Journal of Nepal Biotechnology Association, 7 (1), 2026: 50-55
DOI: https://doi.org/10.3126/jnba.v7i1.92081

Contents available at https://www.nepjol.info/index.php/jnba

Journal of Nepal Biotechnology Association

ISSN: 1728-1628

Journal Homepage: https://www.nepalbiotech.org/nba-journal

Research Article

Detection and Expression of blaKPC-2 and fimH Genes in *Klebsiella pneumoniae* Clinical Isolates

Anil Kumar Sah[1], Bikesh Kumar Sharma[2], Manish Bhatt[2], Milan Gharti Magar[2], Neha Limbu Begha[2], Prekshya Tiwari[2], Nilima Shrestha[1], Biraj Lohani[2], Subodh Sharma[1], Sharad Hari Gajuryal[3], Avinash Chandra[3], Rabin Pahari[2], Megha Raj Banjara[4]

[1]Annapurna Research Center, Maitighar, Kathmandu, Nepal
[2]SANN International College, Kathmandu, Nepal
[3]Annapurna Neurological Institute and Allied Sciences, Maitighar, Kathmandu, Nepal
[4]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

ARTICLE INFO

ARTICLE HISTORY

Received: 17/02/2026
Revised: 15/03/2026
Accepted: 16/03/2026

CORRESPONDENCE

Anil Kumar Sah

Annapurna Research Center, Maitighar, Kathmandu, Nepal
Email: anilshah4in@gmail.com
https://orcid.org/0000-0001-5428-8503

ABSTRACT

Klebsiella pneumoniae cause several serious infections, pneumonia, urinary tract infections and bloodstream infections, especially in a immune-compromised patient. There has been rise in antibiotics resistant *K. pneumoniae* in recent years. This study aimed detection and expression of blaKPC-2 and fimH genes in carbapenemase producing *K. pneumoniae* that can form biofilms in clinical samples. A total of 608 clinical samples were processed for *K. pneumoniae* using standard microbiological procedure. Antibiotic resistance was checked using the modified Kirby-Bauer disc diffusion method, and carbapenemase production was tested using a modified carbapenem inactivation method in conjunction with EDTA modified carbapenem inactivation method. In order to detect the presence and expression of resistant genes, bacterial DNA and RNA were extracted. A total of 30 isolates were positive for *K. pneumoniae* among which 21 (70%) were multi-drug resistant and 18 (85.71%) were carbapenemase producers in MDR isolates. A total of 14 isolates (66.67%) produced biofilm, of which 6 (28.57%) were strong, 6 (28.57%) were moderate and 2 (9.52%) were weak. Both blaKPC-2 and fimH genes were detected in 19 isolates (90.47%) and were actively expressed in 16 (76.19%). These findings show the blaKPC-2 and fimH genes are common in clinical isolates and are actively being expressed. In conclusion, there is urgent need to control highly resistant *K. pneumoniae.*

Keywords: blaKPC-2; Biofilm; Carbapenem resistance; fimH; *Klebsiella pneumoniae*

Introduction

Klebsiella pneumoniae is a Gram-negative pathogenic bacterium that causes different infections such as pneumonia, urinary tract infections, wound infections, bacteremia and sepsis. The infections can be serious particularly in immune compromised individuals (Mohamudha et al., 2016; Paczosa & Mecsas, 2016).

Infections by multi-drug resistant (MDR) strains of *K. pneumoniae* are increasing global threats evident by limited available treatments (Pitout et al., 2020). In a meta-analysis done in Nepal, a pooled prevalence of 64% MDR *K. pneumoniae* was reported, which is alarming issue (Odari & Dawadi, 2022). Misuse, overuse and underuse of antibiotics in humans and animals, in addition to unregulated prescription of antibiotics and lack of well-equipped hospitals and clinics may be the driving factors for increasing antimicrobial resistance rates in Nepal (Dahal & Chaudhary, 2018; Acharya & Wilson, 2019; Pokharel & Adhikari, 2020; Rijal et al., 2021). Different mechanisms such as biofilm formation, production of β-lactamases, enzymatic modifications, efflux pumps and reduction of membrane permeability are responsible for developing multi-drug resistant *K. pneumoniae* (Li et al., 2024).

Carbapenems are the most common lastline drug to treat infections caused by MDR *K. pneumoniae*. However increasing rate of carbapenem resistant strains of *K. pneumoniae* is of great concern (Nordmann et al., 2009; Pitout et al., 2015). In 2017, WHO listed carbapenem resistant *K. pneumoniae* in high priority (critical) for which development of new antibiotics is required. *K. pneumoniae* become resistant to carbapenems because of three mechanisms: production of *K. pneumoniae* carbapenemase, efflux pumps and reduction in cell membrane permeability particularly due to ompK35 and ompK36 porins mutation. Carbapenemase enzymes belong to ambler class A (KPC), class B (NDM, IMP, VIM) and class D (OXA-48) (Alizadeh et al., 2020; Li et al., 2025).

Mere detection of resistance genes like blaKPC-2 and blaVIM, as well as virulence factors such as fimH and rmpA, do not show the real impacts of antibiotic resistant strains (Bush & Bradford, 2016; Flores-Mireles et al., 2015). How these strains act clinically depend on gene expressions? In fact, the expression of several genes affects the virulence and resistance level. Hence, we can't ignore the gene expression as it could compromise identification of clinical risk and limit the definite prediction of treatment outcomes (Rasko & Sperandio, 2010).

In addition, the co-expression of resistance and virulence factors, have not been studied enough. This lack of data has constrains on the development of targeted effective infection control and treatment methods (Hall-Stoodley et al., 2004; World Health Organization, 2023). This study aims to detect expression of blaKPC-2 and fimH genes in carbapenemase producing *K. pneumoniae.*

Materials and Methods

Study site and duration

This study was conducted at the Department of Microbiology of the Annapurna Neurological Institute and Allied Sciences (ANIAS) and the Department of Molecular Biology of Annapurna Research Center (ARC), Maitighar, Kathmandu from April to October 2025. Ethical approval was obtained from the Institutional Review Committee (Reg no: 2025/04) at Annapurna Neurological Institute. Written informed consent was obtained from all participants.

Identification of *Klebsiella pneumoniae* from clinical samples

A total of 608 non-duplicate samples including sputum, urine, wound swab, cerebrospinal fluid (CSF) and endotracheal tubes were processed and cultured using standard microbiological techniques. *K. pneumoniae* isolates were identified by studying characteristics on blood agar and MacConkey agar, performing Gram staining and biochemical tests (catalase test, oxidase test, urease test, triple sugar iron agar test and IMViC test).

Antibiotic sensitivity testing

Modified Kirby Bauer Disc diffusion technique was used for antibiotic sensitivity testing of isolated *K. pneumoniae.* In this study, following antibiotics were used: Amikacin (AK), Levofloxacin (LE), Cefixime (CFM), Cefepime (CPM), Cotrimoxazole (COT), Cefotaxime (CTX), Ofloxacin (OF), Gentamicin (GEN), Meropenem (MRP), Piperacillin + Tazobactam (PIT), Doxycycline (DO), Polymyxin B (PB), Clindamycin (CL), Tigecycline (TGC), Ciprofloxacin (CIP), Ceftazidime (CAZ), Linezolid (LZ), Ceftriaxone (CTR) and Nitrofurantoin (NIT). MDR is acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Identification of carbapenemase producers

Meropenem resistant isolates were screened for carbapenemase production modified carbapenem inactivation method (mCIM) in combination with modified EDTA carbapenem inactivation method (eCIM) described by Tsai et al. (2020).

Biofilm formation test

Isolates forming biofilm were screened using the Congo red agar (CRA) method (Freeman et al., 1989).

Extraction of nucleic acid

Alkaline hydrolysis method was used for the extraction of plasmid DNA from isolates (Sambrook & Russell, 2001). The extracted plasmid DNA was subsequently used as templates for amplification of blaKPC-2 gene. For the amplification of fimH gene, chromosomal DNA was extracted using the Quick-DNA/RNA Miniprep Kit (ZYMO Research, USA). Finally, total RNA was extracted from the isolates using Vision Purify Viral RNA Extraction Kit (Vision Biotechnology, Turkey) to detect gene expression.

Gene identification

blaKPC-2 and fimH genes were amplified by PCR using primers, forward: 5'-GATACCACGTTCCGTCTGG-3' and reverse: 5'-GCAGGTTCCGGTTTTGTCTC-3' (Hindiyeh et al., 2008) for blaKPC-2 gene, and forward: 5'-GTGCCAATTCCTCTTACCGTT-3' and reverse: 5'-TGGAATAATCGTACCGTTGCG-3' (Hojati et al., 2015) for fimH gene.

For amplification of each gene, 5 µl DNA template was added to 10 µl mixture containing 6.5 µl master mix, 2.5 µl nuclease free water and 0.5 µl each of respective reverse and forward primers. PCR was performed in Proflex Thermocycler using optimized conditions (Table 1).

The PCR products were resolved by agarose gel electrophoresis in 1.5% agarose gel stained with ethidium bromide run at 70 V for 40 minutes, followed by analysis in gel doc system.

cDNA synthesis and gene expression

The cDNA from isolated RNA was synthesized using the Vision cDNA Synthesis Kit (Vision Biotechnology, Turkey), following manufacturer's instructions. cDNA was used as a template for amplification of blaKPC-2 and fimH genes using specific primers and optimized conditions as explained above. The amplified products were documented using agarose gel electrophoresis and gel doc system.

Table 1: Optimized conditions for amplification of blaKPC-2 and fimH genes.

Gene	Optimized conditions for PCR					Length of PCR product (bp)
	Initial denaturation	Denaturation	Annealing	Extension	Final extension	
blaKPC-2	94°C for 3 min	94°C for 1 min (35 cycles)	60°C for 1 min (35 cycles)	72°C for 1 min (35 cycles)	72°C for 5 min	246
fimH	96°C for 3 min	96°C for 30 sec (35 cycles)	64°C for 1 min (35 cycles)	72°C for 1 min (35 cycles)	72°C for 5 min	164

Results and Discussion

Out of 608 total clinical samples, 150 were cultured positive among which 30 (20%) samples tested positive for *K. pneumoniae.* In a subset of 30 isolates, 21 (70%) *K. pneumoniae* were found to be MDR and 18 (85.7%) isolates were confirmed to be carbapenemase producers by mCIM and eCIM methods. Consistent with our study finding, the recent study conducted in Nepal reported 73.5% MDR *K. pneumoniae* (Neupane et al., 2025). Meanwhile, Pyakurel et al. (2021) reported 77.6% carbapenemase producing *K. pneumonia*, which is higher than our findings.

Out of 21 MDR isolates, 14 (66.67%) showed biofilm production on CRA, which is lower than finding of Pradhan et al. (2019) (Figure 1). Among the 21 isolates, 19 (90.47%) isolates were found to be harbouring fimH gene. fimH gene was detected in 6 strong biofilm forming isolates, 5 moderate and 2 weak biofilm forming isolates. Meanwhile, fimH gene was also detected in 6 non-biofilm forming isolates (Figure 2). Presence of fimH genes only does not necessarily produce biofilms, there are so many other genes responsible for it. These results showed a significant role of fimH genes in production of biofilms, which is consistent with several other studies (Alwan et al., 2024; Ashwath et al., 2022). However, no statistically significant association was observed between biofilm formation strength and either fimH gene detection ($p = 0.710$) or fimH gene expression ($p = 0.660$) (Table 2). These findings suggest that although fimH is widely distributed among clinical isolates, its expression alone may not determine biofilm phenotype, highlighting the involvement of additional virulence determinants in biofilm formation.

Among 21 MDR isolates of *Klebsiella pneumoniae*, the blaKPC-2 gene was detected in 19 (90.47%) isolates, while 16 (76.19%) demonstrated gene expression. Phenotypic carbapenemase production was observed in 18 isolates, whereas 19 (90.47%) and 16 (76.19%) were positive for blaKPC-2 gene detection and expression respectively. This detection rate is higher than the recent report in Nepal (Khadka et al., 2025). No statistically significant association was observed between

carbapenemase production and the presence of the blaKPC-2 gene (p = 0.544), indicating that gene carriage alone does not necessarily correspond to phenotypic resistance. However, a significant association was found between carbapenemase production and blaKPC-2 gene expression (p = 0.001), suggesting that transcriptional activation of this carbapenemase gene plays a crucial role in resistance phenotype (Table 3). These findings indicate that while the gene is widely distributed among *Klebsiella pneumoniae* isolates, blaKPC-2 expression is strongly correlated with phenotypic carbapenemase activity, highlighting the importance of transcriptional regulation in antimicrobial resistance.

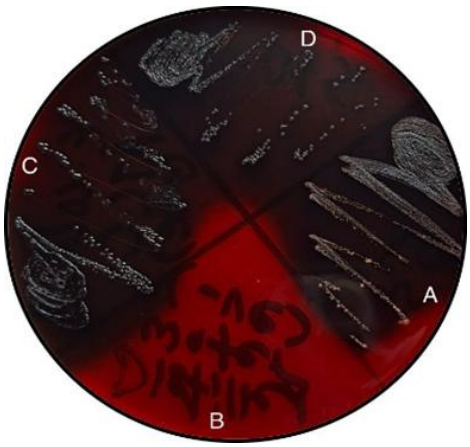

Figure 1: Biofilm Production on CRA: A: Positive control (*E. coli* ATCC 25922), B: Negative control (Distilled water), C&D: Samples (Strong category).

The co-existence of blaKPC-2, and fimH expression and biofilm formation highlights a concerning convergence of resistance and virulence factors in *K. pneumoniae* isolates. Such findings could assist infection control efforts, antimicrobial resistance regulation, and therapy.

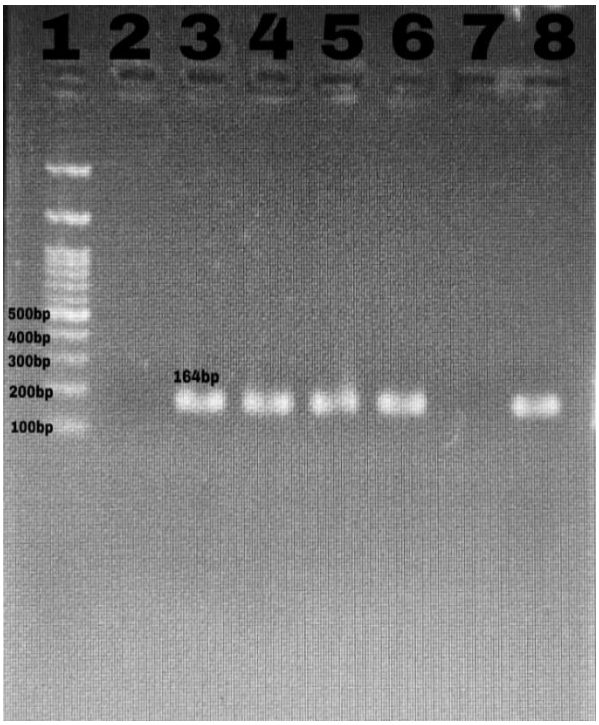

Figure 2: Gel image showing fimH gene expression. 1: Ladder (100 bp); 2: Negative control; 3: Positive control; 4, 5, 6 & 8: Samples (fimH +ve); 7: Blank

Table 2: Pattern of fimH gene detection, gene expression on congo red test (Biofilm production).

		fimH gene detection				fimH gene expression			
		Positive	Negative	Total	p-value	Positive	Negative	Total	p-value
Congo Red Test	Strong	6	0	6	0.71	4	2	6	0.66
	Moderate	5	1	6		5	1	6	
	Weak	2	0	2		1	1	2	
	No-biofilm	6	1	7		6	1	7	
	Total	19	2	21		16	5	21	

Table 3: Pattern of carbapenemase production test for blaKPC-2 gene detection and expression.

		blaKPC-2 gene detection				blaKPC-2 gene expressionn			
		Positive	Negative	Total	p-value	Positive	Negative	Total	p-value
Carbapenemase production test	Positive	16	2	18	0.544	16	2	18	0.001
	Negative	3	0	3		0	3	3	
	Total	19	2	21		16	5	21	

Conclusion

This study investigated blaKPC-2 and fimH genes expression in carbapenemase producing *K. pneumoniae*. The study revealed high prevalence of both genes and most of the gene-positive isolates were also found to possess active expression of both genes, along with co-expression in a majority of isolates. In addition, carbapenemase production was also common among these isolates, and nearly all of them were also multidrug-resistant. The association of resistance and

virulence genes along with a high prevalence of carbapenem resistance and biofilm formation ability among these isolates indicates a serious clinical problem and highlights the need for strict infection control and judicious use of antibiotics in Nepal.

Acknowledgements

We are thankful to department of Microbiology, Annapurna Neuro Hospital and Department of Biotechnology, SANN International College for cooperating with us throughout the research. Lastly, we sincerely thank patients and their family for their support and cooperation for sample collection. We are also grateful to all who directly or indirectly contributed to the successful completion of this research.

References

Alizadeh, N., Ahangarzadeh Rezaee, M., Samadi Kafil, H., Hasani, A., Soroush Barhaghi, M. H., Milani, M., & Ghotaslou, R. (2020). Evaluation of resistance mechanisms in carbapenem-resistant Enterobacteriaceae. *Infection and Drug Resistance*, *13*, 1377-1385.

Alwan, A. M., Abdallah, H. N., & Hamzah, A. S. (2024). Molecular Analysis of FimH Gene in Biofilm-Producing and Multidrug Resistant Klebsiella pneumoniae. *Hilla University College Journal For Medical Science*, *2*(3), 25-31.

Bush, K., & Bradford, P. A. (2016). β-Lactams and β-lactamase inhibitors: An overview. *Cold Spring Harbor Perspectives in Medicine*, *6*(8), a025247.

Dahal, R. H., & Chaudhary, D. K. (2018). Microbial infections and antimicrobial resistance in Nepal: current trends and recommendations. *The open microbiology journal*, *12*, 230.

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*, *13*, 269–284.

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology*, *2*, 95–108.

Khadka, S., Adhikari, R. P., Khanal, L. K., Pandey, R., Sah, A. K., & Sapkota, J. (2025). Study on blaKPC gene among Klebsiella pneumoniae from the Clinical Specimens in a Tertiary Care Hospital, Kathmandu, Nepal. *NMCJ*, *27*(2), 149-54.

Li, J., Shi, Y., Song, X., Yin, X., & Liu, H. (2025). Mechanisms of antimicrobial resistance in Klebsiella: advances in detection methods and clinical implications. *Infection and Drug Resistance*, *18*, 1339-1354.

Li, Y., Kumar, S., & Zhang, L. (2024). Mechanisms of antibiotic resistance and developments in therapeutic strategies to combat Klebsiella pneumoniae infection. *Infection and Drug Resistance*, *17*, 1107-1119.

Mohamudha, P. R., Belgode, N. H., Laura, M., & Michael, R. M. (2016). Carbapenem resistance mechanisms among blood isolates of *Klebsiella pneumoniae* and Escherichia coli. *African Journal of Microbiology Research*, *10*(2), 45–53.

Neupane, B., Devkota, M. D., Pokhrel, B. M., Rimal, S., & Banjara, M. R. (2025). Phenotypic characteristics and carbapenemase genes in Klebsiella pneumoniae from patients at Upendra Devkota Memorial National Institute of Neurological and Allied Sciences, Kathmandu, Nepal. *BMC Infectious Diseases*, *25*(1), 1119.

Nordmann, P., Cuzon, G., & Naas, T. (2009). The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. *The Lancet infectious diseases*, *9*(4), 228-236.

Odari, R., & Dawadi, P. (2022). Prevalence of Multidrug-Resistant Klebsiella pneumoniae Clinical Isolates in Nepal. *Journal of tropical medicine*, *2022*(1), 5309350.

Paczosa, M. K., & Mecsas, J. (2016). *Klebsiella pneumoniae*: Going on the Offense with a Strong Defense. *Microbiology and Molecular Biology Reviews*, *80*(3), 629–661.

Pitout, J. D., Peirano, G., Kock, M. M., Strydom, K. A., & Matsumura, Y. (2019). The global ascendency of OXA-48-type carbapenemases. *Clinical Microbiology Reviews*, *33*(1), 10-1128.

Pitout, J. D., Nordmann, P., & Poirel, L. (2015). Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. *Antimicrobial Agents and Chemotherapy*, *59*(10), 5873-5884.

Pokharel, S., & Adhikari, B. (2020). Antimicrobial resistance and over the counter use of drugs in Nepal. *Journal of Global Health*, *10*(1), 010360.

Rasko, D. A., & Sperandio, V. (2010). Anti-virulence strategies to combat bacteria-mediated disease. *Nature Reviews Drug Discovery*, *9*, 117–128.

Rijal, K. R., Banjara, M. R., Dhungel, B., Kafle, S., Gautam, K., Ghimire, B., ... & Ghimire, P. (2021). Use of antimicrobials and antimicrobial resistance in Nepal: a nationwide survey. *Scientific Reports*, *11*(1), 11554.

World Health Organization. (2023). *Antimicrobial resistance: global report on surveillance*. World Health Organization.