

## Original Article

## Prevalence & antibiogram of *Pseudomonas aeruginosa* at S.S.G. Hospital, Baroda, Gujarat, India

Jignasha Tadvi\*, T. B. Javadekar\*\*, Rachana Bhavsar\*, Nirav Garala\*\*\*

\* Resident, \*\* Prof. & Head, Dept. of Microbiology, Government Medical College and S.S.G.Hospital, Baroda

\*\*\*Asst. Professor, Dept. of Obs. & Gynec., Government medical college, Rajkot

DOI: 10.5455/jrmds.20153310

### ABSTRACT

**Background:** *Pseudomonas aeruginosa* is a gram negative bacterium that continues to be a major cause of opportunistic nosocomial infections, causing around 9-10% of hospital infections. It is hard to treat because of intrinsic resistance of the species and its ability to further resistance to multiple groups including  $\beta$ -lactams, aminoglycosides and fluoroquinolones.

**Aims:** This study was undertaken to determine the prevalence of *Pseudomonas* and their susceptibility pattern at S.S.G. Hospital, BARODA.

**Materials and Methods:** Between March 2015 to May 2015, 150 strains of *P. aeruginosa* were isolated from different clinical specimens. The samples were selected on the basis of their growth on Mac Conkey and nutrient agar medium with oxidase positive. Colonies were subjected to biochemical tests to identify species. Antimicrobial susceptibility of all the isolates was performed by disc diffusion (Kirby –Bauer) method according to CLSI guidelines.

**Results:** Majority of isolates of *P. aeruginosa* were obtained from specimens of blood, pus, wound, sputum, tracheal aspirates, pleural fluid, ICD fluid, bile fluid. The prevalence of pathogen was 4.15% and 98% pathogens were sensitive Piperacillin+Tazobactam followed by Meropenem (93.33%), Levofloxacin (92.66%), Cefazidime (82%), Cefoperazone(81.33%), Piperacillin (80.66%), Amikacin(56%), Gentamicin(54.66%).

**Conclusion:** The results confirmed the occurrence of drug resistant strains of *P. aeruginosa*. Meropenem, Levofloxacin and Piperacillin+Tazobactam were found to be the most effective antimicrobial drugs. It is rational treatment regimens prescription by the physicians to limit the further spread of antimicrobial resistance among the *P. aeruginosa* strains.

**Key words:** *Pseudomonas aeruginosa*, Prevalence, Antimicrobial sensitivity

### INTRODUCTION

Antimicrobial agents have been the only easily and widely used therapeutic option available to counter the infections caused by diverse microbial agents. However, microbial populations have developed various strategies to overcome these antimicrobial agents - a major contributing factor in the development of anti-microbial resistance worldwide. *Pseudomonas aeruginosa* is a ubiquitous and versatile human opportunistic pathogen and has implications on morbidity, mortality and healthcare costs both in hospitals and in the community [1]. The development of resistance to all available antibiotics in some organisms may preclude the effectiveness of any antibiotic regimen [2, 3]. Infections caused by *P. aeruginosa* are frequently life-threatening and difficult to treat as it exhibits intrinsically high resistance to many antimicrobials

[4] and the development of increased, particularly multi-drug resistance in health care settings [4, 5]. Mechanisms that cause antimicrobial drug resistance and multi-drug resistance in *P. aeruginosa* are due to acquisition of resistance genes (e.g. those encoding beta-lactamase [6] and amino-glycoside modifying enzymes [7] via horizontal gene transfer and mutation of chromosomal genes (target site, efflux mutations) are the target of the fluoroquinolones particularly ciprofloxacin [8]. Biofilm formation in *P. aeruginosa*, particularly in the case of pulmonary infections in patients with cystic fibrosis, contributes to its resistance to antimicrobial agents [9]. Hyper mutable (or mutator) strains of *P. aeruginosa* exhibiting increased mutation rates are common in chronic infections such as those that occur in the lungs of cystic fibrosis patients [10]. Increase in the frequency of multi-drug resistant (MDR) strains of *P.*

*aeruginosa* has severely limited the availability of therapeutic options. On-going studies on current antimicrobial resistance profiles of *P. aeruginosa* 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 therapeutic treatment options. This study was designed to find out the prevalence and current antimicrobial susceptibility patterns of *P. aeruginosa* strains in a centrally located tertiary care hospital in S.S.G.Hospital, Baroda.

#### MATERIALS AND METHODS

##### Study duration and Sample size:

This prospective study was conducted from March 2015 to May 2015 at S.S.G. Hospital, Baroda. During these period total 3618 samples (blood, pus/wound, sputum, tracheal aspirates, pleural fluid, Inter costal drainage (ICD) fluid, and bile fluid) were tested, of which 1901 samples showed growth. Out of 1901, 150 *Pseudomonas* isolated from various clinical samples were tested.

Ethical clearance: All these samples were a part of diagnosis. So ethical consideration is not necessary.

##### Isolation and identification of *Pseudomonas*

The samples were selected on the basis of their growth on routine culture media like Mac-Conkey agar, Nutrient agar. A battery of tests were performed that included 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* [11].

The *Pseudomonas* isolates were subjected to susceptibility testing by disc diffusion technique according to the Clinical Laboratory Standards International (CLSI) guidelines with quality controls (*P. aeruginosa* ATCC 27853) [11].

##### Susceptibility test for *Pseudomonas*

Anti-microbial susceptibility tests were done by the Kirby-Bauer disk diffusion method as per the recommendations of National Committee for Clinical Laboratory Standards (NCCLS) [12].

The antimicrobials tested included Piperacillin (100 µg), Piperacillin+Tazobactam (100/10 µg), Amikacin (30 µg), Cefoperazone (75 µg), Levofloxacin (5 µg), Ceftazidime (30 µg), Gentamicin (10 µg), Meropenem (10 µg)

#### RESULTS

150 strains of *P. aeruginosa* were isolated from 3618 samples. It shows the prevalence rate is 4.15%.

Table-1 shows the clinical isolates of *Pseudomonas aeruginosa* in different clinical samples in which *pseudomonas* is more commonly isolates from blood and pus/ wound samples followed by tracheal aspiration, sputum, ICD fluid, pleural fluid, and bile fluid.

**Table 1: Distribution of specimens of *P. aeruginosa* clinical isolates**

Source of specimen	Number (% , n=150)
<b>Pus</b>	34 (22.67%)
<b>Sputum</b>	5 (3.33%)
<b>Wound</b>	44 (29.33%)
<b>Tracheal aspirate</b>	6 (4%)
<b>Blood</b>	54 (36%)
<b>Pleural fluid</b>	2 (1.33%)
<b>Bile</b>	1 (0.67%)
<b>ICD fluid</b>	4 (2.67%)
<b>Total</b>	150 (100%)

**Table 2: Antimicrobial susceptibility patterns of *P. aeruginosa* clinical isolates**

Antibiotic	Sensitive no. (% , n=150)
<b>Piperacillin</b>	121 (80.66%)
<b>Piperacillin+Tazobactam</b>	147 (98%)
<b>Amikacin</b>	84 (56%)
<b>Cefoperazone</b>	122 (81.33%)
<b>Ceftazidime</b>	123 (82%)
<b>Levofloxacin</b>	139 (92.66%)
<b>Gentamicin</b>	82 (54.66%)
<b>Meropenem</b>	140 (93.33%)

Table-2 shows the sensitivity pattern of *P. aeruginosa*. 98% *P. aeruginosa* were sensitive Piperacillin+Tazobactam which is the most sensitive drug followed by Meropenem (93.33%), Levofloxacin (92.66%) , Ceftazidime (82%), Cefoperazone(81.33%), Piperacillin (80.66%), Amikacin(56%), Gentamicin(54.66%).

#### DISCUSSION

*Pseudomonas aeruginosa* is a major cause of nosocomial infections worldwide. In this study, a total of 150 isolates of *Aeruginosa* were isolated and identified from various clinical sources, from the hospitalized patients and their antimicrobial susceptibility patterns were determined. The

distribution of specimens of *P. aeruginosa* may vary with each hospital as each hospital facility has a different environment associated with it. In this study majority of the *Pseudomonas aeruginosa* isolates were more from exudative specimens of blood (36%) followed by wound (29.33%), pus (22.66%), tracheal aspiration (4%), sputum (3.33%), ICD fluid (2.66%), pleural fluid (1.33%), and bile fluid (0.66%). These results are comparable to similar results had been obtained in different studies in India reported by Mohanasoundaram [13] and Arora et al [14] respectively.

In this study, 150 strains of *P. aeruginosa* were isolated from 3618 samples as shown in table-1. It shows the prevalence rate is 4.15%. This study was comparable to the similar study in Afghanistan and Greece shows the prevalence rate is 6.67% and 16.6% respectively. [15, 16]

Antibiotic susceptibility testing data for Piperacillin (100 µg), Piperacillin+Tazobactam (100/10 µg), Amikacin (30 µg), Cefoperazone (75 µg), Levofloxacin (5 µg), Ceftazidime (30 µg), Gentamicin (10 µg), Meropenem (10 µg) were compiled. Majority of *Pseudomonas* isolates were susceptible to Piperacillin+Tazobactam (98%), Meropenem (93.33%) and Levofloxacin (92.66%) and followed by Ceftazidime (82%), Cefoperazone (81.33%), Piperacillin (80.66%), Amikacin (56%), Gentamicin (54.66%). So, Piperacillin+Tazobactam, Meropenem, Levofloxacin continue to remain the mainstay for treatment for *Pseudomonas* infections. The resistance profiles of *P. aeruginosa* to the eight anti-microbial agents tested varied among the isolates investigated. This is consistent with a report published in 2002 in Mangalore, India [17] but other studies have showed varying degrees of resistance to imipenem in recent years [13, 14, 18, 19]. High resistance to aminoglycosides had been reported in studies done in India [13, 14], Bangladesh [20], Turkey [21] and Malaysia [22]. Similarly higher rates of resistance to fluoroquinolones such as ciprofloxacin (40.5%) had been reported in a study done in North Kerala, India [23] and ciprofloxacin resistance (92%) was shown in a study from Malaysia [19]. Amikacin alone tested showed a resistance rate of 44% in this study whereas Piperacillin+Tazobactam drug showed a lower resistance of 2% only, which makes the combination drug the preferred choice against *P. aeruginosa* infections. Thus, emphasis should be given towards use of combined antibiotics in the treatment of *pseudomonas* infections [24].

*Aeruginosa* strains in this study exhibited a high rate of resistance to the third generation cephalosporin drug – Ceftazidime (18%). A much

higher resistance to ceftriaxone of 75%, 86% and 93.9% had been reported in studies done in India [14], Bangladesh [20] and Nepal [24]. This study revealed that chloramphenicol had the highest rate of resistance (72.41%) to *P. aeruginosa* strains suggesting that this drug should no longer be included in the treatment regimen for *P. aeruginosa* infections in this population group. A study done in Kano, Nigeria [25] demonstrated a much higher rate of resistance (97.7%) of *P. aeruginosa* isolates to chloramphenicol.

This study has a few limitations. First, including the community acquired isolates of *Aeruginosa* along with hospital isolates would have provided a much better picture of resistance patterns of strains in this geographical area. Second, it is essential to conduct a large scale study with newer anti-*pseudomonas* agents. Third, molecular typing and plasmid profile of the *P. aeruginosa* isolates would provide the much needed details about the strains and lastly extended spectrum beta-lactamase (ESBL) producing *P. aeruginosa* which have become a major cause of nosocomial infections.

## CONCLUSION

The prevalence of *Pseudomonas aeruginosa* is 4.15% among clinical isolates of various clinical samples. Active screening and compliance with recommended infection control practices play an important role in the control of hospital acquired infection. Results of the present study clearly demonstrated the occurrence of resistance to various antipseudomonal agents among the *P. aeruginosa* isolates. We suggest a more restricted and a more rational use of this drug in this hospital setting. Piperacillin+Tazobactam, Levofloxacin with beta-lactamase inhibitors are the preferred drugs for optimal management of infections caused by *P. aeruginosa*. Regular anti-microbial susceptibility monitoring is essential for local, regional and national level isolates. This would help and guide the physicians in prescribing the right combinations of anti-microbials to limit and prevent the emergence of multi-drug resistant strains of *P. aeruginosa*.

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**Corresponding Author:**

Dr. Jignasha Tadvi,  
A/3, 400, Vaikunth-1 Society,  
Near Bapod Jakatnaka,  
Waghodia road, Vadodara- 390019,  
E-mail: [dr.jignasha.tadvi@gmail.com](mailto:dr.jignasha.tadvi@gmail.com)

Date of Submission: 13/09/2015

Date of Acceptance: 30/09/2015

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**How to cite this article:** Tadvi J, Javadekar TB, Bhavsar R, Garala N. Prevalence & antibiogram of *Pseudomonas aeruginosa* at S.S.G. Hospital, Baroda, Gujarat, India. *J Res Med Den Sci* 2015;3(3):204-7.

**Source of Support:** None

**Conflict of Interest:** None declared