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Phenotypic detection of ESBL-producing *Escherichia coli* from faecal samples of stray dogs in Kirtipur Municipality, Kathmandu, Nepal

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ESBL, *Escherichia coli*, Faecal carriage, Multidrug resistance, Stray dogs

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ABSTRACT

Extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* represent an increasing public health concern due to its ability to inactivate third-generation cephalosporins and frequent association with multidrug resistance. Antimicrobial resistance has been widely investigated in clinical settings in Nepal, but limited information is available in stray animal populations. Stray dogs that are exposed to contaminated waste and sewage may serve as reservoirs of resistant bacteria and contribute to environmental dissemination. This cross-sectional study was conducted from June to December 2025 in Kirtipur Municipality, Kathmandu, to assess fecal carriage of ESBL-producing and multidrug-resistant (MDR) *E. coli* among stray dogs. Thirty fresh fecal samples were collected non-invasively and processed for culture using standard microbiological procedures. Isolates were identified by colony characteristics, Gram staining and biochemical tests. Antimicrobial susceptibility testing was done by the modified Kirby–Bauer disk diffusion method following CLSI 2024 guidelines, and ESBL production was confirmed phenotypically using the combination disk method. From the 30 samples, 52 morphologically distinct *E. coli* isolates were isolated. The dog-level carriage rate of phenotypically confirmed ESBL E.coli was 16.7% (5/30), and MDR was 23.3% (7/30). At the isolate level, 9.6% (5/52) were phenotypically confirmed as ESBL-producers and 13.5% (7/52) were MDR. The presence of antibiotic-resistant *E. coli* in stray dogs indicates that these animals might contribute to the circulation of antimicrobial resistance in urban environments.

Introduction

Antimicrobial resistance (AMR) has become a significant public health problem globally due to treatment failure. Particularly, extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* have the ability to hydrolyze third-generation cephalosporins, and also they are frequently resistant to multiple antimicrobial classes, thereby limiting therapeutic options (Suarez-Yana et al., 2024). Detection of these bacteria in clin-

ical and environmental settings indicates that resistance is not only confined to hospitals. Therefore, detection of AMR from the ecological perspective has become increasingly important.

In recent years, animals have been recognized as potential reservoirs and disseminators of antibiotic resistant bacteria (Salgado-Caxito et al., 2021). Particularly, dogs are important components when considering the human–animal interface. In most cases, companion animals are monitored through veterinary care, but the stray dogs live without medical monitoring and are continuously exposed to environmental contaminants such as sewage, discarded food, and waste materials. These might facilitate colonization with antibiotic resistant bacteria. The fecal shedding of ESBL-producing *E. coli* by stray dogs therefore raises concern, especially in densely populated urban environments where close human contact is common.

In Nepal, most published data on antimicrobial resistance have focused on clinical isolates from hospitals or food (Acharya et al., 2017; Adhikari et al., 2025; Devkota et al., 2018; Katuwal et al., 2026; Pudasaini et al., 2026; Tiwari et al., 2024). Though a limited number of animal-based studies exist, information regarding antibiotic resistant bacteria in stray dog populations remains scarce (Sharma et al., 2025). Therefore, we aimed to examine whether stray dogs in Kirtipur, Kathmandu harbor ESBL-producing and multidrug resistant *E. coli*. By isolating and characterizing faecal *E. coli* from stray dogs in Kirtipur Municipality, this study aims to provide baseline data that may contribute to a broader understanding of antimicrobial resistance dynamics within a One Health framework.

Materials and Methods

Study Design

This cross-sectional study was conducted for a period of six months from June 2025 to December 2025 at the Kirtipur Municipality, Kathmandu District, Nepal, to assess the faecal carriage of ESBL-producing *E. coli* among stray dogs residing in this area. Since the registered population list for stray dogs was not available, simple random sampling was not possible. Therefore, purposive sampling was done for the collection of samples from different locations like market areas, residential streets, and public parks to ensure the diverse representation of the local stray dog population.

Study Population and Sample Collection

Freshly passed stool samples were collected from 30 stray dogs by non-invasive procedures after natural defecation using sterile containers, taking precautions not to contaminate the samples with the environment. All samples were transported to the microbiology laboratory immediately and processed within 2 hours of collection.

Isolation of *Escherichia coli*

Each fecal sample was inoculated onto MacConkey agar and incubated aerobically at 37 °C for 18–24 hours. Lactose-fermenting colonies were further selected for analysis. To ensure representation of phenotypic diversity, multiple morphologically distinct colonies from each sample were sub-cultured (Tille, 2020).

Identification of Isolates

Identification of *E. coli* was done on based colony characteristics, Gram staining, and biochemical tests, including catalase, oxidase, indole, methyl red, Voges–Proskauer, citrate utilization, triple sugar iron (TSI) agar, and motility tests (Acharya et al., 2025).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done by modified Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Interpretation of zone of inhibition was done according to CLSI 2024 guidelines (CLSI, 2024). Isolates were categorized as either susceptible, intermediate, or resistant.

Phenotypic Detection of ESBL Production

Isolates showing reduced susceptibility to third-generation cephalosporins were subjected to confirmatory testing using the combination disk method. An increase in zone diameter in the presence of clavulanic acid was considered as confirmed ESBL producers (CLSI, 2024).

Definition of Multidrug Resistance

Multidrug resistance (MDR) was defined as resistance to at least one antimicrobial agent in three or more different antimicrobial classes (Magiorakos et al., 2012).

Ethical approval

Ethical approval for this study was obtained from the Ethical Review Committee, Institute of Science and Technology, Tribhuvan University (Approval Reference Number: ERCIOST-25-0013) on 8 May 2025. Faecal samples were collected non-invasively from stray dogs in public places following natural defecation, without restraining or handling the animals. All procedures were conducted in accordance with institutional and national guidelines for the ethical treatment of animals. No animals were harmed or disturbed during the study.

Data Analysis

Data were entered into Microsoft Excel and analyzed descriptively. Results were expressed as frequencies and percentages. The statistical association between ESBL production and MDR status was calculated using Fisher's exact test.

Results

Isolation of *Escherichia coli*

Out of 30 fecal samples processed for culture, 52 morphologically distinct *E. coli* isolates were recovered.

Antimicrobial Susceptibility Pattern

The antibiotic susceptibility pattern showed a variation among the isolates. The highest resistance rates were observed against the antibiotics, namely ampicillin and tetracycline. In contrast, gentamicin and cefotaxime had higher susceptibility. Overall, 7 isolates (13.5%) were found to be multidrug resistant (Table 1).

Table 1

Antimicrobial susceptibility of E. coli isolates from stray dogs (n = 52)

Antibiotic Class	Antibiotic Tested	Susceptible (n, %)	Intermediate (n, %)	Resistant (n, %)
Beta-lactams	Ampicillin	30 (57.7%)	10 (19.2%)	12 (23.1%)
Cephalosporins	Cefotaxime	37 (71.2%)	10 (19.2%)	5 (9.6%)
Aminoglycosides	Gentamicin	42 (80.8%)	5 (9.6%)	5 (9.6%)
Fluoroquinolones	Ciprofloxacin	40 (76.9%)	7 (13.5%)	5 (9.6%)
Tetracyclines	Tetracycline	35 (67.3%)	10 (19.2%)	7 (13.5%)
Sulfonamides	Trimethoprim-sulfamethoxazole	33 (63.5%)	12 (23.1%)	7 (13.5%)

Detection of ESBL-Producing *E. coli*

Upon initial screening for ESBL production, 8 isolates (15.4%) were identified as potential ESBL producers. Out of the potential ESBL producers, 5 isolates (9.6%) were identified as confirmed ESBL producers. The dog-level carriage rate of phenotypically confirmed ESBL E.coli was 16.7% (5/30), and MDR was 23.3% (7/30). At the isolate level, 9.6% (5/52) were phenotypically confirmed as ESBL-producers and 13.5% (7/52) were MDR (Table 2).

Table 2

Distribution of MDR and ESBL-producing E. coli isolates (n = 52)

Isolate Category	Isolate level (n=52)		Dog carriage level (n=30)	
	Number	Percentage	Number	Percentage
Presumptive ESBL producers	8	15.4	8	26.7
Confirmed ESBL producers	5	9.6	5	16.7
Multidrug resistant	7	13.5	7	23.3

ESBL production and multidrug resistance

Table 3

Multidrug resistance and ESBL production by E. coli isolates (n=30)

ESBL production	Multidrug resistance		p-value
	MDR	Non-MDR	(Fisher's Exact test)
ESBL positive	5	0	< 0.001
ESBL negative	2	23	< 0.001

At the host level, a strong association was observed between ESBL production and multidrug resistance. Out of the 30 dogs sampled, 5 (16.7%) carried *E. coli* that were both ESBL producers and multidrug-resistant. Statistical analysis using Fisher's exact test showed that the presence of ESBL-producing strains was significantly associated with an MDR profile ($p<0.001$) (Table 3).

Discussion

This study identified multidrug-resistant (13.5%) and ESBL-producing (9.6%) *E. coli* among the bacterial isolates obtained from faecal samples of stray dogs in Kirtipur Municipality. Although the overall prevalence was not high, the detection of antibiotic resistant bacteria in free-roaming animals is quite noteworthy. In our study, these dogs were not sampled based on their clinical illness or prior antimicrobial exposure, suggesting that resistant organisms may be circulating silently within the urban environment. Compared with the global context, our findings appear consistent with the trends reported in companion and stray animals. A scoping review and meta-analysis by Salgado-Caxito et al., estimated a pooled prevalence of ESBL-producing *E. coli* in dogs and cats of approximately 6.87%, with higher values observed in certain Asian regions (Salgado-Caxito et al., 2021). The prevalence observed in this study falls within that reported range. However, comparisons should be interpreted cautiously, due to the differences in sampling methods, laboratory methods, and animal health status which can substantially influence reported rates.

Few studies in the regional context have reported the higher prevalence of ESBL producing bacteria in canine populations. For instance, Tudu et al. (2022) reported nearly 30% of isolates to be the ESBL producers among those isolated from the diarrheic dogs in Kolkata. This contrasts with our findings might be due to the differences in host condition and antibiotic exposure (Tudu et al., 2022). Diseased animals are more likely to have received antibiotic treatment, which can select for antibiotic resistant strains. In contrast, the dogs sampled in this study were free-roaming and not selected based on clinical presentation. This distinction may partly explain the comparatively lower prevalence observed here.

In the context of Nepal, antimicrobial resistance has been increasingly reported across human, food, water and other environmental sectors (J.Acharya, Shrestha, et al., 2024; Dahal et al., 2025). The implementation of the ESBL *E. coli* Tricycle

surveillance project in Kathmandu demonstrated high levels of ESBL positivity in wastewater and notable carriage in human and poultry samples (J.Acharya, Shrestha, et al., 2024). Such findings underscore the environmental dimension of resistance dissemination. The identification of ESBL- producing E.coli in stray dogs could add another very important component to this ecological network and suggests that resistance determinants may be shared across different interconnected reservoirs.

The multidrug resistance rate identified in this study (13.5%) was lower than rates commonly reported in hospital-based isolates within Nepal, where MDR proportions often exceed 50% (Acharya et al., 2025). This difference may be due to the varying levels of antibiotic selection pressure. Hence, the presence of MDR strains in stray dogs should not be overlooked. These animals frequently inhabit public spaces, potentially contributing to environmental contamination and indirect human exposure.

The finding that all ESBL-positive isolates in our study were also MDR strains is consistent with the literature, suggesting that ESBL genes are frequently located on mobile genetic elements (plasmids) that also carry resistance determinants for other antibiotic classes, such as aminoglycosides and fluoroquinolones (Acharya et al., 2025). This co-carriage facilitates the emergence of highly resistant superbugs in the urban environment, complicating potential treatment outcomes if these strains are transmitted to humans.

This study had several limitations. The sample size was relatively small and confined to a single municipality, which may limit the generalization of this study to the country level. Additionally, characterization of antibiotic resistance was based solely on phenotypic methods. Molecular identification of specific ESBL genes such as blaCTX-M and blaTEM would provide greater insight into resistance mechanisms and potential transmission pathways. Hence, more studies incorporating molecular tools and expanded geographic sampling would help to strengthen the understanding of antimicrobial resistance patterns in animal reservoirs.

Despite these limitations, this study contributes preliminary data on ESBL and MDR carriage in stray dogs in Kathmandu, an area where published information remains limited. Continued surveillance integrating human, animal and environmental components will be essential to clarify the dynamics of resistance dissemination in Nepal.

Conclusion

The presence of antibiotic resistant and ESBL-producing *E. coli* in stray dogs indicates that these animals might contribute to the circulation of antimicrobial resistance in urban environments.

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Artificial Intelligence (AI) Use and Disclosure

In the preparation of this manuscript, AI tool-chatGPT was used for checking grammar.

Similarity and Originality Confirmation

The manuscript is original, not under review elsewhere, and complies with TUJ publication ethics.

Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

Data available from the corresponding author on request.

References

Acharya, J., Jha, R., Gompo, T. R., Chapagain, S., Shrestha, L., Rijal, N., Shrestha, A., Koirala, P., Subedi, S., Tamang, B., Kattel, H. P., Khaniya, B., Shrestha, B., Karki, A., Adhikari, R. P., Kayastha, S., Pradhan, P., Shrestha, S. D., Raghubanshi, B. R., & Samuel, R. (2024). Prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in humans, food, and environment in Kathmandu, Nepal:Findings from ESBL *E. coli* Tricycle Project. International Journal of Microbiology, 2024, 1094816. https://doi.org/10.1155/2024/1094816

Acharya, J., Shrestha, A., Rijal, N., Jha, P., Jha, R., Rijal, K. R., Sharma, S., Banjara, M. R., & Ghimire, P. (2024). Comparison of Beta-lactamase Resistance Gene Detection in MDR *Escherichia coli* Isolates in Nepal. Tribhuvan University Journal of Microbiology, 11(1), 9–19. https://doi.org/10.3126/tujm.v11i1.81363

Acharya, J., Shrestha, A., Rijal, N., Jha, R., Rijal, K. R., Sharma, S., Banjara, M. R., & Ghimire, P. (2025). Multidrug resistant *Escherichia coli* isolated at National Public Health Laboratory, Nepal. Journal of Nepal Health Research Council, 23(01), 31–41. https://doi.org/10.33314/jnhrc.v23i01.5067

Acharya, M., Joshi, P. R., Thapa, K., Aryal, R., Kakshapati, T., & Sharma, S. (2017). Detection of metallo-β-lactamases-encoding genes among clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital, Kathmandu, Nepal. BMC Research Notes, 10(1), 718. https://doi.org/10.1186/s13104-017-3068-9

Adhikari, S., Sharma, S., Adhikari, S., Shrestha, S., & Bhatta, D. R. (2025). mecA and PVL genes in methicillin-resistant Staphylococcus aureus from clinical specimens: A cross-sectional hospital based study from Nepal. Iranian Journal of

Microbiology. https://doi.org/10.18502/ijm.v17i1.17806

CLSI. (2024). Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100. Clinical and Laboratory Standards Institute.

Dahal, C., Adhikari, S., Regmi, R. S., Sapkota, S., Adhikari, N., Sharma, S., Banjara, M. R., Chalise, B. S., Ghimire, P., & Rijal, K. R. (2025). Detection of plasmid-mediated mcr-1 gene in multidrug-resistant *Escherichia coli* from clinical specimens at a tertiary hospital in Nepal. Infectious Diseases & Immunity, 5(2), 112–119. https://doi.org/10.1097/ID9.0000000000000157

Devkota, S. P., Sharma, S., Bhatta, D. R., Paudel, A., Sah, A. K., & Kandel, B. P. (2018). Prevalence of the blaNDM gene among metallo-β-lactamase-producing Gram-negative isolates from western Nepal. Journal of Global Antimicrobial Resistance, 12, 3–4. https://doi.org/10.1016/j.jgar.2017.11.003

Katuwal, A., Dhakal, H. P., Gurung, B., Adhikari, S., Sharma, S., Rijal, K. R., Banjara, M. R., & Ghimire, P. (2026). Antimicrobial Resistance in Bacterial Isolates from Patients Attending a Cancer Center. Journal of Nepal Health Research Council, 23(04), 785–794. https://doi.org/10.33314/jnhrc.v23i04.4733

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases, 18(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Pudasaini, B., Thapa, P., Chaudhary, A. N., & Sharma, S. (2026). Extended Spectrum Beta Lactamases (ESBL) producing Salmonella Isolated from Raw Goat Meat and Water from Slaughterhouses in Chitwan, Nepal. Nepalese Journal of Agricultural Sciences, 30, 111–118. https://doi.org/10.3126/nepjas.v30i1.89059

Salgado-Caxito, M., Benavides, J. A., Adell, A. D., Paes, A. C., & Moreno-Switt, A. I. (2021). Global prevalence and molecular characterization of extended-spectrum β-lactamase producing-*Escherichia coli* in dogs and cats—A scoping review and meta-analysis. One Health, 12, 100236. https://doi.org/10.1016/j.onehlt.2021.100236

Sharma, S., Regmi, S., Aryal, S., Sharma Chalise, B., Gurung, K., Thapa, J., Adhikari, S., Sharma, S., Rijal, K. R., Poudel, P., & Ghimire, P. (2025). Extended Spectrum β-Lactamases Producing and Biofilm Forming Multidrug Resistant Klebsiella Pneumoniae from Bacterial Coinfection and Secondary Infection in Patients with Coronavirus Disease At A Tertiary Care Hospital, Kathmandu, Nepal. Journal of Institute of Science and Technology, 30(2), 105–114. https://doi.org/10.3126/jist.v30i2.82555

Suarez-Yana, T., Salgado-Caxito, M., Hayer, J., Rojas-Sereno, Z. E., Pino-Hurtado, M. S., Campaña-Burguet, A., Caparrós, C., Torres, C., & Benavides, J. A. (2024). ESBL-producing *Escherichia coli* prevalence and sharing across seabirds of central Chile. The Science of the Total Environment, 951, 175475. https://doi.org/10.1016/j.scitotenv.2024.175475

Tille, P. M. (2020). Bailey & Scott's diagnostic microbiology.Churchill Livingstone

Tiwari, A., Poudel, P., Khanal, S., Lekhak, S., Adhikari, S., Sharma Regmi, R., Sharma, S., Panta, O. P., & Karki, T. B. (2024). Emergence of mcr-1 Gene in Colistin-Resistant *Escherichia coli* Isolates from Chicken in Chitwan, Nepal. Foodborne Pathogens and Disease, 21(7), 403–408. https://doi.org/10.1089/fpd.2023.0151

Tudu, R., Banerjee, J., Habib, M., Bandyopadhyay, S., Biswas, S., Kesh, S. S., Maity, A., Batabyal, S., & Polley, S. (2022). Prevalence and molecular characterization of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* isolated from dogs suffering from diarrhea in and around Kolkata. Iranian Journal of Veterinary Research, 23(3), 237–246. https://doi.org/10.22099/IJVR.2022.42543.6176