

Original Article

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF PSEUDOMONAS SPECIES ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: *Pseudomonas aeruginosa* is the commonest agent causing opportunistic nosocomial infections, contributing to mortality and morbidity worldwide. Along with its intrinsic and acquired resistance mechanisms, infections caused by *Pseudomonas* species further lead to treatment failure. This study was done to determine the prevalence and antimicrobial susceptibility pattern of different species of *Pseudomonas* isolated from various clinical samples by phenotypic methods.

Methods: This study involved the examination of clinical samples for various species of *Pseudomonas* by using different standard biochemical tests. Their antimicrobial susceptibility profile was performed by the Kirby Bauer disc diffusion method. Analysis of the antibiogram pattern was done to study the multi-drug resistance among *Pseudomonas* species.

Results: Out of the 1249 bacterial isolates, *Pseudomonas* species accounted for 12.9%. *Pseudomonas* species showed maximum resistance to gentamicin (31.4%), followed by ciprofloxacin (30.2%) and showed the least resistance to colistin and polymyxin B. 46 isolates out of the 162 (28%) were found to be Multi-Drug Resistant (MDR) *Pseudomonas aeruginosa*.

Conclusion: The present study highlights that *Pseudomonas* species remains a major cause of hospital-acquired infections. Multidrug resistance was observed in most of the strains, which makes the therapeutic options more difficult. Surveillance of antimicrobial resistance and strict infection control measures are essentially to be practiced in managing and control of infections caused by *Pseudomonas*.

Keywords: *Pseudomonas aeruginosa*, Antimicrobial susceptibility, Multidrug resistance

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INTRODUCTION

The genus *Pseudomonas*, being one of the most complex bacterial genera, contains more than 140 species, among which most of them are saprophytic. Around 25 species are associated with humans to cause opportunistic infections. Some of the important clinically relevant species are *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *P. mallei*, *P. pseudomallei*, *P. maltophilia* and *P. putrefaciens* [1, 2]. *Pseudomonas aeruginosa* is the most commonly identified one from the clinical specimens of hospital admitted patients [3]. Even though it is a commensal of microflora in healthy humans, it is a commonly encountered causative agent of infections seen in hospitalized patients, particularly in burns, respiratory diseases, catheterized and immunocompromised patients. It is one of the commonest gram-negative bacteria that takes advantage of an individual's weakened immune status to cause infection by its tissue-damaging toxins. Infections caused by *Pseudomonas aeruginosa* can infect any anatomical site; hence it can be isolated from various body fluids such as wounds, urine, blood, wounds, eye or ear swabs, and sputum. It is also a grave concern to cancer and burn patients. It can cause infections such as Urinary Tract Infections (UTI), respiratory infections, particularly Ventilator-Associated Pneumonia (VAP) in debilitated patients, bone and joint infections, dermatitis, otitis media, bacteremia and other numerous variety of systemic infections [4, 5].

It is ubiquitous in nature. It has the ability to thrive and colonize anywhere, being widely distributed in the environment-soil, vegetation, water bodies, sewage, hospitals and even on the moist sites of the skin of healthy individuals. And its ability to resist the antibacterial and antiseptic agents makes it more complex. This organism is hard to treat because of its acquired and intrinsic resistance [1, 6]. The defense mechanism of *Pseudomonas aeruginosa* makes it immune to many antibiotics by different means such as chromosomally encoded genes, restricted outer membrane

permeability, production of antibiotic inactivating enzymes or by the efflux system that pumps antibiotics out of the cell [7, 8].

Keeping in view the knowledge about the ability of *Pseudomonas* spp. to thrive in myriad of habitat, its etiology as well as the pathology and its wide range of mechanisms of resistance to antibiotics, this study was aimed to isolate and characterize various *Pseudomonas* species from diverse clinical samples of a tertiary care hospital and to analyze its antibiotic susceptibility profile.

MATERIALS AND METHODS

This perspective cross-sectional study was conducted for a period of 3 mo from December 2019 to February 2020 in the clinical microbiology laboratory at Saveetha Medical College. Clinical samples received in the laboratory were subjected to gram staining and were inoculated on to blood agar and macconkey agar. In addition to these, chocolate agar was used for culturing respiratory samples and blood. All the culture Media were incubated aerobically at 37 °C overnight. Routine biochemical tests were used to identify *Pseudomonas*. These included colony morphology, pigment production, motility test, oxidase test, catalase test, growth at 41 °C, nitrate reduction, gelatin hydrolysis, arginine dihydrolase and sugar fermentation test.

The isolated species were subjected to an Antibiotic susceptibility test by the Kirby Bauer disc diffusion method recommended by the Clinical Laboratory Standard Institute (CLSI) guidelines. Individual isolates were inoculated on Mueller Hinton Agar plates at a concentration of 10⁶ Colony Forming Units (CFU) by lawn culture method. Discs impregnated with antibiotics Amikacin (30mcg), Gentamicin (10mcg), Ceftazidime (30mcg), Ciprofloxacin (5mcg), Imipenem (10mcg), Meropenem (10mcg), Ertapenem (10mcg), Ofloxacin (5mcg), Cefepime (30mcg), Cefepime (30mcg), Piperacillin-tazobactam (100/10mcg), Tigecycline (15mcg),

Colistin (10mcg) and Polymyxin B (300mcg) were placed. Plates were examined after 24 h of incubation at 37 °C.

RESULTS

During the study period, a total of 1249 bacterial isolates were obtained, in which 162 isolates were found to be Pseudomonas species.

Prevalence of Pseudomonas species was 12.9%. Pseudomonas species were predominantly recovered from exudates (pus samples, wound swabs, tissues and body fluids) followed by respiratory samples (sputum, bronchoalveolar lavage, endotracheal aspirates), blood samples and urine. The distribution of clinical samples from which Pseudomonas species were isolated is given in fig. 1.

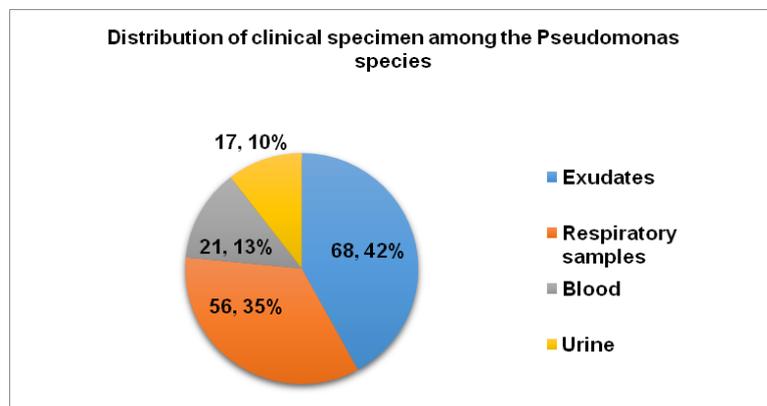


Fig. 1: Distribution of clinical specimen types among the Pseudomonas species isolated, pseudomonas isolates were more from male patients (109; 67%) than from female patients (53;33%) [table 1]

Table 1: Distribution of male and female patients among IP-OP departments

Patients	IP	OP	Total
Male	98	11	109 (67.3%)
Female	47	6	53 (32.7%)
Total	145 (90%)	17 (10%)	

Pseudomonas infections were observed highest in the age group 46-60 y (36%) followed by 61-75 y (28%) and 31-45 y (20%). Age-wise distribution is given in table 2.

Table 2: Age-wise distribution of Pseudomonas isolates

Age group	No. of Pseudomonas spp isolated
0-15 y	1
16-30 y	18
31-45 y	33
46-60 y	58
61-75 y	45
76-90 y	7

Almost 90% of the Pseudomonas species were found out from inpatients in which it was highest from surgical wards (19%), ICU (17%) followed by medicine wards (14%) and Chest and TB medicine (10%). Detailed distribution of Pseudomonas species from various other departments are given in the fig. 2.

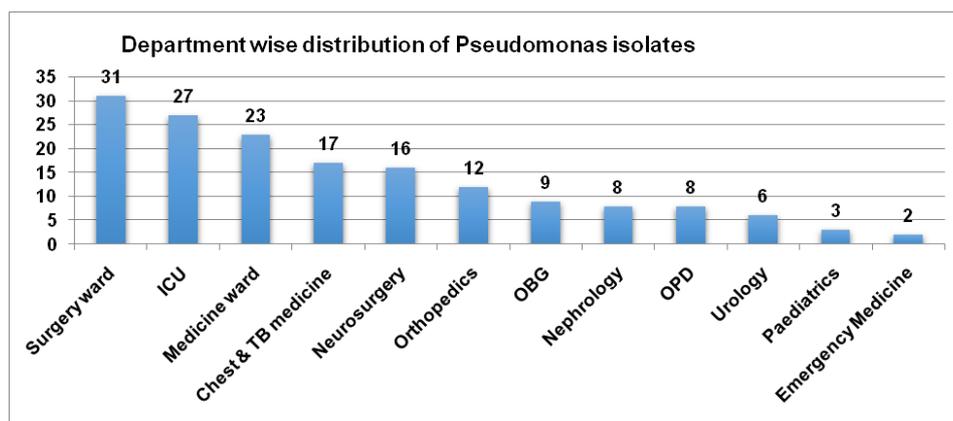


Fig. 2: Department wise distribution of Pseudomonas isolates

Out of the total isolates, *Pseudomonas aeruginosa* was the most prevalent species, which accounted for 87% followed by *Pseudomonas stutzeri* (6%) and *Pseudomonas putida* (4%).

Pseudomonas fluorescens (2%) and *Pseudomonas maltophilia* (1%) were the least isolated species (fig. 3: Distribution of various species of *Pseudomonas* isolated from clinical specimens).

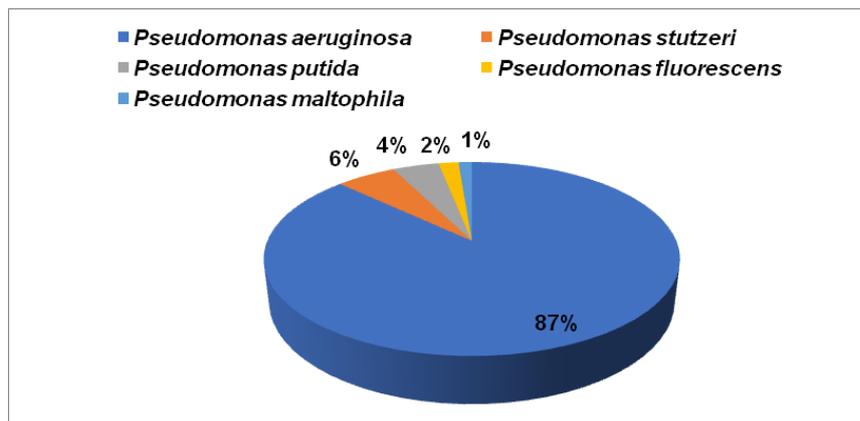


Fig. 3: Distribution of various species of *Pseudomonas* isolated from clinical specimens

Most of the strains were resistant to fluoroquinolones, aminoglycosides, cephalosporins and carbapenem group of drugs. The highest level of resistance was observed against Gentamicin, which is an aminoglycoside with 51 strains to be resistant, followed by ciprofloxacin, a fluoroquinolone group of drug (49; 30.2%), ceftazidime (45; 27.7%) cefoperazone-sulbactam (41;25.3%), amikacin (40;24.6%), meropenem (31; 19.1%),

cefepime (31; 19.1%), imipenem (30; 18.5%), piperacillin-tazobactam (29; 17.9%) and ertapenem (27; 16.6%). Most of the strains showed sensitivity towards Colistin and Polymyxin B.

Forty-six strains (28%) of *Pseudomonas* spp were multidrug-resistant (MDR) i. e, strains resistant to three or more than three different group of drugs. And interestingly, all the 46 strains were *Pseudomonas aeruginosa*.

Table 3: Antimicrobial resistance pattern of *Pseudomonas* spp

Antibacterial drug	% of isolates showing resistance	% of isolates showing sensitivity
Amikacin	24.6%	75.4%
Gentamicin	31.4%	68.6%
Ceftazidime	27.7%	72.3%
Ciprofloxacin	30.2%	69.8%
Imipenem	18.5%	81.5%
Meropenem	19.1%	80.9%
Ertapenem	16.6%	83.4%
Ofloxacin	14.1%	85.9%
Cefaperazone-sulbactam	25.3%	74.7%
Cefepime	19.1%	80.9%
Piperacillin-tazobactam	17.9%	82.1%
Tigecycline	13.5%	86.5%
Colistin	3.1%	96.9%
Polymyxin B	3.7%	96.3%

DISCUSSION

Pseudomonas aeruginosa is the most common gram-negative, non-fermenting bacteria isolated from clinical specimens that pose serious challenge for the treatment of nosocomial infections. The main objective of our study was to investigate the epidemiological data of *Pseudomonas* spp. isolated from various clinical samples and to analyze the antimicrobial resistance patterns against routinely used antibiotics.

In our study, highest percent (19%) of *Pseudomonas* spp was obtained from surgery units, which is in concordance with the result of Sharma *et al.* [3] with 33%. Most of the patients infected by *Pseudomonas* spp are male (109; 67.3%) when compared to the females, which is in close concordance with the study of Josef *et al.* [6], (113 males; 67.3%). Polymyxin B and colistin shows the highest percentage of sensitivity and percentage of resistance being low(3.7%, 3.1%), which also supports other studies that are made throughout India, making them the ideal antimicrobial agent. 46 *Pseudomonas* (28%) were MDR strains. Several studies with similar MDR rates were reported-24.4% by Josef *et al.* [6], 25.35% by

Qayoom *et al.* [9] and 24% by Sharma *et al.* [3]. High percentage of MDR isolated from clinical samples is worrisome. To contain the spread of drug resistance, appropriate laboratory detection, control of patient-to-patient transmission and judicious use of antibiotics should be done.

CONCLUSION

It is inevitable that the antibiotic susceptibility pattern of bacterial pathogens like *P. aeruginosa* in specialized clinical units should be continuously monitored so as to minimize the resistance to in use routine antibiotics. Judicious usage of antibiotics and creating a standard antibiotic policy that supports the clinician to choose the appropriate drug of choice helps in preventing drug resistance, whereas proper infection control measures and timely identification of resistant pathogen can help in reducing the spread of multidrug-resistant strains.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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