

The Frequency of *Pseudomonas aeruginosa* Clinical isolates in a Tertiary Care Hospital

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ABSTRACT

Background:

Pseudomonas aeruginosa is a nosocomial pathogen distributed worldwide. This study examined the antimicrobial resistance patterns of *P. aeruginosa* clinical isolates procured from hospitalized patients.

Aims and objectives:

To study the Resistance pattern of *Pseudomonas aeruginosa* isolates from miscellaneous samples.

Materials and methods:

A total number of 125 *Pseudomonas* strains were isolated from 904 miscellaneous samples (Sputum, HVS, Pus, Ear swab) between January 2016 to March 2016. Samples were obtained from the hospitals and processed in our central Microbiology lab. Antimicrobial susceptibility test were performed by Kirby Bauer disc diffusion method as per the recommendations of NCCLS guidelines.

Result:

P. aeruginosa were isolated from 125 miscellaneous samples such as sputum (49%) followed by HVS (27%), pus and wound swab (22%) & ear swab (2%). Most effective antibiotic was the Aminoglycosides drugs– Amikacin (93.6%), Gentamicin (76%), followed by Carbapenem drug- Imipenem (80%) and Fluoroquinolones drug- Ciprofloxacin (72.8%). Our results reveal high resistance to Penicillin drug- Amoxicillin (95.2%) and 1st, 3rd generation Cephalosporins (Cefazoline (92.8%), Cefotaxime (88.8%)).

Conclusion:

This study implicates that, the organism isolated from various clinical specimens were highly sensitive to Amikacin, which is followed by Imipenem, Gentamicin, Ciprofloxacin. In hospitalized patients, the emergence and spread of the resistance can be reduced by regular monitoring of drug resistance and strict infection control measures should be ensured.

Key words: Antimicrobial resistance, Clinical isolates, *Pseudomonas aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa is one of the important organism responsible for drug-resistant nosocomial infections. Aerobic, oxidase positive, non-fermenter. Day by day *Pseudomonas aeruginosa* is becoming a very common pathogen isolated from various clinical samples. It belongs to the genus *Pseudomonas*, which is widely distributed in nature. Although it is considered as a contaminant, it may colonize in healthy humans without causing disease, but sometimes, it is potential to act as a pathogen which can be identified without any doubt due to its positive disease impact in isolated clinical samples and also an agent of nosocomial infection.

It is regularly a cause of nosocomial pneumonia, nosocomial urinary tract infections, surgical site infections, infections of severe burns and also for the infectious patients undergoing either chemotherapy for neoplastic diseases or antibiotic therapy. Multiple factors contribute to make *Pseudomonas aeruginosa* as a nosocomial pathogen, for example, injudicious administration of broad spectrum antibiotics, instrumentation, and intrinsic resistance of microorganisms to numerous antimicrobial agents¹.

P. aeruginosa is found almost everywhere that is in water, in soil and also on plants. It can also be present in tap water found in patient rooms. It can be isolated from various body fluids such as sputum, urine, wounds, and eye or ear swabs and from blood, because it can infect almost any external part or organ of the body. Strains of *P. aeruginosa* which are Multidrug-resistant (MDR) are often isolated from the patients suffering from nosocomial infections, especially from those who are present in the intensive care unit².

Pseudomonas aeruginosa is notoriously difficult organism to control with antibiotics or disinfectants and has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several epidemiological studies track its emergence as multi-drug-resistant *Pseudomonas aeruginosa* (MDRPA) strains in clinical isolates. According to the CDC, the overall incidence of *P. aeruginosa* infections in US hospitals averages

about 0.4 percent (4 per 1000 discharges) and this bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections³. Due to prolonged hospital stay these patients are at high risk of acquiring nosocomial infection. In this situation topical antimicrobial agents play a limited role that reduces the incidence of septic complication but the incidence of bacterial colonization were not decreased⁴.

Pseudomonas aeruginosa is one of the most common nosocomial pathogens in humans and is often a major problem. Though rare in the normal flora of humans, it is isolated frequently from hospitalized patients. *Pseudomonas aeruginosa* is an important microorganism which causes problems clinically as a result of its high resistance to antimicrobial agents⁵.

Pseudomonas aeruginosa is a virulent agent having a tendency to develop resistance to majority of the antibiotics available for the treatment. It is a leading cause of life-threatening nosocomial infections⁶. It's intrinsic resistance to many antimicrobial agents and development of multidrug resistance imposes severe therapeutic problem for clinicians⁷.

MATERIALS AND METHODS

Sample collection:

This investigation was carried out in the Department of Microbiology in SSSMC & RI, Thiruporur. It is a tertiary care centre, referral and teaching hospital. This study was conducted between January 2016 to March 2016. During this period, totally 904 samples were collected from various wards of the hospital.

Inclusion criteria:

Various clinical specimens- Miscellaneous samples like (Sputum, HVS, Pus, Ear swab) of all age patients having Clinical infection were received for culture & sensitivity test.

Exclusion criteria:

Urine and Blood samples were excluded from this study.

Specimens:

Specimens were collected from patients who were hospitalized. A total of 904 clinical specimens were investigated for bacterial culture and identification. Only one isolate from each patient was considered in the study.

Sample processing:

The samples were selected on the basis of their growth on routine Mac Conkey medium (Non-lactose fermenting pale colonies) & on Brain Heart Infusion Agar (greenish pigmented colonies) which were oxidase test positive.

Confirmation of *Pseudomonas* species:

The specimens were collected from the hospitalized patients admitted in different wards of the hospital. These were processed for bacterial species identification by standard microbiological procedures. Specimens were taken from various sources like sputum, HVS, pus/wound swab, ear swab and were inoculated on routine culture media like Mac-Conkey agar, blood agar and eosin-methylene blue agar. A variety of tests were performed that includes gram's staining, colony morphology, motility tests, sugar fermentation tests and biochemical tests such as oxidase test, urease test and IMViC

(Indole, Methyl Red, Voges-Proskauer and Citrate) tests for the confirmation of the isolates as *Pseudomonas aeruginosa*⁸.

Antibiotic susceptibility:

The susceptibility test for *Pseudomonas aeruginosa* isolates were performed by Kirby Bauer method as per the recommendations of National Committee for Clinical Laboratory Standards guidelines (NCCLS, 1998). Antimicrobial susceptibility tests were done by disc diffusion methods on Muller Hinton agar plates⁹. The routine antibiotic sensitivity testing were done for the following drugs such as Cefazoline, Cefotaxime, Ciprofloxacin, Imipenem, Amikacin, Gentamicin, Amoxicillin.

Ethical consideration:

All these samples were a part of routine diagnosis. So, ethical consideration is not necessary.

RESULT**Table 1: Distribution of *Pseudomonas aeruginosa* among various clinical samples**

DURATION	SPUTUM	HVS	PUS	EAR SWAB	Total
January	16	2	10	2	30
February	23	6	5	-	34
March	22	26	12	1	61
Total	61	34	27	3	125
Percentage%	49%	27%	22%	2%	100%
Male = 56 = 45% Female= 69 = 55%					

Totally 904 samples were tested and identified by standard microbiological procedures, out of these, 125 samples showed growth of *Pseudomonas aeruginosa*. They were isolated and tested for antibiotic sensitivity.

Of these 125 strains of *P. aeruginosa*, 69 (55%) were from females and 56 (45%) were from males. Strains of *Pseudomonas aeruginosa* isolated from samples of sputum (49%) was found to be the highest, followed by HVS (27%), pus and wound swab (22%) & Ear swab (2%) as shown in the **Table 1**.

Pseudomonas aeruginosa isolated from various samples were sensitive to Amikacin (93.6%), Imipenem (80%), Gentamicin (76%),

Ciprofloxacin (72.8%), Cefotaxime (11.2%), Cefazoline (7.2%), Amoxicillin (4.8%).

Antimicrobial susceptibility patterns of *P. aeruginosa* varied markedly with the antibiotic tested. *P. aeruginosa* isolates showed maximum resistance to Amoxicillin (95.2%), Cefazoline (92.8%) and cefataxime (88.8%) and the least resistance to amikacin (6.4%).

All isolates were sensitive to the aminoglycosides drugs - Amikacin, Gentamicin and carbapenem drug- imipenem, where as all the isolates were resistance to the Penicillin drug-Amoxicillin and first, third generation of cephalosporin drugs- cefazoline, Cefotaxime as shown in **Table 2**.

Table 2: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*

Antibiotic Name	Sensitive (%)	Resistant (%)
Amikacin	93.6	6.4
Imipenem	80	20
Gentamicin	76	24
Ciprofloxacin	72.8	27.2
Cefotaxime	11.2	88.8
Cefazoline	7.2	92.8
Amoxicillin	4.8	95.2

DISCUSSION

In this study, totally 125 isolates of *P. aeruginosa* were isolated from various clinical samples of hospitalized patients and identification was done by standard bacteriological procedures and their antimicrobial susceptibility patterns were determined.

The dissemination of *P. aeruginosa* isolates may differ from each hospital based on the hospital facility. In our study, 49% of the *P. aeruginosa* isolates were obtained from Sputum, followed by 27% HVS, 22% Pus, 2% Ear swab. Moreover, our results had been obtained from different studies in India as described by Mohanasoundaram¹⁰ and Arora et al.,^{11,12} respectively.

In the present study, *P. aeruginosa* isolates were more among females 69(55%) than males 56(45%). This result was significantly different from that obtained by Humodi A. Saeed et al., in which *P. aeruginosa* isolates were more among males 37(54%) than females 31(46%)¹³.

A former study described by Bhatta DR et al.,¹⁴ has shown that Amikacin (81.4%) was found to be sensitive among the examined strains of *P. aeruginosa*. Similarly in the current study, Amikacin (93.6%) was proved to be the most effective drugs for *P. aeruginosa* strains investigated.

Nadeem Sajjad Raja et al., also reported Amikacin was the most effective drug tested among the Aminoglycosides, while Gentamicin was the least effective drug, and this study revealed both Aminoglycosides drugs – Amikacin (93.6%) Gentamicin (80%) to be effective¹⁵.

But in contrast to this study, high resistance to Aminoglycosides had been reported in studies done by Mohanasoundaram KM and Arora, et al.^{10,11}. Reports from Russia, contrarily indicates that the antimicrobial resistance to Gentamicin (75%) of *P. Aeruginosa* strains had shown to be increased intensively¹⁶.

In the present study, *P. aeruginosa* isolates were found to be sensitive to the carbapenem drug- Imipenem. This is consistent with a report published in 2002 in Mangalore, India¹⁷. But other studies have showed varying degrees of resistance to Imipenem in recent years^{18,19}.

Similarly, Hogardt M, Schmoldt S. et al., reported that (89.8%) of *P. aeruginosa* strains exhibited susceptibility to Fluoroquinolones such as Ciprofloxacin²⁰. But in contrary, the incidence of Ciprofloxacin resistance among *P. aeruginosa* has been reported in a range between 30% and 40%,²¹ and also Ciprofloxacin resistance (92%) was shown in a study from Malaysia²².

Like our study, Amoxicillin were found to have natural resistance to strains of *pseudomonas aeruginosa* which has been proved by Marilyn Porras Gómez et al.²³. A study conducted by Farida Anjum et al.,²⁴ the susceptibility pattern of *pseudomonas aeruginosa* against various antibiotics, Cefazoline has shown 99% of resistance for clinical isolates. Watankunakorn reported that the majority of *P. aeruginosa* were resistance to cefotaxime²⁵. Similarly in our study, first generation cephalosporin drug- Cefazoline (92.8%) and third generation cephalosporin drug - Cefotaxime (88.8%) were found to be similar resistance to different clinical isolates.

CONCLUSION

Pseudomonas aeruginosa remains as an important cause of nosocomial infections in various clinical isolates and also opportunistic infections in immune compromised individuals. We found that there is an increased level of incidence of *Pseudomonas aeruginosa* in tertiary care hospital. This study implicates that, the organism isolated from various clinical specimen is highly resistant to the following drugs such as Amoxicillin, Cefazoline, Cefotaxime. In our study, Aminoglycoside drug-

Amikacin was the effective anti-pseudomonal drug against all isolates of *P. aeruginosa* and followed by Imipenem, Gentamycin, and Ciprofloxacin. Hence the emergence of resistance in microbes can be prevented by implication of strict guidelines for antibiotic suggesting and suitable infection control measures. This study might support the clinicians in prescribing the right combinations of antimicrobials to limit the growth of multi drug resistant strains of *P. aeruginosa*

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