ABSTRACT

Introduction
In this era of modern medicine, antimicrobial resistance can be regarded as a major health calamity. The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains poses therapeutic challenges and lead to treatment failure in hospitalized patients. This study was conducted to determine various types of β-lactamases among MDR *P. aeruginosa* isolates recovered from hospitalized patients.

Methods
This study was conducted at Tribhuvan University Teaching Hospital, Maharajgunj, Nepal. The clinical samples collected from inpatients were processed for detection of *P. aeruginosa* isolates and antibiotic susceptibility profile was determined. The MDR strains were identified and ceftazidime-resistant isolates were subjected for detection of extended-spectrum-β-lactamase (ESBL), metallo-β-lactamase (MBL), and *Klebsiella pneumoniae* carbapenemase (KPC).

Results
A total of 161 *P. aeruginosa* isolates were recovered during the study period encompassing 73.3% (n=118) MDR isolates. The MDR isolates included 50.0% (n=59) from lower respiratory tract infections; and 39.8% (n=47) were from the intensive care unit patients. The MDR isolates showed a high resistance profile towards piperacillin, cephalosporins, and fluoroquinolones (>85%). Resistance to carbapenems and aminoglycosides were up to 80% and 60% respectively. Extended spectrum-β-lactamase, MBL, and KPC mediated resistance were seen in 34.7%, 43.6%, and 14.4% MDR isolates, respectively.

Conclusion
Multidrug resistance as well as resistance mediated by β-lactamases production were high among *P. aeruginosa* isolates. Therefore, early detection of antimicrobial resistance and rational use of antibiotics play a critical role to fight against this MDR pathogen.

Keywords
Metallo-β-lactamase, multidrug resistance, *Pseudomonas aeruginosa*
INTRODUCTION

*Pseudomonas aeruginosa* is one of the emerging nosocomial micro-organisms responsible for a vast variety of infections in hospitalized patients. Various anti-Pseudomonal antibiotics are available for the treatment of these infections but the selection of appropriate antimicrobials is quite challenging because of its ability to gain resistance to multiple classes of antibiotics.1 *Pseudomonas aeruginosa* symbolizes a phenomenon of antimicrobial resistance and practically all known resistance mechanisms are seen in *P. aeruginosa* isolates. Multidrug-resistant (MDR) phenotypes among *P. aeruginosa* are now common due to the simultaneous expression of different resistance mechanisms.2

Carbapenem-resistant *P. aeruginosa* (CRPA) remains an important cause of hospital-acquired infections (HAIs) and has been prioritized as a critical pathogen requiring urgent discovery, research, and development of new antibiotics by the World Health Organization (WHO).3 Among the various mechanisms of carbapenem resistance in *P. aeruginosa*, the production of carbapenemase enzymes is the most worrying mechanism due to their easily transmissible properties to other bacterial pathogens, increasing prevalence, and their association with resistance to other antimicrobial categories leading to MDR phenotypes.4 The isolates producing extended-spectrum-β-lactamase (ESBL) and metallo-β-lactamase (MBL) enzymes show resistance to a variety of β-lactam antibiotics and cause serious infection outbreaks in healthcare settings.5

Therefore, this study was designed to detect different types of β-lactamases among MDR *P. aeruginosa* isolated from hospitalized patients in a tertiary care university hospital.

METHODS

This study was conducted from January 2017 to December 2017 among the hospitalized patients of Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. The ethical approval for the study was obtained from the Institutional Review Committee of the Institute of Medicine with reference number 262(6-11-E)2/073/074. In this study, all consecutive and non- replicate isolates of *P. aeruginosa* recovered from hospitalized patients were included. The various specimens collected from hospitalized patients were cultured on chocolate agar (CA) plate, MacConkey agar (MA) plate, and blood agar (BA) plate and incubated at 37°C for 24 to 48h. *Pseudomonas aeruginosa* isolates were confirmed by employing different biochemical tests and growing on selective media according to the American Society for Microbiology (ASM).6

The antibiotic susceptibility testing of *P. aeruginosa* was performed by the Kirby-Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines (Performance Standards for Antimicrobial Susceptibility Testing, M100S Document, 26th Edition, 2016).7 The antibiotic susceptibility was validated by testing of the reference strain *P. aeruginosa* (ATCC 27853) against all tested antimicrobial agents. The guidelines recommended by Magiorakos et al.8 were implemented to identify the MDR and extensively drug-resistant (XDR) isolates.

The MDR *P. aeruginosa* isolates resistant to ceftazidime disk were considered as potential ESBL producers and further tested by the combination disk (CD) method for confirmation of ESBL production.7 Modified Hodge test (MHT) was used to detect the production of carbapenemase enzymes in MDR *P. aeruginosa* isolates resistant to either meropenem and/or imipenem. After incubation, a cloverleaf shape indention growth of *Escherichia coli* ATCC 25922 within the disk diffusion zone along with the growth of test organism streak was considered as a positive MHT test. A negative MHT test showed inhibition of *E. coli* ATCC 25922 growth along with the growth of test organism streak.9 The MDR *P. aeruginosa* isolates resistant to any carbapenem group of antibiotics were also used to detect and differentiate MBL and KPC enzymes. The detection of MBL-producing, KPC-producing, and KPC+MBL co-producing isolates was identified by using a combination of four meropenem disks recommended by Tsakris et al.10 The MDR *P. aeruginosa* isolates harboring at least two types of β-lactamases were characterized as multitype β-lactamases producing isolates.

The data generated during the study were analyzed by using a 16.0 version of Statistical Package for the Social Sciences (SPSS). Pearson’s correlation test was applied to correlate variables where the data with a p-value ≤ 0.05 (95% CI) was considered statistically significant.

RESULTS

During the study period, a total of 161 non-duplicate *P. aeruginosa* isolates were recovered from an equal number of consecutive samples. Out of the total 161 *P. aeruginosa*, 73.3% (n=118) were MDR, and about half of the isolates (50.9%) were XDR. Out of 118 MDR *P. aeruginosa* isolates, 66.9% (n=79) were isolated from male patients and 33.1% (n=39) were recovered from female patients, with male to female ratio of 2.02. The highest number of MDR isolates (n=25) were from the male patients of the age group 16-32 years (Table 1).

The majority of cases (39.8%) were from intensive care units followed by the neurology department (22.0%) and the minor number was from the
maternity ward (0.8%). Similarly, most of the MDR 
*P. aeruginosa* isolates (50.0%) were recovered 
from lower respiratory tract infections followed by 
pus and swab specimens (20.3%) while the least 
number was isolated from each blood and catheter 
tips samples (1.7%) (Table 2).

The antibiotic profile showed that the beta-lactams 
were found to be less effective against MDR 
*P. aeruginosa* as the highest rate of resistance was 
mediated towards ceftazidime (96.6%), cefepime 
(94.9%), and piperacillin (96.6%). More than 85% 
of the isolates were resistant to fluoroquinolones 
(ofloxacin, ciprofloxacin, and levofloxacin), and 
72.9% to gentamicin. About 80% of the isolates 
were resistant to carbapenems, whereas amikacin 
showed promising efficacy (49.2% isolates were 
susceptible). Polymyxin B was found to be the 
most effective regimen against MDR 
*P. aeruginosa* as resistance was not documented towards this 
antibiotic (Table 3).

The antibiotic resistance mediated by \( \beta \)-lactamase 
enzymes was frequently seen in this study. Resistance due to ESBL and MBL production 
was observed in 34.7% and 63.6% MDR isolates, 
respectively. The highest rate of both ESBL and MBL 
mediated resistance was seen in MDR isolates 
from catheter tips (100% each). The KPC mediated 
antibiotic resistance was reported in 14.4% of MDR 
isolates, with the highest rate of isolates being 
from body fluid samples (21.4%). MBL with ESBL 
and MBL with KPC type \( \beta \)-lactamases production 
were documented in 14.4% and 7.6% isolates, 
respectively (Table 4).

Among carbapenem-resistant MDR isolates 
(n=99), MHT was seen positive in 67.7% (n=67) 
and negative in 32.3% (n=32) isolates. Among the 
MBL producing MDR isolates, 78.7% were MHT 
positive. Additionally, 33.3% of MBL non-producing 
MDR isolates were MHT positive. Similarly, all 
KPC producers and MBL+KPC co-producing MDR 
isolates were MHT positive (Table 5).

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**Table 1. Distribution of MDR Pseudomonas aeruginosa isolates by gender and age group of patients**

| Age group (Years) | Number (%) |
|-------------------|------------|
|                    | Female     | Male | Total |
| ≤15               | 06 (5.1)   | 12 (10.2) | 18 (15.3) |
| 16-32             | 08 (6.8)   | 25 (21.2) | 33 (28.0) |
| 33-48             | 06 (5.1)   | 16 (13.5) | 22 (18.6) |
| 49-64             | 12 (10.2)  | 7 (5.9)   | 19 (16.1) |
| ≥65               | 7 (5.9)    | 19 (16.1) | 26 (22.0) |
| Total             | 39 (33.1)  | 79 (66.9) | 118 (100) |

**Table 2. Ward and specimen wise distribution of MDR Pseudomonas aeruginosa isolates**

| Wards         | Number of isolates in different specimens |
|---------------|------------------------------------------|
|               | LRTS* | Pus/swabs | Urine | Body fluids | Blood | Catheter tips | Total (%) |
| ICU*          | 32    | 5         | 1     | 7           | 1     | 1            | 47 (39.8) |
| Neurology     | 17    | 2         | 7     | 0           | 0     | 0            | 26 (22.0) |
| Surgery       | 4     | 8         | 6     | 5           | 0     | 1            | 24 (20.3) |
| Respiratory   | 4     | 0         | 1     | 0           | 0     | 0            | 5 (4.2) |
| Pediatrics    | 0     | 2         | 1     | 1           | 0     | 0            | 4 (3.4) |
| Orthopedics   | 1     | 1         | 1     | 0           | 0     | 0            | 3 (2.6) |
| Burn          | 0     | 3         | 0     | 0           | 0     | 0            | 3 (2.6) |
| ENT*          | 0     | 3         | 0     | 0           | 0     | 0            | 3 (2.6) |
| Nephrology    | 1     | 0         | 0     | 1           | 0     | 0            | 2 (1.7) |
| Maternity     | 0     | 0         | 0     | 0           | 1     | 0            | 1 (0.8) |
| Total (%)     | 59 (50.0) | 24 (20.3) | 17 (14.4) | 14 (11.9) | 2 (1.7) | 2 (1.7) | 118 (100) |

*aLower respiratory tract specimens (sputum, bronchoalveolar lavage, and endotracheal aspirate); *bIntensive care units; *cEar, nose, and throat
DISCUSSION

In Nepalese hospitals, antibiotic resistance among micro-organisms is a major problem.11 In the present study, we have isolated a highest number of MDR *P. aeruginosa* isolates from lower respiratory tract infections (50.0%). The highest recovery rate of *P. aeruginosa* from lower respiratory tract infections in our study also correlates with other studies done in various countries.12,13 We documented a higher number of MDR *P. aeruginosa* (39.8%) isolates from ICU patients. In another study, *P. aeruginosa* was also documented to be the usual pathogen isolated from ICU patients than that of other hospital wards.14

An increasing trend of resistance to multiple classes of antibiotics has been documented in *Pseudomonas aeruginosa*, which is the crucial reason for treatment failure in hospitalized patients.15 Multidrug-resistant *P. aeruginosa* isolates were found highly resistant to several classes of antibiotics. From previous literature, an elevated rate of resistance against carbapenems, fluoroquinolones, and third-generation cephalosporins has been documented in this bacterium.13 As a consequence of the arbitrary use of antibiotics, these agents have decreased their usefulness in Nepal.11 Among the β-lactam group, 96.6% MDR isolates were found to be resistant to ceftazidime, 94.9% to cefepime, 96.6% to piperacillin, 78.8% to piperacillin-tazobactam, 83.9% to meropenem, and 78.8% to imipenem. Mishra et al.16 from Nepal have documented a lower rate of resistance towards β-lactams. The findings of our study correlate with the observations of Parajuli et al.,11 and Ansari et al.15 from Nepal, and Behera et al.17 from India. We identified a resistance rate of 97.5% to ciprofloxacin, 95.8% to ofloxacin, and 88.1% to levofloxacin, which is higher than that reported by Mishra et al.16 in 2008 and Anil et al.18 in 2013. Parajuli et al.11 from Nepal reported a similar rate of fluoroquinolone resistance in *P. aeruginosa* (up to 90%). The increasing trend of resistance was also reported against aminoglycosides, being resistance rate up to 72.9% to gentamicin and 50.8% to amikacin and is higher than that reported by Mishra et al.16 in 2008.
but similar to the report of Parajuli et al.\textsuperscript{11} in 2017 in ICU patients. In this study, polymyxin B was found to be 100\% effective against MDR \textit{P. aeruginosa}, which could be used as a therapeutic option against MDR \textit{P. aeruginosa} isolates. However, it could arise detrimental consequences in hospitalized patients due to its toxicity. Other studies also found polymyxin B as the most effective regimen against serious pseudomonal infections.\textsuperscript{11,16}

The rate of MDR in \textit{P. aeruginosa} isolates has dramatically increased (73.3\% MDR isolates) and 50.9\% isolates were XDR, which is higher than those reported by Mishra et al.\textsuperscript{16} (65.9\% MDR) from Nepal in 2008, Prakash et al.\textsuperscript{19} (31.7\% MDR) from India in 2014, Flamm et al.\textsuperscript{20} (24.9\% MDR) and Langeveld et al.\textsuperscript{21} (37.3\% MDR) from the USA in 2004 and 2017 respectively, and Pereira et al.\textsuperscript{22} (39.9 \% MDR and 2.9\% XDR) from Portugal in 2015. The rate of MDR \textit{P. aeruginosa} is in an upward trend throughout the world, especially in developing countries causing a life-threatening situation.

In this study, we observed 34.7\% ESBL producing MDR isolates. Umadevi et al.\textsuperscript{23} from India reported only 13\% ESBL producing \textit{P. aeruginosa} isolates. Likewise, in two other studies from India\textsuperscript{24} and Brazil,\textsuperscript{25} low rates of ESBL production of about 21\% were observed. The variation in the prevalence of ESBL from country to country and hospital to the hospital may be ascribed to antibiotic prescribing habits and the presence of pathogens harboring ESBL genes.

World Health Organization published a list of antibiotic-resistant bacteria and categorized carbapenem-resistant \textit{P. aeruginosa} (CRPA) as a critical pathogen (priority first).\textsuperscript{3} The emergence of MBLs in \textit{P. aeruginosa} is becoming a serious summons as these enzymes possess high hydrolytic activity against all \beta-lactam classes.\textsuperscript{23,26} Among a total of 118 MDR isolates, we identified 63.6\% isolates as MBL producers which is much higher than previous studies carried out by different authors from Nepal.\textsuperscript{16,16} However, a lower rate of MBL production was reported by Joseph et al.\textsuperscript{27} (20\%) from India, Rafiee et al.\textsuperscript{28} (37.3\%) from Iran, and only 3.3\% by Mishra et al.\textsuperscript{16} from Nepal in 2008. We have found 14.4\% KPC-producing isolates and they were also co-producing MBL enzymes. Falahat et al.\textsuperscript{29} reported 12\% KPC-producing \textit{P. aeruginosa} from Iran. We have also identified a few MDR isolates harboring a multitype of \beta-lactamase enzymes. MBL with ESBL enzymes was identified in 14.4\% and MBL with KPC was seen in 7.6\% MDR isolates. Besides, 14.36\% co-producer of ESBL and MBL was documented in \textit{P. aeruginosa} by Chaudhary and Payasi\textsuperscript{30} from India while only 3.4\% co-producer of ESBL and MBL was reported by Ansari et al.\textsuperscript{15} from Nepal. In the present study, we found infections in hospitalized patients with highly resistant \textit{P. aeruginosa} isolates. This is due to the high prevalence of MDR strains and the production of various beta-lactamas that can contribute resistance to different classes of antibiotics.

**CONCLUSION**

According to the findings of this study, infections caused by MDR \textit{P. aeruginosa} in hospitalized patients are common. MDR isolates had a higher rate of ESBL and MBL production and these isolates were only completely susceptible to potentially toxic antibiotics like polymyxin B, which is a major concern for hospitalized patients. Therefore, early detection of antimicrobial resistance and rational use of antibiotics play a critical role to fight against this MDR pathogen.

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**CONFLICT OF INTEREST**

None declared.

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