Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Various Clinical Samples at a Tertiary Care Hospital

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**Abstract**

*Pseudomonas aeruginosa* is a Gram-negative, rod-shaped and asporogenous bacterium. It has a pearlescent appearance and grape-like odour. *P. aeruginosa* grows well at 25°C to 37°C, and its ability to grow at 42°C helps distinguish it from many other *Pseudomonas* species. It has been known to cause a variety of other infections like pneumonia, urinary-tract infection, skin and soft-tissue infections, in severe burns and in infections among immunocompromised individuals. Objectives of the study is to isolate & identify *Pseudomonas aeruginosa* & other species (spp) from various clinical samples; to find out their distribution in various areas in the hospital; to study their antibiotic resistance pattern.

It is a Lab based retrospective study conducted between the study period of April 2018-March 2019 at the Department of Microbiology ESIC Medical College, Sanath Nagar, Hyderabad. A total of 10,988 samples were received at Department of Microbiology laboratory between the study period out of which 2488 samples were found to be culture positive. *Pseudomonas aeruginosa* & spp was isolated in 142 samples (5.8%) out of total 2488 samples. The prevalence of *Pseudomonas aeruginosa* & spp. in our hospital setting was maximal in the In patient department (Wards & ICU’s). The strains isolated from out patient department were mostly found to be sensitive to most of the antibiotics. Highest resistance was noted in *Pseudomonas aeruginosa* & *Pseudomonas spp* tolevofloxacin (47.3%, 45.5%), ciprofloxacin (45.7%, 39.1%), ceftazidime (38.7%, 30.4%). Whereas least resistance was encountered with Tobramycin, Netilmicyn & piperacillin tazobactam followed by Carbapenams. Regular bacteriological identification and antimicrobial susceptibility surveillance *P. aeruginosa* is needed to identify the antimicrobial resistance pattern in the setting of our hospital.

**Keywords**

*Pseudomonas aeruginosa*, Antibiotic resistance, Levofloxacin

**Article Info**

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**Introduction**

*Pseudomonas aeruginosa* is a Gram-negative, rod-shaped and asporogenous bacterium. It has a pearlescent appearance and grape-like odour. *P. aeruginosa* grows well at 25°C to 37°C, and its ability to grow at 42°C helps distinguish it from many other *Pseudomonas* species. *P. aeruginosa* is a ubiquitous microorganism which has the ability to
survive under a variety of environmental conditions.\textsuperscript{1}

\textit{Pseudomonas aeruginosa} is implicated in a wide variety of infections & is gaining increasing resistance over the past few years. \textit{Pseudomonas aeruginosa} has been implicated in various respiratory infections, especially cystic fibrosis, where it has tendency to persist due to formation of bio-films. It has been known to cause of variety of other infections like pneumonia, urinary-tract infection, skin and soft-tissue infections, in severe burns and in infections among immunocompromised individuals.

Independent risk factors have been identified for multi drug-resistant (MDR) or pan resistant P.aeruginosa infection like prior to use of antibiotics, history of \textit{P. aeruginosa} infection or colonization within the previous year, length of hospital stay, being admitted as in-patient or in the intensive care unit (ICU), mechanical ventilation, malignant disease and history of chronic obstructive pulmonary disease.\textsuperscript{2,3,4}

The antibiotic resistance mechanisms include the acquisition of extended-spectrum β-lactamases, carbapenemases, aminoglycoside-modifying enzymes and 16S ribosomal ribonucleic acid methylases. Mutational changes causing the up-regulation of multi-drug efflux pumps, depression of ampC, modification of antimicrobial targets and changes in the outer membrane permeability barrier are also described. \textsuperscript{5}

This study has been undertaken to check the antimicrobial susceptibility of \textit{Pseudomonas aeruginosa} \&\textit{Pseudomonas species} isolated from various samples in the hospital setting. The main objectives of this study include to isolate \& identify \textit{Pseudomonas aeruginosa} \& other spp from various clinical samples (blood, urine, respiratory sample, pus, body fluids). To find out their distribution in various areas in the hospital and also to study their antibiotic resistance pattern.

Materials and Methods

Study area

Department of Microbiology ESIC Medical College, Sanath Nagar, Hyderabad.

Study design

It is a Lab based retrospective study conducted between the study period of April 2018 to March 2019. Permission from institutional ethics committee was obtained.

Inclusion criteria

All clinically significant \textit{Pseudomonas aeruginosa} isolates will be included in the study.

Non-duplicate isolates were taken

Exclusion criteria

Repeated isolates from the same patient were excluded

A total of 142 \textit{Pseudomonas aeruginosa} \& other \textit{Pseudomonas spp} were isolated from different clinical specimens including wound swab/ pus, body fluids, urine, respiratory samples (bronchial wash, sputum, tracheal aspiration, throat pleural fluid) & blood. The isolates were identified by colony morphology, Gram’s staining and biochemical test according to standard laboratory test methods. The antibiotic susceptibility patterns of \textit{Pseudomonas isolates} were analyzed by carrying out by disc diffusion method (Kirby-Bauer) in Muller-Hinton agar media according to the Clinical & Laboratory Standards Institute (CLSI) 2019.
Pseudomonas ATCC 27853 was used as control strain. The results of susceptibility test were categorized into susceptible and resistant.

**Statistical analysis**

The data was entered into excel spread sheet and statistical analysis was done by applying descriptive statistics to generate percentages.

**Results and Discussion**

A total of 10,988 samples were received at Department of Microbiology laboratory between the period of April 2018- March 2019 out of which 2488 samples were found to be culture positive. *Pseudomonas aeruginosa & spp* was isolated in 142 samples (5.8%) out of total 2488 samples.

The prevalence of *Pseudomonas aeruginosa & spp.* in our hospital setting was maximal in surgery wards (46, 32.4 %), followed by Pulmonology ward (28, 19.7%), outpatient department (17, 12%) surgical ICU(13, 9.15%). The area wise distribution of *Pseudomonas aeruginosa & spp* is depicted in Fig 1.

The maximum number of *Psuedomonas spp* isolates was from pus & body fluids (51.4%), followed by respiratory samples (36.6%) & urine (7.04%). The findings of these are depicted in the table 1.

| Specimen                                           | No. of isolates (n =142) |
|----------------------------------------------------|--------------------------|
| Urine                                              | 10 (7.04%)               |
| Pus & body fluids                                  | 73 (51.4%)               |
| Respiratory samples (sputum, swab, tracheal aspirate, tissue) | 52 (36.6%)               |
| Blood                                              | 5 (6.25%)                |
| CSF                                                | 2 (2.5%)                 |

**Table.1 Shows the percentage of *Pseudomonas* isolates from various samples**

| S.no | Medication                   | *P.aeruginosa* | *P.species* |
|------|------------------------------|----------------|-------------|
|      |                              | S%             | R%          | S%           | R%           |
| 1.   | Amikacin (AMK)               | 88.8           | 11.2        | 91.6         | 8.4          |
| 2.   | Ceftazidime(CAZ)             | **61.3**       | **38.7**    | 69.6         | 30.4         |
| 3.   | Ciprofloxacin(CIP)           | **54.3**       | **45.7**    | 60.9         | 39.1         |
| 4.   | Gentamicin(GEN)              | **67.7**       | **32.3**    | 76.1         | 23.9         |
| 5.   | Imipenem(IPM)                | 72.6           | 27.4        | 81           | 19           |
| 6.   | Levofloxacin(LVX)            | **52.7**       | **47.3**    | 54.5         | 45.5         |
| 7.   | Meropenem(MEM)               | 65.4           | 29.6        | 77.6         | 22.4         |
| 8.   | Piperacillin Tazobactam (TZP)| 87.2           | 12.8        | 81           | 19           |
| 9.   | Piperacillin(PIP)            | 85             | 15          | 77.7         | 22.3         |
| 10.  | Tobramycin(TOB)              | 91.8           | 8.2         | 88.8         | 11.2         |
| 11.  | Netilmicin(NET)              | 90.1           | 9.9         | 75.6         | 24.4         |
| 12.  | Ticarcillin Clavulanate(TCC) | **86**         | **14**      | 81.8         | 18.2         |
Highest resistance was noted in *Pseudomonas aeruginosa* & *Pseudomonas spp* to levofloxacin (47.3%, 45.5%), ciprofloxacin (45.7%, 39.1%), ceftazidime (38.7%, 30.4%). Whereas least resistance was encountered with Aminoglycosides (Tobramycin, Netilmicin) & piperacillin tazobactam followed by Carbapenams. The susceptibility pattern of *Pseudomonas aeruginosa* & *spp* is depicted in the table 2.

*Pseudomonas aeruginosa* has emerged as a difficult to treat pathogen due to the various ways in which it evades the antimicrobial agents. The various ways in which they have developed resistance is due to the production of Extended beta lactamase (ESBL), metalobeta lactamases (MBL), Amp C. Out of 2488 culture positive samples.

*Pseudomonas aeruginosa* & *spp* was isolated in 142 samples (5.8%). In a study done by Sukesh, K et al the total number of *Pseudomonas* isolates were 32 out of 716 (4.4%).

Area wise distribution showed the prevalence of *Pseudomonas aeruginosa* & *spp* in our hospital setting in the In patient Department was maximal in surgery wards (46, 32.4%), followed by Pulmonology ward (28, 19.7%), surgical ICU (13, 9.15%). In the Out patient department the number of *Pseudomonas* strains isolated were (17, 12%).

In a study done by Minu Kumari et al the prevalence of *Pseudomonas* *spp* was maximal in neurosurgery ward (590, 31%), followed by surgery ward (417, 22%), surgical ICU (367, 19%), neurosurgical ICU (284, 15%), follow up outpatient department (163, 9%), emergency department (39, 2%) and orthopaedics ward (37, 2%).

In this study, the maximum number of *Psuedomonas spp* isolates were from pus & body fluids (51.4%), followed by respiratory samples (36.6%) & urine (7.04%).

In a study done by Jamshaid AK et al, most of the *Pseudomonas* *spp* isolates were from (pus 57.64%) , followed by urine 24.2%.

In a study by Sukesh K et al out of 100 clinical isolates of *Pseudomonas aeruginosa*, maximum isolates (71%) are isolated from pus/swab followed by 16% from urine, 12%
from sputum and 3% from other samples. In both the studies urine was found to be the most common specimen after pus where *Pseudomonas spp* were isolated, in contrary to our study where respiratory samples were the next most common sample after pus.

In this study resistance to aminoglycoside amongst *Pseudomonas aeruginosa* & *Pseudomonas spp* was as follows, gentamycin (32.3%, 23.9%), and amikacin (11.2% & 8.4%), tobramycin (8.2%, 11.2%) respectively. Resistance to aminoglycosides was seen to be quite high in other parts of the world, 70.70% by Lutfu Savas et al. and 69.86% by Agandi KM et al.\(^9,10\) were documented, where as lower rate were reported from Pakistan (6.73% by Nadeem et al. and 24% by Jamshaid et al.)\(^11,8\) similar to our study.

Resistance amongst the flouroquinolone group was as follows to *Pseudomonas aeruginosa* & *Pseudomonas spp*; ciprofloxacin (45.7%, 39.1%), and levofloxacin (47.3% & 45.5%) respectively.

It has been observed in the study done by Hoque MM et al the resistance rate for ciprofloxacin was 54.04% which is similar to our study.\(^12\)

Study done by Alyahawi A et al, documented a resistance of 34% to levofloxacin.\(^19\)

Dash et al observed that in majority (77.7%) of isolates were resistant, to more than 3 groups of antimicrobials, in which resistance to levofloxacin was found to be 36.1%.\(^5\)

Slightly higher resistance to levofloxacin in our study may be due increased usage of this antimicrobial in our setting.

In a study done by Cetin Kilinic et al, it was determined that resistance to piperacillin-tazobactam was seen to be quite high (57.9%).\(^13\) In our study, on the contrary it was found to be quite low 12.8% & 19% for *Pseudomonas aeruginosa* & *Pseudomonas spp* respectively which is in accordance with other studies as determined by SENTRY Antimicrobial Surveillance Program (1997–2007).\(^14\) In this study it was concluded that, piperacillin/tazobactam remained a very active beta-lactam when tested in vitro against clinical isolates of *P. aeruginosa*.\(^14\)

In our study resistance to ceftazidime was found to be 38.4% for *Pseudomonas aeruginosa* isolates. Inan et al reported resistance against ceftazidime as 34%.

In a study carried out in Turkey the most effective antibiotics were carbapenems (imipenem and meropenem) and the resistance rates were detected as 15% and 20.4%.\(^9\) Inan et al also reported resistance to Imipenem as 26% which is comparable to our study where we documented resistance in *Pseudomonas aeruginosa* strains to Imipenem & Meropenem to be 27.4% & 29.6% respectively.\(^16\)

Gamal F. Gad reported amikacin as the most active drug against *P. aeruginosa* followed by meropenem, cefepime and fluoroquinolones.\(^16\) In an India study done by MuktiKesh Dash et al, Imipenem, meropenem and piperacillin/tazobactam were most effective drugs observed which showed resistant rates of 6.4%, 8% and 11.3% respectively.\(^17\)

In this present study, the most effective antimicrobials were the aminoglycoside group, followed by β-lactamase inhibitor combination (Piperacillin-Tazobactam Ticarcillin-clavulinate), Carbapenems, 3\(^{rd}\) generation cephalosporin and fluoroquinolones.
Lower rate of resistance to aminoglycoside group of drugs, β-lactam+β lactamase inhibitor combination (Piperacillin-Tazobactam Ticarcillin-clavulanate). Carbapenems may have been seen due to their lesser use in our set up.

In conclusion, regular bacteriological identification and antimicrobial susceptibility surveillance *P. aeruginosa* is needed to identify the antimicrobial resistance pattern in the setting of our hospital. Therefore each hospital must analyse their typical flora and analyse the antimicrobial susceptibility on a regular basis, so that there may be rational use of antimicrobial, decreasing the chances of spread of further antibiotic resistance in the community. Hospitals harbour many antimicrobial resistant strains, so utmost care must be taken to further enhance the resistance pattern in our health care systems by prudent antimicrobial stewardship.

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