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Metallo-β-lactamase-Producing Clinical Isolates of *Klebsiella pneumoniae* from Cancer Patients Visiting Nepal Cancer Hospital, Lalitpur

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ABSTRACT

Background

The emergence of metallo-beta-lactamase (MBL)-producing bacterial pathogens, particularly *Klebsiella pneumoniae* (*K. pneumoniae*) poses a significant challenge in the treatment of infections in immunocompromised populations such as cancer patients. This study aims to investigate the prevalence of MBL-producing *K. pneumoniae*, identify associated risk factors for MBL production, and determine their antimicrobial susceptibility pattern among cancer patients in Nepal.

Methods

A hospital-based, cross-sectional study was conducted on cancer patients from June 2023 to May 2024 in a tertiary care cancer hospital of Nepal. Samples received at the hospital were processed as per standard protocol for isolation, phenotypic identification and antibiotic susceptibility testing following CLSI guideline (2021).

Results

Out of 3,504 cancer patients, 241 non-duplicate cases of *K. pneumoniae* infection were identified. Among these isolates, the majority were obtained from urine samples 101(42%), followed by sputum samples 61(25.3%). A total of 104 isolates (43.2%) were confirmed as MBL (metallo-β-lactamase) producers. These MBL-producing isolates exhibited significantly higher resistance to most of the tested antibiotics compared to non-MBL producers. Statistical analysis revealed that prolonged hospitalization, prior use of broad-spectrum antibiotics, and the presence of fever were significantly associated with MBL production among *K. pneumoniae* isolates in cancer patients.

Conclusions

This study demonstrates a high prevalence of MBL-producing *K. pneumoniae* among cancer patients in Nepal, with these strains showing significantly elevated resistance to commonly used antibiotics underscoring the need for targeted infection control strategies and strict antimicrobial stewardship in oncology settings.

Keywords: metallo-beta-lactamase; *Klebsiella pneumoniae*; cancer patients; Nepal; antimicrobial resistance.

Correspondence: Dr. Prakash Ghimire, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Email: prakash.ghimire@cdmi.tu.edu.np, Phone: +977-9851066710. **Article received**: 2025-08-06. **Article accepted**: 2025-010-12. **Article published**: 2025-12-28.

INTRODUCTION

K. pneumoniae bacteremia is more common and associated with higher mortality in cancer patients.[1] The global rise of antibiotic-resistant bacteria poses a growing challenge for healthcare providers.[2] Excessive antibiotics use in humans, poor infection control, and increased global travel have accelerated the spread of multidrug-resistant (MDR) strains.[3,4] *K. pneumoniae* is of particular concern because its production of metallo-β-lactamases (MBLs) confers resistance to most β-lactam antibiotics, including carbapenems.[5] These enzymes belong to Ambler class B and require 1 or 2 zinc ions for enzyme activity.[6] Clinically important MBLs such as NDM, IMP, and VIM are plasmid-mediated and can easily transfer between bacterial species like *K. pneumoniae*, facilitating the rapid spread of resistance..[7] There are limited treatment options for the infections caused by MBL producing bacteria.[8] This study investigates the prevalence, risk factors, and antimicrobial resistance patterns of MBL-producing *K. pneumoniae* among cancer patients in a tertiary care hospital in Nepal.

METHODS

This study was conducted at Nepal Cancer Hospital and Research Center, a tertiary care cancer hospital in Lalitpur, Nepal, over a period of 12 months from June 2023 to May 2024. Clinical samples were collected from cancer patients, including wound swabs, pus, blood, urine, sputum, fluid and tips. The ethical approval for the study was obtained from Nepal Health Research Council (NHRC), Nepal (Ref. No. 112/2023) prior to the commencement of the work. Bacterial identification was performed using standard microbiological techniques, including Gram staining and biochemical tests.[9] Antibiotic susceptibility testing was done using the disk diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2021)[10] except for antibiotic tigecycline. The combined disk test (CDT) was used to detect MBL-positive isolates. For this purpose, two disks of imipenem and imipenem plus EDTA 0.5 M (ethylenediaminetetraacetic acid) were placed on the cultured plate. After incubation at 35 °C for 16-18 h, an increase of ≥7 mm in the inhibition zone between the imipenem-EDTA disk and imipenem alone was considered as MBL producing isolates.[11,12] Data were entered and analyzed using SPSS version 16. The resistance patterns of MBL and non MBL *K. pneumoniae* to various antibiotics were compared using chi-square test, with a p-value <0.05 considered statistically significant.

RESULTS

A total of 241 cases of *K. pneumoniae* infection in patients with cancer were identified during the study period. The mean age of the study population was 59.6 years and patients with male gender comprised 50.6% of cases. Those with hematologic malignancy comprised 5.8% of cases. Hundred and twenty-five patients (51.9% of cases) were exposed to certain antibiotics prior to culture and 164 patients showed fever (68% of cases) (Table 1).

Table 1. Demographic and clinical characteristics of cancer patients with *Klebsiella pneumoniae* infection. (n=241)

Variable	Frequency (%)
Gender	
Female	119(49.4)
Male	122(50.6)
Age (Years)	
Up to 15	5(2,1)
16-45	36(14.9)
46-60	74(30.7)
More than 60	126(52.3)
Type of Cancer	
Solid	227(94.2)
Hematologic	14(5.8)
Exposure to antibiotics in the past six months	
No	116(48.1)
Yes	125(51.9)
Symptom (fever)	
No	77(32)
Yes	164(68)

Among total *K. pneumoniae* isolates, 42% (101) of isolates were obtained from urine sample followed by sputum sample 61 (25.3%). The isolates were predominantly obtained from medical ward 94 (39%) followed by daycare 51(21.2%) and surgical

ward 40 (16.6%) (Figure 1).

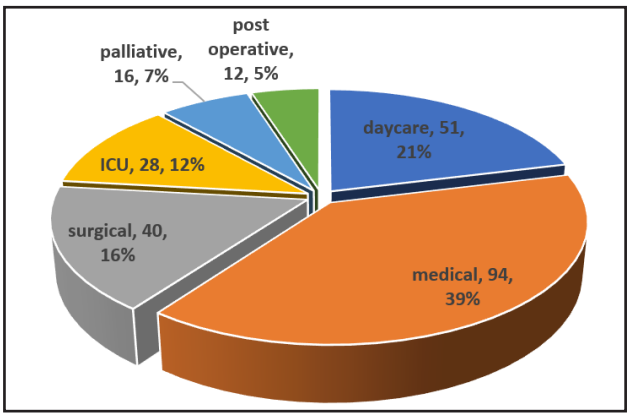

Figure 1. Ward wise distribution of *K. pneumoniae* isolates. (n=241)

Table 2. Sample wise distribution of *K. pneumoniae* isolates. (n=241)

Sample	Frequency (%)
Body fluid	17(7)
Blood	9(3.7)
Tips	25(10.4)
Pus	28(11.6)
Sputum	61(25.3)
Urine	101(42)

Among 241 isolates of *K. pneumoniae*, 104 (43.2%) isolates were found to produce MBLs. Both non MBL and MBL *K. pneumoniae* exhibited high resistance to commonly used antibiotics. The MBL-producing isolates showed significantly higher resistance rates

Table 3. Antibiotic susceptibility pattern of non-MBL *K. pneumoniae* (n=137) and MBL *K. pneumoniae.* (n=104)

Antibiotic	non-MBL Sensitive n(%)	Resistant n(%)	MBL Sensitive n(%)	MBL Resistant n(%)	p-value
Amikacin	126(92.0)	11(8.0)	-	104(100)	< 0.001
Gentamicin	116(84.7)	21(15.3)	3(2.9)	101(97.1)	< 0.001
Tobramycin	102(74.5)	35(25.5)	1(1.0)	103(99.0)	< 0.001
Amoxicillin-clavulanic acid	1(0.7)	136(99.3)	-	104(100)	1
Piperacillin-Tazobactam	89(65.0)	48(35.0)	1(1.0)	103(99.0)	< 0.001
Ciprofloxacin	85(62.0)	52(38.0)	-	104(100)	< 0.001
Ofloxacin	81(59.1)	56(40.9)	1(1.0)	103(99.0)	< 0.001
Norfloxacin (n= 107)	32(66.7)	16(33.3)	1(1.7)	58(98.3)	< 0.001
Levofloxacin	84(61.3)	53(38.7)	1(1.0)	103(99.0)	< 0.001
Cefuroxime	48(35.0)	89(65.0)	-	104(100)	< 0.001
Cefoxitin	49(35.8)	88(64.2)	-	104(100)	< 0.001
Cefotaxime	60(43.8)	77(56.2)	-	104(100)	< 0.001
Cefixime	75(54.7)	62(45.3)	-	104(100)	< 0.001
Cefepime	79(57.7)	58(42.3)	-	104(100)	< 0.001
Cefpodoxime	65(47.4)	72(52.6)	-	104(100)	< 0.001
Ceftazidime	61(44.5)	76(55.5)	-	104(100)	< 0.001
Doxycycline	104(75.9)	33(24.1)	58(55.8)	46(44.2)	< 0.001
Tetracycline	98(71.5)	39(28.5)	16(15.4)	88(84.6)	< 0.001
Meropenem	112(81.8)	25(18.2)	-	104(100)	< 0.001
Imipenem	112(81.8)	25(18.2)	-	104(100)	< 0.001
Doripenem	111(81.0)	26(19.0)	-	104(100)	< 0.001
Aztreonam	112(81.8)	25(18.2)	-	104(100)	< 0.001
Nitrofurantoin (N = 92)	17(42.5)	23(57.5)	-	52(100)	< 0.001
Tigecycline	132(96.4)	5(3.6)	86(82.7)	18(17.3)	< 0.001
Colistin	137(100)	-	104(100)	-	1
Polymyxin B	137(100)	-	104(100)	-	1

to all antibiotics except Amoxicillin clavulanic acid, Colistin and Polymyxin B compared to non-MBL producers. Furthermore, colistin and polymyxin B resistant was not found in both non MBL and MBL producers (Table 3).

Chi-square analysis revealed that prolonged hospitalization, prior use of broad-spectrum antibiotics, and presence of fever were significantly associated with the MBL-production among the pathogens in cancer patients (Table 4).

Table 4. Risk factors for MBL (n=104) and non-MBL. (n=137)

Variables	MBL n(%)	non-MBL n(%)	p-value
Fever			
No	9(8.65)	68(49.64)	< 0.001
Yes	95(91.35)	69(50.36)	
Exposure to antibiotic in the past six months			
No	42(40.38)	83(60.58)	<0.001
Yes	62(59.62)	54(39.42)	
Duration of hospital stays			
≤48 hrs	54(51.92)	94(68.61)	<0.001
≥48 hrs to 34 days	50(48.08)	43(31.39)	

DISCUSSION

Bacterial resistance to antibiotics is rapidly growing, and investigation of antibiotic resistance profiles and mechanisms is necessary in each region to control the spread of resistant bacteria among cancer patients. In Nepal, a few studies have focused on resistance pattern of bacteria isolated from cancer patients. Bacterial infection caused by *K. pneumoniae* strains are among the most important and prevalent infections that should be considered a serious threat to human health worldwide.[13-15]

In the present study, *K. pneumoniae* was predominantly isolated from urine samples of cancer patients. Similar findings were reported by Jiang A.M. et al. [16], Bora A. et al. [17], and Nepal K. et al.[18] However, these findings contrast with some earlier reports where *Klebsiella* species were reported to be more commonly associated with bloodstream infections in cancer patients.[14] In the present study, *K. pneumoniae* showed different levels of resistance to the tested antibiotics, revealing that tigecycline and doxycycline are the most effective antibiotics. Similar result was reported by Tang HJ et al [19] and Kumar S et al [20]. In contrast, resistance to beta-lactam antibiotics such as cefoxitin and cefuroxime was high. This high level of resistance to beta-lactam antibiotics may be attributed to the misuse of antibiotics in Nepal as antibiotics are easily available in drug stores without prescriptions at the request of patients.

In addition, treatment of infections caused by *K. pneumoniae* is becoming more difficult due to different antibiotic resistance mechanisms that are being developed.[13] Therefore, in this study, we investigated the antibiotic resistance profiles among *K. pneumoniae* strains isolated from cancer patients. Carbapenems (imipenem and meropenem) and aminoglycosides (gentamicin and amikacin) are last-line antibiotics, which are usually used to treat infection caused by resistant isolates.[21] Our results showed that non-MBL isolates exhibited lower resistance to carbapenems (18.2% to 19%) and aminoglycosides (8% to 25.5%), which is higher than previously reported in India.[14] In contrast, studies from Ethiopia and Taiwan reported that all *K. pneumoniae* isolates were susceptible to meropenem, and 75% were susceptible to amikacin.[15, 22] However, MBL-producing isolates in our study were 100% resistant to both carbapenems and aminoglycosides, which is consistent with the findings of Bora A et al.[17] The study revealed a high prevalence of MBL-producing *K. pneumoniae* in cancer patients in Nepal, with significant antimicrobial resistance patterns. The findings are consistent with similar studies in other regions, indicating that MBL production is a growing threat in healthcare settings worldwide due to the excessive use of carbapenems.[11, 23]

This study revealed that patients who were symptomatic during sample collection, had a history of antibiotic exposure and hospitalized for long duration were more susceptible to infection. This finding is in agreement with several other studies.[24-26] These factors are particularly persistent in cancer treatment centers, where patients undergo frequent surgeries and chemotherapies. The presence of MBL-producing pathogens in cancer patients is alarming,

as these individuals are already at high risk of infection due to their compromised immune status.

CONCLUSIONS

This study highlights the significant prevalence of MBL-producing *K. pneumoniae* in cancer patients in Nepal, underscoring the need for improved infection control measures and antibiotic stewardship programs. Further surveillance and research into the molecular mechanisms of resistance and the development of alternative treatment strategies are essential to combat the growing threat of MDR pathogens.

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