

OCCURRENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF PSEUDOMONAS SPP. IN VARIOUS CLINICAL ISOLATES IN A TERTIARY CARE CENTRE BIHAR, INDIA

Anjoo Anupama¹, Nafisa Rahman², Ranjan Kumar², Amit Kumar³

¹Assistant Professor, Department of Microbiology, JLN MCH, Bhagalpur, Bihar, India

²Tutor, Department of Microbiology, JLN MCH, Bhagalpur, Bihar, India

³Associate Professor, Department of Microbiology, JLN MCH, Bhagalpur, Bihar, India

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Corresponding Author:

Dr. Nafisa Rahman,

Email: nafisarahman26@gmail.com

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Abstract

Background: The present study was undertaken to assess the antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Bihar, India. Due to significant changes in microbial genetic ecology, as a result of indiscriminate use of anti-microbials, the spread of anti-microbial resistance is now a global problem. **Materials and Methods:** Out of 267 culture positive samples, 59 samples of *Pseudomonas aeruginosa* were examined and 10 different types of specimen were collected. Microbial sensitivity testing was done using disk diffusion test with *Pseudomonas* species NCTC 10662, as per CLSI guidelines. **Result:** The highest number of *Pseudomonas* infections was found in urine, followed by pus and sputum. *Pseudomonas* species demonstrated marked resistance against monotherapy of penicillins, cephalosporins, fluoroquinolones, tetracyclines and macrolides. Only combination drugs like Ticarcillin + Clavulanic acid, Piperacillin + Tazobactam, Cefoperazone + Sulbactam, Cefotaxime + Sulbactam, Ceftriaxone + Sulbactam and monotherapy of amikacin showed higher sensitivity to *Pseudomonas* infections. **Conclusion:** From the present study, we conclude that urinary tract infection was the most common hospital acquired infection. Also, co-administration of β -lactamase inhibitors markedly expanded the anti-microbial sensitivity of semi-synthetic penicillins and cephalosporins. The aminoglycoside group of antibiotics - amikacin - demonstrated maximum sensitivity against *pseudomonas* species. Therefore, use of amikacin should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains. Periodic susceptibility testing should be carried out over a period of two to three years, to detect the resistance trends. Also, a rational strategy on the limited and prudent use of anti-Pseudomonal agents is urgently required.

INTRODUCTION

Multiple antibiotic resistances in bacterial populations are a pervasive and growing clinical problem, which is recognized as a threat to public health. Hence, there is a need to conduct area-specific monitoring studies to profile different pathogens responsible for specific infections and their resistance patterns, so as to generate data that would help clinicians to choose the correct empirical treatment.

Pseudomonas aeruginosa (*P. aeruginosa*) is an epitome of opportunistic nosocomial pathogen, which causes a wide spectrum of infections and leads to substantial morbidity in immunocompromised patients. Despite therapy, the mortality due to nosocomial pseudomonal

pneumonia is approximately 70%.^[1] Unfortunately, *P. aeruginosa* demonstrates resistance to multiple antibiotics, thereby jeopardizing the selection of appropriate treatment.^[2] Therefore, the present study was undertaken to find out the antibiotic susceptibility patterns of pathogenic isolates of *Pseudomonas aeruginosa* from various specimens of hospital acquired infections (HAI).

MATERIALS AND METHODS

Our study group comprised of samples, which were clinically suspected cases of bacterial infections. The study was conducted in Department of Microbiology, JLN MCH, Bhagalpur, Bihar between August 2022 to January 2023.

Five hundred and twenty-seven non-duplicate isolates were taken (i.e. multiple isolates of the same species from the same patient were excluded). Two hundred and sixty seven samples obtained from sputum, endo-tracheal Tract secretion, broncho-alveolar lavage, blood, urine, body tissues, pus, semen, cerebro-spinal fluid (CSF), and body fluids (peritoneal fluid) and costal bronchial Secretions (CBS) reported the presence of bacterial infection. Identification of all isolates was carried out by a positive reaction to oxidase and production of pyocyanin.^[3] Culture examination was carried out using Nutrient agar and MacConkey's medium, followed by inoculation by four flame streak method.

Antibiotic susceptibility was confirmed by disk diffusion technique on Muller-Hinton medium (Becton Dickinson Microbiological Systems, Cockysville, MD), performed according to the Clinical Laboratory Standard Institute (CLSI) guidelines.^[3] Paper disks (Hi-media, Mumbai) were impregnated with antibiotics (Sigma Chemical Co., St. Louis, Mo.): Penicillins: ampicillin (10mcg), amoxicillin (20mcg), ticarcillin (75mcg), piperacillin (100mcg); cephalosporins: cephalixin (30 mcg), cefuroxime (30mcg), cefazolin (30mcg), cefotaxime (30mcg), ceftriaxone (30mcg), ceftazidime (30mcg), ceftizoxime (30mcg), cefoperazone (75 mcg), cefpodoxime 10 mcg, cefdinir (5 mcg), cefepime (30 mcg); carbepenems: imipenem (10mcg), meropenem (10 mcg); monobactams: aztreonem (30 mcg); combinations: ampicillin + sulbactam (10/10 mcg), amoxicillin + clavulanic acid (20/10 mcg), piperacillin + tazobactam (100/10 mcg), ticarcillin + clavulanic acid (75/10 mcg), cefoperazone + sulbactam (75/10 mcg), cefotaxime + sulbactam (30/10 mcg), ceftriaxone + sulbactam (30/10 mcg); Aminoglycosides: gentamicin (10 mcg), tobramycin (10 mcg), amikacin (30 mcg), netilmicin (30 mcg); quinolones: ciprofloxacin (5mcg), ofloxacin (5mcg), levofloxacin (5mcg), gatifloxacin (5mcg); tetracyclines: doxycycline (30mcg), minocycline (30mcg); macrolides: azithromycin (15mcg) and miscellaneous: chloramphenicol (30 mcg) respectively. They were incubated overnight at 37°C

in 5-10% CO₂ enriched environment (candle jar). The diameter of the zone of inhibition was measured and compared to that of standard strain and the results were interpreted as sensitive, intermediate resistant or resistant, based on CLSI guidelines.^[4] The category "susceptible" was defined as identification of a strain as susceptible by the disk diffusion method. Quality control strains of Pseudomonas species NCTC-10662 was used to validate the results of the antimicrobial discs. Susceptibility data were compared by using a Chi-square test with statistical package for the social sciences (SPSS) software for Windows, version 12. Both susceptibility and resistance were calculated as percentages with 95% confidence intervals. The analysis was performed on the cross-tabulated values of the presence of the resistant/intermediate/susceptible isolates, according to the categories of the selected variable. A P value of < 0.05 was considered to be statistically significant.

RESULTS

Of the 527 samples subjected to culture sensitivity, 267 reported presence of bacterial infection, thereby suggesting 42.85% as the occurrence level. The percentage occurrence of Pseudomonas in these 267 samples was only 22.08% (59 samples), of which 65.2% (i.e. 36 samples) and 35.75% (i.e. 23 samples) were reported from males and females respectively. Various specimens studied under the present investigation included urine, pus, sputum, blood, endotracheal secretions (ET), semen, and catheter tip (CT), stool, body fluids and body tissues.

The majority of specimens from which P. aeruginosa was isolated consisted of urine, pus and sputum. The acid resistant penicillins such as ticarcillin and piperacillin combinations (R=22.31% and 33.06% respectively) (P< 0.001) had significantly greater antibacterial activity against P. aeruginosa, when compared to their respective monotherapies (R=68.76% and 72.31% respectively).

Table 1: Frequency of specific sites from which P. aeruginosa was isolated

Specimen	Number of Specimens	% of Total
Urine	17	28.81
Pus	18	30.50
Sputum	16	27.11
ET	5	8.47
Semen	1	1.69
CT	1	1.69
Tissue	1	1.69
Total	59	100%

ET: Endo-tracheal tract secretions, CT: Catheter tip

A similar scenario was observed for ampicillin and amoxicillin, where the combination therapy with sulbactam and clavulanic acid (P< 0.001) demonstrated significantly higher antibacterial activity against P. aeruginosa, when compared to their respective monotherapies (R=97.81% in both the cases). The organism showed remarkable resistance against cephalosporin group of antibiotics, ranging from 68.76% for ceftazidime to

96.44% for cephalexin. But the extended-spectrum penicillins and the third generation cephalosporins, in combination with sulbactam, tazobactam and clavulanic acid showed a significant decrease in resistance to *P. aeruginosa*.

Table 2: Antibiotic resistance pattern of *P. aeruginosa* against cephalosporin group of antibiotics

Cephalosporins	Sensitive	Intermediate	Resistant
Cephalexin	6.53	0	96.44
Cefazolin	15.2	5.37	89.39
Cefuroxime	4.17	0	98.26
Cefoperazone	27.69	2.97	74.13
Cefpodoxime	22.31	0	77.69
Cefdinir	22.31	0	77.69
Ceftazidime	31.24	0	68.76
Ceftriaxone	16.94	3.56	79
Ceftizoxime	37.22	0	77.69
Cefotaxime	24.13	3.56	72.31
Cefepime	33.06	0	66.94

Values shown are percentage of total

A notable observation was that piperacillin and ticarcillin combinations demonstrated better antibacterial activity, as compared to cephalosporin combinations ($P < 0.001$). We observed that *P. aeruginosa* was highly sensitive to the carbapenem group of antibiotics like imipenem (75.87%) and meropenem (66.94%), while aztreonem showed 74.13% resistance ($P < 0.001$). On the other hand, aminoglycosides, fluoroquinolones, tetracyclines, macrolides and chloramphenicol did not demonstrate statistically significant susceptibility patterns ($P > 0.05$).

Table 3: Antibiotic resistance patterns of *P. aeruginosa* against different group of antibiotics

Class of drugs	Sensitive	Intermediate	Resistant
Aminoglycosides			
Gentamicin	31.24	0	68.76
Tobramycin	31.24	2.97	67.07
Netilmycin	39.33	3.56	67.01
Amikacin	42.81	3.97	57
Fluoroquinolones			
Ciprofloxacin	27.69	5.37	66.94
Ofloxacin	27.69	5.37	66.94
Levofloxacin	37.51	3.79	65.2
Gatifloxacin	35.7	0	65.2
Tetracyclines			
Doxycycline	9.83	0	97.01
Minocycline	17.01	3.97	85.7
Macrolides			
Azithromycin	9.83	3.56	87.51

In the present study, sensitivity of *P. aeruginosa* was in the 31-84% range for aminoglycosides, while for fluoroquinolones, susceptibility was found to be in the 27-67% range. For tetracyclines, macrolides and chloramphenicol, resistance was found to range between 75 and 91%. All antibiotics used as monotherapy in the present study did not demonstrate statistically significant susceptibility patterns ($P > 0.05$), with the exception of imipenem and meropenem.

DISCUSSION

P. aeruginosa is inherently resistant to many antimicrobial agents, mainly due to the synergy between multi-drug efflux system or a type I AmpC β -lactamase and low outer membrane permeability.^[5-7] The age- and sex-wise distribution of patients diagnosed with infection followed the natural epidemiological pattern.^[8] Out of 59 culture positive specimens isolated, 28.81% were urine

samples, indicating that urinary tract infection (UTI) is the most common HAIS.^[9] It is one of the most important causes of morbidity in the general population and is the second most common cause of hospital visits.^[10,11] The incidence of UTI is greater in women than men, which may be either due to anatomical predisposition or urolithial mucosal adherence to mucopolysaccharide lining or other host factors.^[9,12] Concurrent administration of a β -lactamase inhibitor such as clavulanate or sulbactam markedly expands the spectrum of activity of acid resistant penicillins like ticarcillin and piperacillin. The dose as well as the incidence of toxicity was subsequently reduced with semi-synthetic penicillins like ticarcillin, which makes it the preferred ureidopenicillin against *P. aeruginosa* infections. Our results are in corroboration with the one reported by other workers,^[11] so much so that the overall resistance to various generations of cephalosporins was high on account of the production of extended spectrum β -lactamses (ESBLs) by the bacteria involved.^[13]

In our study, notable resistance (16.94%) to *P. aeruginosa* was observed against carbapenems. The resistance to carbapenems, especially in *P. aeruginosa*, results from reduced levels of drug accumulation or increased expression of pump efflux.^[14,15] The resistance may also be due to the production of metallo- β -lactamases (MBL), which can be chromosomally encoded or plasmid mediated.^[16] The carbapenem hydrolyzing enzyme carbapenamase may be class B-metallo β -lactamases or class D-oxacillinases or class A-clavulanic acid inhibitory enzymes.^[17]

Among the aminoglycosides, amikacin has the highest sensitivity against *P. aeruginosa* [Table 3], which is in corroboration with an earlier report published from India.^[18] Amikacin was designed as a poor substrate for the enzymes that bring about inactivation by phosphorylation, adenylation or acetylation, but some organisms have developed enzymes that inactivate this agent as well. Amikacin seems to be a promising therapy for Pseudomonal infection. Hence, its use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains.^[19] The problem of increasing resistance to *P. aeruginosa* has limited the use of other classes of antibiotics like the fluoroquinolones, tetracyclines, macrolides and chloramphenicol.^[20]

In fact, the irrational and inappropriate use of antibiotics is responsible for the development of resistance of *Pseudomonas* species to antibiotic monotherapy. Hence, there is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of “reserve drugs” to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. An effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management.

CONCLUSION

Hence, based on the observations of present study, we recommend use of either semi-synthetic penicillins like ticarcillin, piperacillin or third generation cephalosporins like cefoperazone, cefotaxime and ceftriaxone along with β -lactamase inhibitors (clavulanate or sulbactam) against *Pseudomonas* species infections, in similar hospital settings. Further, amikacin should be considered as a reserved drug for the treatment of severe nosocomial infections.

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