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ORIGINAL RESEARCH

Molecular Detection of CTX-M Type ESBL Genes in Clinical Isolates of *Klebsiella* Species

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ABSTRACT

Objective: The objective of this study is to determine the prevalence of Extended-Spectrum β-Lactamase (ESBL) production and CTX-M genes among *Klebsiella* species isolated from clinical specimens.

Methods: A total of 1,815 clinical samples—including urine, blood, sputum, pus, and body fluids were collected at Himal Hospital Kathmandu, during 2019–2020. Standard microbiological techniques were used for isolation and identification of bacterial pathogens. Antimicrobial susceptibility testing was performed using the modified Kirby–Bauer disk diffusion method following CLSI (2019) guidelines. ESBL screening was conducted using third-generation cephalosporins, and confirmation was done via the Double Disk Synergy Test (DDST). Molecular detection of the CTX-M gene was performed using PCR with specific primers targeting a 544 bp amplicon.

Results: Among 1,815 clinical samples, urine constituted the majority (65.8%), followed by blood (25.1%). *Escherichia coli* was the predominant isolate (89.1%), while *Klebsiella pneumoniae* (6.2%) and *Klebsiella oxytoca* (0.74%) comprised a smaller proportion. Of the 28 *Klebsiella* spp isolates, the highest antibiotic sensitivity was observed toward Amikacin (60.7%) and Meropenem (57.1%), whereas complete resistance to Amoxicillin (100%) and high resistance to Cefixime (89.3%) and Cefotaxime (75.0%) were recorded. ESBL screening identified 22 (78.6%) potential ESBL producers, of which 18 (64.3%) were confirmed phenotypically. PCR analysis revealed the CTX-M gene in 7 of the 18 ESBL-positive isolates, demonstrating a notable presence of CTX-M–mediated resistance among *Klebsiella* spp.

Conclusion: The findings highlight a concerning prevalence of ESBL production and CTX-M genes in *Klebsiella* species in the study population, underscoring the need for continuous surveillance, rational antibiotic use, and strengthened antimicrobial stewardship programs to limit the spread of multidrug-resistant strains.

Keywords: *Klebsiella* spp, ESBL producer and CTX-M

INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) possess a major global antimicrobial resistance threat because of their ability to hydrolyze extended-spectrum cephalosporins. Over the past two decades, the CTX-M family has become the predominant ESBL type worldwide, surpassing earlier TEM- and SHV-derived ESBLs in prevalence and clinical impact (Edelstein et al., 2003; Pitout, et al., 2019). CTX-M enzymes confer high-level resistance to cefotaxime and are frequently

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co-located with additional resistance determinants on mobile genetic elements, particularly conjugative plasmids, facilitating rapid dissemination among Enterobacterales, including *Klebsiella* species (Zhao & Hu, 2013).

The CTX-M family originated from the chromosomal β-lactamases of environmental *Kluyvera* species, later mobilized into clinically significant bacteria through insertion sequences such as ISEcp1 and ISCR1 (Zhao & Hu, 2013). More than 200 CTX-M variants have now been identified and are classified into distinct phylogenetic groups (e.g., CTX-M-1, CTX-M-2, CTX-M-9 groups), with several—most notably CTX-M-15—demonstrating rapid global spread and strong association with epidemic clones (Vignoli et al., 2016). The widespread dissemination of plasmid-borne *bla* $_{CTX-M}$ gene has been particularly concerning among *Klebsiella pneumoniae*, a major healthcare-associated pathogen implicated in urinary tract infections, pneumonia, septicemia and surgical-site infections. ESBL-producing *K. pneumoniae* infections are associated with prolonged hospital stays, increased morbidity, and limited therapeutic options (Peymani et al., 2017).

In Nepal, various laboratory-based studies and systematic reviews have documented a high burden of ESBL producers among clinical Enterobacteriaceae, with CTX-M–type genes repeatedly reported as a predominant genetic determinant. A national systematic review and meta-analysis and several hospital-based studies have highlighted widespread detection of CTX-M (including CTX-M-15 alleles) among *Klebsiella pneumoniae* across diverse clinical sources and geographic regions of Nepal, underscoring a continuing and possibly expanding reservoir of CTX-M in human clinical isolates (Shyaula et al., 2023).

Although phenotypic methods such as the Kirby–Bauer disc diffusion ESBL screen and combined disc tests remain essential for routine detection, molecular assays are required for definitive confirmation, epidemiologic surveillance and variant characterization (Xie et al., 2025). Polymerase chain reaction (PCR) targeting *bla* $_{CTX-M}$ gene is the most widely employed approach because of its sensitivity, specificity and rapid turnaround time (Edelstein et al., 2003).

Given the increasing global burden of ESBL-mediated resistance and the clinical significance of *Klebsiella* species as reservoirs and disseminators of *bla* $_{CTX-M}$, molecular detection of CTX-M genes remains critical for guiding antimicrobial therapy, informing infection prevention strategies, and strengthening surveillance systems. This study focuses on the molecular detection of CTX-M–type ESBL genes among clinical isolates of *Klebsiella* species, contributing to the understanding of their distribution and epidemiological patterns in the local clinical setting.

METHODS

Materials

All the laboratory experiments were conducted using distilled water and analytical grade chemicals. The culture media, biochemical tests media, stains and antibiotic discs were purchased from HI media Laboratories Pvt. Ltd., India.

Sample collection and processing

This study was conducted at Himal Hospital and Kantipur College of Medical Science, Kathmandu during the year 2019-2020 and analyzed 1815 clinical samples (blood, urine, sputum, and wound swabs) from patients of all ages, including both inpatients and outpatients. The clinical samples were collected using aseptic techniques and were processed following standard microbiological techniques (Forbes et al., 2012). The clinical samples were subjected to Gram's staining, culture on Blood Agar and MacConkey Agar plates for the isolation and were further subjected to biochemical tests for the identification of the isolates.

Antimicrobial susceptibility testing

The antibiotic susceptibility test was performed by modified Kirby–Bauer method of disk diffusion within the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2019).

ESBLs Screening and Confirmation

Screening of ESBLs

Isolates were screened for Extended Spectrum Beta-Lactamase (ESBL) production using the disc diffusion method based on CLSI (2019) guidelines. Antibiotics used for initial screening included Ceftazidime (30µg), Cefotaxime (30µg), and Ceftriaxone (30µg). Isolates showing inhibition zones of ≤22mm (Ceftazidime), ≤27mm (Cefotaxime), or ≤25mm (Ceftriaxone) were considered potential ESBL producers.

Confirmation of ESBLs

Suspected ESBL-producing isolates were confirmed using the Double Disk Synergy Test (DDST) per CLSI, 2019. Mueller-Hinton agar was inoculated with

a 0.5 McFarland suspension of the test organism. Discs of Cefotaxime (30µg), Ceftazidime (30µg), Ceftriaxone ((30µg)) and Cefotaxime-Clavulanic acid (30/10µg), Ceftazidime-Clavulanic acid(30/10µg), and Ceftriaxone-Clavulanic acid (30/10µg) were placed 16–20mm apart. After incubation at 37°C for 18–24 hours, a ≥5mm increase in the inhibition zone for the combination disc compared to the single antibiotic disc confirmed ESBL production. *E. coli* ATCC 25922 was used as the control strain.

Molecular Detection of CTX-M gene

Klebsiella spp was cultured in Luria Bertani (LB) broth at 37°C for 24 hours using an orbital shaker at 120rpm. Plasmid DNA was extracted from 1.5ml of culture via the alkaline lysis method and suspended in TE buffer, then labeled and stored at -20°C. PCR amplification was carried out using 3µl of plasmid DNA, 21µl of master mix, and 0.5µl each of forward and reverse primers in a total volume of 25µl. Detection of the CTX-M gene utilized primers CTX-M F (5′-TTTGCGATGTGCAGTACCAGTAA-3′) and CTX-M R (5′-CTCCGCTGCCGGTTTTTATC-3′), yielding a 544bp product (Edelstein et al., 2003).

Thermal cycling for CTX-M included initial denaturation at 94°C for 15min, followed by 30 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1.5min, extension at 72°C for 1min, and a final extension at 72°C for 7min. PCR products were analyzed via 2% agarose gel electrophoresis stained with ethidium bromide, run at 100V for 70 minutes. A 100bp DNA ladder was used for molecular size estimation, and bands were visualized using a gel documentation system (Edelstein et al., 2003).

Data Analysis

Collected data were entered into Microsoft Excel and analyzed using SPSS version 23.0 and analyzed accordingly.

RESULTS

Out of 1,815 clinical specimens analyzed, the majority were urine samples (65.8%, n=1,194), followed by blood (25.1%, n=455), sputum (5.2%, n=94), pus (3%, n=55), body fluids (0.5%, n=10), and other specimen types (0.4%, n=7). Among the isolates, *Escherichia coli* was the most predominant species, accounting for 89.1%, followed by *Klebsiella pneumoniae* (6.2%), *Proteus* spp (3.96%), and *Klebsiella oxytoca* (0.74%).

Table 1: Distribution pattern of Gram-Negative bacteria in clinical samples

Organism isolated	No. of isolates	Total percentage (%)
Escherchia coli	360	89.1
Klebsiella pneumoniae	25	6.2
Proteus spp	16	3.96
Klebsiella oxytoca	3	0.74
Total	404	100

Antimicrobial susceptibility pattern of *Klebsiella* spp

Among 28 *Klebsiella* spp isolates, the highest sensitivity was detected against Amikacin (60.7%), followed by Meropenem (57.1%) and Gentamicin (46.4%). Moderate sensitivity was detected against Cotrimoxazole (39.3%), Ceftriaxone (35.7%), and fluoroquinolones—Norfloxacin, Levofloxacin, and Ciprofloxacin (32.1% each). The isolates were highly resistant against Amoxicillin (100%), Cefixime (89.3%), Cefotaxime (75.0%), Ofloxacin (71.4%) and Ceftazidime (64.3%) as shown in table 2.

Table 2: Antimicrobial susceptibility pattern of *Klebsiella* spp

Antibiotic used	Sensitive N(%)	Intermediate N(%)	Resistance N(%)
Amikacin	17(60.7)	4(14.3)	7(25)
Amoxicillin	0(0)	0(0)	28(100)
Cefixime	3(10.7)	0(0)	25(89.3)
Ciprofloxacin	9(32.1)	0(0)	19(67.9)
Ceftriaxone	10(35.7)	3(10.7)	15(53.6)
Cotrimoxazole	11(39.3)	1(3.6)	16(57.1)
Ceftazidime	4(14.3)	6(21.4)	18(64.3)
Cefotaxime	5(17.9)	2(7.1)	21(75)
Doxycycline	8(28.6)	4(14.3)	16(57.1)
Gentamycin	13(46.4)	4(14.3)	11(39.3)
Levofloxacin	9(32.1)	4(14.3)	15(53.6)

Antibiotic used	Sensitive N(%)	Intermediate N(%)	Resistance N(%)
Meropenem	16(57.1)	4(14.3)	8(28.6)
Nalidixic acid	5(17.9)	6(21.4)	17(60.7)
Norfloxacin	9(32.1)	1(3.6)	18(64.3)
Nitrofurantoin	5(17.9)	7(25)	16(57.1)
Ofloxacin	6(21.4)	2(7.3)	20(71.4)
Piperacillin/tazobactam	11(39.3)	13(46.4%)	4(14.3)

Screening and confirmation of ESBL producing *Klebsiella* spp

ESBL production was screened using the Kirby-Bauer disc diffusion method in accordance with CLSI guidelines, employing 30µg discs of third-generation cephalosporins (Ceftazidime, Cefotaxime, and Ceftriaxone). Of the 28 *Klebsiella* spp isolates, 22 (78.6%) were screened positive for ESBL production whereas 18 (64.3%) were confirmed as ESBL producers by at least one of the three antibiotics.

Prevalence of CTX-M gene among *Klebsiella* spp

Among 28 *Klebsiella* spp isolates, 18 (64.3%) were confirmed as ESBL producers, with the CTX-M gene detected in 7(38.9%) of these isolates, indicating its prevalence among ESBL-producing strains.

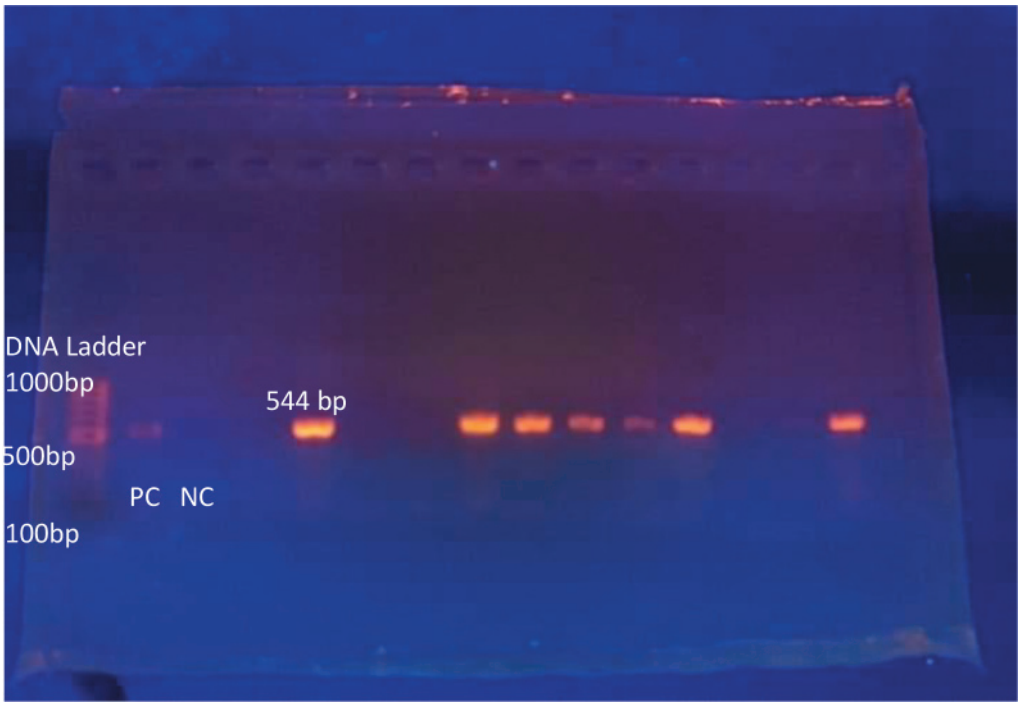

Figure 1: Gel electrophoresis patterns of CTX-M gene producing *Klebsiella* spp

DISCUSSION

In this study, urine was the predominant specimen type (65.8%), followed by blood (25.1%) and sputum (5.2%). This pattern reflects the high burden of urinary tract infections (UTIs) as reported globally and in Nepal, where urine samples typically account for the majority of clinical submissions (Flores-Mireles et al., 2015; Shaikh et al., 2008; Shrestha et al., 2019).

E. coli was the most frequently isolated organism (89.1%), consistent with its established role as the leading cause of UTIs and bloodstream infections worldwide (Tandogdu & Wagenlehner, 2016) and in Nepalese hospitals (Khanal et al., 2022; Mahaseth et al., 2019). Its dominance is likely due to virulence factors such as adhesins and biofilm formation which is responsible for colonization of the urinary tract (Flores-Mireles et al., 2015).

Klebsiella pneumoniae was the second most common isolate (6.2%). Though less prevalent than *E. coli*, it remains clinically significant due to its frequent association with ESBL and carbapenemase production, as reported by previous investigators in Nepal (Mahaseth et al., 2019; Pyakurel et al., 2021; Sharma et al., 2024). Lower prevalence of *Proteus* spp (3.96%)

and *Klebsiella oxytoca* (0.74%) is consistent with prior studies from Nepal where these species contribute less frequently to UTIs but may harbor multidrug resistance (Khanal et al., 2022; Shrestha et al., 2019).

In this study, the 28 *Klebsiella* spp isolates demonstrated the highest sensitivity to Amikacin (60.7%), followed by Meropenem (57.1%) and Gentamicin (46.4%). Moderate sensitivity was observed against Cotrimoxazole (39.3%), Ceftriaxone (35.7%), and fluoroquinolones (32.1%). In contrast, the isolates showed high resistance to Amoxicillin (100%), Cefixime (89.3%), Cefotaxime (75.0%), Ofloxacin (71.4%), and Ceftazidime (64.3%). This susceptibility profile aligns with trends reported in Nepal, where Amikacin and carbapenems remain the most effective agents against *Klebsiella* urinary isolates, whereas third-generation cephalosporins and fluoroquinolones are largely ineffective due to widespread ESBL production (Khanal et al., 2022; Mahaseth et al., 2019; Sharma et al., 2024).

Similar patterns have been reported elsewhere in the world. Studies from India, Europe, and the Middle East countries demonstrate high resistance of *Klebsiella pneumoniae* to β-lactams and fluoroquinolones, with preserved activity of aminoglycosides and carbapenems (Lahlaoui, et al., 2014; Logan & Weinstein, 2017; Pitout et al., 2019). The high resistance to cephalosporins and fluoroquinolones in our study likely reflects the dissemination of ESBL genes such as *bla* $_{CTX-M}$, which hydrolyze third-generation cephalosporins and confer cross-resistance to multiple antibiotic classes (Cantón et al., 2012).

Although Meropenem retained over 50% sensitivity, the emergence of carbapenem-resistant *Klebsiella* strains is a growing concern in Nepal (Mahaseth et al., 2019; Pyakurel et al., 2021). This underscores the importance of routine antimicrobial susceptibility testing and judicious use of broad-spectrum antibiotics to limit further resistance. The moderate susceptibility to Piperacillin/Tazobactam (39.3%) and Gentamicin (46.4%) suggests that these agents could be considered for empirical therapy where carbapenem-sparing strategies are required, but susceptibility-guided therapy remains essential.

Screening for ESBL production using the Kirby-Bauer disc diffusion method revealed that 22 of 28 isolates (78.6%) were screen-positive, and 18 isolates (64.3%) were confirmed as ESBL producers. Molecular characterization demonstrated the presence of the *bla* $_{CTX-M}$ gene in 7 of these confirmed ESBL-producing isolates.

The dominance of CTX-M–type ESBLs among ESBL producers is well documented elsewhere in the world. For instance, studies from Ethiopia reported 95.8% of ESBL-producing isolates harboring CTX-M–type genes, with CTX-M-1 group (particularly CTX-M-15) being most common (Worku et al., 2025). In Iran, 92% of ESBL-producing *Klebsiella pneumoniae* urinary isolates carried *bla* $_{CTX-M}$, highlighting the widespread distribution of this genotype (Maleki et al., 2018). Similarly, recent investigations demonstrate high CTX-M prevalence, with many isolates also co-carrying resistance determinants to aminoglycosides, fluoroquinolones, and sulfonamides (Park et al., 2024).

In Nepal, the predominance of CTX-M–type ESBLs has been reported in multiple studies. Khanal et al., (2022) found that 89.6% of phenotypically confirmed ESBL producers carried blaCTX-M (Khanal et al., 2022), while Mahaseth et al., (2019) reported CTX-M prevalence of 30–50% among ESBL-positive uropathogens (Mahaseth et al., 2019). Pantha et al., (2024) observed ESBL production in 78% of pediatric urinary isolates, with a significant proportion harboring CTX-M genes (Pantha et al., 2024). These findings align with our results and confirm the widespread dissemination of CTX-M–type ESBLs among *Klebsiella* spp in Nepal.

Detection of CTX-M in only 7 of 18 phenotypic ESBL isolates suggests that other ESBL genes, such as blaTEM and blaSHV, may be present in the remaining isolates, a phenomenon reported by previous investigators in Nepal (Chander & Shrestha, 2013; Mahaseth et al., 2019) . The global and local dominance of CTX-M-type ESBLs likely reflects several factors: high transmissibility via plasmids, co-carriage of resistance genes to other antibiotic classes, and selective pressure from widespread cephalosporin use (Cantón et al., 2012).

CONCLUSION

The antimicrobial susceptibility profile of the 28 *Klebsiella* spp isolates revealed limited treatment options, with the highest sensitivity observed for Amikacin, Meropenem, and Gentamicin. Moderate susceptibility to cotrimoxazole, ceftriaxone, and fluoroquinolones further reflects emerging resistance trends. Notably, the isolates exhibited

very high resistance to amoxicillin, third-generation cephalosporins, and ofloxacin underscoring the continued spread of multidrug-resistant *Klebsiella* spp and the need for vigilant antibiotic stewardship and routine resistance monitoring. Similarly, A high proportion of *Klebsiella* spp isolates demonstrated extended-spectrum β-lactamase activity, with 64.3% confirmed as ESBL producers. Molecular analysis further revealed that the CTX-M gene was present in a notable subset of these isolates, underscoring its role in mediating resistance among ESBL-producing *Klebsiella* spp. These findings highlight the growing significance of CTX-M–type β-lactamases and emphasize the need for continued surveillance and effective antimicrobial stewardship.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Cantón, R., González-Alba, J. M., & Galán, J. C. (2012). CTX-M enzymes: origin and diffusion. *Frontiers in Microbiology*, 3, 110.

Chander, A., & Shrestha, C. D. (2013). Prevalence of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae urinary isolates in a tertiary care hospital in Kathmandu, Nepal. *BMC Research Notes*, 6(1), 487.

Clinical and Laboratory Standards Institute. (2019). *Performance standards for antimicrobial susceptibility testing (CLSI supplement M100, 29th ed.)*. Clinical and Laboratory Standards Institute.

Edelstein, M., Pimkin, M., Palagin, I., Edelstein, I., & Stratchounski, L. (2003). Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Antimicrob Agents Chemother, 47*(12), 3724-3732. doi:10.1128/aac.47.12.3724-3732.2003.

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(5), 269-284.

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). *Bailey & Scott's diagnostic microbiology* (12th ed.). Mosby.

Khanal, L. K., Amatya, R., Sah, A. K., Adhikari, R. P., Khadka, S., Sapkota, J., & Rai, S. K. (2022). Prevalence of Extended Spectrum Beta Lactamase producing *Escherichia coli* and *Klebsiella* spp from urinary specimen in a tertiary care hospital. *Nepal Medical College Journal*, 24(1), 75-80.

Lahlaoui, H., Khalifa, A. B. H., & Moussa, M. B. (2014). Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum β-lactamase (ESBL). *Medecine et maladies infectieuses*, 44(9), 400-404.

Logan, L. K., & Weinstein, R. A. (2017). The epidemiology of carbapenem-resistant Enterobacteriaceae: The impact and evolution of a global menace. *Journal of Infectious Diseases*, 215(Suppl 1), S28–S36.

Mahaseth, S. N., Sanjana, R. K., Jha, B. K., & Pokharel, K. (2019). Prevalence of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from urinary tract infected patients attending tertiary care hospital of central Nepal. *Journal of College of Medical Sciences–Nepal*, 15(3), 211–217.

Maleki, N., Tahanasab, Z., Mobasherizadeh, S., Rezaei, A., & Faghri, J. (2018). Prevalence of CTX-M and TEM β-lactamases in *Klebsiella pneumoniae* isolates from patients with urinary tract infection, Al-Zahra hospital, Isfahan, Iran. *Advanced Biomedical Research*, 7(1), 10.

Pantha, S., Parajuli, H., Arjyal, C., Karki, S. T., Shrestha, D., & Thakur, M. (2024). Phenotypic characterization of ESBL-producing urinary isolates of *E. coli* and *Klebsiella* spp in a tertiary care children's hospital in Nepal. *Tropical Medicine & Health*, 52(1), 20.

Park, N., Hur, J. I., Lee, S., & Ryu, S. (2024). Prevalence of CTX-M types among ESBL-producing pathogenic *Escherichia coli* isolates from foodborne diarrheal patients in Gyeonggi-do, South Korea. *Food Science & Biotechnology*, 33(12), 2825–2833.

Peymani, A., Naserpour-Farivar, T., Zare, E., & Azarhoosh, K. (2017). Distribution of blaTEM,

blaSHV, and blaCTX-M genes among ESBL-producing *Pseudomonas aeruginosa* isolated from Qazvin and Tehran hospitals, Iran. *Journal of Preventive Medicine & Hygiene*, 58(2), E155.

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), e00102–19. https://doi.org/10.1128/CMR.00102-19

Pyakurel, S., Ansari, M., Kattel, S., Rai, G., Shrestha, P., Rai, K. R. (2021). Prevalence of carbapenemase-producing *Klebsiella pneumoniae* at a tertiary care hospital in Kathmandu, Nepal. *Infectious Diseases & Health*, 49(1), 78.

Shaikh, N., Morone, N. E., Bost, J. E., & Farrell, M. H. (2008). Prevalence of urinary tract infection in childhood: A meta-analysis. *Pediatric Infectious Disease Journal*, 27(4), 302–308.

Sharma, M., Jha, B., Neupane, S., & Bhatt, C. P. (2024). Prevalence of carbapenemase producing *Klebsiella pneumoniae* causing urinary tract infection in patients visiting Kathmandu Medical College. *Journal of Nepal Medical College*, 13(2), 25–29.

Shrestha, L. B., Baral, R., Poudel, P., & Khanal, B. (2019). Clinical, etiological and antimicrobial susceptibility profile of pediatric urinary tract infections in a tertiary care hospital of Nepal. *BMC Pediatrics*, 19(1), 36. https://doi.org/10.1186/s12887-019-1410-1.

Shyaula, M., Khadka, C., Dawadi, P., & Banjara, M. R. (2023). Systematic review and meta-analysis on extended-spectrum β-lactamases producing *Klebsiella pneumoniae* in Nepal. *Microbiology Insights, 16*, 11786361221145179.

Tandogdu, Z., & Wagenlehner, F. M. (2016). Global epidemiology of urinary tract infections. *Current Opinion in Infectious Diseases*, 29(1), 73–79.

Vignoli, R., García-Fulgueiras, V., Cordeiro, N. F., Bado, I., Seija, V., Aguerrebere, P., Gutkind, G. (2016). Extended-spectrum β-lactamases, transferable quinolone resistance, and virulotyping in extra-intestinal E. coli in Uruguay. *Journal of Infection in Developing Countries*, 10(1), 43–52.

Worku, S., Abebe, T., Abdissa, A., Berhan, A., Alemu, A., Beyene, G. T., Swedberg, G. (2025). Molecular epidemiology of extended-spectrum beta-lactamase producing Gram-negative bacteria among surgical site infection patients in Ethiopia: A multicenter prospective study. *BMC Infectious Diseases*, 25(1), 1091.

Xie, Q., Xu, T., Chen, L., & Shi, C. (2025). Molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* ST15-KL19 isolates from ICU patients in a Chinese tertiary hospital. *Frontiers in Microbiology*, 16, 1673965.

Zhao, W.-H., & Hu, Z.-Q. (2013). Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Critical Reviews in Microbiology*, 39(1), 79–101.