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Epidemiology and antimicrobial resistance of *Escherichia coli* in broiler chickens, farmworkers, and farm sewage in Bangladesh

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Abstract

Background: Antimicrobial resistance (AMR) has become an emerging threat worldwide, and developing countries such as Bangladesh are considered to be at greater risk of disseminating the resistant bacteria between human–animal interfaces.

Objectives: The present study was carried out to determine the prevalence and AMR profile of *Escherichia coli* isolated from broiler chickens, the environment, and farmworkers. This study also aimed to identify the risk factors associated with multidrug-resistant (MDR) *E. coli* infection in broiler chickens. In addition, the presence of carbapenem resistance gene (*NDM-1*) was assessed.

Methods: A total of 114 *E. coli* isolates, recovered from 150 samples (cloacal swabs = 50, farm sewage = 50, and hand washed water of farmworkers = 50) collected from 50 broiler farms, were identified by biochemical examination and polymerase chain reaction (PCR) assay. Antimicrobial susceptibility test was performed for 10 antibiotics by disk diffusion test. Carbapenem resistance gene (*NDM-1*) was detected by PCR. Risk factors were identified through multivariable logistic regression.

Results: The highest prevalence of *E. coli* was recorded in broiler chickens (86%) and the lowest in farmworkers (66%). For MDR *E. coli* infection, 'winter season', 'absence of specific shoes for staff', and 'use of antibiotics without veterinarian's prescription' were the significant risk factors. High resistance of the *E. coli* isolates was observed against levofloxacin (81.6%), doxycycline (78.1%), cefotaxime (78.1%), and ciprofloxacin (70.2%). About 76% of the isolates demonstrated MDR. None of the isolates were positive for the *NDM-1* gene.

Conclusions: The high level and similar pattern of antibiotic resistance in *E. coli* isolates from broiler chickens, farmworkers, and sewage in poultry farms indicates a good possibility of spreading the antibiotic-resistant *E. coli* in such settings.

KEYWORDS
antimicrobial resistance, Bangladesh, broiler chickens, *E. coli*, multidrug resistance

1 | INTRODUCTION

Unquestionably, antibiotics have become the most important solution to many infectious diseases; however, the recent emergence of antimicrobial resistance (AMR) both in the field of human and veterinary medicine has become a significant public health concern worldwide (Ferri et al., 2017; Palma et al., 2020). Exaggerated use of antibiotics in the production facilities of food animals not only for therapeutic purposes but also for growth promotion or prophylaxis purposes is thought to be the crucial factor behind this (Agyare et al., 2019). When bacteria in the guts of animals are exposed to various antimicrobial agents with sub-therapeutic concentrations and frequencies, they acquire resistance to the antimicrobial agents that have been used through selective pressure (Scott et al., 2002). In intensively reared food animals such as poultry, where antibiotics are often administered as growth promoters to whole flocks, the antibiotic selection pressure in bacteria is high resulting in a high concentration of resistant bacteria in their faecal flora (Marshall & Levy, 2011). Being an essential part of the endogenous microflora, *Escherichia coli* can easily gain resistance against antimicrobials that are consumed by poultry birds (Hussain et al., 2017). Moreover, the potentiality of *E. coli* to transfer antibiotic resistance determinants to its other strains as well as different bacteria is well known (Rasheed et al., 2014).

In Bangladesh, poultry farming is expanding day by day and the sector contributes 14% of the total value of livestock output (Hamid et al., 2017). About 37% of the total meat production comes from this industry (Hamid et al., 2017). With the considerable expansion of poultry farming, farmers are more inclined to use antibiotics at sub-therapeutic doses for growth promotion and infection prevention (K. S. Islam et al., 2016). This tendency is worsening the scene of AMR by increasing the selection of resistant bacteria (Ayukekbong et al., 2017).

Food animals, especially poultry as well as poultry houses, serve as an important reservoir of *E. coli* and thus a potential source of human infection by its pathogenic strain (Stromberg et al., 2017). Antimicrobial-resistant *E. coli* can be transmitted from poultry to humans directly or via food. These resistant bacteria may cause colonization in the human gastrointestinal tract and may also contribute resistance genes to human endogenous microflora. Several studies reported the spread of antimicrobial-resistant *E. coli* from chickens to humans in various countries (Amir et al., 2019; Norizuki et al., 2017). The likelihood of transmission of antimicrobial-resistant *E. coli* among humans, animals, and the environment is a crucial threat to public health. Therefore, more focus should be given to the people who are occupied in poultry farming to reduce the risk of transmission of AMR.

In Bangladesh, variable prevalence (61.67%–82%) and high rate of AMR of *E. coli* in cloacal swab of broiler chickens have been reported (Akond et al., 2009; Hossain et al., 2008; Jakaria et al., 2012; Sarker et al., 2019). But there is a scarcity of studies identifying the risk factors for multidrug-resistant (MDR) *E. coli* infection in broiler chickens. Furthermore, reports on the AMR pattern of *E. coli* in one health setting (poultry farm–workers–farm sewage) in Bangladesh are lacking. Notably, at present, the emergence and global spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is considered a major public health threat (Hansen, 2021; Jean et al., 2015). Among different carbapenem resistance genes, *bla*NDM-1, *bla*OXA-1, and *bla*OXA-47 have been detected in different bacteria isolated from environmental samples, household water supply, and hospital samples in Bangladesh (Begum & Shamsuzzaman, 2016; M. Islam et al., 2012; Talukdar et al., 2013; Toleman et al., 2015). However, the presence of these genes particularly *blaNDM-1* in commercial poultry farm settings is yet to be investigated. Therefore, a cross-sectional study was conducted in broiler farms in two selected districts (Mymensingh and Gazipur) of Bangladesh with the aim to determine the prevalence of *E. coli* and assess their antimicrobial resistance profile in broiler chickens, farm sewage, and farmworkers. Risk factors associated with MDR *E. coli* infections in broiler chickens were also identified. Additionally, the presence of carbapenem resistance New Delhi metallo β-lactamase-1 (*NDM-1*) gene was assessed.

2 | MATERIALS AND METHODS

2.1 | Study area and design

A cross-sectional study was conducted in commercial broiler farms in Mymensingh and Gazipur districts from December 2018 to April 2019. To get representative data, five upazilas from each district and five broiler farms from each upazila were randomly selected. The selected Upazilas were Fulbaria, Trishal, Ishwarganj, Muktagacha, and Sadar of Mymensingh, and Kapasia, Kaliganj, Kaliakoir, Sreepur, and Sadar of Gazipur district.

2.2 | Sample collection

Cloacal swabs were collected from 10 birds in each farm and pooled to make one sample. Additionally, farm sewage ($n = 50$) and hand washed water of farmworkers ($n = 50$) were collected from the respective farms. While collecting the cloacal swab, cloacae of broiler chickens were moistened with alcohol dipped cotton and then swabs were collected using sterile swab sticks with 1 ml Buffered Peptone Water (BPW). Farm sewage samples were collected into a sterile falcon tube following aseptic measures. To obtain the hand washed water samples, hands of the workers were washed with 100 ml of sterile distilled water and collected into sterile falcon tube and sealed. Then, the samples were transported in a cool box to the laboratory within no more than 2 h of collection.

2.3 | Definition of human traffic control system

Based on the presence of foot-bath, a fence around the farm, gate, and restriction in the entry, the human traffic control system was graded as good, moderate, and poor. The presence of each item was scored as

1, and the total score was 4. A good human traffic control system was considered when the score was 4, moderate when 3, and poor when <3 score.

2.4 | Data collection

A structured questionnaire was developed using KoboCollect (mobile data collection app) and pre-tested prior to collecting data from the farms. Demographic data of the farmers, flock data along with data on antibiotic usage were collected through face-to-face interviews of the farmers, and geo-location of the farm was also recorded. Farmers who have participated voluntarily were included in this study and written consent was obtained from them before collecting the data.

2.5 | *E. coli* isolation and identification

E. coli was isolated and identified based on standard bacteriological procedure (ISO, 2001). First, samples were pre-enriched with 1 ml sterile BPW, then transferred into nutrient broth (NB), and incubated at 37°C for 24 h. The culture was then streaked onto MacConkey agar and incubated at 37°C for 24 h. Three presumptive *E. coli* colonies were then sub-cultured to obtain pure culture, and identification was performed using Gram staining and biochemical tests including catalase, oxidase, indole, methyl red, Voges-Proskauer test, and sugar fermentation test using triple sugar iron agar. Biochemically confirmed isolates were then subjected to PCR assay by using *malB* promoter gene-specific primers as described earlier (Wang et al., 1996). Bacterial DNA was extracted by the boiling method as described earlier (Dashti et al., 2009). Extracted DNA was measured using nanodrop spectrometer (NanoDrop One; Thermo Fisher Scientific, USA) and more than 100 ng/μl concentration of DNA was maintained to use for PCR assay. The nucleotide primer sequences were *ECO-1* forward: 5′-GAC CTC GGT TTA GTT CAC AGA-3′ and *ECO-2* reverse: 5′- CAC ACG CTG ACG CTG ACC A-3′. Amplification was done in a 25 μl reaction volume containing 12.5 μl of master mix (Biolabs, USA), 0.5 μl (50 pmol) forward, 0.5 μl (pmol) reverse primer, 11 μl nuclease-free water (Life Technologies, USA), and 0.5 μl DNA template in Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific Inc., USA). The PCR conditions were initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 7 min. PCR amplified products were subjected to gel electrophoresis (1.5% agarose) with ethidium bromide fluorescence, and visualized by using ultraviolet (UV) transilluminator.

2.6 | Antimicrobial susceptibility testing

Antimicrobial susceptibility of *E. coli* was performed by the disk diffusion test on 10 antimicrobials belonging to six different classes. In brief, after adjusting the turbidity of bacterial suspension equivalent to 0.5 McFarland standard, 150 μl test suspension was inoculated on to Mueller–Hinton agar plates, and then antibiotic disks were placed and incubated at 37°C for 18–24 h. Antibiotic disks, procured from Biomaxima (Poland) and Oxoid (UK), were *tetracyclines*: doxycycline (DO, 30 μg), *polymyxins*: colistin (CT, 10 μg), *penicillin β-lactamase inhibitors*: amoxyclav (AMC, 30 μg), *fluoroquinolones*: ciprofloxacin (CIP, 5 μg), levofloxacin (LEV, 5 μg), *cephalosporins*: ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), cefotaxime (CTX, 30 μg), *carbapenems*: imipenem (IPM, 10 μg) and meropenem (MEM, 10 μg). The results of the antimicrobial susceptibility test were interpreted according to the guidelines of Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2018). MDR isolates were defined as per the guidelines proposed by Magiorakos et al. (2012) with some modifications. Magiorakos et al. (2012) used the term 'non-susceptible' to denote 'resistant' combining both resistant and intermediate isolates. Here, we have considered only the resistant isolates excluding the 'intermediate' ones. Thus, isolates resistant to at least 1 antimicrobial agent in ≥3 antimicrobial classes were classified as MDR.

2.7 | Detection of *NDM-1* gene

The carbapenem resistance *NDM-1* gene was detected by PCR using specific primers as described earlier (M. Islam et al., 2012). The primer sequences were *NDM-1* forward: 5′-CTT CCA ACG GTT TGA TCG TC-3′ and *NDM-1* reverse: 5′-TAG TGC TCA GTG TCG GCA TC-3′ with fragment size of 465 bp. The PCR was run with reaction volume of 25 μl containing 12.5 μl of master mix, 0.5 μl (10 pmol) forward, 0.5 μl (10 pmol) reverse primer and 11 μl nuclease-free water and 0.5 μl DNA templates. The thermal conditions were initial denaturation at 95°C for 7 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 68°C for 1 min and final extension at 68°C for 7 min. The PCR products were then run on 1.5% agarose gel as described elsewhere.

2.8 | Statistical analysis

The SPSS version 22.0 software was used for the statistical analysis. Descriptive analysis was performed to calculate the prevalence of *E. coli* and resistance percentages. Any significant differences in the prevalence of *E. coli* and their resistance percentages among different types of samples were analyzed by chi-square test (*Z*-test for proportions) and Fisher's exact test (wherever appropriate). Risk factors associated with MDR *E. coli* infection in broiler chickens were identified through univariable and multivariable logistic regression. Variables bearing *p*-values less than 0.3 in the univariable analysis were included in the multivariable logistic regression (backward selection) analysis, and the Hosmer–Lemeshow test was performed to assess the fit of the final model.

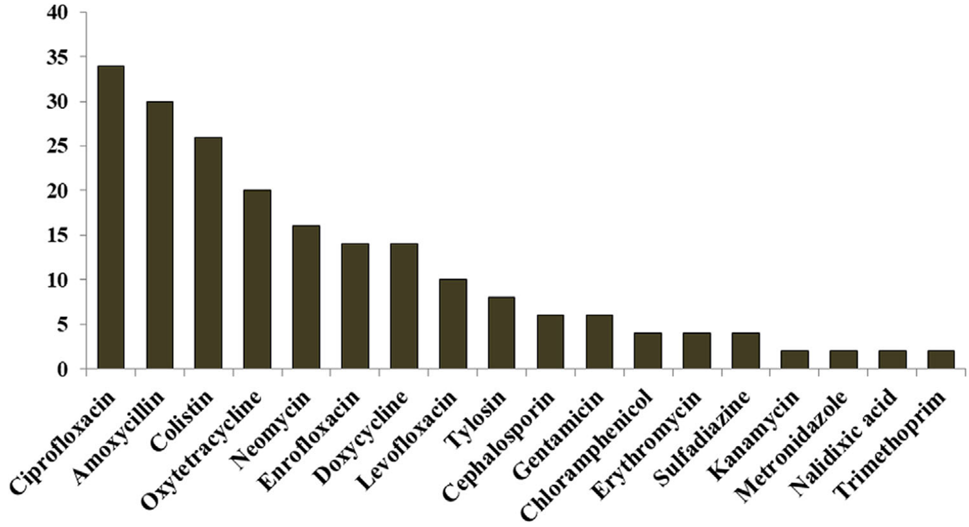

FIGURE 1 Most commonly used antibiotics in the broiler farms

3 | RESULTS

3.1 | Biosecurity and management practices

The findings of the questionnaire survey revealed that none of the farms were registered with the Department of Livestock Services, Bangladesh. The frequency of farmers with more than 5 years of experience in poultry farming was higher in Gazipur district compared with Mymensingh district. Most of the farms had a flock size of ≤2000 in both the districts (Table 1).

Most of the farmers sold the litter materials as manure. The majority of the farmers (96%) responded that they separate the sick birds from the healthy birds and bury the dead birds. Almost all the farmers used to clean waterer and feeder daily or every alternate day. The wastes produced in the farms were found to be evacuated into the drain (52%) or directly into the adjacent ponds (46%). Regarding the use of antibiotics, four farmers claimed that they did not use any antibiotics on their farms. Around half of the farmers used antibiotics for therapeutic purposes. The highest percentage of farmers used antibiotics according to the suggestion of feed dealers (56%), followed by company representatives (38%), while only 14% of farmers followed the prescription of veterinarians. However, the frequency of farmers who follow the prescription of veterinarians was comparatively higher in the Mymensingh district (20%) compared with the Gazipur district (5.7%) (Table 1). Ciprofloxacin (34%) was the highest used antibiotic, followed by amoxycillin (30%) and colistin (26%) in the farms (Figure 1).

3.2 | Prevalence and distribution of *E. coli*

Of the 150 samples examined, 114 isolates of *E. coli* were recovered, and all the isolates were confirmed positive in PCR assay as the target amplicon size of 585 bp was observed. Thus, the overall prevalence of *E. coli* was 76% (Table 2). Roughly similar prevalence was observed in both the study districts (78.7% and 73.3% in Gazipur and Mymensingh, respectively). Among the three types of samples, the highest prevalence was observed in cloacal swab samples (86%) and the lowest in hand washed water samples (66%). Significant ($p < 0.05$) difference was observed in the prevalence of *E. coli* between these two types of samples (Table 2).

3.3 | Antibiotic resistance profile of *E. coli*

The antimicrobial susceptibility test revealed that the highest resistance was observed in case of levofloxacin (81.6%), cefotaxime (78.1%), and doxycycline (78.1%), followed by ciprofloxacin (70.2%) (Table 3). The lowest percentage of resistance was found in ceftazidime (1.8%) along with ceftriaxone (7.9%) and colistin (14.9%). Notably, imipenem (65.8%) and meropenem (50.9%) belonging to carbapenem have shown significant resistance considering the fact that they are not used in poultry practices in Bangladesh. Resistance percentage based on sample types is illustrated in Figure 2. Almost a similar percentage of resistance was observed among the three types of samples in case of levofloxacin, doxycycline, cefotaxime, colistin and ceftriaxone. However, farm sewage isolates showed a considerably high resistance percentage to amoxyclav and imipenem than the other two types of isolates.

3.4 | Multidrug resistance of *E. coli*

In total, about 76% of *E. coli* isolates exhibited multidrug resistance which is 78.8%, 76.3%, and 74.4% in case of hand washed water, farm sewage, and cloacal swab, respectively (Table 4). There was no significant difference in MDR percentages among these three types of samples. About 70% isolates were resistant to 4–7 antibiotics, while 27% and 3.5% were resistant to 1–3 and 8–9 antibiotics, respectively

TABLE 1 Demographic and flock health management information of 50 broiler chicken farms in Mymensingh and Gazipur districts

Variables		Districts		Total (N = 50)
		Mymensingh, number of farms (%) (N = 25)	Gazipur, number of farms (%) (N = 25)	
Registered		0	0	0
Farming experience (years)	≤5	19 (76)	13 (52)	32 (64)
	>5	6 (24)	12 (48)	18 (36)
Flock size (number of birds)	≤2000	23 (92)	21 (84)	44 (88)
	>2000	2 (8)	4 (16)	6 (12)
Other poultry house within 500 m	Yes	15 (60)	19 (76)	34 (68)
	No	10 (40)	6 (24)	16 (32)
Using of farm premises	Only for poultry farming	20 (80)	19 (76)	39 (78)
	Integrated with other farming	5 (20)	6 (24)	11 (22)
Human traffic control system†	Poor (<3)	18 (72)	12 (48)	30 (60)
	Moderate (≥3)	7 (28)	13 (52)	20 (40)
Use of water sanitizer in drinking water		0	9 (36)	9 (18)
Disposal of litter	Compost	3 (12)	2 (8)	5 (10)
	Sold as manure	15 (60)	20 (80)	35 (70)
	Throw into nearby pit	9 (36)	9 (36)	18 (36)
	To biogas plant	0	3 (12)	3 (6)
Separation of sick birds		24 (96)	24 (96)	48 (96)
Management of dead bird	Burial	24 (96)	22 (88)	46 (92)
	Thrown away	3 (12)	7 (28)	10 (20)
	To garbage bin	0	3 (12)	3 (6)
Cleaning of feeder	Daily	8 (32)	6 (24)	14 (28)
	Alternate day	16 (64)	13 (52)	29 (58)
	Once a week	1 (4)	0	1 (2)
	Twice a week	0	6 (24)	6 (12)
Cleaning of waterer	Daily	19 (76)	21 (84)	40 (80)
	Alternate day	5 (20)	2 (8)	7 (14)
	Once a week	1 (4)	0	1 (2)
	Twice a week	0	2 (8)	2 (4)
Waste management	Compost pit	0	1 (4)	1 (2)
	Pond	16 (64)	7 (28)	23 (46)
	Drainage system	9 (36)	17 (68)	26 (52)
Use of antibiotics		23 (92)	23 (92)	46 (92)
Purpose of antibiotics use	Therapeutic	15 (65.2)	8 (34.8)	23 (46)
	Preventive and therapeutic	6 (26)	6 (26)	12 (24)
	Therapeutic and growth promoter	1 (4.34)	2 (8.7)	3 (6)
	Preventive, therapeutic, and growth promoter	1 (4.34)	7 (30.4)	8 (16)
By whom suggestion antibiotics were used	Company representatives	7 (28)	12 (34.3)	19 (38)
	Experienced farmer	0	2 (5.7)	2 (4)
	Feed dealer	9 (36)	19 (54.3)	28 (56)
	Self	4 (16)	0	4 (8)
	Veterinarian	5 (20)	2 (5.7)	7 (14)

Note: N = number of farms.
†Total score = 4 (foot-bath: 1, fence around the farm: 1, Gate: 1, and entry restricted: 1).

TABLE 2 District and sample type wise prevalence of *E. coli*

Variables	Total number of samples	Number of samples positive	Prevalence (%)
Districts			
Mymensingh	75	55	73.3*
Gazipur	75	59	78.7*
Total	150	114	76
Sample type			
Cloacal swab	50	43	86*
Farm sewage	50	38	76*,**
Hand washed water	50	33	66**
Total	150	114	76

*,** Values with different superscripts within the same column differ significantly ($p < 0.05$).

TABLE 3 Antibiotic resistance profile of *E. coli* isolates to 10 antibiotics

	Cloacal swab (N = 43)			Farm sewage (N = 38)			Hand washed water (N = 33)			Total (N = 114)		
Antibiotic name	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Levofloxacin	83.7	14	2.3	78.9	2.6	18.4	81.8	6.1	12.1	81.6	7.9	10.5
Ciprofloxacin	65.1	25.6	9.3	73.7	5.3	21.1	72.7	12.1	15.2	70.2	14.9	14.9
Ceftazidime	4.7	0	95.3	0	7.9	92.1	0	3	97	1.8	3.5	94.7
Ceftriaxone	7	20.9	72.1	10.5	13.2	76.3	6.1	15.2	78.8	7.9	16.7	75.4
Cefotaxime	74.4	9.3	16.3	81.6	10.5	7.9	78.8	9.1	12.1	78.1	9.6	12.3
Amoxyclav	11.6	32.6	55.8	39.5	39.5	21.1	9.1	57.6	33.3	20.2	42.1	37.7
Colistin	16.3	0	83.7	13.2	0	86.8	15.2	0	84.8	14.9	0	85.1
Doxycycline	79.1	18.6	2.3	76.3	13.2	10.5	78.8	21.2	0	78.1	17.5	4.4
Imipenem	62.8	25.6	11.6	73.7	10.5	15.8	60.6	21.2	18.2	65.8	19.3	14.9
Meropenem	46.5	14	39.5	52.6	13.2	34.2	54.5	6.1	39.4	50.9	11.4	37.7

Abbreviations: I, intermediate; N, number of isolates; R, resistant; S, susceptible.

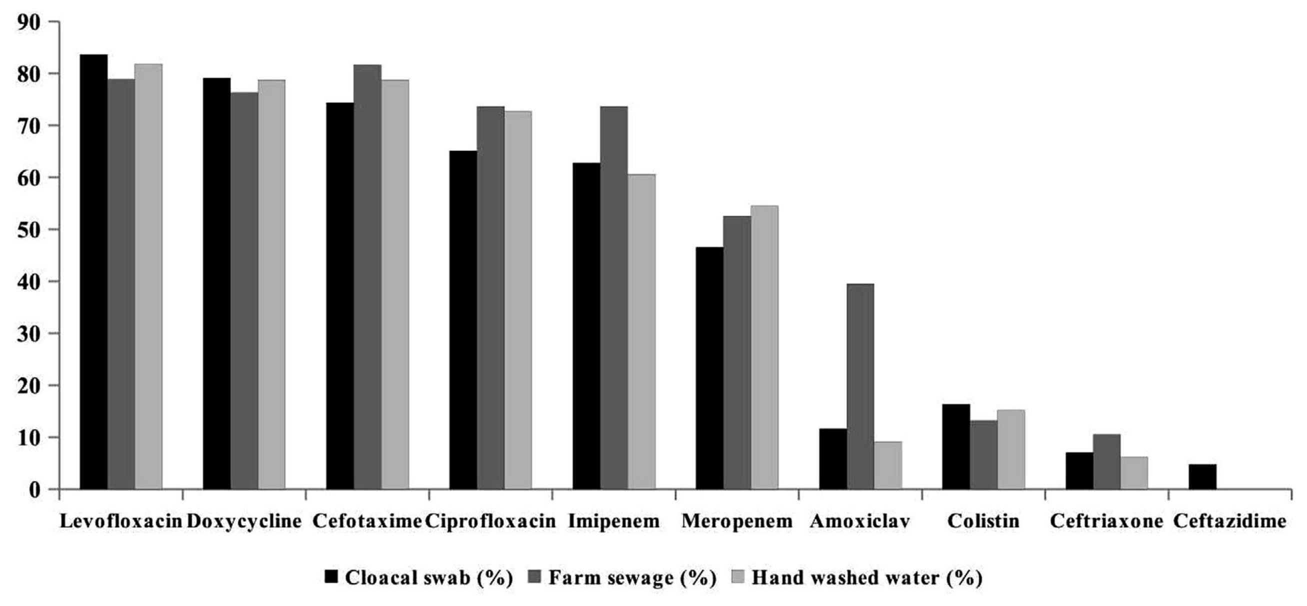

FIGURE 2 Resistance profile of *E. coli* isolated from three types of samples

TABLE 4 Multidrug resistance (MDR)[†] was observed among different types of *E. coli* isolates

Sample type	Number of isolates	Percentage	*p*-Value
Cloacal swab (N = 43)	32	74.4	0.906
Farm sewage (N = 38)	29	76.3	
Hand washed water (N = 33)	26	78.8	
Total (N = 114)	87	76.3	

Note: N = number of isolates.
[†]MDR when isolates are non-susceptible to at least one antimicrobial agent in ≥3 antimicrobial categories.

(Table 5). High resistance percentages were observed against more than 6 antibiotics in case of farm sewage isolates (52.6%), followed by hand washed water isolates (45.4%). Although, cloacal swab isolates exhibited higher resistance of 51.2% to 4–6 antibiotics. Alarmingly, one farm sewage isolate showed resistance to nine among 10 tested antibiotics.

In terms of antibiotic class, 72.8% of the total isolates were resistant to 3–5 classes of antibiotics (Table 5). In cloacal swab isolates, resistance to 3–4 antibiotic classes was 58.2%, and more than half of the isolates showed resistance to 4–5 antibiotic classes in case of farm sewage (52.6%) and hand washed water (57.6%). Of note, three farm sewage isolates and one cloacal swab isolate were found resistant to all six antibiotic classes.

All three types of isolates showed a similar trend of resistance to the classes of antibiotics used (Table 6). The highest resistance was observed against quinolones (84.2%) and cephalosporins (80%), followed by tetracyclines (78.1%) and carbapenems (68.4%), while lower resistance was found in polymyxins (14.9%) and penicillin β-lactamase inhibitors (20.2%) (Table 6).

3.5 | Risk factors for MDR *E. coli* infection in broilers

The present study revealed the significant risk of infection with MDR *E. coli* in broiler chickens in the winter season (Tables 7 and 8). Significant risk of MDR *E. coli* infection was also observed in the broiler chickens of those farms where antibiotics were used without prescription of veterinarians and whose farm personnel did not use specific shoes in the farm.

3.6 | Phenotypic resistance pattern of *E. coli* isolated from chickens

The distribution of phenotypic resistance patterns among the broiler chicken isolates is illustrated in Figure 3. The most common resistance pattern was levofloxacin–ciprofloxacin-cefotaxime–doxycycline–imipenem–meropenem found in five isolates. The pattern of levofloxacin-ciprofloxacin-cefotaxime-doxycycline and levofloxacin–ciprofloxacin–cefotaxime–colistin–doxycycline–imipenem–meropenem were found in four isolates followed by levofloxacin-doxycycline in three isolates of broiler chickens.

TABLE 5 The distribution of phenotypic resistance of *E. coli* isolates to number of antibiotics and antibiotic classes

	Number (%) of resistant isolates			
Number of antibiotics	Cloacal swab (*n* = 43)	Farm sewage (*n* = 38)	Hand washed water (*n* = 33)	Total (*n* = 114)
1	1 (2.3)	4 (10.5)	4 (12.1)	9 (7.9)
2	8 (18.6)	3 (7.9)	2 (6.1)	13 (11.4)
3	4 (9.3)	3 (7.9)	2 (6.1)	9 (7.9)
4	10 (23.3)	4 (10.5)	7 (21.2)	21 (18.4)
5	5 (11.6)	4 (10.5)	3 (9.1)	12 (10.5)
6	7 (16.3)	8 (21.1)	11 (33.3)	26 (22.8)
7	6 (14)	10 (26.3)	4 (12.1)	20 (17.5)
8	2 (4.7)	1 (2.6)	0	3 (2.6)
9	0	1 (2.6)	0	1 (0.9)
Number of antibiotic classes				
1	3 (7)	5 (13.2)	4 (12.1)	12 (10.5)
2	8 (18.6)	4 (10.5)	3 (9.1)	15 (13.2)
3	11 (25.6)	6 (15.8)	7 (21.2)	24 (21.1)
4	14 (32.6)	10 (26.3)	16 (48.5)	40 (35.1)
5	6 (14)	10 (26.3)	3 (9.1)	19 (16.67)
6	1 (2.3)	3 (7.9)	0	4 (3.5)

Note: *n* = number of isolates.

TABLE 6 Antibiotic class-wise resistance percentage of three types of *E. coli* isolates (tetracyclines: doxycycline; polymyxins: colistin; penicillin β-lactames inhibitors: amoxyclav; fluoroquinolones: levofloxacin and ciprofloxacin; cephalosporins: ceftazidime, ceftriaxone, and cefotaxime; carbapenems: imipenem, meropenem)

	Number (%) of resistant isolates			
Antibiotic classes	Cloacal swab ($n = 43$)	Farm sewage ($n = 38$)	Hand washed water ($n = 33$)	Total ($n = 114$)
Fluoroquinolones	37 (86)	31 (81.6)	28 (84.8)	96 (84.2)
Cephalosporins	33 (76.7)	31 (81.6)	26 (78.8)	90 (80)
Penicillin β-lactames inhibitors	5 (11.9)	15 (39.5)	3 (9.1)	23 (20.2)
Polymyxins	7 (16.3)	5 (13.2)	5 (15.2)	17 (14.9)
Tetracyclines	34 (79.1)	29 (76.3)	26 (78.8)	89 (78.1)
Carbapenems	28 (65.1)	28 (73.7)	22 (66.7)	78 (68.4)

Note: n = number of isolates.

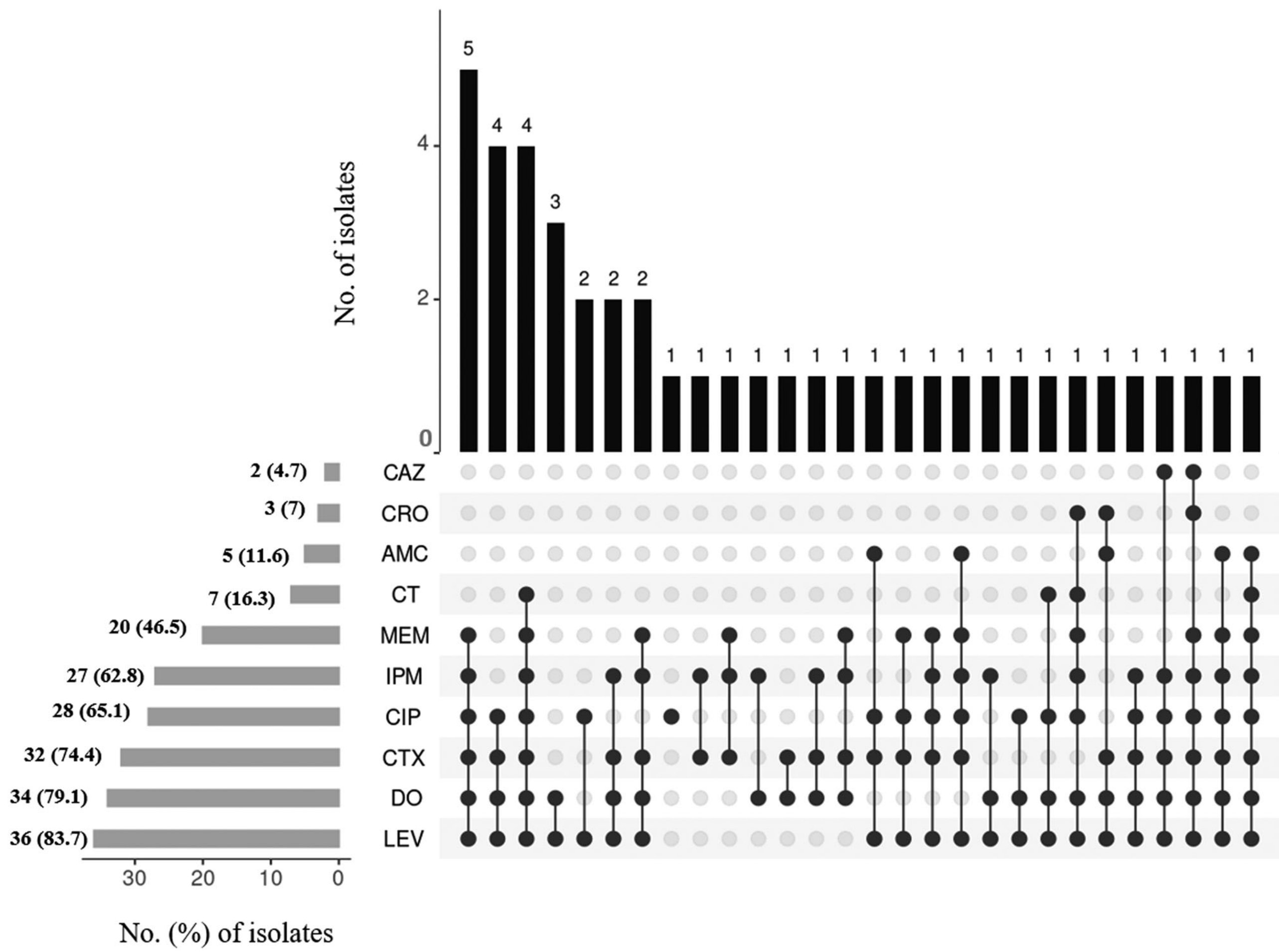

FIGURE 3 An UpSet plot summarizing the phenotypic resistance pattern of *E. coli* isolates in broiler chickens. The horizontal bars at the bottom left of the figure show the total number of isolates with percentages resistant to each antimicrobial agent. Joined black circles to the right of these bars indicate that the same phenotypic resistance pattern was common to the number of isolates shown at the top of each vertical bar. Abbreviations: AMC, amoxyclav; CT, colistin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; DO, doxycycline; IPM, imipenem; LEV, levofloxacin; MEM, meropenem

TABLE 7 Risk factors for multidrug-resistant *E. coli* infection in broiler chickens: results of univariable logistic regression

Variables		OR	95% CI	*p*-Value
Experience of farming (years)[‡]	≤5 (*n* = 28)	3.07	0.74–12.62	0.121
	>5 (*n* = 15)	Ref.	–	–
Season[‡]	Winter (December–February) (*n* = 24)	5.09	1.12–23.14	0.035
	Pre-monsoon (March) (*n* = 19)	Ref.	–	–
Use of farm premises	Only for poultry farming (*n* = 33)	Ref.	–	–
	Integrated with other farming (*n* = 10)	1.5	0.27–8.45	0.646
Distance of natural water body (m)[‡]	<20 (*n* = 29)	2.13	0.52–8.77	0.295
	≥20 (*n* = 14)	Ref.	–	–
Age of birds (days)	1–14 (*n* = 13)	Ref.	–	–
	15–35 (*n* = 30)	1.46	0.34–6.23	0.609
Flock size	≤1500 (*n* = 35)	Ref.	–	–
	>1500 (*n* = 8)	0.49	0.09–2.53	0.397
Human traffic control system[†]	Poor (*n* = 25)	0.73	0.18–3.02	0.669
	Moderate (*n* = 18)	Ref.	–	–
Specific shoes for staff[‡]	Yes (*n* = 18)	Ref.	–	–
	No (*n* = 25)	3.34	0.8–13.94	0.098
Litter condition[‡]	Wet (*n* = 10)	3.91	0.44–35.15	0.223
	Dry (*n* = 33)	Ref.	–	–
Litter turning[‡]	Alternate day (*n* = 16)	Ref.	–	–
	Once a week (*n* = 18)	6.22	1.06–36.57	0.043
	Twice a week (*n* = 9)	2.72	0.43–17.42	0.29
Involvement of farmer with other livestock farms[‡]	Yes (*n* = 18)	2.35	0.53–10.52	0.263
	No (*n* = 25)	Ref.	–	–
Other poultry farms within 500 m	Yes (*n* = 30)	0.42	0.08–2.32	0.322
	No (*n* = 13)	Ref.	–	–
Management of dead birds	Burial (*n* = 33)	Ref.	–	–
	Put into garbage bin and thrown away (*n* = 10)	0.75	0.16–3.59	0.715
Use of baits to control rodents[‡]	Yes (*n* = 14)	Ref.	–	–
	No (*n* = 29)	0.37	0.07–2	0.25
Drinkers maintenance	Daily (*n* = 34)	Ref.	–	–
	Not daily (*n* = 9)	1.26	0.22–7.23	0.795
Use of antibiotics	Yes (*n* = 40)	1.50	0.12–18.36	0.749
	No (*n* = 3)	Ref.		
Purpose of antibiotics use	Treatment (*n* = 19)	Ref.		
	Others[#] (*n* = 21)	1.48	0.37–5.96	0.583
Follow veterinarian's prescription[‡]	Yes (*n* = 7)	Ref.		
	No (*n* = 33)	11.25	1.75–72.5	0.004

Note: *n* = number of farms.
Abbreviations: CI, confidence interval; OR, odds ratio.
[†]Total score = 4 (foot-bath: 1, fence around the farm: 1, gate: 1, and entry restricted: 1).
[‡]Variables included in the multivariable logistic model.
[#]Other purposes of antibiotics use include preventive and growth promotion.

TABLE 8 Risk factors for multidrug-resistant *E. coli* infection in broiler chickens: results of multivariable logistic regression

Variables		OR	95% CI	*p*-Value
Season	Winter (December–February) ($n = 24$)	8.39	1.10–63.92	0.040
	Pre-monsoon (March) ($n = 19$)	Ref.	–	–
Specific shoes for staff	Yes ($n = 18$)	Ref.	–	–
	No ($n = 25$)	8.62	1.19–62.61	0.033
Follow veterinarian's prescription	Yes	Ref.	–	–
	No	18.53	1.97–173.9	0.011

Note: n = number of farms.
Abbreviations: CI, confidence interval; OR, odds ratio.

3.7 | Carbapenem resistance *NDM-1* gene

A total of 114 isolates were screened for the presence of carbapenem resistance *NDM-1* gene. But none of the isolates were found to be positive for the *NDM-1* gene.

4 | DISCUSSION

This study documents the updated data on the prevalence along with the AMR profile of *E. coli* in poultry farms at one health perspective that can be used to establish an integrated AMR surveillance system and can also facilitate the evaluation of interventions used to prevent and control AMR. The overall prevalence of *E. coli* was 76% and the highest prevalence (86%) was observed in cloacal swab samples which is higher than the previous reports of Hossain et al. (2008), Akond et al. (2009), and Sarker et al. (2019) in Bangladesh. However, Jakaria et al. (2012) and Bashar et al. (2011) reported 82% and 100% prevalence of *E. coli* in broiler chickens, respectively. Although the reason behind such differences in the prevalence of *E. coli* is unclear, several factors can contribute to such variations such as regional differences, sample collection techniques, season, and bacterial identification methods. The lower prevalence (66%) in hand washed water samples found in the present study was corroborated by Akond et al. (2009) who reported a lower prevalence of *E. coli* in hand washed water samples compared to cloacal swab samples. Although there was no report of the prevalence of *E. coli* in farm sewage samples in the study area, the current study revealed 76% prevalence, which was lower than the prevalence of *E. coli* isolated from river and sewage water (Nahar et al., 2019; Uddin et al., 2019).

While exploring the risk factors for MDR *E. coli* infection in broiler chickens, three potential risk factors namely 'winter season', 'absence of specific shoes for staff', and 'use of antibiotics without prescription of veterinarians' were identified. In winter, the birds generally gather together to share the heat allowing to increase the stocking density thus the spread of bacterial infection. Moreover, the stocking density of birds was previously reported as a risk factor for the occurrence of antibiotic-resistant *E. coli* in broilers (Persoons et al., 2011). Another variable that was identified as a risk factor for MDR *E. coli* infection was the absence of specific shoes for the farm staff. The use of no separate shoes allows *E. coli* to transmit among diverse sources including the human living places. Shared materials between humans and animals may contribute to the transmission of antibiotic-resistant bacteria from animals to humans and vice versa (Roess et al., 2013). One of the major concerns is the over-the-counter sale of antibiotics without prescription that promote irrational use, overuse, and misuse of antibiotics in the animal health as well as human health sectors in most of the developing countries including Bangladesh (Hassan et al., 2021; Kalam et al., 2021; Kumar et al., 2013; Masud et al., 2020). Subsequently, indiscriminate use of antibiotics without prescription contributes to the development and spread of antimicrobial resistance (McEwen & Collignon, 2018; Singer et al., 2003).

In the antimicrobial susceptibility study, we used 10 antibiotics of six different classes. The challenge was not negligible as four (meropenem, ceftriaxone, colistin, and ciprofloxacin) of the antibiotics tested in this study are classified by the World Health Organization as extremely important antibiotics in human medicine, and the other six (levofloxacin, ceftazidime, cefotaxime, amoxyclav, doxycycline, and imipenem) are classified as highly important antibiotics (World Health Organization, 2017). The *E. coli* isolates in this study exhibited resistance to all classes of the tested antibiotics. High resistance was observed against fluoroquinolones (84.2%), followed by cephalosporin (80%) and tetracycline (78.1%). Among fluoroquinolones, 81.6% and 70.2% of isolates showed resistance to levofloxacin and ciprofloxacin, respectively. A recent study reported 83% resistance to levofloxacin in *E. coli* isolated from cloacal swab of broiler chickens, which is almost similar to our present finding (Al Azad et al., 2019). A similar high resistance percentage to ciprofloxacin was reported in the previous studies conducted by Akond et al. (2009) (100%) and Bashar et al. (2011) (82%) in poultry birds. Fluoroquinolones particularly ciprofloxacin are widely available and often considered as the most frequently used antibiotic in poultry production in Bangladesh, which was also observed in the present study (K. S. Islam et al., 2016). The increasing tendency to use antibiotics as a preventive measure might be the reason behind this high resistance to fluoroquinolones.

Among cephalosporins, the resistance to cefotaxime was remarkably higher (78.1%) compared to the other two antibiotics of that group. High resistance to cefotaxime was previously reported in broiler farms abroad (Vinueza-Burgos et al., 2019) and the household water supply in Bangladesh (Talukdar et al., 2013). In addition, the reports on prevalence of cefotaxime resistance genes in poultry, wild birds, and the environment of Bangladesh corroborate the high resistance of cefotaxime found in the present study (Haque et al., 2014; Hasan et al., 2012). The emergence of such resistance genes in poultry production facilities may influence the spread of resistant bacteria among animals and humans. Resistance to tetracycline was found 78.1%, which is lower than the report of Sarker et al. (2019) and Azad et al. (2017) who reported that all of the *E. coli* isolates from broiler chickens were resistant to tetracycline. Bacterial resistance to tetracycline is of plasmid nature, and the existence of a wide variety of genetic determinants leads to the persistent acquisition of resistance genes by conjugation or transformation (Miles et al., 2006).

It is very alarming to note that in the carbapenem group, 65.8% and 50.9% of the isolates were resistant to imipenem and meropenem, respectively, although there was no record of using those critically used antibiotics in the poultry farms. In the previous studies, 100% susceptibility of *E. coli* to carbapenem was reported in isolates from poultry (Bashar et al., 2011), household water (Talukdar et al., 2013), and humans (Lina et al., 2014). However, in a recent study, 31.66% and 10% resistance to meropenem and imipenem, respectively, were found in *E. coli* isolated from cloacal swab of poultry (Sobur et al., 2019). Carbapenem antibiotics are considered as 'last-line agents' as they are used to treat infections due to MDR bacteria that are non-responsive to other classes of antibiotics (Kamata et al., 2015). Generally, the emergence of carbapenem-resistant strain could occur through the acquisition of carbapenem-resistance genes via plasmid-mediated horizontal gene transfer. However, in this study, none of the *E. coli* isolates (both carbapenem-resistant and non-resistant) harboured *NDM-1* gene, a carbapenem resistance gene. The presence of a carbapenemase is not the only determinant for the resistance to carbapenems. Combinations of different other mechanisms can be responsible for the resistance such as activity of extended-spectrum beta-lactamases or ampicillinase C enzymes and decreased permeability of outer membrane (Guerra et al., 2014). Some other important carbapenemases such as Verona imipenemase (VIM), Imipenemase (IMP), *K. pneumoniae* carbapenemase (KPC), and oxacillinase-48 (OXA-48) are responsible for the resistance in gram negative bacteria (Hansen, 2021; Jean et al., 2015), which were not investigated in this study. In addition, not all carbapenemases mediate resistance to all carbapenems (EFSA Panel on Biological Hazards, 2013; Miriagou et al., 2010). Besides, irrational use of antibiotics which allows the long-term contact between bacteria and antibiotics may contribute to the development and spread of carbapenem resistance (Ye et al., 2018).

In the present study, about 76% of *E. coli* isolates exhibited multidrug resistance, of which 78.8% isolates from hand washed water, 76.3% isolates from farm sewage, and 74.4% form cloacal swab. Variable percentages (64%–100%) of MDR were reported in *E. coli* from poultry and its products in Bangladesh (Akhtar et al., 2016; Parvin et al., 2020; Sarker et al., 2019). Concerningly, four isolates from our study showed resistance to all six classes of antibiotics, which poses a risk for the transmission of MDR *E. coli* from poultry production facilities to human surroundings. It was speculated that the frequent use of antibiotics for preventive purposes or growth-promoting agents in Bangladesh could reflect this high percentage of MDR *E. coli* in poultry farms (K. S. Islam et al., 2016). The present study also reported the irrational use of antibiotics as more than half of the respondents use antibiotics based on the suggestion of feed dealers.

The similar phenotypic resistance pattern of *E. coli* isolates from three different sources observed in the current study implies that there was a possibility of transmission of resistant *E. coli* between broiler chickens, farmworkers, and the environment. Therefore, the data presented in the current study could act as baseline epidemiological evidence. Further research on genomic and phylogenic relatedness combining with the presented data could help us to accurately identify the transmission networks of *E. coli* in broiler farms.

5 | CONCLUSION

A very high level of antibiotic resistance of *E. coli* particularly to fluoroquinolones and cephalosporin, was observed, and the isolates from broiler chickens, farmworkers, and sewage showed similar pattern of resistance phenotype. Moreover, the existence of high rates of MDR *E. coli* was documented, which pose a potential threat to public health. Finally, the data generated in the present study could be useful in formulating and implementing concerted interventions to reduce AMR in human-animal-environmental interface in Bangladesh.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

No animal experimentation was done in this study. However, cloacal swabs were collected very softly to avoid any discomfort to the broiler chickens. Farmers who have participated voluntarily were included in this study and the written consent was obtained from them before sampling and data collection.

AUTHOR CONTRIBUTIONS

Conceptualization, investigation, methodology, and writing-original draft: Amit Kumar Mandal. *Data curation, formal analysis, investigation, methodology, and writing-review & editing*: Sudipta Talukder. *Investigation and Methodology*: Md. Mehedi Hasan and Syeda Tanjina Tasmim. *Data curation, project administration, validation, and writing-review & editing*:

Mst. Sonia Parvin. *Investigation, project administration, and validation*: Md. Yamin Ali: *Conceptualization, data curation, funding acquisition, investigation, project administration, resources, supervision, validation, and writing-review & editing*: Md. Taohidul Islam:

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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