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Research article

Prevalence and Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Various Clinical Samples in a Tertiary Care Hospital

Surya Narayan Mahaseth[1], Lokeshwar Chaurasia[2], Brajesh Jha[3], Raj Kumari Sanjana[3]

Department of Microbiology
Janaki Medical College, Janakpur, Nepal

[1] Assistant professor, Department of Microbiology, Janaki Medical College, Janakpur, Nepal
[2] Assistant professor, Department of Pharmacology, Janaki Medical College, Janakpur, Nepal
[3] Associate professor, Department of Microbiology, College of Medical Sciences, Bharatpur, Chitwan, Nepal

ABSTRACT

B**ackground and Objectives:** *Pseudomonas aeruginosa* is one of the leading causes of hospital-acquired infections. Increased resistance in this organism continues to pose a significant threat to patient care because of limited therapeutic options. The main objective of this study was to find out the prevalence and current antimicrobial susceptibility pattern of *P. aeruginosa* isolates obtained from various clinical samples at a tertiary care hospital.

Material and Methods: The study was conducted in a tertiary care hospital in Bharatpur, Chitwan, Nepal on 453 isolates of *Pseudomonas aeruginosa* from various clinical samples. The colonies which were grown on culture media were identified by different standard biochemical tests. Antimicrobial susceptibility testing was done using Kirby–Bauer disc diffusion method and the results were interpreted according to the CLSI guidelines. Quality control of the test was done by standards ATCC strain of *P. aeruginos*a 27853.

Results: This present study revealed the prevalence rate of *P. aeruginosa* was 11.29%. Piperacillin-tazobactam was the most sensitive chemotherapeutic agent with 94.26% susceptibility rate, followed by imipenem 89.40% and levofloxacin 88.08%. Amikacin showed better susceptibility rate 67.33% than that of gentamicin 48.78%; the susceptibility rate to cephalosporin and aztreonam was relative very low. Most of the *P. aeruginosa* strains were isolated from clinical samples like sputum 206, urine 81, respiratory secretion 76, and pus 35. Out of 453 clinical isolates, 167(36.86%) clinical isolates of *P. aeruginosa* were found to be MDR.

Conclusion: Most of the *P. aeruginosa* strains were isolates from sputum, urine, respiratory secretions and pus samples and were found to be MDR. Piperacillin-tazobactam was the most sensitive chemotherapeutic agent followed by Imipenem, levofloxacin.

Key words: *Pseudomonas aeruginosa*, Antibiotic Susceptibility Testing, Antipseudomonal Agents, Tazobactam, Aztreonam, Multi Drug Resistance (MDR)

INTRODUCTION

Pseudomonas aeruginosa is a clinically important, motile, aerobic, Gram negative

opportunistic bacterium, which is responsible for one of the leading causes of nosocomial and community acquired infections [1]. It is also responsible for a variety of systemic infections like urinary tract infections, respiratory tract infections, gastrointestinal tract infections, dermatitis, bacteremia, soft tissue infections, bone and joint infections [2]. It is one of the important bacterial pathogens isolated from various samples. Despite advances in medical and surgical care and introduction of wide variety of antimicrobial agents having anti-pseudomonal activities, life-threatening infections caused by *P. aeruginosa* continues to cause complications in nosocomial infections [3].

Pseudomonas aeruginosa has naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharide (LPS). There are variety of mechanisms involved in the resistance of *P. aeruginosa* such as efflux pumps [4], production of ESBLs (extended spectrum beta-lactamases) [5], MDR, target site or outer membrane modification [3], biofilm formation and aminoglycoside modifying enzymes [6]. ESBLs are an important reason of bacterial resistance throughout the world [5]. Microbiologists, clinicians, infection control professionals and scientists engaged in finding new antimicrobial agents are now facing distinctive challenge to ESBL producing isolates.

In addition to its intrinsic resistance to various antibiotics, it also readily acquires resistance to the potentially active agents [7]. Since some of the resistance markers are carried by plasmids, the threat to human health is compounded by the possibility of transmission of markers to other Gram-negative pathogens [8]. Resistance to anti-pseudomonal antibiotics is increasing worldwide. This situation has been compounded by the lack of new classes of anti-pseudomonal drugs [9]. Therefore, knowledge of current drug resistance pattern of the common pathogenic bacteria in a particular region is useful in clinical practice. Hence, the present study was conducted to find out prevalence and the antimicrobial susceptibility pattern of *P. aeruginosa* isolates obtained from various clinical samples at Microbiology department in a tertiary care hospital, Bharatpur, Chitwan, Nepal.

MATERIAL AND METHODS

The study was conducted at Department of Microbiology, Janaki Medical College Teaching Hospital, Janakpurdham, Nepal with approval from the Institutional Review/Ethical Committee. All the clinical samples including sputum, pus, urine, respiratory samples, tissue and blood received in the Microbiology laboratory for culture and sensitivity were processed. After initial Gram staining, they were inoculated on blood agar and MacConkey's agar. The inoculated culture plates were incubated aerobically at 37°C for 24 hours [10].

Identification: Following the appearance of bacterial growth, only isolates of *Pseudomonas aeruginosa* were included in this study. The standard biochemical tests were applied for identification.

Details of the patient: Demographic details including age, sex, ward/critical unit were noted.

Antibiotic susceptibility test: Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (MHA) plates by Kirby-Bauer disc diffusion method, according to Clinical Laboratory Standards Institute (CLSI)

guidelines. All *Pseudomonas aeruginosa* isolates were subjected to antimicrobial susceptibility test as per CLSI guidelines [11].

Following antibiotic discs from HI- MEDIA were used for antibiotic susceptibility test: piperacillin (100µg), piperacillin + tazobactum (100/10µg), ticarcillin + tazobactum (75/10µg), ceftazidime (30µg), cefotaxime (10µg), imipenem (10µg), meropenem (10µg), amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), levofloxacin (5µg) and aztreonam (50µg).

Pseudomonas aeruginosa, ATCC 27853 strains, was used for quality control.

RESULTS

The present study was carried out in the Microbiology laboratory, College of Medical Sciences and Teaching Hospital (COMS-TH), Bharatpur, Chitwan, Nepal for 1 year. A total of 9139 samples were received for culture and sensitivity in Bacteriology Diagnostic Laboratory, of which 4013 samples showed growth on culture. Among 4013 cultured organism, 453 isolates were identified as *P. aeruginosa* (11.29%). The samples from which we cultured were sputum, urine, pus, wound swab, blood, respiratory secretions and others. Out of 439 isolated *P. aeruginosa*, the most common samples given positive growth were sputum 206 (45.47%), urine 81 (17.88%), respiratory secretions 76 (16.78%), pus 35 (7.73%), wound swab 21 (4.63%), blood 14 (3.09%) and other samples 20 (4.41%) as shown in Table 1.

Among 453 cases of *P. aeruginosa*, 282(62.25%) cases were isolated from male patients and 171(37.75%) were from female patients as shown in table 2.

Table 1: Distribution of total isolates of *P. aeruginosa* strains from different samples

Clinical samples	Cases	Percentage (%)
Sputum	206	45.47
Urine	81	17.88
Pus	35	7.73
Wound swab	21	4.63
Respiratory secretions (E.T. tip/ bronchial wash etc)	76	16.78
Blood	14	3.09
Others (CSF/semen/foley's tip)	20	4.41
Total	**453**	**100**

Table 2: Sex-wise distribution of P. aeruginosa strains

Gender	Number of samples	Percentage (%)
Male	282	62.25
Female	171	37.75
Total	**453**	**100**

Table 3 shows majority of the isolates (81.46%) were recovered from patients attending inpatient departments than from outpatient departments (18.54%). Maximum number of *P. aeruginosa* was recovered from patients who were more than 40 upto 60 years of age (33.99%) followed by those who were more than 60 years of age (24.94%) as shown in table 4.

Table 3: Distribution of *P. aeruginosa* strains among outpatient department (OPD) and inpatient department (IPD)

Department	Number of isolates	Percentage (%)
IPD	369	81.46
OPD	84	18.54
Total	**453**	**100**

Table 4: Age-wise distribution of isolates of *P. aeruginosa*

Age in years	Total samples	Percentage (%)
0-20	75	16.56
21-40	111	24.50
41-60	154	33.99
>60	113	24.94
Total	**453**	**100**

Table 5 shows antibiotic sensitivity patterns of *P. aeruginosa*. We found that the most sensitive drug was piperacillin-tazobactam 94.26% (427) followed by imipenem 89.40% (405), levofloxacin 88.08% (399), ticarcillin + tazobactam 87.86% (398), meropenem 83.0% (376) and piperacillin 74.83% (339). In our study we also found *P. aeruginosa* MDR Strains from clinical isolates as shown in table 6.

studies done in India 9.28% [12], 13% [13]. A similar study done in Kathmandu [14], Nepal in 2013 showed 17.05% *P. aeruginosa* isolates. A higher prevalence rate (32-40%) was also documented by other studies [15, 16].

In our study, we found that most of the cases belonged to older age group of 41-60 years (33.99%) followed by elderly age group of >60 years (24.94%). This could be explained

Table 5: Antimicrobial susceptibility of *P. aeruginosa* isolates (n = 453) to various antibiotics

S.No	Name of antibiotics	Sensitivity number of samples	Sensitivity %	Resistant number of samples	Resistance %
1.	Piperacillin	339	74.83	114	25.16
2.	Piperacillin+ tazobactum	427	94.26	26	5.74
3.	Ticarcillin+ tazobactum	398	87.86	55	12.14
4.	Meropenem	376	83.00	77	16.99
5.	Imipenem	405	89.40	48	10.60
6.	Gentamicin	221	48.78	232	51.21
7.	Amikacin	305	67.33	148	32.67
8.	Ciprofloxacin	230	50.77	223	49.23
9.	Levofloxacin	399	88.08	54	11.92
10.	Aztreonam	282	62.25	171	37.75
11.	Ceftazidime	232	51.21	221	48.78
12.	Cefotaxime	241	53.0	212	46.80

Table 6: Distribution of MDR *P. aeruginosa* isolates among clinical specimens

S.No.	Clinical samples	Total number of samples	Number of MDR strains	Percentage (%) of MDR isolation
1.	Sputum	206	85	41.26
2.	Urine	81	21	25.92
3.	Pus	35	13	37.14
4.	Wound swab	21	12	57.14
5.	Respiratory secretions	76	28	36.84
6.	Blood	14	03	21.43
7.	Others	20	05	25.00
	Total	453	167	36.86

DISCUSSION

In this study, a total of 453 strains of *P. aeruginosa* were isolated and identified from various clinical samples from the outpatient and inpatients and their antimicrobial susceptibility patterns were determined. This present study revealed the prevalence rate of *P. aeruginosa* was 11.29% which is almost comparable to the prevalence rate shown in

as due to prolonged hospitalization, decreased immunity and other associated comorbidities in these age groups. A study done in Ahmadabad [17], India, showed that 29% patients were aged between 31 and 45 years. Duration of hospital stay is directly proportional to infection which was much higher 81.46% in inpatients than in outpatients 18.54%. Gender-wise, male

patients 62.25% constituted a larger group in our study. Other studies from India, Nepal have also shown similar findings [17, 18]. The distribution of specimen of *P. aeruginosa* may vary with each hospital as each hospital has a different environment and facility associated within. The most common source of the isolate in our study was sputum (45.47%) followed by urine (17.88%) and only 7.73% of the isolates were recovered from pus. Shresthaet al. also reported maximum percentage of *P. aeruginosa* from sputum, urine (36.27% each) and pus (9.8%) [18]. Other studies reported maximum isolation from pus followed by urine and sputum [19].

We studied resistance pattern of *P. aeruginosa* against 12 antimicrobial agents; *P. aeruginosa* isolates were found most sensitive to piperacillin + tazobactam (94.26%) followed by imipenem (89.40%) and this may be due to the restricted use of piperacillin + tazobactam and imipenem in this hospital. Imipenem is a carbapenem antibiotic which is highly active against *P. aeruginosa*. Our study showed only 10.60% of resistance to imipenem which was almost similar to the resistance rate revealed by study done in a different university of Kathmandu (9.1% and 15.4%) [20, 21]. Higher rate of resistance was seen in study done by Taneja et al. (36.4%) [22] and Chaudhary et al. (66.5%) [23].

In our study, levofloxacin 11.92% resistant followed by ciprofloxacin 49.23% resistant, while meropenem only 16.99% resistant and amikacin 32.67% resistant were detected to be the most effective drugs for routine use against the *P. aeruginosa* strains investigated. Among the quinolones and aminoglycosides, our study detected 49.23% resistance to ciprofloxacin which is comparable to the study done by Yadav et al. who reported 49%

resistance to ciprofloxacin [13]; similarly higher rate of resistance had also been reported from Kerala, India (40.5%) [24], and from Malaysia (92%) [25]. An earlier study reported from Kathmandu, Nepal showed amikacin was 81.4% sensitive [26], In this study, we got amikacin 67.33% sensitive among *P. aeruginosa*. In other study, high percentage of resistance to aminoglycosides had been reported from India [27], Bangladesh [28]. In our study, piperacillin + tazobactam showed 94.26% sensitive whereas piperacillin alone showed 74.83% sensitive followed by ticarcillin + tazobactam 87.86% sensitive. In other study, piperacillin alone tested showed a resistance rate of 25.16% reported from Chhattisgarh, India [13]. Therefore, it indicates beta-lactamase inhibitor markedly expands the spectrum of activity of drug. Thus, it determines that the combination drug should be the preferred choice against *P. aeruginosa*.

We found that *P. aeruginosa* was 48.78% resistant to ceftazidime, third-generation cephalosporin drug and 46.80% resistant to cefotaxime. Much higher resistance to third-generation cephalosporin 95%, 75%, and 86%, had been reported in studies done in Nepal [26], India [27] and Bangladesh [28].

We found sensitivity to levofloxacin was 88.08%. Thus, levofloxacin should be included in the treatment regimen for the *P. aeruginosa*. Another significant finding in this study was the rate of multidrug resistance (MDR), which was found to be 36.86%; similarly, MDR rate of 19.6% from Malaysia [29] and 20.69% from Kathmandu, Nepal [21] had been reported. A higher rate of MDR had been reported from studies conducted from Nepal (89.4%) [26] and 100% MDR *P. aeruginosa* isolates from Iran [30]. This study has a few limitations that are molecular

typing, plasmid profile and study of mechanism of development of MDR strain, which would provide much-needed detail information and there should also be analysis of ESBL-producing *P. aeruginosa* which is a major cause of nosocomial infection with MDR strains in hospital [31, 32].

CONCLUSION

The prevalence and sensitivity of *P. aeruginosa* often varies between communities, hospitals in the same community and among different patient populations in the same hospital. In our study, we found results that clearly indicate the evolution of MDR stains and the occurrence of resistance to various anti-pseudomonal agents among the *P. aeruginosa* isolates. We suggest a more restricted and more rational use of drugs in the treatment of *P. aeruginosa* infection in hospital setting. Increase in antibacterial resistance in *P. aeruginosa* is a cause of concern. Regular antimicrobial susceptibility monitoring is essential for local, regional and national level isolates. This would help prescribing the right combination of chemotherapeutic agent and prevent the emergence of MDR strains of *P. aeruginosa.*

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Correspondence to:

Dr Surya Narayan Mahaseth
Assistant professor
Department of Microbiology
Janaki Medical College
Janakpur, Nepal.
Email ID: dosurya@gmail.com
Contact number: +977-9851012818