STUDY ON *BLA*KPC GENE AMONG *KLEBSIELLA PNEUMONIAE* FROM THE CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL, KATHMANDU, NEPAL

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ABSTRACT

Multidrug resistance (MDR) among *Klebsiella pneumoniae*: a common nosocomial pathogen, has been a great concern for managing its infections effectively worldwide. Production of KPCs (*Klebsiella pneumoniae* carbapenemases) is one of the most important mechanisms in this bacterium, making it resistant to carbapenems: the most commonly used antibiotics for treating MDR organisms. This study was done to detect KPC genotypes (*bla*KPC-1, *bla*KPC-2, *bla*KPC-3) among *K. pneumoniae* isolates and to determine their antimicrobial susceptibility patterns.A descriptive cross-sectional study was conducted from September 2023 to February 2024 in a total of 82 non-repetitive clinical isolates of *K. pneumoniae* at Clinical Microbiology Laboratory of Nepal Medical College Teaching Hospital (NMCTH), Nepal. More than one-third (30/82; 36.58%) of the isolates were MDR. Out of the total isolates, 18 (21.95%) were resistant to carbapenems. Of these, 14 (77.77%) isolates possessed *bla*KPC-2 gene. All isolates were susceptible to Polymyxin and Colistin sulphate. Almost half of the isolates were resistant to Cephalophorins (Cefixime-43.90%, Ceftriaxone-41.0% and Ceftazidime-43.9%). Presence of KPC producing *K. pneumoniae* isolates in our set-up, with higher level of resistance to multiple antibiotics highlights the need for continuous monitoring and endorsement of control strategies to tackle the challenges created by this bacterial pathogen.

KEYWORDS

Klebsiella pneumoniae, KPC, *bla*KPC

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INTRODUCTION

Klebsiella spp. are gram negative bacilli, ubiquitously found in nature including water, soil and animals, that can also easily colonize on medical devices and common in healthcare environment.[1] Among the *Klebsiella* spp., *K. pneumoniae* is the most important pathogen and World Health Organization (WHO) has included this bacterium among the ESKAPE pathogens, that are highly virulent and MDR bacteria responsible for majority of hospital acquired infections worldwide.[2] Management of antimicrobial resistance in multi-drug-resistant- *K. pneumoniae* (MDR-KP) is a major challenge for clinicians, as the optimal treatment option for their infections is still not well established.[3,4] Combination therapies including high-dose Meropenem, Colistin, Fosfomycin, Tigecycline and Aminoglycosides are widely used, however emergence of MDR-KP with various evolving resistance mechanisms has limited their effectiveness.[3-5] Carbapenems are one of the major options for treatment of MDR-KP infections but these days, the emergence of carbapenem-resistant *K. pneumoniae* (CR-KP) has become a global health concern and is listed by WHO as a critical priority.[2,4-6] The prevalence of CR-KP is increasing, possibly due to the increasing clinical use of carbapenems in recent years.[7]

The mechanisms of resistance to carbapenems may be related to the combination of decrease in bacterial outer membrane permeability, increasing production of extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases (AmpC) and expression of β-lactamases like Carbapenemases.[8] Of these mechanisms, the production of carbapenemases is the most important mechanism of resistance in Enterobacteriaceae such as *K. pneumoniae*.[9,10]

The most frequently reported carbapenemases in *K. pneumoniae* are ambler molecular class A (*K. pneumoniae* carbapenemases-KPCs), class B (verona integrated metallo β-lactamase-VIM, imipenemase-IMP, New Delhi metallo β -lactamase-NDM), and class D (OXA-48-like) types.[9-12] The KPC type β-lactamases, encoded by KPC-gene are considered the most prevalent mechanism for carbapenem resistance in *K. pneumoniae*[9,10] and it can widely spread due to its location on various plasmids.[10] Of the various KPC types reported, KPC-2 and KPC-3 remain the most commonly identified variants worldwide.[13]

KPC producing *K. pneumoniae* has substantially increased globally in the recent years.[9,10] A study done in Nepal showed that 8.3% of the *K. pneumoniae* isolates has KPC gene.[14] However, the prevalence of KPC producing *K. pneumoniae* and the detection of the associated gene has not yet been accessed fully in Nepal. So, this study was undertaken to ascertain the present scenario of the presence of KPC genotypes among *K. pneumoniae* isolates and to determine their antimicrobial susceptibility patterns.

MATERIALS AND METHODS

All the clinical samples (pus, blood, urine, stool, sputum, pleural fluid, CSF) received for culture and sensitivity in the Clinical Microbiology laboratory of Nepal Medical College Teaching Hospital (NMCTH) from September 2023 to February 2024 were processed following standard protocols. Culture of the sample and identification of the organism was done following standard microbiological techniques.[15] Identification of isolated organisms was done based on colony morphology, Gram staining and a battery of biochemical tests results. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] Isolates that are resistant to at least one each from three different classes of antimicrobial agents were regarded as MDR in this study.[17] Broth microdilution (BMD) method was used for the susceptibility testing against Polymyxin-B and Colistin sulphate, as recommended by both CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[16,18] The minimum inhibitory concentration (MIC) was determined as the lowest antibiotic concentration at which no visible bacterial growth is observed. An MIC of ≤2 µg/mL for Colistin was considered as susceptible, while an MIC >2 µg/mL as resistance. Same Colistin breakpoints were used for interpretation as suggested by EUCAST. Carbapenem susceptible and resistant isolates were chosen by convenient sampling and subjected to polymerase chain reaction (PCR) for the detection of KPC genotypes (bla_{KPC-1}, bla_{KPC-2}, bla_{KPC-3}) at Annapurna Research Center, Kathmandu.

Detection of bla_{KPC} gene:[19]

The bacterial DNA was extracted by using DNA extraction kit (GeneDireX, Taiwan). PCR amplification was performed using primers for bla_{KPC} that included forward primer-CGTCTAGTTCTGCTGTCTTG and reverse primer CTTGTCATCCTTGTTAGGCG. A volume of 1.5 µL of ready DNA was added to a final volume of 30µL PCR mixture comprising 12.5 µL of 2 × master mix, including 1 × PCR buffer, 1.5

mmol/L MgCl2, 0.15 mmol/L dNTP and 1.25 IU Taq DNA polymerase, 0.7µL of 0.8 µmol/L each primer and 14.6µL of sterile distilled water. PCR program was performed as follows: 94 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 31 s and extension at 73 °C for 1 min, with a final extension at 72 °C for 5 min. The PCR products were visualized by electrophoresis in 1.5% agarose gels stained with ethidium bromide.

RESULTS

A total of 82 *K. pneumoniae* were isolated from various clinical samples during the study period. Most of the isolates were resistant to cephalosporins and all of the isolates were susceptible to Polymyxin (Table 1). More than one-third (30/82; 36.58%) of the isolates were MDR. Out of the total 82 isolates, 18 isolates were resistant to carbapenem group of antibiotics. All the carbapenem resistant isolates were MDR which accounted for 60% MDR isolates. The MDR isolates were commonly resistant to Cephalosporins, Aminoglycosides and Fluoroquinolones group of antibiotics (Table 2).

Table 1: Resistance pattern of *K. pneumoniae* isolates (n=82)

Antibiotic	*n* of isolates (%)
Piperacillin	26 (31.71%)
Piperacillin/Tazobactam	22 (26.83%)
Cefixime	36 (43.90%)
Ceftriaxone	34 (41.00%)
Ceftazidime	36 (43.90%)
Ciprofloxacin	28 (34.14%)
Ofloxacin	30 (36.58%)
Cotrimoxazole	18 (19.15%)
Amikacin	26 (31.70%)
Imipenem	18 (21.95%)
Meropenem	18 (21.95%)
Polymyxin-B (MIC>2µg/ml)	0 (0.00%)
Colistin (MIC >2µg/ml)	0 (0.00%)

Table 2: Resistant pattern of MDR isolates (n=30)

Antibiotic	*n* of isolates (%)
Piperacillin	26 (86.67%)
Piperacillin/Tazobactam	22 (73.33%)
Cefixime	30 (100.00%)
Ceftriaxone	30 (100.00%)
Ceftazidime	30 (100.00%)
Ciprofloxacin	24(80.00%)
Ofloxacin	26 (86.67%)
Cotrimoxazole	16 (53.33%)
Amikacin	24(80.00%)
Imipenem	18 (60.00%)
Meropenem	18 (60.00%)
Polymyxin-B (MIC>2µg/ml)	0 (0%)
Colistin (MIC >2µg/ml)	0 (0%)

Table 3: KPC producers among *K. pneumoniae* isolates

	K. pneumoniae isolates subjected to PCR	KPC producers
Carbapenem resistant	18	14
Carbapenem susceptible	23	0
Total	**41**	**14**

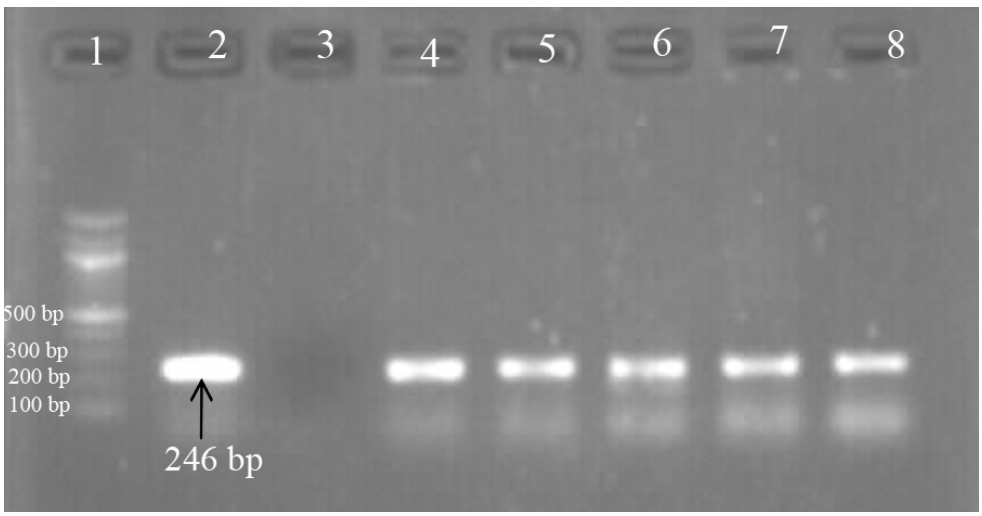

Fig. 1: PCR for the detection of KPC gene in *Klebsiella pneumoniae*. 1-Ladder; 2-Positive Control; 3-KPC Negative Control (amplicon size 246 bp); 4-8: *K.pneumoniae* KPC positive

A total of 41 isolates (which included all the 18 carbapenem resistant isolates and 23 carbapenem susceptible isolates) were subjected to PCR for the detection of $blac_{KPC}$ gene. None of the carbapenem susceptible (n=23) isolates possessed bla_{KPC} gene. Out of the 18 carbapenem resistant isolates, 14 (77.77%) possessed bla_{KPC-2} gene (Table 3, Fig 1). Bla_{KPC-1} and bla_{KPC-3} gene were not detected in any of the isolates.

DISCUSSION

K. pneumoniae is a common pathogen for community and hospital-acquired infections, such as pneumonia, urinary tract infection, and bacteremia. The emergence of antimicrobial resistance in *K. pneumoniae* poses a serious threat to public health, particularly with the rise of CRKP.[9,10,20]

In this study, out of the total 82 *K. pneumoniae*, 18 (21.9%) were resistant to carbapenems that aligns with a systematic review and meta-analysis done by Lin *et al.*[21] who has reported the global prevalence of CR-KP as 28.69% however, in the same review highest prevalence of 66.04% in south Asia and lowest (14.29%) in North America was reported. In a study done in Nepal in 2018, out of 88 isolates of *K. pneumoniae*, 58 (65.9%) were resistant to one of the carbapenems.[22] Another study done in India by Sokhi *et al.*[23] has shown the prevalence of 14.90%. These variations may be due to multiple factors like antibiotic prescribing practices, infection control strategies applied in different geographical settings.[24,25] Our study shows a lower prevalence of carbapenem resistance among *K. pneumoniae* having almost similar findings as from the developed countries. This could be due to the post – COVID period as some studies have shown that the AMR rates of restricted and noncommunity-used antimicrobials have declined in the post-COVID period.[26]

A study done in India in 2020 showed that, 3 out of 45 (6.66%) carbapenem resistant isolates harboured KPC gene.[23] Similar finding (8.3%) was shown by Baral *et al.*[14] in a study done in Nepal, however this study showed quite higher prevalence (77.77%) comparing to these findings. Most of the bla_{KPC} gene harbouring isolates were from critically ill inpatient who had been under treatment with multiple antibiotics promoting selective pressure to acquire resistance in this case. Similarly, the presence of high-risk clones and carbapenemase genes like bla_{KPC}, *OXA-48* varies geographically, contributing to regional differences in resistance patterns.[24,25] Poor hospital hygiene, overcrowding, and limited surveillance also facilitate the nosocomial spread of CRKP. This finding suggests judicious treatment following antibiotic stewardship guidelines and hospital infection control practices to be improved to control spread of such resistant bacteria.

Studies have shown that even the carbapenem susceptible isolates may harbor bla_{KPC} genes among *K. pneumoniae* because there might be a chance of expression of these genes in the isolates without being phenotypically expressed.[14,27] In this study, both the cabapenem resistant and susceptible isolates were subjected to PCR for detection of bla_{KPC} gene, however none of the carbapenem susceptible isolates found to harbor bla_{KPC} genes.

In a study, a remarkable level of resistance profile for almost all tested antibiotics was reported among carbapenemases-producing organisms and has shown higher rate of resistance (>80.00%) against Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Cefepime, Ceftazidime and Ceftolozane/Tazobactam, Ciprofloxacin, Trimethoprim/Sulfamethoxazole while a slightly lower rate of resistance against aminoglycosides (≈ 60.00%).[28] In this study also, similar findings were seen among the carbapenem resistant isolates.

The production of KPCs confers high-level resistance to conventionally used carbapenems and other β-lactams / β-lactamase inhibitors combinations making them ineffective for their infections. In our study, the high prevalence of KPC producing *K. pneumonaie* highlights the urgent need to adopt alternative therapeutic options and strengthen control measures. Recently approved novel antibiotics like Ceftazidme/Avibactam, Meropenem/Vaborbactam (Carbapenem/BLI), Imipenem/Relebactam (Carbapenem/BLI) has shown better effectiveness against KPC-producing *K. pneumoniae*.[29] Similarly, combinations of two carbapenems may also be another option having the better synergistic action where one act as a suicide enzyme inhibitor restoring the activity of other carbapenem by lowering the enzymatic activity.[30] In addition to alternative therapeutics, different preventive strategies like stringent infection prevention practices, implementation of antimicrobial stewardship, active surveillance, use of molecular diagnostic tools are to be promoted to reduce the transmission rate and clinical impact of these pathogens.[24]

Antibiotic resistance is increasing worldwide and we are running out of treatment alternatives. The resistance mechanism varies among organisms for different antibiotics but the presence of antibiotic resistance genes (ARGs) is the root cause of bacterial resistance.[31] Early detection of these sorts of resistance genes, such as KPC would be a beneficial tool for identifying infections and aiding in their control and prevention. Also, these genes can widely spread due to their location on various plasmids. As a result, infection management for organisms harbouring such genes is challenging. Therefore, it is important to detect KPC gene to know its occurrence and take measures for infection control so as to reduce the prevalence of such infections and improve the overall quality of health care.

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REFERENCES

1. Bengoechea JA, Sa Pessoa J. *Klebsiella pneumoniae* infection biology: living to counteract host defences. *FEMS Microbiol Rev* 2019; 43: 123-44. DOI: 10.1093/femsre/fuy043
2. WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: WHO; 2017.
3. Masalane NS, Bester LA, Ismail A *et al*. Drug resistant *Klebsiella pneumoniae* from patients and hospital effluent: a correlation?. *BMC Microbiol* 2025; 25: 284. DOI: https://doi.org/10.1186/s12866-025-03987-5
4. Matteo Bassetti, Elda Righi, Alessia Carnelutti, Elena Graziano , Alessandro Russo. Multidrug-resistant *Klebsiella pneumoniae*: challenges for treatment, prevention and infection control. *Expert Rev Anti Infect Ther* 2018; 16: 749-61. DOI: 10.1080/14787210.2018.1522249
5. Al-Zalabani A, Althobyane OA, Alshehri AH et al. Prevalence of *Klebsiella pneumoniae* Antibiotic Resistance in Medina, Saudi Arabia, 2014-2018. *Cureus* 2020; 12: e9714. DOI: 10.7759/cureus.9714
6. Al-Zahrani IA and Alsiri BA. The emergence of carbapenem-resistant *Klebsiella pneumoniae* isolates producing OXA-48 and NDM in the Southern (Asir) Province, Saudi Arabia. *Saudi Med J* 2018; 39: 23-30. DOI: 10.15537/smj.2018.1.21094
7. Hu Y, Liu C, Shen Z *et al*. Prevalence, risk factors and molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in patients from Zhejiang, China, 2008–2018. *Emerg Microbes Infect* 2020; 9: 1771–9. DOI: 10.1080/22221751.2020.1799721
8. Alhazmi WA. Review on the carbapenem resistance mechanisms of *Klebsiella pneumoniae*. *J Pharmaceutical Res Int'l* 2021; 33: 352–7. DOI: 10.9734/jpri/2021/v33i39B32212.
9. Nargis, Rehman T, Ali L, Khan H, Madina. Detection of KPC-2 gene in *K. pneumoniae*, *E. coli* and *P. mirabilis* isolated from urine sample of urinary tract infection patients. *Pakistan J Med Health Sci* 2021; 15: 2292-95. DOI: https://doi.org/10.53350/pjmhs211572292
10. Ghasemnejad A, Doudi M, Amirmozafari N. The role of the bla_{KPC} gene in antimicrobial resistance of *Klebsiella pneumoniae*. *Iran J Microbiol* 2019; 11: 288-93. DOI: https://doi.org/10.18502/ijm.v11i4.1465
11. Candan ED, Aksöz N. *Klebsiella pneumoniae*: characteristics of carbapenem resistance and virulence factors. *Acta Biochim Pol* 2015; 62: 867–74. DOI: 10.18388/abp.2015_1148
12. Lee CR, Lee JH, Park KS *et al*. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options and detection methods. *Front Microbiol* 2016; 7: 895. DOI: 10.3389/fmicb.2016.00895
13. Munoz-Price LS, Poirel L, Bonomo RA *et al*. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 2013; 13: 785–96. DOI: 10.1016/S1473-3099(13)70190-7.
14. Baral R, Tuladhar R, Manandhar S *et al*. Detection of bla_{KPC} gene among carbapenemase producing *Klebsiella pneumoniae* isolated from different clinical specimens at tertiary care hospital of Nepal. *BMC Microbiol* 2024; 24: 144. DOI: https://doi.org/10.1186/s12866-024-03301-9
15. Garcia LS. Clinical microbiology procedures handbook: American Society for Microbiology Press; 2010. DOI: 10.1128/9781555817435S.
16. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 34th ed. CLSI supplement M100. CLSI, 2024.
17. Magiorakos AP, Srinivasan A, Carey RB *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268–81.

18. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters, version 2024. http://www.eucast.org.

19. Bina M, Pournajaf A, Mirkalantari S, Talebi M, Gholamreza I. Detection of the *Klebsiella pneumoniae* carbapenemase (KPC) in *K. pneumoniae* isolated from the clinical samples by the phenotypic and genotypic methods. *Iran J Pathol* 2015; 10: 199–205.

20. Li S, Feng X, Li M, Shen Z. In vivo adaptive antimicrobial resistance in *Klebsiella pneumoniae* during antibiotic therapy. *Front Microbiol* 2023; 14: 1159912. DOI: 10.3389/fmicb.2023.1159912.

21. Lin XC, Li CL, Zhang SY, Yang XF, Jiang M. The global and regional prevalence of hospital-acquired carbapenem-resistant *Klebsiella pneumoniae* Infection: a systematic review and meta-analysis. *Open Forum Infect Dis* 2023; 11: ofad649. DOI: 10.1093/ofid/ofad649

22. Pyakurel S, Ansari M, Kattel S *et al*. Prevalence of carbapenemase-producing *Klebsiella pneumoniae* at a tertiary care hospital in Kathmandu, Nepal. *Trop Med Health* 2021; 49: 78. DOI: 10.1186/s41182-021-00368-2.

23. Sokhi A, Agarwal P, Maheshwari M, Chakravarti A. Detection of *Klebsiella pneumoniae* carbapenemase (KPC) producing *Enterobacteriaceae* isolates from various clinical samples in a rural health setup. *J Pure Appl Microbiol* 2020; 14: 397-401. DOI: 10.22207/jpam.14.1.41

24. WHO. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2023.

25. Logan LK and Weinstein RA. The epidemiology of carbapenem-resistant *Enterobacteriaceae*: the impact and evolution of a global menace. *J Infect Dis* 2017; 215: S28–S36.

26. Taleb MH, Elmanama AA, Taleb AH, Tawfick MM. Pre- and post-COVID-19 antimicrobial resistance profile of bacterial pathogens, a comparative study in a tertiary hospital. *J Infect Dev Ctries* 2023; 17: 597-609. DOI: 10.3855/jidc.17791.

27. Seecoomar GD, Marmol BC, Kwon DH. Promoter deletions of *Klebsiella pneumoniae* carbapenemase (KPC) - encoding genes (bla_{KPC-2}) and efflux pump (AcrAB) on β-lactam susceptibility in KPC-producing Enterobacteriaceae. *FEMS Microbiol Lett* 2013; 348: 120-6. DOI: 10.1111/1574-6968.12273

28. Del Rio A, Puci M, Muresu N *et al*. Comparison of genotypic and phenotypic antimicrobial profile in carbapenemases producing *Klebsiella pneumoniae*. *Acta Biomed* 2023; 94: e2023201. DOI: 10.23750/abm.v94i5.14412.

29. Theuretzbacher U, Carrara E, Conti M, Tacconelli E. Role of new antibiotics for KPC-producing Klebsiella pneumoniae. *J Antimicrob Chemother* 2021; 76: i47-i54. DOI: 10.1093/jac/dkaa497.

30. Allander L, Vickberg K, Lagerbäck P, Sandegren L, Tängdén T. Evaluation of In Vitro activity of double-carbapenem combinations against KPC-2-, OXA-48- and NDM-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Antibiotics (Basel)* 2022; 11: 1646. DOI: 10.3390/antibiotics11111646.

31. Jian Z, Zeng L, Xu T *et al*. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *J Basic Microbiol* 2021; 61: 1049-70. DOI: 10.1002/jobm.202100201.