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# Isolation and Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* from Various Clinical Samples at a Tertiary Care Hospital

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**A B S T R A C T**

*Pseudomonas aeruginosa* is a classical opportunistic pathogen with innate resistance to many antibiotics and disinfectants. Clinical samples which yielded Pseudomonas aeruginosa were included in this study. Antibiogram of the isolates was determined by Kirby-Bauer disc diffusion method. A total of 339 pseudomonas aeruginosa were isolated from various clinical samples during the period of study. Majority of the isolates in our study were obtained from Pus / Wound swabs followed by other samples. Resistance to amoxicillin +Clavulinic acid (74.3%) was high in our study followed by resistance to ciprofloxacin, ceftazidime and amikacin. This hospital data will help in formulating antibiotic polices and in implementation of better infection control strategies. Periodic antimicrobial surveillance to monitor the resistance patterns of these organisms is thus required.

## Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen present in a variety of environments.[13] It can infect almost any external site or organ. It can cause community or hospital acquired infections.[14] Most community acquired infections are mild and superficial, but in hospital patients infections are more common, more severe and more varied.[2] The pseudomonads are a heterogenous group that have in common an inability to ferment lactose.[17] In the clinical setting it is the most commonly encountered gram negative species that is not a member of the family enterobacteriaceae and is an uncommon member of the normal human flora.[14] The importance of the bacillus as a disease causing agent was not recognized till recently, when it established itself as one of the most troublesome agents causing nosocomial infections.[16] The importance of this species derives from the widespread distribution of its strains in nature,[6] high intrinsic resistance to many antibiotics at levels attainable in body tissues[5] and the number of pathogenicity factors that they can produce.[6] It has a notorious propensity to

mutate to even more resistant strains during therapy. During three decades, the impact of resistance was minimized by the rapid development of potent antipseudomonal agents. However the situation has recently changed with the worldwide selection of strains carrying resistance to beta lactams, fluroquinolones and aminoglycosides. Further this situation has been compounded by the lack of development of new classes of antipseudomonal drugs for nearly two decades. Physicians now resort to drugs such as colistin and polymyxin which were discarded years ago. [17]

Hence this study was undertaken to know the rate of isolation of *Pseudomonas aeruginosa* from various clinical samples at our center and their susceptibility to commonly used antibiotics.

## Materials and Methods

This study was carried out in the Department of Microbiology, Alluri Sitaramaraju Academy of Medical Sciences, ASRAMS, Eluru from January to December 2017. During this period various clinical samples like pus/wound swab, urine, bronchial wash, sputum, pleural fluid, synovial fluid, tips and aural swabs were analyzed for the isolation of *Pseudomonas aeruginosa* by direct gram staining and culturing on Blood agar, MacConkey agar and Nutrient agar. Samples of all age groups and both sexes were included in the study.

## Characterization of bacterial isolates

The samples were selected on the basis of following characteristics:

- Growth on the routine MacConkey medium –NLF
- Typical fruity grape like odor
- Gram negative slender bacilli on gram stain

- Oxidase positive
- Actively motile in hanging drop preparation

Further identification of *Pseudomonas aeruginosa* done according to standard microbiological methods.[14,21]

- Indole production test – Negative
- Citrate utilization test – Positive
- Urea hydrolysis test – Negative
- Triple Sugar Iron medium – K/K with no gas and no H2S
- Oxidative reaction in Hugh Leifson media (O/F test)
- Ability to grow at 42°C

## Antimicrobial susceptibility testing

All confirmed *Pseudomonas aeruginosa* isolates were subjected to antimicrobial susceptibility testing on Muller Hinton agar plates by Kirby-Bauer disc diffusion method according to CLSI guidelines. [3]

Antimicrobial discs used for testing in the study are

Amikacin 30μg
Amoxyclav 20/10 μg
Ciprofloxacin 5 μg
Ceftazidime 30 μg
Imipenem 10 μg
Piperacillin + Tazobactum 100/10 μg

## Results and Discussion

A total of 339 *Pseudomonas aeruginosa* isolates were obtained from various clinical samples during the period of the study. Of them 176 were from inpatients and 163 were from outpatients. Distribution of the samples is shown in chart -I. Maximum number of isolates was obtained from Pus / Wound swabs followed by urine, aural swabs, sputum and other samples. Isolation rate of
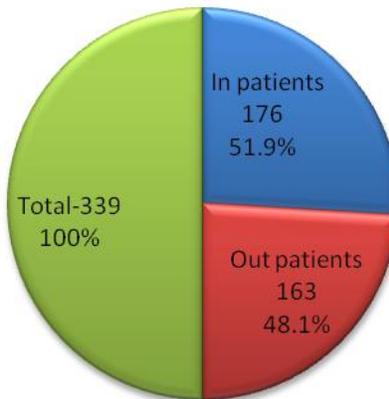
*Pseudomonas aeruginosa* from various clinical samples is shown in the chart -II. 324(95.6%) isolates in our study were monomicrobial where as 15(4.4%) of isolates in our study were polymicrobial. Most of them were associated with *Klebsiella* and most of them were from pus / wound swabs. Highest sensitivity in our study was for Imipenem, followed by piperacillin + Tazobactum. Most of the isolates in our study were resistant to amoxycilin + clavulinic acid, whereas amikacin, ciprofloxacin and ceftazidime showed intermittent level of resistance. Antibiotic susceptibility pattern of our isolates is shown in the table 1.

**Table.1** Antimicrobial susceptibility profile of *Pseudomonas aeruginosa*

| Name of the antimicrobial agent | Sensitive isolates n (%) | Resistant isolates n (%) |
|---|---|---|
| **Amikacin** | 227 (66.9%) | 112(33.0%) |
| **Amoxycillin+clavulunic acid** | 87 (25.7%) | 252(74.3%) |
| **Ceftazidime** | 217(64.0%) | 122(35.9%) |
| **Ciprofloxacin** | 203(59.9%) | 136(40.1%) |
| **Piperacillin+Tazobactum** | 319(94.1%) | 20(5.9%) |
| **Imipenem** | 334(98.5%) | 05(1.5%) |



Chart : I  Distribution of Culture positive Pseudomonas.aeruginosa

In patients 176 51.9%

Out patients 163 48.1%

Total-339 100%

**Chart.II** Isolation rate of *Pseudomonas aeruginosa* in various clinical samples
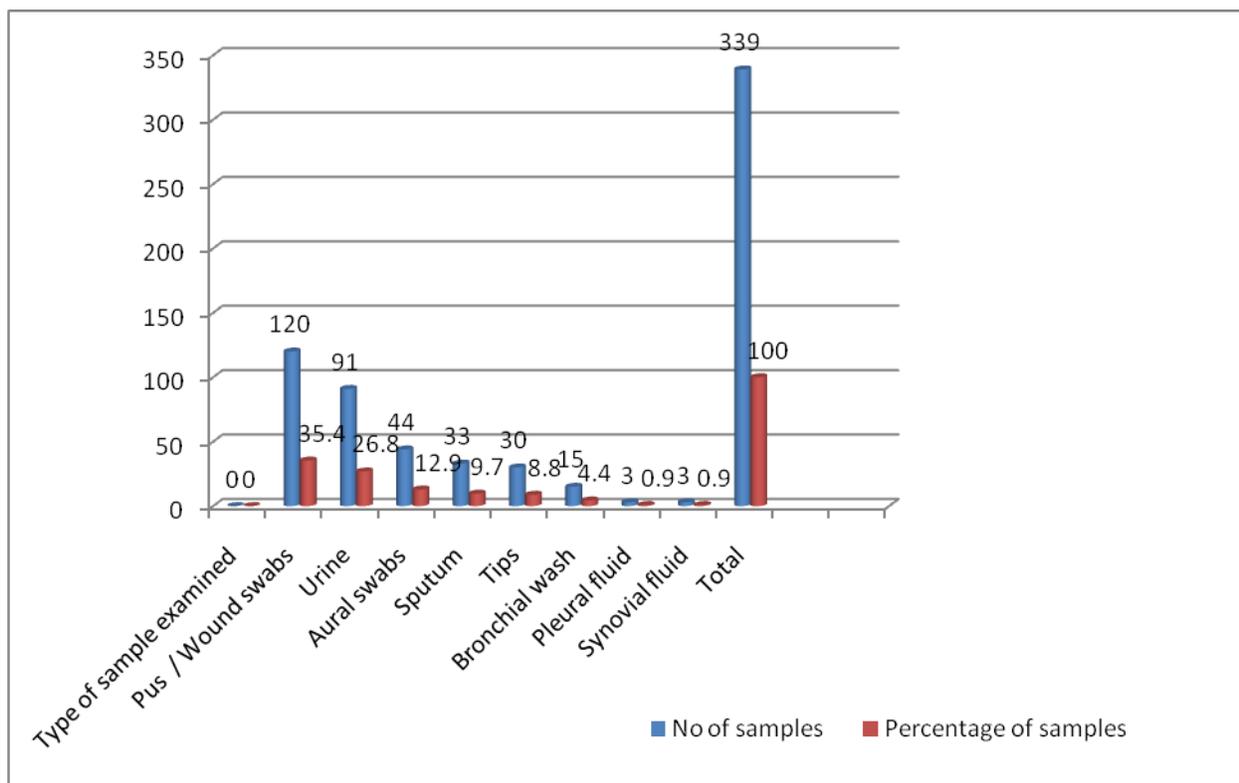


*Pseudomonas aeruginosa* is the pseudomonad most frequently recovered from clinical specimens. It is the most clinically significant among the NFBs. It is an important cause of hospital acquired infections because of its ability to persist and multiply in moist environment of the hospital wards, bathrooms, kitchens, equipment and antiseptic or disinfectant solutions which is of particular importance in cross-infection. The antimicrobial therapy for *Pseudomonas* infection is frustrating because the bacteria are typically resistant to most antibiotics. In our study out of the 339 *Pseudomonas* isolated from various clinical samples 51.9% were from inpatients and rest from the outpatients. Isolation from inpatients was more in comparison to outpatients which is similar to other studies. Whereas study by Muktikesh Dash *et al.,* showed isolation rates of 70% from in patients which is higher than our study.[9] In our study maximum isolates of

*Pseudomonas aeruginosa* were from pus /wound swabs that is 35.4%, which is similar to the study conducted by Chithra Jayaprakash *et al.,* which showed 37.04% isolation [1] and Hoque *et al.,* 38.38%.[4] Where as Saroj Golia *et al.,* reported 55.83% isolation which is much higher than our study.[18] Our study showed 26.9% isolation from urine samples, which is similar to the study conducted by Hoque *et al.,* that is 28.28% [4] and Premanadham *et al.,* 25.12%.[10] Whereas Saroj Golia *et al.,* reported 5% [18] and Chithra Jayaprakash *et al.,* reported 8.15% [1] which is much lower than our study. Isolation rates from the sputum samples in our study were 9.7% where as K.M. Mohanasoundaram *et al.,* reported 8.8% isolation rates from sputum samples.[7] Our study showed that 12.9% of isolates were from aural swabs similar to the study conducted by Saroj Golia *et al.,* that is 8.33% [18] where as Mirzadi Sadaf *et al.,*

reported 30%, higher rate of isolation than our study.[8] Isolation rate of *Pseudomonas* from bronchial wash in our study was 4.4% lower than that reported by Hoque MM *et al.,* 11.62% [17] 0.9% of isolates in our study were from pleural fluid similar to Hoque MM *et al.,* that is 0.50%.[4] *Pseudomonas aeruginosa* is inherently resistant to many antimicrobial agents, thus posing a great challenge in community acquired and nosocomial infections. In our study maximum resistance was noted to amoxicillin+clavulinic acid 74.3% which is similar to that reported by Igbalajobi *et al.,* 76.2%,[12] where as Walaa M. Saeed *et al.,* reported much lower resistance to the drug 25%.[20] Next resistant drug in our study was ciprofloxacin 40.1% which is similar to the study conducted by Nishi Tiwari *et al.,* 43%.[11] 33.0% of *Pseudomonas aeruginosa* isolates in our study were resistant to amikacin which is similar to the studies conducted by Nishi Tiwari *et al.,* 41%,[11] whereas Saroj Golia *et al.,* reported only 13.3% of resistance to amikacin much lower than our study.[18] Rajat Rakesh M *et al.,* reported 43% resistance to ceftazidime which is similar to our study 35.9%.[15] Resistance to Piperacillin +Tazobactum varied in different studies, our study reported only 5.9% resistance similar to Rajat Rakesh *et al.,* 4% [15] whereas Nishi Tiwari *et al.,* reported little higher level of resistance to the drug 26%.[11] Most of the isolates in our study were sensitive to Imipenem 98.5% which is similar to most other studies. Shenoy S *et al.,* [19] and Saroj Golia *et al.,* [18] reported 100% susceptibility to Imipenem where as Nishi Tiwari *et al.,* reported 23% resistance to the drug.[11] This study shows that clinical isolates of *Pseudomonas aeruginosa* are becoming resistant to commonly used antibiotics and slowly gaining resistance to newer drug combinations also.

In conclusion, this study reveals the rate of isolation of *Pseudomonas* from various clinical samples and their tendency towards antibiotic resistance. Though resistance to commonly used drugs against *Pseudomonas* was not that high in our study, but is a cause of concern. This studies guide clinicians in the choice of antimicrobial therapy. They also emphasize the need for rational use of antimicrobials and the need to minimize and preserve the use of reserve drugs, the need for institutional antibiotic policies and adherence to infection control practices to reduce the resistance and morbidity.

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