has been reported world-wide (Orrett FA. 2004) and this is a serious therapeutic problem in the management of disease due to these organisms. The resistance profile of *Pseudomonas aeruginosa* to the eight antimicrobial agents tested varied among the isolate investigated. One streaking feature in our study was that all the *P. aeruginosa* isolates were found to be sensitive to Imipenem and Meropenem. This may due to restricted use of Imipenem and Meropenem in our hospital. Amikacin (18.2%) and Ciprofloxacin (31.7%) show very low resistance and proved to be the most effective drugs for routine use among the *P. aeruginosa* strains investigated in this study. An earlier study reported from Nepal, shown Amikacin (81.4%) and Ciprofloxacin (70.3%) are high sensitive drugs against *P. aeruginosa* (Koirala P *et al.*, 2010).

Murase, et al. 1995 in their study showed that there is distinct difference in the sensitivity pattern of isolates of *P. aeruginosa* from specimen to specimen. Piperacillin alone tested showed a resistance rate of (53.9%) in this study wears beta –lactams/ beta-lactams inhibitor drug Cefoperazone-Sulbactum showed a lower resistance of (36.5%) only. The emphasis should be given towards use of combined antibiotics in the treatment of Pseudomonal infections (Bhandari S *et al.*, 2012). Similar resistance rate for Piperacillin (54.6%) has been reported in the study done by Shenoy et al. 2002. Relatively low Piperacillin resistance (11.5%) had been reported in patient isolates of *P. aeruginosa* in a study from Saudi Arabia (Al –Tawfiq JA. 2007).

Table.1 Sex Wise Distribution of Cases

Sex	Total no.	Percentage (%)
Male	78	61.90%
Female	48	38.09%
Total	126	100%

Table.2 Age Distribution of Cases

Age (Years)	No. of Isolates	Percentage (%)
< 20	17	13.49%
21 – 40	57	45.23%
41 – 60	21	16.66%
>60	31	24.60%
Total	126	100%

Table.3 Distribution of *P. aeruginosa* from Different Clinical Samples

Source of specimen	No. of Specimens	Percentage (%)
Pus	54	42.85%
Sputum	32	25.39%
Urine	23	18.25%
Tracheal Aspirate	11	8.73%
BAL	06	4.76%
Total	126	100%

Table.4 Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Different Clinical Samples

Antibiotic	No. of Isolate Resistance	% Resistance
Amikacin	23	18.25
Piperacillin	68	53.96
Ceftriaxone	94	76.60
Cefoperazone-	46	36.50
Sulbactum	38	30.15
Ciprofloxacin	40	31.74
Co-Trimoxazole	91	72.22
Imipenem	00	00
Meropenem	00	00

Fig. 1: Sex wise distribution of cases

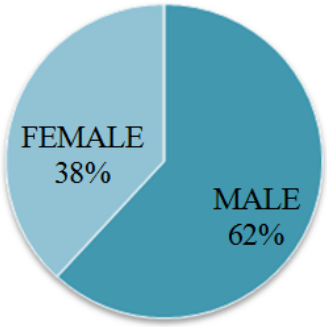


Fig. 2: Age Distribution of cases

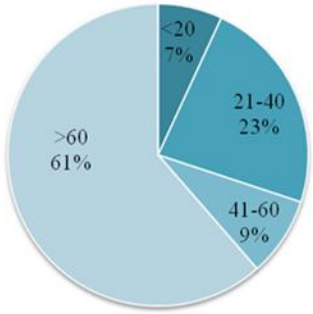


Fig. 3: Distribution of P. aeruginosa from different clinical samples

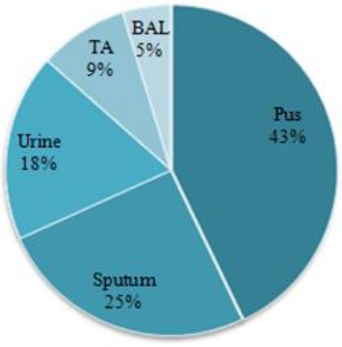
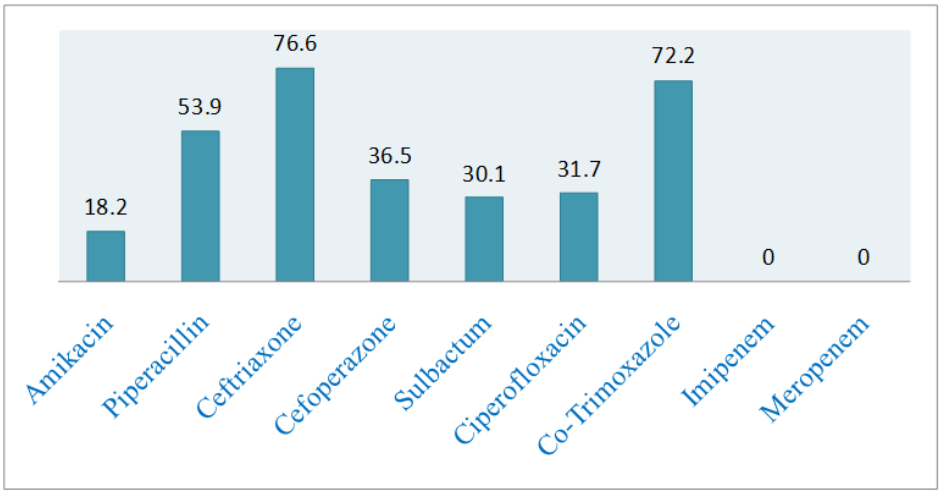


Fig.4 Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Different Clinical Samples



A study of Bhandari S *et al.,* 2012 showed *P. aeruginosa* isolates obtained from intensive care unit (ICU) of National Heart Centre has high Cefoperazone-Sulbactum sensitivity rate of (84.8%) and another study of Ahmed SM *et al.,*. 2012 has been showed low resistance in Cefoperazone-Sulbactum (11.1%). The rate of resistance for Co-Trimoxazole on the present study was (72.2%). In contrast, a study of Rashid A *et al.,* 2007 has been showed rate of resistance for Co-Trimoxazole to be (93.5%) in wound swabs and Pus Isolates, while a study of Nwankwo EOK *et al.,*. 2010 showed *P. aeruginosa* isolates (100%) resistance to Co-Trimoxazole. *Pseudomonas aeruginosa* strains in this study exhibited a high rate of resistance to the third generation cephalosporin drug- Ceftriaxone (76.6%). A much high resistance to Ceftriaxone of (75%) had been reported in study done by Arora D *et al.,* 2011. Lesser rate of resistance to Ceftriaxone (40%) had been reported in another study of Ramana BV *et al.,* 2012.

Our study thus indicates that *P. aeruginosa* is becoming resistant to commonly used antibiotics due to excessive consumption of antibiotics exerting selected pressure on bacteria, frequently used invasive devices and severs under laying diseases. The empirical antibiotic treatment should be avoided and treatment should be carried out using antibiotic susceptibility test and efforts should be made to prevent spread of resistant bacteria.

In conclusion, Result of the present study clearly demonstrated the occurrence of resistance to various antipseudomonal agents among the *P. aeruginosa* isolates. Imipenem and Meropenem was the only antipseudomonal drugs against which all isolates of *P. aeruginosa* were fully sensitive. We suggest a more restricted and a more rational use of these drugs in this hospital setting. Amikacin, Ciprofloxacin and semi–synthetic penicillin with beta – lactamase inhibitors are the preferred drugs for optimal management of infection caused by *Pseudomonas aeruginosa*. Regular anti-microbial susceptibility monitoring is essential of local, regional and national level isolates. This would held and guide the physicians in prescribing the right

combination of anti-microbial to limit and prevent the emergency of multi-drug resistant strains of *Pseudomonas aeruginosa*.

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