Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* Isolates from Tertiary Care Hospitals in Kathmandu

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ABSTRACT

Background

Antimicrobial resistance, caused by *Pseudomonas aeruginosa* (*P. aeruginosa*), poses a global health threat, limiting treatment options and increasing morbidity and mortality rates due to its intrinsic and multidrug resistance.

Objective

To determine the antimicrobial resistance patterns of *P. aeruginosa* isolates from patients visiting or admitted to tertiary care hospitals in Kathmandu.

Method

A cross-sectional study was conducted at Bir Hospital and Tribhuvan University Teaching Hospital (TUTH) from December 2021 to December 2022. Isolates were identified and tested for antibiotic susceptibility following standard microbiological guidelines.

Result

The antimicrobial resistance of 200 *P. aeruginosa* isolates increased from low to high levels, as per the recommended anti-pseudomonal antibiotics by the Clinical and Laboratory Standards Institute (CLSI), from 0% to 94%. piperacillin/tazobactam exhibited significantly lower resistance at 18(9%) and while considerably higher resistance was observed with ceftazidime at 188(94%) compared to different antibiotics, followed by amikacin 34(17%), imipenem 58(29%), ciprofloxacin 42(21%), aztreonam 51(25.5%), and fosfomycin 44(22%). No resistance was observed to colistin and polymyxin B. *P. aeruginosa* resistant to carbapenem was accounted for 33.5% of the total, and multidrug resistance categories included multidrug resistance (MDR) at 39.0%, extensively drug resistance (XDR) at 13.5%, and *P. aeruginosa* difficult-to-treat (DTR PA) at 4.6%.

Conclusion

Most of the isolates were resistant to anti-pseudomonal antibiotics; however, colistin, polymyxin B, amikacin, doripenem, piperacillin/tazobactam, and fosfomycin were effective against MDR *P. aeruginosa*. Regular surveillance measures are essential to manage antimicrobial resistance.

KEY WORDS

Antimicrobial resistance, Difficult-to-treat Pseudomonas aeruginosa, Extensively drug resistance, Multidrug resistance

INTRODUCTION

Antimicrobial resistance, which is now prevalent, appears to have been uncommon prior to the introduction of antibiotics. Antibiotic resistance among *P. aeruginosa* infections is a growing global issue and a threat to public health, posing multiple therapeutic obstacles. Additionally, the susceptible strains of *P. aeruginosa* can acquire drug resistance during treatment with a frequency that is relatively high.[1]

P. aeruginosa has developed resistance to all classes of antimicrobial agents, including penicillins, cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. This emergence of resistance has limited therapeutic options, resulting in increased rates of morbidity and mortality and increased costs associated with treating patients infected with *P. aeruginosa*. Its high intrinsic antibiotic resistance and propensity to develop multidrug resistance pose significant therapeutic challenges.[2]

This study aimed to determine the antimicrobial resistance patterns of *P. aeruginosa* isolates from patients visiting or admitted to tertiary care hospitals in Kathmandu.

METHODS

A cross-sectional study using consecutive sampling was conducted at Bir Hospital and Tribhuvan University Teaching Hospital (TUTH) from December 2021 to December 2022. A total of 200 clinical isolates of *P. aeruginosa* were consecutively obtained from clinical specimens (e.g., Blood, Urine, Pus, Sputum and others) at different wards. Convenience sampling was done, and the sample size was calculated using the formula.[3]

Where,

n= minimum required sample size

Z= 1.96 at 95% Confidence Interval (CI)

p= prevalence of *Pseudomonas aeruginosa* isolates, 6.2%.[4,5]

q= 1-p

d= margin of error, 3.5%

$n=z^2 \times p \times q/d^2$

$=1.962 \times 0.062 \times 0.938/0.0352$

=183

The required minimum sample size calculated was 183.

Identification of isolated organisms: The clinical isolates were identified using standard procedures. On blood agar, Mac Conkeys agar, and cysteine lactose electrolyte-deficient agar, were inoculated. Isolates were identified by colony morphology, gram staining, and biochemical assays such as catalase, oxidase, sulphide, indole, motility, citrate, urea, TSI reaction, pyocyanin production.[6-8]

Inoculums for Antimicrobial Sensitivity Testing (AST): An overnight culture was incubated on a stirring water immersion for two hours, and the turbidity of the inoculums was matched with a 0.5 MacFarland standard suspension. The preparation of McFarland standards required the addition of precise volumes of 1% sulfuric acid and 1.175% barium chloride. The McFarland standard 0.5 contains 99.5 ml of 1% sulfuric acid and 0.5 ml of barium chloride at 1.175% concentration. Comparable turbidity was observed between the standard and a bacterial suspension containing 1.5x108 CFU/ml.[9]

Antibiotic Susceptibility Test: AST was performed by using the Clinical and Laboratory Standards Institute (CLSI).[10-12] The antimicrobial testing was conducted using the modified Kirby-Bauer disc diffusion technique. For this purpose, an evaluation of 17 antimicrobial agents with anti-pseudomonal capabilities was selected from eight separate antibiotic classes.[13] The antibiotic discs that were used for testing are as follows: amikacin (AK, 30 μg), gentamycin (GEN, 10μg), tobramycin (TOB, 10 μg) nettilin (NET 30 μg), imipenem (IMP, 10 μg), meropenem (MRP 10 μg), doripenem (DOR 10 μg), ceftazidime (CAZ, 30 μg), cefepime (CPM, 30 μg), ciprofloxacin (CIP, 5 μg), levofloxacin (LE, 5 μg), piperacillin/tazobactam (100 μg/10 μg), ticarcillin/clavulanic acid (TCC, 75/10 μg), aztreonam (AT, 30 μg), fosfomycin (FO, 200 μg), polymyxin B (PB, 300 units), and colistin (CL, 10 μg) [Hi Media Laboratories Pvt. Ltd., Mumbai, India]. Zone sizes obtained were measured and interpretation was made according to CLSI guidelines.[14,15]

Colistin and Polymyxin B Susceptibility Test: The susceptibility of colistin was assessed using the colistin broth disc elution (CBDE) technique. In summary, there were four MacCartney bottles that were labelled as growth control (GC), with concentrations of 1 µg/ml, 2 µg/ml, and 4 µg/ml. Every bottle held 10 millilitres of CAMHB. Subsequently, colistin discs with a concentration of 10 µg each, produced by HiMedia Laboratories Pvt. Ltd. in India, were inserted into each container, resulting in concentrations of 0 µg/ml, 1 µg/ml, 2 µg/ml, and 4 µg/ml. Following vortexing for a duration of 1 minute, the bottles were subsequently incubated at ambient temperature for a period of 30 minutes to facilitate the elution of colistin from the discs. The inoculum was prepared by applying fresh colonies obtained from an overnight culture on Mueller Hinton agar (MHA, HiMedia Laboratories Pvt. Ltd.). These colonies were then mixed with normal saline to achieve a concentration corresponding to a 0.5 McFarland standard. After adding 50 µl of inoculum to each container, a gentle vortex was applied. The level of transparency of the development in the tubes was measured after a 24-hour incubation period at a temperature of 35 degrees Celsius. Furthermore, a similar approach was employed to evaluate the susceptibility of polymyxin B.[12]

P. aeruginosa ATCC 27853 and *E. coli* ATCC 25922 were used as the control organisms for antibiotic sensitivity and were kindly provided by the National Public Health Laboratory, Teku, Kathmandu.

All the data collected were analyzed using MS Excel and Statistical Software SPSS version 26.0. The frequency of MDR *P. aeruginosa* and the percentage of resistant antibiotics, and the chi-square value were calculated. Results were considered significant if the P-value was less than 0.05.

Ethical approval for the study was obtained from the Ethical Review Board, National Health Research Council, Kathmandu, Nepal (Reg. no. 78/2021), Institutional Review Board, NAMS, Bir Hospital (Ref. no. 481/2078/79), and Institutional Review Committee, IOM, Maharajgunj (Ref. no. 95 (6-11) E2 79/80).

RESULTS

The antibiotic susceptibility of *P. aeruginosa* was evaluated against a list of 17 antimicrobial drugs. The susceptibility of the genera was assessed in a study involving 200 isolates (Table 1). The findings indicate that there was no observed resistance to colistin and polymyxin B. *P. aeruginosa* demonstrated varying degrees of resistance to a range of antibiotics, with ceftazidime exhibiting the highest resistance rate at 94%. This was followed by ticarcillin/clavulanic acid (48.5%), levofloxacin (33%), cefepime at 29.5%, imipenem at 29%, aztreonam at 25.2%, meropenem at 25%, tobramycin at 24%, gentamycin at 23%, nettilin at 22.7%, fosfomycin at 22%, ciprofloxacin at 21%, doripenem at 19%, amikacin at 17%, and piperacillin/tazobactam at 9%.

Multi-drug resistance (MDR) is characterized by the resistance of a microorganism to at least one antimicrobial agent from each of three distinct classes of antibiotics. A total of 78 (39%) *P. aeruginosa* isolates exhibited multi-drug resistance (MDR), while 27 (13.5%) had extensively drug resistance (XDR). Additionally, 9 (4.5%) isolates were found to have *P. aeruginosa* difficult-to-treat resistance (DTR PA). The distribution of these resistance patterns is visually represented in figure 1.

Table 2-3 demonstrates that the incidence of *P. aeruginosa* strains exhibiting resistance to MDR *P. aeruginosa* was greater in comparison to bacteria that shown susceptibility to Non-MDR *P. aeruginosa* for the majority of the antibiotics examined. Additionally, Table 4 illustrates the percentage of *P. aeruginosa* strains obtained from different sources.

DISCUSSION

The susceptibility of the isolates was assessed against eight antimicrobial classes known to be effective against *P aeruginosa* in order to know the current antimicrobial

Table 1. Antibiotic Susceptibility Pattern of *P. aeruginosa* (n=200)

Antimicrobial Category	Antimicrobial agents	Resistance (%)	Intermediate (%)	Sensitive (%)
Aminoglycosides	Gentamycin	46(23)	5(2.5)	149(74.5)
	Tobramycin	48(24)	6(3)	146(73.4)
	Amikacin	34(17)	9(4.5)	157(78.5)
	Nettilin	44(22.7)	5(2.5)	151(75.5)
Antipseudomonal carbapenems	Imipenem	58(29)	22(11)	120(60)
	Meropenem	50(25)	5(2.5)	145(72.5)
	Doripenem	38(19)	6(3)	156(78)
Antipseudomonal cephalosporins	Ceftadizime	188(94)	9(4.5)	3(1.5)
	Cefepime	59(29.5)	21(10.5)	120(60)
Antipseudomonal fluoroquinolones	Ciprofloxacin	42(21)	15(7.5)	143(71.5)
	Levofloxacin	66(33)	5(2.5)	129(64.5)
Antipseudomonal penicillins + β- lactamase inhibitors	Ticarcillin/clavulanic acid	97(48.5)	65(32.5)	38(19.5)
	Piperacillin/tazobactam	18(9)	33(16.5)	149(74.5)
Monobactams	Aztreonam	51(25.2)	68(34)	81(40.5)
Phosphonic acids	Fosfomycin	44(22)	40(20)	116(58)
Polymyxins	Colistin	0(0)	0(0)	200(100)
	Polymyxin B	0(0)	0(0)	200(100)

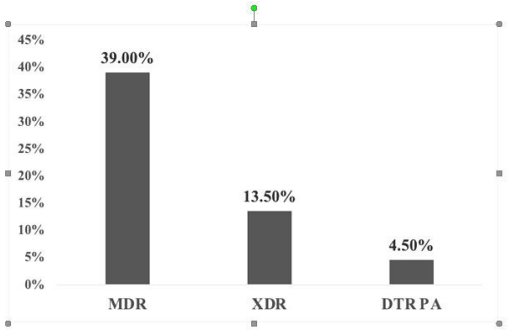

Figure 1. Incidence of MDR, XDR and DTR PA in *P. aeruginosa* Isolates (n=200)

resistance pattern of the organism. These classes include aminoglycosides, carbapenems, broad-spectrum cephalosporins from the third and fourth generations, extended-spectrum penicillin, combinations of these penicillin with β-lactamase inhibitors, monobactams, fluoroquinolones, phosphonic acid and polymyxins. According to Magiorakos et al., the isolates that demonstrated resistance to three or more classes of antimicrobial agents were classified as multidrug-resistant (MDR) organisms. The definitions for multidrug resistance (MDR) were formulated by considering just the aspect of acquired antimicrobial resistance, whereas intrinsic resistance was not specifically addressed. XDR was characterised by the absence of susceptibility to at least one agent in all but two or fewer antimicrobial categories, indicating that bacterial isolates remained sensitive to just one or two categories. On the other hand, PDR was

Table 2. Resistance of MDR and non-MDR P. aeruginosa to various antimicrobial categories/antimicrobial agents

Antimicrobial Category	Antimicrobial agents	Non-MDR n (%)	MDR n (%)	χ^2	p-value
Aminoglycosides	Gentamycin	0/122 (0)	46/78 (59.6)	93.440	0.000
	Tobramycin	1/122 (0.8)	47/78 (60.3)	92.153	0.000
	Amikacin	0/122 (0)	34/78 (43.6)	64.072	0.000
	Nettilin	1/122 (0.8)	43/78 (55.1)	81.779	0.000
Antipseudomonal carbapenems	Imipenem	10/122 (8.2)	48/78 (61.5)	65.751	0.000
	Meropenem	3/122 (2.5)	47 (60.3)	84.770	0.000
	Doripenem	0/122 (0)	38/78 (48.7)	73.378	0.000
Antipseudomonal cephalosporins	Ceftadizime	110/122 (90.2)	78/78 (100)	8.162	0.004
	Cefepime	6/122 (4.9)	53/78 (67.9)	90.890	0.000
Antipseudomonal fluoroquinolones	Ciprofloxacin	0/122 (0)	42/78 (53.8)	83.155	0.000
	Levofloxacin	2/122 (1.6)	64/78 (82.1)	139.148	0.000
Antipseudomonal penicillins + β- lactamase inhibitors	Ticarcillin/clavulanic acid	35/122 (28.7)	62/78 (79.5)	49.156	0.000
	Piperacillin/ tazobactam	0/122 (0)	18/78 (23.1)	30.938	0.000
Monobactams	Aztreonam	2/122 (1.6)	49/78 (62.8)	93.748	0.000
Phosphonic acids	Fosfomycin	23/122 (18.9)	21/78 (26.9)	1.806	0.179
Polymyxins	Colistin	0/122 (0)	0/122 (0)	-	-
	Polymyxin B	0/122 (0)	0/122 (0)	-	-

defined as the absence of susceptibility to all agents in all antimicrobial categories.[13] DTR PA was characterised by the presence of *P aeruginosa* strains that demonstrate a lack of susceptibility to each of the following agents: The antibiotics piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin are often used in clinical settings.[16]

The present investigation revealed that the prevalence of resistance to aminoglycosides was 28.5%. Specifically, resistance rates were seen at 23% for gentamycin, 17% for amikacin, 24% for tobramycin, and 22.7% for nettilin was found to be lower than the rates previously reported in Nepal and Saudi Arabia.[17-19] The current study was to assess and compare the effectiveness of amikacin, gentamicin, tobramycin, and nettilin resistance in *P. aeruginosa*. The results revealed that amikacin had a more pronounced inhibitory impact on *P. aeruginosa* compared to the other aminoglycosides.

The present study has shown a significant level of antimicrobial resistance (94.5%) in *P. aeruginosa* against

Table 3. Antimicrobial susceptibility profiles of MDR *P. aeruginosa*

Antimicrobial Category	Antimicrobial agents	MDR (n = 78)		χ^2	p-value
		Susceptible n (%)	Resistant n (%)		
Aminoglycosides	Gentamycin	32(41)	46(59.6)	2.513	0.113
	Tobramycin	31(39.7)	47(60.3)	3.282	0.070
	Amikacin	44(56.4)	34/(43.6)	1.282	0.258
	Nettilin	35(44.9)	43/(55.1)	0.821	0.365
Antipseudomonal carbapenems	Imipenem	30(38.5)	48/(61.5)	4.154	0.042
	Meropenem	31(39.7)	47(60.3)	3.282	0.070
	Doripenem	40(51.3)	38(48.7)	0.051	0.821
Antipseudomonal cephalosporins	Ceftadizime		78(100)		
	Cefepime	25(32.1)	53(67.9)	10.051	0.002
Antipseudomonal fluoroquinolones	Ciprofloxacin	36(46.2)	42(53.8)	0.462	0.497
	Levofloxacin	14(17.9)	64(82.1)	32.051	0.000
Antipseudomonal penicillins + β- lactamase inhibitors	Ticarcillin-clavulanic acid	16(20.5)	62(79.5)	27.128	0.000
	Piperacillin-tazobactam	60(76.9)	18(23.1)	22.615	0.000
Monobactams	Aztreonam	29(37.2)	49(62.8)	5.128	0.024
Phosphonicacids	Fosfomycin	57(73.1)	21(26.9)	16.615	0.000
Polymyxins	Colistin	0/122(0)	0/122(0)	-	-
	Polymyxin B	0/122(0)	0/122(0)	-	-

Table 4. Proportion of *P. aeruginosa* strains recovered from various sources in relation to MDR

Source	MDR PA, n (%)	Non-MDR PA, n (%)	χ^2	p-value
Blood (n=6)	4/78(5.1)	2/122(1.6)	0.667	0.414
Urinary tract (n=53)	18/78(23.1)	35/122(28.7)	5.453	0.020
Wounds (n=75)	34/78(43.6)	40/133(32.8)	0.486	0.485
Respiratory tract (n=66)	22/78(28.2)	45/122(36.9)	7.896	0.005

cephalosporin antibiotics. Ceftazidime exhibited the highest level of resistance among the antibiotics tested, with a non-susceptibility of 94.0%. This observation aligns with previous studies conducted by Manandhar et al. and Shidiki et al.[20,21]

The findings of the present investigation indicate that a significant proportion of *P. aeruginosa* strains, including 33.5%, have shown resistance against carbapenem drugs, including imipenem, meropenem, and doripenem. Similar rates of carbapenem resistance were observed in the United States with a range of 10-30%.[22] Relatively increased resistance to imipenem was identified in the context of resistance to carbapenems, which include meropenem, imipenem, and doripenem. These carbapenems are known for their clinically relevant β-lactamases, which exhibit the broadest range of action.

The present investigation revealed a resistance rate of 33.5% against both ciprofloxacin and levofloxacin. However,

when examined separately, the resistance rates were reported as 21% for ciprofloxacin and 33% for levofloxacin. Similarly, prior research conducted by other scholars has shown a resistance rate of 36.8% and 46% for ciprofloxacin and levofloxacin, respectively, exceeding the rate seen in the current study.[23]

In this study, antipseudomonal penicillin combined with a beta-lactamase inhibitor resistance was 55.5%. The observation of resistance to the combination of piperacillin with tazobactam was documented. According to Baniya et al., the prevalence rate of piperacillin/tazobactam resistance in Nepal was found to be 45.9%, which was seen to be somewhat higher compared to the findings of recent research.[24] There were frequent reports on the prevalence of resistance to extended-spectrum penicillin, namely ticarcillin in conjunction with the β-lactamase inhibitor clavulanic acid.

The current study revealed a decreased prevalence of aztreonam resistance in Nepal compared to previously documented rates.[23-25] In a study conducted in France, it was stated that the resistance to fosfomycin was higher (33.3%) compared to the findings of our investigation, which indicated a lower resistance rate.[26]

Many studies have figured varying degrees of resistance to colistin and polymyxin B. This investigation revealed a notable observation: none of the isolates exhibited resistance to colistin and polymyxin. But Yadav et al. and Kaur et al. figured out all isolates were completely susceptible to colistin and polymyxin B, which was consistent with our study.[25,27] This finding provides confirmation that colistin and polymyxin B should be considered as the empirical treatment for severe pseudomonal infections.

Several studies have conducted assessments of varying proportions of multidrug-resistant (MDR) cases in Nepal, with reported figures ranging from 21% to 89%.[23-25,27-34] In the present investigation, a total of 78 strains (39%) were identified as multi-drug resistant (MDR). Research conducted in Nepal has found a comparable incidence of multidrug-resistant (MDR) cases. However, the identification of trends in MDR prevalence is complicated by variations in resistance levels across various time periods and geographical regions. The increase in antibiotic resistance in Nepal may be attributed to the limited availability of completely effective antibiotics in research and the prevalence of drug-resistant strains in hospitalized patients and invasive areas. This situation further worsens the failure of infection control measures and increases the risk of transmission caused by medical intervention.[23]

The Kirby-Bauer disk diffusion susceptibility test and CBDE test categorized 200 *P. aeruginosa* strains into two groups: 78 (39%) MDR strains resistant to non-susceptible to $\geq$ 1 agent in $\geq$ 3 antimicrobial categories and 122 (61%) non-MDR strains susceptible all or non-susceptible to $\leq$ 2 antimicrobial categories. The research observed notable variations in drug sensitivity between multi-drug resistant (MDR) and non-MDR strains of *P. aeruginosa*, as shown by their distinct antibiotic resistance profiles. The prevalence of *P. aeruginosa* strains exhibiting multidrug resistance (MDR) was higher compared to non-MDR bacteria across the majority of tested antibiotics. In this study, it was found that colistin, polymyxin B, amikacin, doripenem, piperacillin/tazobactam, and fosfomycin were significantly effective antibiotics against the MDR *P. aeruginosa*.

P. aeruginosa exhibits resistance to various antimicrobial agents and expresses diverse molecular epidemiology to various established classes of antibiotics, including β-lactams, fluoroquinolones, tetracycline, and aminoglycosides. The resistance mechanism is due to complex chromosomally encoded genes and the inherent capacity for biofilm formation. The main mechanisms behind *P. aeruginosa* resistance include enzymatic modification, impermeability resistance, efflux system, and modification in the outer membrane. The enzymatic modification involves the modification of aminoglycoside enzymes, while impermeability resistance is due to lipopolysaccharide (LPS) present in the cell wall. The drug efflux system includes outer membrane channel-forming protein (OMF), resistance nodulation division (RND), and membrane fusion protein.[35]

CONCLUSION

Most of the isolates were resistant to anti-pseudomonal antibiotics. However, colistin, polymyxin B, amikacin, doripenem, piperacillin/tazobactam, and fosfomycin were found to be very effective against MDR *P. aeruginosa*. Periodic surveillance of *P. aeruginosa* resistance patterns is required to provide up-to-date information on the efficacy of antipseudomonal antibiotics commonly employed nationwide. The high resistance rate identified in this study against antipseudomonal antibiotics underscores the urgent need for focused measures to control antimicrobial resistance.

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