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ORIGINAL RESEARCH

Assessment of Antibiotic Resistance of *Escherichia coli* Isolated from Poultry Droppings in the Kathmandu Valley

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ABSTRACT

Objective: To assess antibiotic resistance in *Escherichia coli* isolated from chicken droppings from selected poultry farms within Kathmandu valley.

Methods: Stool samples of chicken from 21 different farms were collected aseptically and transported to Central Department of Microbiology laboratory. Samples were cultured in MacConkey and M-Endo agar to isolate *E. coli* and was confirmed phenotypically using biochemical tests. The isolates were tested for antimicrobial susceptibility by modified Kirby-Bauer disk diffusion method following CLSI guidelines.

Results: *E. coli* were isolated from all 104 dropping samples. *E. coli* isolates were resistant to ampicillin/sulbactam (91.34%), cefoxitin (98.07%), chloramphenicol (77.88%), ciprofloxacin (83.65%), contrimoxazole (79.81%), gentamicin (65.38%), levofloxacin (83.65%), and tetracycline (100%). While less proportions of *E. coli* were resistant to ceftriaxone (16.35%), colistin (3.85%) and imipenem (25%). Out of 104 isolates, 94 (90.38%) were multidrug resistant (MDR). Frequency of cleaning of the coop ($p = 0.0017$), farm size ($p < 0.001$), farm operation ($p<0.001$), water source ($p < 0.001$) and common diseases of poultry ($p < 0.001$) were found significantly associated with MDR *E. coli*.

Conclusion: High prevalence of MDR *E. coli* was found on chicken within Kathmandu valley, which might be introduced into humans through food chain. Therefore, adherence of biosecurity measures for reducing the use of antibiotics in the poultry farms is suggested.

Keywords: *Escherichia coli,* antibiotic susceptibility, poultry, stool samples

INTRODUCTION

Antimicrobial resistance is a critical global health problem wherein, over time, pathogens develop the ability to evade treatment, making infections increasingly difficult to manage. Responsible for 700,000 deaths globally in 2019, this figure is projected to rise to 10 million by 2050, surpassing cancer mortality. AMR arises through either intrinsic resistance, whereby it forms a part of bacterial physiology, or through acquired resistance due to genetic modifications. Horizontal gene transfer-enabling conjugation, transformation, and transduction-allows for the spread of resistance genes through mobile genetic elements such as plasmids, phages, and genomic islands, significantly accelerating the global spread and impact of resistance (Luiken et al., 2022).

Gram-negative bacterium of the Enterobacteriaceae family, *Escherichia coli* is widely recognized as an indicator for antimicrobial resistance due to its wide dissemination and extraordinary ability to carry resistance genes on mobile genetic elements, which can then be transferred to pathogenic strains. In the context of poultry, APEC acts as the primary etiological agent of colibacillosis, further solidifying its dual role as both a disease-causing microorganism and a reservoir of resistance-associated genetic determinants (de Mesquita Souza Saraiva et al., 2021).

Poultry meat is one of the fastest-growing agricultural

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sub-sectors in Nepal, sharing around 4% in the nation's GDP. It stands globally in the position of 112nd in the production of chicken meat and 92nd in egg production. The production of poultry meat, as recorded in 2021/22, has reached 65,387 tons with an average growth rate of 6.6% annually (Adhikari et al., 2023). In Nepal, 14 antibiotic types are available for veterinary use, among which tetracycline, enrofloxacin, neomycin, doxycycline, levofloxacin, colistin, and tylosin are primarily used for treating colibacillosis (Bhattarai et al., 2024). Nepal is among the 30 countries with a high burden of AMR. The amount spent on veterinary medicine and feed supplement sales is very high, and almost 13% of veterinary expenditure goes towards antibiotics. The most frequently used antibiotics are tetracycline, enrofloxacin, neomycin-doxycycline, levofloxacin, colistin, and tylosin. Ampicillin, amoxicillin, ceftriaxone, and gentamicin are commonly misused in clinical and veterinary practices (Bhattarai et al. 2024).

Poultry and their production environments are reservoirs for both resistant bacteria and genes for health consequence to humans. Fresh broiler litter sample stored at room temperature for about two months reduced the potential pathogens to less than 83% (Kyakuwaire et al., 2019). Poor hygiene and biosecurity in poultry favor pathogen emergence and excessive use of antimicrobials. Antimicrobial residues, derived from either administration or excretion by chickens, often concentrate in litter, with associated environmental and health risks. Non-standardized treatment practices and a lack of professional training among farm personnel further increase the inappropriate use of antimicrobials, which are often used not only for therapeutic reasons but also as growth promoters and for prophylaxis. Poor selection of antimicrobials, inappropriate dosages, and prolonged duration of use enhance AMR development, increasing the growing global threat (Moffo et al., 2022; Salam et al., 2023). The monitoring of antimicrobial resistance in poultry could provide the information of AMR that could potentially transmit to human. Further, this can provide AMR status on poultry which is one of the biggest meat industry in the country.

METHODS

Study design

This was a field and laboratory based cross-sectional study. Stool samples from different breeds of chicken were taken from farms within and out of Kathmandu valley area and were processed at the laboratory of Central Department of Microbiology, Tribhuvan University, Kritipur, Kathmandu. These stool samples were collected from 5 different sites within the farm where the activity of the chicken was most.

Study sites and duration

There were 21 different farms within inside and outside of Kathmandu valley, these locations included Changunagar, Chandragiri and Kritipur area. The study was conducted from July to August 2025.

Sample collection

A total of 5 sites within a farm was taken as a point for sample collection. Fresh feces of the chicken was collected in sterile swab and brought to the Central Department of Microbiology on Stuart transport medium.

Isolation and identification of *E. coli* from samples

Fresh fecal swab was brought in the laboratory and cultured on MacConkey agar and M-Endo agar to isolate *E. coli* which were confirmed through biochemical series of test including catalase test, oxidase test, sulphur indole motility (SIM) test, triple sugar iron agar (TSIA) test, urease, citrate utilization, and methyl-red and Voges Proskauer (MR and VP) test (Cheesbrough, 2006).

Antibiotic susceptibility test

Depending on farms antimicrobial treatments were applied at chick, all *E.coli* isolates were subjected to AST using the disc diffusion test on Mueller-Hinton agar, against 12 antibiotics (Hi-media) and inhibition zones were interpreted following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024). The following antibiotics discs were used: ampicillin sulbactam (10/10µg), gentamicin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), tetracycline (30 µg), colistin (25 µg), imipenem (10 µg), levofloxacin (5 µg), cefoxitin (30 µg), ceftriaxone (30 µg), and co-trimoxazole (25 µg).

Statistical analysis

All the data were entered using Epicollect5 and analysis and graphical representation was done with R with tidyverse package. Antibiotic resistance of *E. coli* was presented using percentages and comparison was done using chi-square test. p-value less than 0.05 was considered significant.

RESULTS

A total of 21 farms were chosen at random, 16 (76.2%) from Kathmandu whereas 5 (23.8%) from Bhaktapur. These farm included farmers who were in this business for 10 years having flocks of 0-6 weeks (85.72%) with many farm having 1000 plus chickens. These farms were properly fenced with mostly (95.24%) having proper flooring for the chicken. Though chicken are changed every 45 days (life cycle in broiler), these coops are often cleaned or whenever new batch of chicks to be introduced. It was observed that most of the farms sell the manure as fertilizer with few using it themselves too. These farms were supplied with tap water from local tap water where most of the house using the same water supply as the one in the coops for their day to day use. Chronic respiratory diseases (CRD) (61.90%) and *E. coli* infection (71.43%) seemed to be common infection around these farms and these farms were mostly vaccinated with rani-khet (Newcastle Disease) and gambaro (Infectious Bursal Disease/IBD) vaccine which are usually recommended through veterinarian or through the dealer who supplies these farm with chicks. Among these farms, there seemed to have decent biosecurity whereas only 5 farms with local breed of chicken and few with broiler were found to follow the maximum biosecurity practices which includes visitors control, use of proper gears, pest and rodents control and isolation of sick chicken.

Out of 104 isolates from stool samples for the chicken, 94 (90.38%) were MDR. There was no association between biosecurity of the chicken (p = 0.218) and vaccine use (p = 0.755) with MDR *E. coli*. Frequency of cleaning of the coop (p = 0.0017), farm size (p <0.001), farm operation (p<0.001), water source (p < 0.001) and common diseases of poultry (p < 0.001) were found significantly associated with MDR *E. coli* (Table 1).

Table 1: Poultry farm characteristics of poultry and isolation of MDR *E. coli*

Factors	Variables	MDR (%) (n=94)	Non-MDR (%) (n=10)	p-value
Farm size				
	100-1000	5 (5.3)	10 (100.0)	< 0.001
	1000-2000	39 (41.4)	0	
	2000-4000	50 (53.2)	0	
Farm operation				
	Fenced	0	5 (50.0)	<0.001
	Fenced with proper flooring	94 (100.0)	5 (50.0)	
Frequency of coop cleaned				
	Sometimes	30 (31.9)	10 (100.0)	0.0017
	Every flock change (>45 days later)	64 (68.1)	0	
Water source				
	Tap water	64 (68.1)	5 (50.0)	< 0.001
	Hand pump	5 (5.3)	5 (50.0)	
	Others	25 (26.5)	0	
Most common diseases				
	Rani khet	25 (26.5)	10 (100.0)	< 0.001
	E. coli	25 (26.5)	10 (100.0)	
	Gambaro	69 (73.4)	5 (50.0)	
	CRD	59 (62.7)	5 (50.0)	
	Others	39 (41.5)	0	
Vaccine use				
	Rani khet	89 (94.6)	10 (100.0)	0.7557
	Gambaro	89 (94.6)	10 (100.0)	
	Others	5 (5.3)	0	
Bio-security				
	Use of gumboot	40 (42.5)	10 (100.0)	0.2186
	Visitor control	84 (89.3)	10 (100.0)	
	Pesticide use	89 (94.6)	10 (100.0)	
	Isolation of sick chicken	20 (21.3)	5 (50.0)	

The antibiotics used in poultry as reported by farmers included levofloxacin (66.6%), enrofloxacin (42.8%), doxycycline (42.8%), ciprofloxacin (38.1%) (Table 2).

Table 2: Antibiotics used on chicken as reported by farmers

Antibiotics	Number (%) (n=21)
Neomycin	2 (9.5)
Enrofloxacin	9 (42.8)
Levofloxacin	14 (66.6)
Tetraycline	3 (14.2)
Doxycyline	9 (42.8)
Colistin	6 (28.5)
Ciprofloxacin	8 (38.1)
Streptopenicillin	1 (4.7)
Others	10 (47.6)

A total of 104 *E. coli* were isolated from 21 different farms consisting of 3 different breeds of chicken i.e., broiler, local and giriraj. High percentages of *E. coli* were resistant towards ampicillin/sulbactam (91.34%), cefoxitin (98.07%), chloramphenicol (77.88%), ciprofloxacin (83.65%), co-trimoxazole (79.81%), gentamicin (65.38%), levofloxacin (83.65%) and tetracycline (100%). While less proportions of *E. coli* were resistant to ceftriaxone (16.35%), colistin (3.85%) and imipenem (25%) (Table 3).

Table 3: Antibiotic susceptibility pattern of *E. coli* (n=104)

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin/Sulbactam	9 (8.66)	95 (91.34)
Cefoxitin	2 (1.93)	102 (98.07)
Ceftriaxone	87 (83.65)	17 (16.35)
Chloramphenicol	23 (22.12)	81 (77.88)
Ciprofloxacin	17 (16.35)	87 (83.65)
Co-trimoxazole	21 (20.19)	83 (79.81)
Colistin	100 (96.15)	4 (3.85)
Doxycycline	63 (60.58)	41 (39.42)
Gentamicin	36 (43.62)	68 (65.38)
Imipenem	78 (75)	26 (25)
Levofloxacin	17 (16.35)	87 (83.65)
Tetracycline	0 (0)	104 (100)

Comparing antibiotic resistance pattern of isolates according to types of chicken, it was observed MDR *E. coli* were isolated from broiler and giriraj breed of chicken, whereas there were no MDR isolate among local breed of chicken stool. Biosecurity measures were more commonly practiced on broiler farm than giriraj and local breed farms (Figure 1).

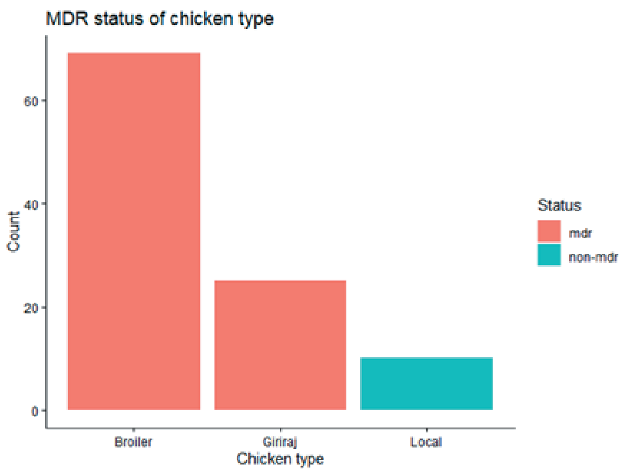

Figure 1: MDR status among different breeds of chicken

DISCUSSION

The results of this survey showed that antibiotics were commonly administered to broilers as compared to local and giriraj breeds of poultry. The antibiotics commonly used in the farms were levofloxacin, enrofloxacin, doxycycline, and ciprofloxacin. This over-reliance on antibiotic further consolidates AMR, particularly in the absence of stringent regulatory and biosecurity measures (Poudel et al., 2024).

This study revealed diverse antimicrobial sensitivity of *E. coli* isolated from chicken's droppings. In particular, it was found that none of the antibiotics were seen to be 100% effective. The resistance rates were 100% for tetracycline, over 90% for ampicillin/sulbactam and Cefoxitin, and above 80% for ciprofloxacin, levofloxacin, and co-trimoxazole showing similar trends as reflected in previous study from Nepal (Khanal et al., 2017). The resistance to antibiotics and used antibiotics were found correlated and antibiotics pressure could result resistance of *E. coli*. A universal trend in the resistance to tetracycline, a common antibiotic used both for therapeutic and prophylactic purposes in Nepalese poultry, represents a particularly alarming situation that calls for immediate action.

Considering the resistance for each antibiotic, resistance to ceftriaxone and colistin were relatively low comparing with other antibiotics as well as reports of the previous studies (Shrestha et al., 2017; Joshi et al., 2019; Dawadi et al., 2021), reflecting their limited use in poultry or their limited use only to critical human infections. The national active surveillance protocol specifically identifies colistin as a highest priority antimicrobial requiring immediate monitoring in animal health sector (Ministry of Agriculture and Livestock Development, 2024). Imipenem resistance significantly exceeds the sporadic detection rates in Nepal of less than 5% in poultry. However, despite low application in Nepalese veterinary medicine, a 25% resistance rate to imipenem might point toward co-selection or horizontal gene transfer of resistance determinants from other commonly used same class of antibiotics, as increasingly reported in poultry.

From the survey, most of the farms seem to emphasize biosecurity, visitor restriction, pesticides use for rodent control but isolation of sick chicken and gumboot use received less attention. WOAH's Terrestrial Animal Health Code recommends a weekly spot-cleaning and full litter removal/disinfection between broiler flocks-every 4-6 weeks-to keep moisture below 20%, thus minimizing ammonia levels and persistence of pathogens (WOAH, 2025). Frequency of cleaning coop shows reduction rate of respiratory and gastrointestinal pathogen outbreaks decreasing antimicrobial use. Untreated surface water harbors fecal coliforms and *Salmonella*, causing gastrointestinal/respiratory pathogens outbreaks. Ranikhet (ND) and gambaro (IBD) were most prevalent viral diseases pointing as one of the reason for frequent use of antimicrobials.

Although broiler farms tend to have better biosecurity but it did not substantially help in explaining their association with MDR occurrence. While the precise data on the use of antibiotics in such poultry is lacking, over-reliance on these antibiotics accelerates the selection of resistant strains and thus poses a serious risk to human and animal health (Mana et al., 2022). The implication is that overuse of antimicrobials outweighs the effects of a good biosecurity system, a pattern that is very similar to other intensive poultry production system (Khanal et al., 2017).

The data showed that around 90% tested *E. coli* isolates were MDR, indicating an extensive presence of strains with high resistance in commercial poultry. The rate of MDR was higher compared to previously reported study from Nepal (Bhattarai et al., 2024). The widespread presence of MDR *E. coli* in poultry droppings poses a significant public health challenge. Its application as organic fertilizer and the high consumption of poultry meat pose a risk of resistance transfer through bacteria and mobile genetic elements to the human food chain and the environment, alarming AMR in Nepal. Therefore, immediate actions of enhanced antimicrobial stewardship, regular AMR surveillance, restricted use of critical antibiotics in poultry, vaccination, and better farm management/ biosecurity are required to minimize this threat for the protection of both human and poultry health.

This study was limited to antibiotic resistance in single bacteria *E. coli* from limited number of poultry farms in Kathmandu valley. We did not explore antibiotic resistant genes that can transfer to bacterial communities.

CONCLUSION

E. coli isolated from chicken droppings were resistant to most commonly used antibiotics. Broiler breed of chicken with poor treatment may lead to development of antimicrobial resistant flora within them, which through food chain might be transmitted to human. Hence, a proper monitoring of antibiotic resistance of bacterial isolates and appropriate treatment of chicken are important for protecting poultry and human health. Adherence of biosecurity measures for reducing the use of antibiotics in the poultry farms is suggested.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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