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Carbapenemase Producing Multi Drug Resistant *Klebsiella pneumoniae* from a Referral Hospital in Nepal

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ABSTRACT

Background: Carbapenem resistant *Klebsiella pneumoniae* (CRKP) are gradually emerging and thus limiting the options for treatment. Carbapenemases hydrolyze the wide spectrum of β-lactam antibiotics. This study aimed to determine the multidrug resistant (MDR) carbapenemase producing *K. pneumoniae* from a referral hospital in Nepal.

Methods: The clinical isolates collected from Tribhuvan University Teaching Hospital, Maharajgunj were cultured, and identified using biochemical tests. The antibiotic susceptibility test was performed using Kirby Bauer disk diffusion method following CLSI guidelines. Production of CRKP (KPC and MBL) was determined by phenotypic methods.

Results: From different clinical specimens, 377 *K. pneumoniae* were isolated and identified phenotypically. Among total *K. pneumoniae*, 80.6% were MDR. The carbapenem resistant *K. pneumoniae* were 46.2%, among them 22.5% were only KPC producer, 10.6% were only MBL producer and 13.0% were both KPC and MBL producer. The CRKP positive isolates were significantly higher in urine (p=0.037), blood (p=0.006), sputum (p=0.032) samples as compared to other samples.

Conclusions: The high prevalence of CRKP emphasizes the need for continuous surveillance among the patients to detect the resistant strain and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance among *K. pneumoniae*.

Keywords: Carbapenemase producer; CRKP; *Klebsiella pneumoniae;* multidrug resistant.

INTRODUCTION

Antimicrobial resistance is a global public health problem, and *Klebsiella pneumoniae* has been recognized as one of the major contributor.[1] *K. pneumoniae* is the second leading cause of deaths globally and a first leading cause of deaths in Southeast Asia from blood stream infections as reported in 2019.[2] Carbapenem-resistant *K. pneumoniae* (CRKP) has been identified by World Health Organization as a top three priority for the control.[3,4] Carbapenemase-producing *K. pneumoniae* was first identified in USA in 1996[5] and KPC-2 in China in 2007 and a carbapenemase-resistance cassette was identified in 2013 in 13.4% of *K. pneumoniae* isolated from the hospital patients.[6] CRKP are significantly associated with higher mortality rate and high hospital expenditure.[7] Remarkably, the mortality rate among patients with pneumonia caused by *K. pneumoniae* is about 50%.[8] A meta-analysis showed that the incidence of CRKP colonization ranges from 2 to 73% with a pooled incidence of 22.3%.[9] The prevalence of extended-spectrum ß lactamases (ESBL) in *K. pneumoniae* was 23% and MDR was 55% in Nepal.[10] The MBL activity was reported in 19% of *K. pneumoniae* isolates from a national public health laboratory in Nepal.[11] Other carbapenemases reported in *K. pneumoniae* include VIM, IMP, NDM.[12]

K. pneumoniae is one of the most common isolate in various clinical specimens and carbapenem resistance is of concern.[13] Updated carbapenem resistance information is needed to reveal the prevailing

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carbapenem resistance in *K. pneumoniae* from clinical isolates. This information is useful for rationale treatment of carbapenem resistant *K. pneumoniae*. Hence, this study was undertaken to determine the rate of carbapenemase producing MDR *K. pneumoniae* isolates from different clinical specimens from the biggest referral hospital of Nepal located in Kathmandu.

METHODS

This was a hospital based cross-sectional study. The *K. pneumoniae* isolates were collected from Tribhuvan University Teaching Hospital and further processing was carried out at the laboratory of Central Department of Microbiology, Kirtipur.

The ethical review for this study was taken from the Nepal Health Research Council Ethical Review Board (ERB) (Regd. No.: 293-2020). Permission was obtained from Tribhuvan University Teaching Hospital to conduct the study. Written informed consent was obtained from the patients before sample and data collection.

Different non-duplicate clinical samples including urine, pus, blood, sputum, swab and body fluids were collected from the patients in the microbiology laboratory of the Tribhuvan University Teaching Hospital, Kathmandu from April 2021 to April 2024. Primary isolation and identification was performed in the hospital laboratory and only the *K. pneumoniae* isolates were further processed in the laboratory of Central Department of Microbiology, Tribhuvan University.

The samples were cultured on MacConkey agar. The mucoid lactose fermenting colonies were sub-cultured on nutrient agar. The Gram staining was performed and Gram negative bacilli were further tested using biochemical tests. *K. pneumoniae* were confirmed by biochemical tests. The *E. coli* ATCC 25922 was used as quality control strain for the tests and *K. pneumoni*ae ATCCBAA-1705 was used as control strain for KPC positive (Serine carbapenemase producer).[14]

Antibiotic susceptibility tests were performed by Kirby Bauer's disc diffusion method (CLSI, 2020). Antibiotics discs used were Gentamicin (30 µg), Amikacin (30µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Ceftazidime (30 µg), Cefixime (5 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Imipenem (10 µg), Meropenum (10 µg), Amoxicillin/ Clavulanate (20/10 µg), Ampicillin Sulbactam (10/10 µg), Aztreonam (30 µg), Cholaramphenicol (30 µg), Colistin Sulphate (10 µg), Tigecycline (15 µg), Doxycycline (30 µg), Polymyxin B (300 µg), Pipeacillin Tazobactam (100/10 µg), Cefoperazone / Sulbactam (75/30 µg), Clavulanic acid and Ceftazidime (30/10 µg), Cotrimoxazole (25 µg), Nitofurantoin (300 µg), and Amoxycillin (10 µg). The results were interpreted as per the Clinical Laboratory Standards Institute recommendations.[14] *K. pneumoniae* ATCC 700 603 strain was used as quality control strain for antimicrobial susceptibility tests.[14] The minimum inhibitory concentration of colistin against *K. pneumoniae* was determined using the colistin broth disc elution method .[14]

Carbapenemase production was screened by disk diffusion method. Each of the isolate with a reduced susceptibility to meropenem or imipenem with inhibition zone diameter of ≤22 mm were identified as potential carbapenemase producer.[14]

The phenotypic detection of the carbapenemase production was performed by the modified Carbapenem Inactivation Method (mCIM) as described by CLSI. Suspension of one loopful of *K. pneumoniae* from an overnight culture on a blood agar plate was made into 2 ml trypticase soya broth in two tubes. One tube was devoid of EDTA (mCIM), while the other was supplemented with EDTA (eCIM). The Meropenem (10 µg) disc was immersed in each tube and incubated for 4 hours at 35°C. After incubation, the disc was removed from the suspension using a 10 µl inoculating loop and placed on a Mueller-Hinton agar plate inoculated with a carbapenem-susceptible *E. coli* ATCC 29522. Then, the results were read after 18-24 hours of incubation at 35°C. An inhibition zone diameter of less than 18 mm was confirmed as CRKP positive and a zone of inhibition ≥19 mm was confirmed as CRKP negative. If zone of inhibition is ≥ 5mm for eCIM than that of mCIM, the isolate was considered as metallo-ß-lactamase positive.[14]

To detect KPC (*Klebsiella pneumoniae* carbapenemase) and MBL (metallo-beta-lactamase) production in bacterial isolates, a disc diffusion method was employed using meropenem discs in conjunction with either phenyl boronic acid (PBA) or EDTA. The stock solution of PBA in the concentration of 20 mg/ml was prepared by dissolving PBA in dimethyl sulfoxide (DMSO). Twenty microliters (400µg of PBA) from this solution was dispensed onto meropenem disc. The stock solution of EDTA was prepared by dissolving anhydrous EDTA in distilled water at concentration of 0.1M. Ten microliters (292 µg of EDTA) from this solution was dispensed onto meropenem disc. These discs were kept on MHA inoculated with test strain viz. one disc of meropenem using without any inhibitor, one disc of meropenem with

PBA and one disc of meropenem with EDTA. The agar plate was incubated overnight at 37° C. The zone of inhibition around these meropenem discs with inhibitor added were compared with that around the meropenem disc alone.[15]

An isolate was confirmed as KPC producing if there is a ≥5 mm increase in the inhibition zone around the meropenem disk with boronic acid compared to the disk containing meropenem alone.[15] The isolate was considered MBL producing when the growth inhibitory zone diameter around the meropenem disc with EDTA was found to be ≥5mm than that of meropenem alone.[15]

Data were entered into Micro-soft Excel and analyzed using SPSS version 25. The distribution of *K. pneumoniae* in different clinical samples, antibiotic susceptibility with different antibiotics, percentages of MDR, KPC and MBL were calculated. Chi-square test was used to assess association between sample wise distribution of CRKP, and antibiotic susceptibility between MDR and CRKP isolates. P-value = 5% was considered statistically significant.

RESULTS

In this study, 377 *K. pneumoniae* were identified, of which 120 (31.8%) were from urine, 122 (32.4%) were from sputum, 53 (14.1%) were from pus, 37 (9.8%) were from blood, 24 (6.4%) were from body fluids, and 21 (5.6%) were from wound swabs.

The CRKP positive isolates were detected 67.6% in blood samples, 54.1% in sputum samples and 42.9% in wound swab samples. At least one third of isolates from each sample type had CRKP (Table 1). The CRKP positive isolates were significantly higher in urine (p=0.037), blood (p=0.006), and sputum (p=0.032) samples as compared to other samples.

Table 1. Distribution of *K. pneumoniae* in various clinical samples.

Clinical samples	CRKP positive (%)	CRKP negative (%)	p-value
Urine	46 (38.3)	74 (61.7)	0.037
Pus	20 (37.7)	33 (62.3)	0.185
Blood	25 (67.6)	12 (32.4)	0.006
Sputum	66 (54.1)	56 (45.9)	0.032
Wound swab	9 (42.9)	12 (57.1)	0.755
Body fluids	8 (33.3)	16 (66.7)	0.193

Among *K. pneumoniae* isolates, 80.6% were multidrug resistant and 46.2% were CRKP. Among CRKP isolates, 22.5% were KPC producers, 10.6% were MBL producers and 13% were both KPC and MBL producers (Table 2).

Table 2. Prevalence of carbapenem resistant *K. pneumoniae* (CRKP).

Characteristics	Number (%)
MDR	304 (80.6)
CRKP	174 (46.2)
KPC only	85 (22.5)
MBL only	40 (10.6)
KPC and MBL both	49 (13.0)

Bacteria resistant to three or more than three different class of antibiotics were considered as MDR.

Among MDR *K. pneumoniae* isolates, majority of isolates were resistant to ceftazidime, ceftriaxone, amoxyclav, cefepime, ampicillin sulbactam, and cotrimoxazole. Among non-MDR isolates, 97.3% were amoxicillin resistant and 30.8% were nitrofurantoin resistant (Table 3).

Table 3. Antibiotic susceptibility status of MDR and non-MDR *K. pneumoniae*.

Antibiotics	MDR			Non-MDR			p-value
	S	I	R	S	I	R	
Gentamicin	76 (25)	8 (2.6)	220 (72.4)	69 (94.5)	1 (1.4)	3 (4.1)	<0.001
Amikacin	79(26.0)	14(4.6)	211(69.4)	68(93.2)	2(2.7)	3(4.1)	<0.001
Ciprofloxacin	19(6.3)	12(3.9)	273(89.8)	62(84.9)	3(4.1)	8(11.0)	<0.001
Levofloxacin	46(15.1)	12(3.9)	246(80.9)	67(91.8)	3(4.1)	3(4.1)	<0.001
Ceftazidime	14(4.6)	10(3.3)	280(92.1)	66(90.4)	1(1.4)	6(8.2)	<0.001
Ceftriaxone	11(3.6)	5(1.6)	288(94.7)	62(84.9)	2(2.7)	9(12.3)	<0.001
Cefepime	15(4.9)	19(6.3)	270(88.8)	64(87.7)	4(5.5)	5(6.8)	<0.001
Imipenem	104(34.2)	14(4.6)	186(61.2)	71(97.3)	2(2.7)	0(0.0)	<0.001
Meropenem	94(30.9)	8(2.6)	202(66.4)	70(95.9)	1(1.4)	2(2.7)	<0.001
Amoxyclav	9(3.0)	8(2.6)	287(94.4)	55(75.3)	5(6.8)	13(17.8)	<0.001
Ampicillin Sulbactum	39(12.8)	12(3.9)	253(83.2)	70(95.9)	2(2.7)	1(1.4)	<0.001
Aztreonam	57(18.8)	9(3.0)	238(78.3)	71(97.3)	1(1.4)	1(1.4)	<0.001
Chloramphenicol	108(35.5)	35(11.5)	161(53.0)	62(84.9)	8(11.0)	3(4.1)	<0.001
Tigecycline	199(65.5)	59(19.4)	46(15.1)	68(93.2)	4(5.5)	1(1.4)	<0.001
Doxycycline	144(47.4	39(12.8)	121(39.8)	71(97.3)	0(0.0)	2(2.7)	<0.001
Piperacillin Tazobactam	74(24.3)	8(2.6)	222(73.0)	70(95.9)	1(1.4)	2(2.7)	<0.001
Cefoperazone / Sulbactam	70(23.0)	20(6.6)	214(70.4)	72(98.6)	1(1.4)	0(0.0)	<0.001
Cotrimoxazole	37(12.2)	4(1.3)	263(86.5)	65(89.0)	2(2.7)	6(8.2)	<0.001
Cefoxitin	83(27.3)	3(1.0)	218(71.7)	70(95.9)	1(1.4)	2(2.7)	<0.001
Nitrofurantoin	17(11.4)	14(9.4)	118(79.2)	13(33.3)	14(35.9)	12(30.8)	<0.001

The resistance rates of CRKP were more than 80% for gentamicin, amikacin, ciprofloxacin, levofloxacin, ceftazidime, ceftriaxone, cefipime, amoxyclav, ampicillin sulbactam, aztreonam, piperacillin tazobactam, cefoperazone, cotrimoxazole, cefoxitin, and nitrofurantoin. For non-CRKP isolates, the resistance to amoxicillin was 99.5% (Table 4).

Table 4. Antibiotic susceptibility status of CRKP and non-CRKP isolates.

Antibiotics	Zone diameter break points (mm)	CRKP	Non-CRKP	p-value	CRKP			Non-CRKP			p-value
		Mean zone of inhibition ±SD (mm)	Mean zone of inhibition ±SD (mm)		S	I	R	S	I	R	
Gentamicin	15	3.30±7.08	12.86±8.8	<0.001	25(14.4)	2(1.1)	147(84.5)	120(59.1)	7(3.4)	76(37.4)	<0.001
Amikacin	17	3.11±6.64	12.30±9.02	<0.001	23(13.2)	4(2.3)	147(84.5)	124(61.1)	12(5.9)	67(33.0)	<0.001
Ciprofloxacin	21	2.36±5.95	12.47±10.93	<0.001	8(4.6)	4(2.3)	162(93.1)	73(36.0	11(5.4)	119(58.6)	<0.001
Levofloxacin	17	4.41±6.5	12.87±9.7	<0.001	12(6.9)	5(2.9)	157(90.2)	101(49.8)	10(4.9)	92(45.3)	<0.001
Ceftazidime	21	2.05±5.05	11.87±9.0	<0.001	3(1.7)	2(1.1)	169(97.1)	77(37.9)	9(4.4)	117(57.6)	<0.001
Ceftriaxone	19	1.73±5.05	11.86±10.08	<0.001	6(3.4)	0(0.0)	168(96.6)	67(33.0)	7(3.4)	129(63.5)	<0.001
Cefepime	25	3.91±6.74	15.48±9.88	<0.001	2(1.1)	6(3.4)	166(95.4)	77(37.9)	17(8.4)	109(53.7)	<0.001
Amoxyclav	18	0.4±2.24	9.34±8.56	<0.001	1(0.6)	0(0.0)	173(99.4)	63(31.0)	13(6.4)	127(62.6)	<0.001
Ampicillin Sulbactum	15	0.90±3.33	10.91±8.40	<0.001	5(2.9)	1(0.6)	168(96.6)	104(51.2)	13(6.4)	86(42.4)	<0.001
Aztreonam	21	7.75±8.51	17.34±8.60	<0.001	19(10.9)	0(0.0)	155(89.1)	109(53.7)	10(4.9)	84(41.4)	<0.001
Chloramphenicol	18	8.61±9.18	14.10±10.73	<0.001	55(31.6)	19(10.9)	100(57.5)	115(56.7)	24(11.8)	64(31.5)	<0.001
Colistin	-	13.86±1.23	13.78±1.20	0.509	-	-	-	-	-	-	-
Tigecycline	18	17.18±2.4	18.282.86	<0.001	113(64.9)	32(18.4)	29(16.7)	154(75.9)	31(15.3)	18(8.9)	0.035
Doxycycline	10	9.97±7.55	13.56±6.83	<0.001	82(47.1)	17(9.8)	75(43.1)	133(65.5)	22(10.8)	48(23.6)	<0.001
Polymyxin B	-	14.21±1.48	14.30±1.16	0.548	-	-	-	-	-	-	-
Piperacillin Tazobactam	21	5.01±6.21	16.92±9.13	<0.001	9(5.2)	2(1.1)	163(93.7)	135(66.5)	7(3.4)	61(30.0)	<0.001
Cefoperazone / Sulbactam	21	4.79±6.61	17.45±10.20	<0.001	9(5.2)	6(3.4)	159(91.4)	133(65.5)	14(6.9)	56(27.6)	<0.001
Cotrimoxazole	16	2.49±6.79	10.10±11.03	<0.001	15(8.6)	4(2.3)	155(89.1)	87(42.9)	2(1.0)	114(56.2)	<0.001
Cefoxitin	18	2.03±5.18	14.01±10.18	<0.001	15(8.6)	2(1.1)	157(90.2)	138(68.0)	2(1.0)	63(31.0)	<0.001
Nitrofurantoin	17	6.06±6.5	11.48±6.21	<0.001	7(8.5)	6(7.3)	69(84.1)	23(21.7)	22(20.8)	61(57.5)	<0.001

Most of the carbapenemase-producing *K. pneumoniae* (82.14%) were susceptible to colistin with ≤1µg/ml, 1.8% had MIC ≤2 (intermediate) and 9.4% had MIC ≤ 4 (resistant) and 6.5% had MIC ≥4.

DISCUSSION

From 377 clinical isolates of *K. pneumoniae,* 80.6% were MDR. Studies have revealed different percentages of MDR cases in Nepal ranging from 21% to 89%.[16-22] These all investigations from Nepal found a substantial proportion of MDR cases, indicating an alarming situation. A large number of patients with MDR bacteria admitted to the hospital are improperly treated based on empirical practices and without complete information on the antibiogram pattern. Further, there is also transmission of antimicrobial resistance among the bacteria through horizontal gene transfer. Lack of antibiotic stewardship and poor hygiene practices can also contribute to the spread of resistant bacteria.

Nearly half of the *K. pneumoniae* were CRKP positive. Similar results have been reported for CRKP since last few years in Nepal .[23,24] This high rates of CRKP could be attributed to overuse and misuse of carbapenem group of antibiotics, further increasing to the selection and spread of CRKP strains.

Although majority of specimens processed in the hospital were sputum and urine, there were significantly higher rates of MDR isolates in urine and blood samples, as compared to other samples. Previous study mentioned that *K. pneumoniae* is frequently associated with respiratory and urinary tract infections and CRKP infections are often linked to invasive procedures, immunocompromised patients and pneumonia.[25] In this study, CRKP detections were found higher in blood, sputum and wound swabs.

Among carbapenem resistant *K. pneumoniae*, 22.5% were KPC producer, 10.6% were MBL producer and 13% were both KPC and MBL producer. Resistance to carbapenems is accountable to various mechanisms such as, production of ß-lactamases including metallo-ß-lactamases, efflux pumps and inhibiting the entry of antibiotics.[25]

The growing practice of treating infections brought on by multidrug resistant isolates directly with carbapenem is now a serious issue.[26] Increasing resistance to carbapenems was also reported in other study. [27]

The study found significant differences in antibiotic response between MDR and non-MDR *K. pneumoniae* isolates. Among MDR *K. pneumoniae* isolates, majority of isolates were resistant to ceftazidime, cefixime/ ceftriaxone, amoxyclav, cefepime, ampicillin sulbactam and cotrimoxazole. The lowest resistance percentages of MDR *K. pneumoniae* were for polymixin B and colistin as determined by broth disc dilution method. Colistin and Polymyxin B are considered as equivalent agents and MIC value of colistin can be used to predict MIC of polymyxin B and vice versa.[14] This study showed that colistin, polymyxin B and tigecycline were effective against MDR *K. pneumoniae* as compared to other antibiotics.

CRKP were found statistically significantly associated with resistance to antibiotics of different classes. This may lead to difficulty in treating the patients leading to mortality. Although data on mortality attributable to antibiotic resistance specially CRKP infections are not available from Nepal, the studies reported that mortality rates due to CRKP infections ranged from 23 to 75%, with older patients experiencing higher mortality rates of 33–50% [13, 28, 29,] A comprehensive understanding of the clinical characteristics, risk factors, and outcomes of CRKP infections is crucial for diagnostic and surveillance efforts and can assist clinicians in formulating effective treatment strategies.[30]

The limitations of the study include that minimum inhibitory concentration value of carbapenem was not determined. This study was limited to single hospital for isolate collection. Colistin and polymixin B were not assessed for MIC determination. The strength of this study includes involving large number of *K. pneumoniae* isolates and reporting carbapenem resistance, types of carbapenem resistance and antibiotic resistance pattern.

CONCLUSIONS

The relatively high frequency of carbapenemase producing *K. pneumoniae* suggests major concern about the emergence of carbapenem resistant *K. pneumoniae* as a potential health threat. The majority of *K. pneumoniae* isolates were obtained from sputum and urine samples. Among CRKP K. *pneumoniae*, KPC was the predominant followed by MBL, and KPC and MBL both indicating an alarming threat. Doxycycline, cotrimoxazole, imipenem and meropenem are effective antibiotics against carbapenem resistant *K. pneumoniae*. Routine surveillance of antibiotic resistance pattern and the detection of carbapenemase production can guide appropriate antibiotic therapy thus preventing the further spread of resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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