Original Article

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Prevalence of Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Clinical Specimens in Tertiary Care Hospital

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ABSTRACT

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen, which causes nosocomial infections in human. The rapid increase in drug resistance of this pathogen is a global concern. The aim of this study was to determine the clinical burden of *P.aeruginosa*, its antibiotic susceptibility pattern along with metallo-ß-lactamase (MBL) detection.

Methods: The descriptive cross-sectional study was conducted in Upendra Devkota Memorial National Institute of Neurological and Allied Sciences from January to August 2021. Isolation and identification of *P. aeruginosa* from clinical specimens was performed by using standard laboratory procedure. All bacterial isolates were phenotypically screened for multidrug resistance using Kirby Bauer disc diffusion method. All the multidrug resistant *P.aeruginosa* were phenotypically screened for MBL producer by Imipenem-EDTA combined disc diffusion test (CDDT).

Results: A total of 770 samples were processed of which 36 isolates of *P. aeruginosa* were obtained. *P.aeruginosa* was isolated mainly from tracheal aspirates, sputum, blood and urine. Among 36 isolates, 50% were found to be multidrug resistant (MDR). More percentage of *P.aeruginosa* isolates were found resistant to aztreonam, ofloxacin and levofloxacin (52.8%). Furthermore, this study reveals antibiotics like piperacillin/tazobactam and carbapenem were found to be good choice for the treatment of infection caused by this organism. Among MDR isolates 66.7% were found to be MBL producer.

Conclusions: The data in this study highlights the prevalence of multidrug resistant, MBL producer, and colistin resistant *P.aeruginosa* in clinical specimens. In this study, carbapenems and piperacillin/tazobactam were found to be most effective antimicrobial drugs for empirical therapy in *P.aeruginosa* infections.

Keywords: Metallo-ß-lactamase; multidrug resistant; prevalence; *Pseudomonas aeruginosa*.

INTRODUCTION

Pseudomonas aeruginosa, a ubiquitous microorganism, persist both in community and hospital settings.[1] It rarely causes infections in immunocompetent individuals but is reported as major opportunistic nosocomial pathogen causing high rate of morbidity in immunocompromised patients.[1,2] Several life threatening nosocomial diseases caused by *P.aeruginosa* includes: pneumonia, respiratory tract infections, urinary tract infections, septicaemia, burn wound infections and multi-organ failure.[3,4]

Infections caused by *P.aeruginosa* are difficult to treat due to its ability to change from acute to chronic infection and build up multidrug resistance (MDR) via multiple mechanisms such as efflux pumps overexpression, low permeability of outer membrane protein (D2 porin), over-expression of the chromosomally encoded AmpC cephalosporinase, drugs modification, and mutation(s) at the target site of the drug. The bacteria also develop antibiotic resistance through the acquisition of resistance genes carried on mobile genetic elements via plasmid.[5,6] The prevalence of MDR *P.aeruginosa* is increasing worldwide nowadays that ranges between 15-30%.[7,8] Such increment of resistance rate is becoming more global and economy challenges in post antibiotic era.[1]

Therefore, it is important to study susceptibility pattern of *P.aeruginosa* isolates to some commonly available

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antibiotics in hospital settings to initiate appropriate antimicrobial therapy. The objective of this study was to determine the characteristics and pattern of MDR *P.aeruginosa* isolated from clinical samples. We also detected MBL producers among MDR *P.aeruginosa* isolates.

METHODS

This study was hospital based cross-sectional study. The study was conducted in microbiology department of Upendra Devkota Memorial National Institute of Neurological and Allied Sciences (UDM-NINAS), Bansbari, Kathmandu from January to August 2021. The study was approved by Institutional Review Committee of Upendra Devkota Memorial National Institute of Neurological and Allied Sciences (UDM-NINAS), Kathmandu, Nepal.

All age group of both genders visiting hospital during our study period were included in this study. Whereas, those samples which were not properly labelled, improperly transported with visible signs of contamination and lacked patients complete information were excluded.

A total of 770 clinical specimens including tracheal aspirates, pus, urine, Foley's tip, sputum, throat swab, blood, cerebrospinal fluid (CSF), pleural fluid, CVP tip from indoor as well as outdoor patients were processed for isolation and identification of possible bacterial pathogens. Aseptic conditions were maintained throughout all the steps of sample collection, storage and processing.

All the clinical specimens were inoculated directly into blood agar, macConkey agar and chocolate agar. *P.aeruginosa* colonies were identified on the basis of colony characteristics on the respective media. Colonies showing typical *P.aeruginosa* characteristics (i.e; flat, leaf-like, irregular margin along with sweet grape-like odor) on culture and morphology on gram staining were transferred to nutrient agar and incubated at 37°C for 24 hours. Further, identification was done by pyocyanin (blue-green) pigmentation and conventional biochemical tests such as catalase test, oxidase test, triple sugar iron (TSI) media, sulphide indole motility (SIM) media, Simmon's citrate media, Chirstensen's urea, oxidation and fermentation media, and methyl red/voges-proskauer broth. Similarly, *P. aeruginosa* was separated from other *Pseudomonas* spp by observing growth on Cetrimide agar at 42°C for 24 hours.[9]

Antibiotic susceptibility tests of all the isolates were performed using the Mueller-Hinton Agar by Kirby Bauer disc diffusion method as recommended by CLSI 2020 guidelines.[10] Few colonies from the culture plate were inoculated into 2 ml peptone water and was incubated at 37°C for 2 hours. Turbidity was then compared with 0.5 Mc Farland Standard. A sterile cotton swab was dipped into broth and was rotated several times and pressed firmly on the inner side of the tube above the broth level to remove excess inoculums from the swab. Then it was inoculated into MHA plate surface by streaking over the entire plate three times, turning the plate 60 degree between streaking. Then commercially available antibiotic disc of 6mm in diameter were applied making close contact with inoculated plate. Therefore, the antibiotics that were used includes; gentamicin (GEN,30 µg), amikacin (AK, 10 µg), ciprofloxacin (CIP, 5 µg), ceftazidime (CAZ, 30 µg), cefepime (CPM, 30 µg), aztreonam (AT, 30 µg), imipenem (IPM,10 µg), piperacillin (PI,30 µg), piperacillin-tazobactam (PIT, 100/10 µg), meropenem (MRP, 10 µg), ofloxacin (OF, 30 µg), Levofloxacin (LEV, 30 µg) and colistin (CL,10 µg). Further, sensitivity to colistin was also determined by determining minimum inhibitory concentration (MIC) value by broth dilution method.

All the multidrug resistant *P.aeruginosa* were subjected for MBL detection. Phenotypic confirmatory test for MBL producers were carried out by using Imipenem-EDTA combined disc diffusion test (CDDT).

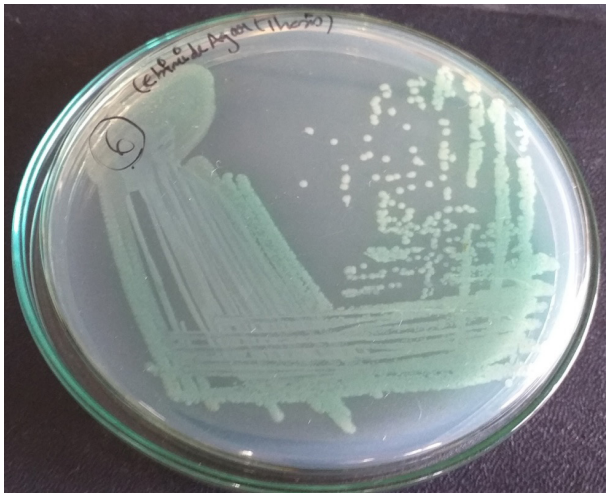
Two imipenem discs were placed on agar plate's containing lawn culture of test organism. 4µl of 0.5 M EDTA solution was applied to one of the imipenem disc, placed 25 mm apart (center-center) and the plate was incubated at 37°C. After 24 hours of incubation, an increase of ≥7 mm in the zone diameter of imipenem-EDTA disc as compared to imipenem disc alone was considered to be positive test for the presence of MBL.

Data obtained were analyzed using SPSS version 18. The $p<0.05$ was considered statistically significant.

RESULTS

Out of total 770 clinical specimen investigated, 36 (4.6%) of *P.aeruginosa* were isolated and identified. Tracheal aspirates (36.1%), sputum (25%), blood (13.9%) and urine (13.8%) were predominant sources of specimens of *P.aeruginosa* clinical isolates (Table 1).

Of these 36 isolates, the highest number of *P.aeruginosa* was obtained from male patients 26 (72.7%). Infections in indoor patients were found to be greater which accounts 35 (97.2%). In addition, most of them belonged to the age group of 40-59 years (16 44.4%) followed by 20-39 years (12, 33.3%) (Table 2).



Photograph 1: P.aeruginosa isolates on Cetrimide agar plate.

Photograph 2. Biochemical results of *P.aeruginosa*. Tubes containing biochemical media from left to right includes; 1st and 2nd : fermentation and oxidation test (oxidative), 3rd: sulphide indole agar (indole negative, motile, and sulphide negative), 4th and 5th: MR/VP (both negative), 6th: Simmon's citrate agar (positive), 7th: Triple sugar iron agar (Alkaline/Alkaline with no H_2S production), and 8th: Urease (negative).

Altogether 36 isolates of *P.aeruginosa* were tested against 12 different antibiotics. Among these antibiotics tested, more number of *P.aeruginosa* were found sensitive towards Piperacillin/tazobactam (66.7%), Imipenem (66.67%) and meropenem (66.7%) followed by Ciprofloxacin (52.7%), Cefepime (50%), Ceftazidime (50%), Gentamicin (50%), Piperacillin (50%) and Amikacin (50%) (Table 3). Among 36 isolates, 50% were found to be multidrug resistant.

Among 36 total clinical isolates, 2 (5.6%) were found to be colistin resistant. MICs of colistin for *P.aeruginosa* isolates were found to be ranged between 1(µg/ml) to 8(µg/ml). Highest MIC value was found to be 8 µg/ml in two isolates (Table 4).

Table 1.Distribution of *P.aeruginosa* isolates among clinical specimens.

Type of sample	Number of sample processed	Samples showing growth *P. aeruginosa* (n=36)	
		Number	Percent
Tracheal aspirates	63	13	36.1
Sputum	48	9	25
Urine	296	5	13.8
Pus	30	1	2.8
Wound swab	5	1	2.8
Blood	128	5	13.9
Cvp tip	19	1	2.8
Foley's tip	27	1	2.8
Others	616	0.0	0.0
Total	770	36	100.0

Table 2. Clinical and socio-demographic characteristics of the patients.

S.N.	Status of patients	Number (%)
1.	Male	26(72.2)
2.	Female	10(27.8)
	Gender	
1.	In-patients	35(97.2)
2.	Out-patients	1(2.8)
	Age distribution (years)	
1.	≤19	3(8.3)
2.	20-39	12(33.3)
3.	40-59	16(44.4)
4.	≥60	5(13.9)

Table 3. Antibiotic susceptibility pattern of *P.aeruginosa*.

Name of antibiotics	Sensitive no (%)	Resistant no (%)
Piperacillin	18(50)	18(50)
Piperacillin/Tazobactam	24(66.7)	12(33.3)
Cefepime	18(50)	18(50)
Ceftazidime	18(50)	18(50)
Aztreonam	17(47.2)	19(52.8)
Imipenem	24(66.7)	12(33.3)
Meropenem	24(66.7)	12(33.3)
Gentamicin	18(50)	18(50)
Amikacin	18(50)	18(50)
Ciprofloxacin	19(52.8)	17(47.2)
Levofloxacin	17(47.2)	19(52.8)
Ofloxacin	17(47.2)	19(52.8)

Table 4. MIC value of colistin among *P.aeruginosa* isolates.

S.N.	MIC value (µg/ml)	No. of *P.aeruginosa* isolates (%)
1.	1	28 (77.8)
2.	2	6 (16.7)
3.	4	0
4.	8	2(5.6)

In this study, CDDT phenotypic method was applied to identify metallo-ß-lactamase (MBL) producing strains of *P.aeruginosa*. Prevalence of MBL producing *P.aeruginosa* isolates was found to be 33.3%.

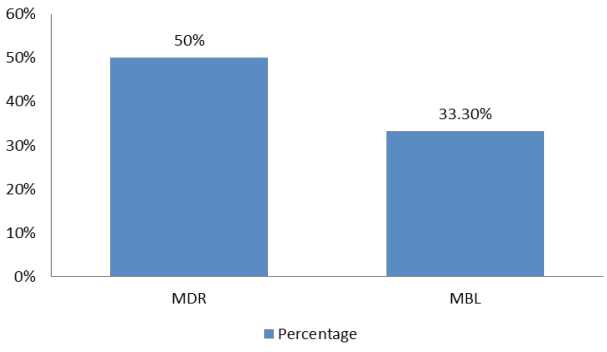
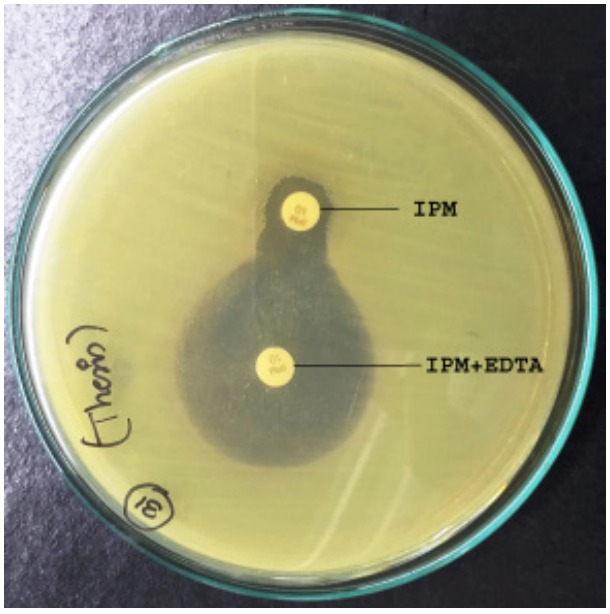


Figure 1: Percentage of MDR and MBL producer *P.aeruginosa* isolates



Photograph 3. Detection of MBL by Double Disc Synergy Test (Zone of inhibition≥7 mm for EDTA disc compared to imipenem disc indicates positive test.

DISCUSSION

In this study, a total of 36 *P.aeruginosa* were isolated and identified from various clinical specimen, from hospitalized patients and their antimicrobial susceptibility pattern along with MBL producers were determined.

This study revealed that the prevalence of *P.aeruginosa* was 4.6%, which is almost similar to other studies conducted in Nepal in recent years.[7,11,12] However, few studies from Nepal reported variable prevalence rate from 2.2% to 17.05%.[13-15] The differences in prevalence rate might be due to exact timing of sampling, different microbiological methodologies and geographic location.[16]

As each hospital has a different environment and facility available within, the distribution of *P.aeruginosa* in specimen varies.[17] The most common source of the isolate in our study was respiratory (tracheal aspirates and sputum) samples followed by blood. In recent years, Pokharel et al also reported maximum percentage of *P.aeruginosa* from respiratory sample. It indicates that burden of respiratory infection by *P.aeruginosa*

is increasing. The ability of *P.aeruginosa* to undergo recombination might have contributed to continuous evolution of them in lungs of immunocompromized patients.[18] Among respiratory sample highest prevalence of *P.aeruginosa* in tracheal aspirates infers that it is one of the predominant organism causing infections in tracheostomized patient.

In our study, we found that most of the cases belonged to elderly age group of 40-59 years followed by older age group of 20-39 years. This could be explained as due to prolonged hospitalization, decreased immunity and other associated co-morbidities in these age groups.[17] Gender-wise, distribution of *P.aeruginosa* was higher in male patients compared to female patients with male to female ratio being 2.6:1. Other studies from Nepal have also shown similar findings.[6,11,15]

P.aeruginosa causes both community and hospital acquired infection. In our study, highest numbers of cases were found in inpatients as compared to outpatients. Findings of previous study conducted in Nepal agree with present study indicates that *P.aeruginosa* are prone to cause hospital acquired infection.[1] The use of equipment i.e; dialysis tubing and respiratory equipment that are susceptible to colonization, decreased immunity and prolonged hospital stay of inpatients might be the predisposing factor.[15]

Increasing resistance to different anti-pseudomonal drugs particularly among hospital strains has been reported world-wide and this is a serious therapeutic problem in the management of disease due to these organisms.[14] In this study, in order to determine the antibiotic susceptibility pattern of *P.aeruginosa* about 12 different antibiotics were tested via Kirby Bauer disc diffusion method. Nearly all *P.aeruginosa* isolates were susceptible to colistin (94.4%). This may be due to the restricted use of colistin in this hospital. Apart from colistin, *P.aeruginosa* isolates were found mostly sensitive to piperacillin-tazobactam, imipenem and meropenem.

In this study, about three forth of the isolates were sensitive to piperacillin/tazobactam whereas, two forth isolates were only susceptible to piperacillin alone; which is comparable to the previous studies.[7,17] This result indicates that beta-lactamase inhibitor markedly expands the spectrum of drugs and combination drug should be the preferred choice against *P.aeruginosa*. As carbapenems are considered as last line drug for *P.aeruginosa*; about three forth isolates were sensitive to carbapenems. This finding is consistent with studies done in recent years.[7,19] However, higher sensitivity (100%) to these drugs reported in previous years in Nepal and Pakistan indicates nowadays resistance to carbapenem drugs is developing gradually, as it is being used now in several hospitals in our locality.[14,20]

We found that highest proportion of *P.aeruginosa* were resistant to Levofloxacin, Ofloxacin and Aztreonam. Aztreonam, not being able to hydrolyse by carbapenemase enzyme plays vital role in treatment of carbapenem resistant *P.aeruginosa* infections. In this study, resistance to aztreonam was found to be higher than the rates reported in Northern Cyprus and in Nepal that ranges from 33%-50%,[11,21] this shows resistance is developing gradually because of its increase use in hospitals. Similarly, we identified resistance rate to levofloxacin, ofloxacin and ciprofloxacin is higher in comparison to the previous studies conducted in Nepal.[11,19,22-24] We also found that among these three fluroquinolones, ciprofloxacin is more effective against *P.aeruginosa* than levofloxacin and ofloxacin.

In our study, about two forth *P.aeruginosa* isolates were resistance to aminoglycosides, which is higher than the previous studies conducted in Nepal.[7,11,22-25] Inappropriate prescription and injudicious use of antibiotics might be the contributing factors for increase in resistance to aminoglycosides. Similarly, about half of the *P.aeruginosa* were resistant to third generation cephalosporin, which is in accordance to the study conducted by Yadhav et al (2018) but higher than the recent studies conducted by Ullahe et al (2019) and Chand et al (2020).[11,21,24] Likewise, about half of the isolates were also found to be resistant to fourth generation cephalosporin which was lower than the study by Ullahe et al (2019).[21]

Another important finding of this study was the rate of multidrug resistance (MDR), which was found to be 50%. Likewise, the prevalence of MBL producer *P.aeruginosa* was found to be 33.3%, which is nearly supported by previous study, carried out in Nepal.[25] However, some studies in other countries have recorded various percentages of MBL-producing *P.aeruginosa* that ranges from 38-69%.[26-29] Differences in prevalence rate might be due to the differences of study period or differences in sample size and study area. Other contributing factors may be antibiotic use pattern or methods used for their detection. There is statistical association between MBL producer and MDR strains (p-value<0.001).

CONCLUSIONS

The result shows the evolution of MDR and MBL

producer strains and occurrence of resistance to various antipseudomonal agents among the *P.aeruginosa* isolates. *Pseudomonas* isolates reveal the highest sensitivity against Piperacillin/tazobactam and carbapenem drugs. Likewise, colistin can be used as reserved drugs for MDR *P.aeruginosa*. Therefore, we suggest a more restricted and more rational use of drugs in the treatment of *P.aeruginosa* infection in hospital settings. Periodic susceptibility testing should be carried out over a period of two to three years to detect the resistance trends in Nepal.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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