



IDENTIFICATION AND ANTIMICROBIAL RESISTANCE IN *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Introduction: *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 resistance to multiple groups of antibiotics including β -lactams, aminoglycosides and fluoroquinolones. This study was undertaken to determine the prevalence of *P. aeruginosa* and its susceptibility pattern isolated from pus samples.

Material and Methods: In this study a total of 57 *P.aeruginosa* isolates were obtained out of 254 pus samples between a period of one year (Feb 2015 to Jan 2016). The isolates were selected on the basis of their growth characteristics on Blood agar, MacConkey agar and Nutrient agar medium. Colonies were subjected to battery of biochemical tests to identify species. Antimicrobial susceptibility testing of all confirmed *P. aeruginosa* isolates was performed by Kirby–Bauer disc diffusion method and results were interpreted according to CLSIs guidelines.

Results: The prevalence of this pathogen was 22.4% and most of the isolates were found to be highly sensitive to Colistin (95.4%), Polymyxin B (95%), Levofloxacin (83.3%), Imipenam (70%), Netilmycin (66%) and Piperacillin+ Tazobactam (64.5%). However, they showed resistance towards Ofloxacin (65%), Piperacillin (64%), Cefazidime (56.3%), Cefoprazone (58%), Cefpime (55%), Aztreonam (53%), Cefaprazone + sulbactam (46%) and Gentamicin (45%). Fourteen (24%) *P. aeruginosa* isolates were Multidrug resistant (MDR) as they were totally resistant to Cephalosporins, aminoglycosides, fluoroquinolones and carbapenems.

Conclusion: High prevalence of *P. aeruginosa* as an opportunistic pathogen has been on the increase with resistance to antimicrobial agents and thus becoming a threat.

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INTRODUCTION

Pseudomonas aeruginosa is one of the most common gram-negative microorganisms identified in the clinical specimens of hospital admitted patients. It is a commensal of human microform in healthy people and is frequently isolated as an opportunistic pathogen in recurrent infections of hospitalized patients.^[1] It can infect almost any external site or organ, and therefore, can be isolated from various body fluids such as sputum, urine, wounds, eye or ear swabs and from blood.^[2] This organism is often hard to treat because of both the intrinsic resistance and acquired resistance i.e. mutations in chromosomal genes, to multiple groups of antimicrobial agents, including β -lactams, aminoglycosides and fluoroquinolones.^[3] An increased resistance of *P.aeruginosa* to β -lactam drugs is because of production of metallo-beta-lactamases i.e. enzymes that efficiently hydrolyze all β -lactams.^[4] The implication of these emerging resistance is in the successful treatment of infections caused by this bacteria

cannot be overemphasized.^[5] It causes infections in hospitalized patients particularly in burns, orthopaedic related infections, respiratory diseases, catheterized and even immunosuppressed patients. Inherent resistance to many antimicrobial agents, contributes substantially to wound related morbidity and mortality worldwide.^[6] Keeping in view the occurrence of *Pseudomonas* spp. in different habitat, its pathology and resistance to antibiotics, this study was aimed to isolate *P.aeruginosa* from pus samples and to determine its antibiotic susceptibility profile.

MATERIAL AND METHODS

The present study was conducted in the Microbiology Laboratory, Nuvjeevan Nursing Home Rajouri. All pus samples received were processed for isolation and identification of *P. aeruginosa* was made according to the Standard microbiological techniques. Blood agar, MacConkey agar and Nutrient agar were used as growth media for the culturing of samples.^[7] The plates were then incubated at 37°C

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for 24 hours to get the growth and were then processed further for identification using standard procedures. *P.aeruginosa* was identified by Gram staining, motility test and biochemical tests like- oxidase test, O/F test, and growth at 420 C.^[8] Antibiotic sensitivity pattern of *P. aeruginosa* isolates to Gentamicin (10 mcg), Ciprofloxacin (5 mcg), Cefotaxime (30 mcg), Ceftazidime (30 mcg), Amikacin (30 mcg), Imipenem (10 mcg), Meropenem (10 mcg), Cefoperazone/ Sulbactam (75/30 mcg), Cefpirome (30 mcg), Aztreonam (50 mcg), Ceftazidime / Clavulanic acid (30/10 mcg), Piperacillin/Tazobactam (100/10 mcg), Piperacillin (100 mcg), Polymyxin (300 u), Colistin (10mcg) was investigated by Kirby-Bauer method on Mueller Hinton Agar (MHA). The final bacterium inoculation conc. was approx 108 cfu/ml that was equal to 0.5 McFarland. MHA plates were incubated overnight at 370, and the diameter of each inhibition zone was measured with special scale supplied by Himedia.

RESULTS

A total of 57 *P. aeruginosa* strains were isolated from 254 pus samples received. Prevalence of *P.aeruginosa* was 22.41%. The highest percentage of isolates was from males (71.9%) and of age group 41-50,61-70 (21%) years each (Table-1). Most of the isolates were found to be highly sensitive to Colistin (95.4%), Polymyxin B (95%), Levofloxacin (83.3%), Imipenam (70%), Netilmicin (66%) and Piperacillin + Tazobactam (64.5%). However, they showed resistance towards Ofloxacin (65%), Piperacillin (64%), Ceftazidime (56.3%), Cefoprazone (58%), Cefpime (55%), Aztreonam (53%), Cefaprazone + sulbactam (46%) and Gentamicin (45%) (Table-2). As the bacterial strains that show resistance to three or more categories of antibiotics are defined as multidrug resistant (MDR) strains, MDR strains of *P.aeruginosa* isolated in this study were 24%. Fourteen *P. aeruginosa* isolates were totally resistant to Cephalosporins, Aminoglycoside, Fluoroquinolones and Carbapenems, showing Multidrug resistance (MDR).

Table 1 Age wise distribution of Pseudomonas aeruginosa isolates

Age group(in years)	No. of isolates (N=57)	Percentage %
<20	07	12.3
21 – 30	11	19.3
31 – 40	05	8.8
41 – 50	12	21.0
51 – 60	04	7.0
61 – 70	12	21.0
> 70	06	10.6

Table 2 Antimicrobial sensitivity pattern of Pseudomonas aeruginosa

Antibiotics	Sensitivity (%)	Resistant (%)
Ceftazidime	43.7	56.3
Cefperazone	42.0	58.0
Cefpime	45.0	55.0
Ceftazidime+clavulanic acid	34.0	66.0
Piperacillin+Tazobactam	64.5	33.5
Cefperazone+sulbactam	54.0	46.0
Piperacillin	36.0	64.0
Aztreonam	47.0	53.0
Imipenem	70.0	30.0
Meropenem	50.0	50.0
Gentamicin	55.0	45.0
Amikacin	58.0	42.0
Netilmicin	66.0	34.0
Polymyxin B	95.5	5.0
Colistin	95.4	4.6
Ciprofloxacin	59.0	41.0

Ofloxacin	35.0	65.0
Levofloxacin	83.3	16.7

DISCUSSION

P.aeruginosa presents a serious therapeutic challenge for treatment of both community acquired and nosocomial infections. Infections caused by *P.aeruginosa* are notoriously difficult to treat due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance. Our study measures the rate of isolation of *P.aeruginosa* (22.44%) as which is quite similar to previous studies as by Tadvi et al.^[9] (22.67%), Viren et al¹⁰ (26.79%), and Ruhil et al¹¹(27.70%). The occurrence of *P.aeruginosa* is found to be higher in males, inpatients in age group >60,41 years and in surgery department, which is same as reported by Viren et. al^[10], Ali Hussein et al¹², Shampa et al¹³ and Rakesh et al.¹⁴ Most of isolates were found to be highly sensitive to Colistin (95.4%), Polymyxin B (95%), Levofloxacin (83.3%), Imipenem (70%), Netilmicin (66%) and Piperacillin + Tazobactam (64.5%), Sensitivity pattern of *P.aeruginosa* nearly coincides with that of Viren et al., Tadvi et al⁹, Ruhil et al.^[11] and Aggarwal et al.^[14] *P.aeruginosa* showed resistance towards Ofloxacin (65%), Piperacillin (64%), Ceftazidime (56.3%), Cefoprazone (58%), Cefpime (55%), Aztreonam (53%), Cefaprazone + sulbactam (46%) and Gentamycin (45%), which was comparable with previous studies done in India as by Arora et al.¹⁶, Jamshaid et al^[7] and Bhatt et al.^[17] In present study prevalence of MDR *P.aeruginosa* was 24.56% which is very much close to the study by Chander et al^[18] (20.69%) and Shampa et al.^[13] (18.00%).

CONCLUSION

It is evident from the study that nowadays *P.aeruginosa* is becoming resistant to cephalosporins, aminoglycosides and even beta lactam (BL) –beta lactamase inhibitor (BLI) combinations. To prevent the spread of the resistant bacteria it is critically important to have strict antibiotic policies. It is desirable 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.

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