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Prevalence of *Escherichia coli* isolated from oropharynx and trachea of clinically sick poultry and antimicrobial resistance pattern of the strains isolated

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A R T I C L E I N F O

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A B S T R A C T

The study was carried out to investigate the isolation of *Escherichia coli* from tracheal and oropharyngeal swab of clinically sick chickens. The antibiotic susceptibility patterns of the isolates to several antimicrobials were determined with a striking emphasis on oxytetracycline. The PCR technique was applied to detect tetA, tetB, and tetC in the tetracycline-resistant isolates. The isolates were initially screened for their resistance patterns against 6 antimicrobials of six different groups using the disc diffusion technique. The results showed that 41% tracheal, 51% oropharyngeal, and 34% samples from both sites were *E. coli* positive respectively. Antimicrobial resistance profiling of the isolates revealed that all the isolates were resistant to oxytetracycline and sulphamethoxazole-trimethoprim, and also 90 %, 82.9%, 63.4%, and 39% resistant to ciprofloxacin, amoxicillin, gentamicin, and colistin respectively. Notably, 82.9% isolates (95% CI 68.4%–91.8%) showed resistance to $\geq$3 groups of antimicrobials that means these were multi-drug resistant. Among the tetracycline-resistant isolates, 85.4% (95% CI 71.2%–93.5%), 29.3% (7.5%–44.6%), and 7.3% (1.8% - 20.1) were positive for tetA, tetB, and tetC genes respectively. The frequency of the isolation of *E. coli* is greater in oropharyngeal than tracheal and both kinds of samples. Commercial poultry with *E. coli* strains has acquired extensive resistance to oxytetracycline. This study suggests a possible association between the tetA gene and oxytetracycline resistance in *E. coli* isolates, but further investigations like knockdown, whole-genome sequencing, and rescue experiments are needed to establish a direct causal relationship.

1. Introduction

In Bangladesh more than 165 million people live and it is the most densely populated nation in the world [1]. The poultry industry in Bangladesh has recognized as a flourishing agricultural industry which can supply regular protein needs and financial aid to a large number of population [2,3]. Based on the reports, this industry provides almost 22%–27% of the country's total meat supply and contributes to the country's gross domestic product (GDP) [4,5,6]. During the prior decade, the poultry sector constrained by several factors like various infectious diseases. Most poultry farmers have no knowledge of proper farming systems and antibiotic usage that contributes to the emergence and transmission of antimicrobial resistance (AMR) to humans through the food supply chain and environmental components [7].

E. coli is one of the most common microbial floras of gastrointestinal tract of human and animal and a frequent microbial contaminant of retail poultry meat [8]. Some strains of *E. coli* like enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC) could spread from animals to humans and even cause illness in commercially farmed chickens [9–12]. Furthermore, various strains of *E. Coli*, as well as enterohemorrhagic *E. coli* O157:H7, Shiga toxin-producing *E. coli*, and others,

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Table 1
Antibiotic resistance profiles of the *E. coli* isolates tested by Bauer-Kirby method (tested samples = 41).

Name of antibiotics	Zone of inhibition (millimeter)			Resistance Pattern		
	Resistant	Inter-mediate	Sensitive	Resistant	Intermediate	Sensitive
TE	≤14	15–18	≥19	41 (100%)	0 (0%)	0 (0%)
SXT	≤10	11–15	≥16	41 (100%)	0 (0%)	0 (0%)
CT	≤10		≥11	16 (39%)	0 (0%)	25 (61%)
CIP	≤15	16–20	≥21	37 (90.2%)	4 (9.8%)	0 (0%)
AML	≤13	14–16	≥17	34 (82.9%)	4 (9.8%)	3 (7.3%)
CN	≤12	13–14	≥15	26 (63.4%)	2 (4.9%)	13 (31.7%)

Note: The Clinical and Laboratory Standards Institute [21] recommendations were used to determine the resistance pattern.

show disastrous impacts on humans by causing different zoonotic diseases [13].

AMR has been recognized as a serious public health concern worldwide and a significant barrier to economic development [14–16]. The overuse and misuse of antibiotics in poultry rearing contribute to the arising and spread of antibiotic-resistant *E. coli*, which can be transmitted to humans through food or direct contact with sick animals [17–19]. Bacteria including *E. coli* have generated multidrug resistance (MDR) due to the haphazard use of antibiotics [10]. Resistance genes have been recognized in food-producing animals and their products [20]. In this systematic review, we have focused on the occurrence, distribution, and patterns of AMR in *E. coli* in poultry, their environment, reservoirs and modes of transmission, as well as the transmission of *E. coli* in poultry with their prevention and control strategies to update our knowledge for adopting effective strategies, and better management systems to decline AMR-related hazards linked to poultry in Bangladesh.

In Bangladesh, *E. coli* infection along with *Mycoplasma gallisepticum*, Infectious bronchitis virus with the involvement of multiple pathogens is often called chronic respiratory disease (CRD). It is at first important to detect the frequency of respiratory infection in poultry caused by *E. coli*. To evaluate it, oropharyngeal swab or tracheal swab can be collected. Unlike oropharyngeal swabs, *E. coli* in tracheal swabs would indicate the presence of true respiratory infection caused by it. The hypothesis is that even if there is any dissimilarity in the frequency of the isolation of *E. coli* both in the oropharyngeal, and in the tracheal swab samples from the same bird showing clinical illness need to be assessed. Another important concern is the extent of resistance already obtained in the strains causing respiratory infections to different antimicrobials, particularly oxytetracycline which is very frequently used in poultry in Bangladesh. Considering the above background, our present research work was conducted to isolate and identify *E. coli* from clinically sick poultry. Moreover, we detected the frequency of the *E. coli* isolates in the oropharyngeal, and tracheal swabs of the same birds suffering from clinical illness, and determined the anti-microbial susceptibility profile of *E. coli* isolates against commonly used antibiotics including oxytetracycline. Finally, we identified the tetracycline resistance-determinant tetA, tetB, and tetC genes in the tetracycline-resistant *E. coli* isolates.

2. Material and methods

2.1. Study area

The present research work was conducted in Chattogram Veterinary and Animal Sciences University, Bangladesh from February 2018 to August 2018. The samples investigated were from different Upazilas in Chattogram district.

2.2. Samples investigated

Both oropharyngeal and tracheal samples were collected from 100 dead birds (including pooled samples), and all the birds sampled had histories of clinical illness. Oropharyngeal or tracheal swabs were collected from a single bird one by one using sterile swabs and pooled all the swabs collected from the birds of the same farm (live or dead or both as supplied from the farm) in a sterile vial containing about 3 ml Stuart's transport medium (from Oxoid) without any antibiotics or chemical added. After collecting the swab samples into the vials, they were kept frozen at −80 °C.

2.3. Isolation and identification of E. coli

At first, a sample was inoculated into a test tube containing buffer peptone water (BPW) (Oxoid Ltd, pH: 6.2 ± 0.0, Basingstoke, Hampshire, UK), and for primary enrichment, incubated at 37 °C. After that, the culture was streaked onto MacConkey agar medium (Oxoid Ltd, pH: 7.4 ± 0.2, Basingstoke, Hampshire, UK) and incubated at 37 °C for 24 h. The growth of *E. coli* was suspected when bright pink-coloured large colonies yielded on a MacConkey agar plate. Then such colonies were streaked onto Eosin Methylene Blue (EMB) agar plate (Merck, pH: 7.1 ± 0.2) and incubated at 37 °C for 24 h "Green metallic sheen" colony morphology yielded on this medium was taken as positive for the growth of *E. coli*.

2.4. Storage of the isolated E. coli

At the final stage, all *E. coli* isolates were cultured in brain heart infusion (BHI) broth (Oxoid Ltd., England) and incubated at 37 °C for overnight. For further investigation of each isolate, 700 μl BHI broth culture was added to 300 μl 15% glycerol in 2 ml sterile in an eppendorf tube and stored at −80 °C.

2.5. Screening of resistance pattern of E. coli isolates against different antibiotics

The Bauer-Kirby method was used to evaluate the antibiotic resistance profiles of *E. coli* isolates. To obtain pure growth, sub-culturing of the preserved organism was done on blood agar and incubated at 37 °C for 24 h 3 or 4 individual colonies from the blood agar were transferred into a tube containing 3 ml of sterile saline solution (0.85% w/v NaCl solution) by using sterile inoculating loop. Then emulsification of the inoculums was done by using vortex machine to avoid clumping of the cells inside test tube. After that, adjustment of the bacterial suspension was done with the turbidity of 0.5 McFarland standards (equivalent to growth of 1–2 × 108CFU/ml). A pre-sterile cotton swab was dipped into the inoculum within 15 min of its preparation, and rotated several times on the inside of the test tube wall with firm pressure for removing excess fluid from the swab. Then rotating the plate at an angle of 60°, the swab was streaked over the entire dry surface of Mueller Hinton agar (Oxoid Ltd., England) in three directions. Finally, the swab was spread around the rim of the agar surface, and the discs were set on the agar surface by using sterile forceps after 15 min of inoculation. Six antibiotic discs were dispensed to the agar surface with an optimum distance between two adjacent discs, and incubated at 37 °C for 18 h. The following antibiotics (with respective disc potencies): TE: Tetracycline (30 μg), CIP: Ciprofloxacin (5 μg), CT: Colistin (10 μg), CN: Gentamycin (10 μg), AML: Amoxycillin (10 μg) and SXT: Sulfamethoxazole-trimethoprim (25 μg)

were used in our present research work. After incubation, by using a ruler, the size of zone of inhibition (in mm) around a disc including the diameter of the disc was measured, and the susceptibility testing of different antibiotics was interpreted as susceptible, intermediate and resistant.

2.6. Criteria for determining resistance patterns

To achieve accurate and trustworthy test findings, clinical laboratories all over the world adopt the Clinical and Laboratory Standards Institute (CLSI) recommendations. Based on the diameter of inhibition zones seen in agar diffusion studies, the [21] recommendations offer defined criteria for classifying bacterial resistance to antibiotics. According to Ref. [21], the following criteria were used in this investigation to identify the resistance patterns:

- ➢ Resistant: Zones of inhibition having a diameter below or equal to the given threshold (Table 1).
- ➢ Intermediate: Zones of inhibition that fall within the CLSI recommendations' specified range (Table 1).
- ➢ Sensitive: Zones of inhibition that are larger or equal to certain threshold (Table 1).

Polymerase chain reaction (PCR) to detect the presence of genes associated with the resistance against oxytetracycline.

For detection of the genes associated with the resistance against oxytetracycline, traditional PCR (Polymerase chain reaction) was performed. The detailed procedures that were followed for PCR testing are given below:

2.7. Blood agar preparation and reculturing

Firstly, fresh blood was collected from antibiotic-free cattle in sterile tube containing specific anticoagulant and collected blood was stored at 4 °C till mixed with blood agar base. Based on the manufacturer's instruction, 18.75 gm Blood Agar base (Oxoid Ltd., England) was considered and liquefied in 500 ml distilled water correctly. Then it was boiled to dissolve completely. After complete boiling, the agar was placed in an autoclave for sterilization at 121 °C for 15 min. After sterilization, the agar was kept in water bath at 50 °C for maintaining the temperature. Then 5% bovine blood was added to the agar base and mixed gently. Finally, the prepared blood agar was dispensed in sterile Petri dishes and set at 37 °C for whole night to check for any contamination. Contamination-free agar plates were separated and stored at 4 °C for further use.

The isolated *E. coli* strains stored at −80 °C were thawed gently at room temperature. Then the isolates were inoculated into 5% citrate bovine blood agar and the incubation was done at 37 °C for 24 h. After completion of incubation, 5–6 well-isolated colonies from blood agar were utilized for the DNA extraction method.

2.8. DNA extraction from the isolates

Boiling method was performed for DNA extraction from the recovered isolates. At first, 200 μl deionized water was taken into a 2 ml eppendorf tube. Then a loopfull fresh colonies (about 5–6) was picked from the agar plate, and transferred to the eppendorf tube. After that, to make a homogenous cell suspension, the tubes were vortexed for few seconds, and boiling was done at 99.9 °C for 15 min. Then immediately, the suspensions were placed at −20 °C for 6–7 min for cooling. Finally, centrifugation was done at 10000 rpm for 5 min. About 100 μl of supernatant was collected in another sterile eppendorf tube and preserved at −20 °C until testing.

2.9. Primers used to test resistance genes

E. coli isolates showing resistance to tetracycline were tested for the existence of tetA, tetB, and tetC genes by PCR using the advised primers, as shown in Table 5.

2.10. PCR

PCR conditions included an initial denaturation phase of 94 °C for 2 min, observed by 35 cycles of 45 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s with a last stage of 72 °C for 10 min. Master mix without any DNA template was used for a negative control, while a previously positive strain isolated for a gene investigated was used for as positive control. Finally, the observation of expected PCR band was done under UV transilluminator (BDA digital, biometra GmbH, Germany).

2.11. Statistical analysis

Data analysis was done by STATA 11 (StataCorp, College Station, Texas, USA). To see the difference in the isolation frequency of *E. coli* between the oropharyngeal and cloacal samples collected from the same birds belonging to the same farms, chi-square (χ^2) test was performed. Then to estimate the strength and statistical significance of association of a variable with resistance of *E. coli* isolate to Oxytetracycline,

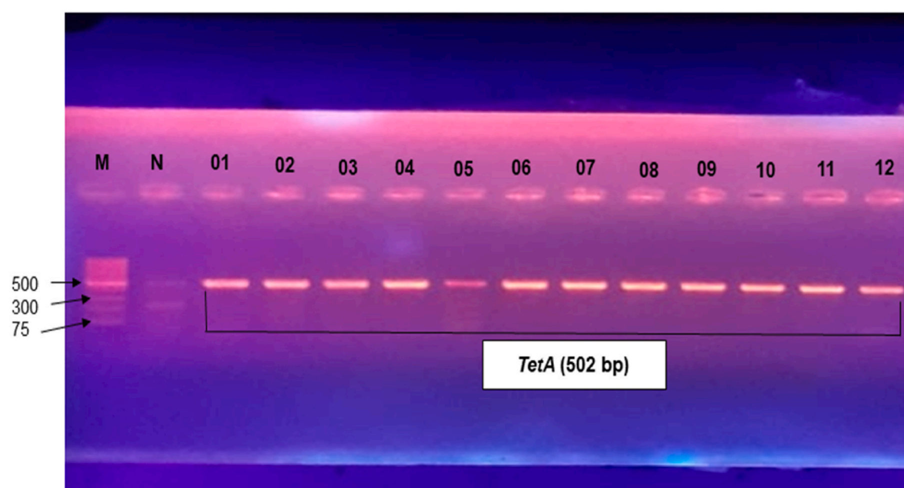

Fig. 1. PCR products showing the *tetA* gene-sized amplicon (502 bp) of a representative number of isolates resistant to oxytetracycline (Legends: M = DNA marker; N=Negative control without DNA; 01–11 = Test samples).

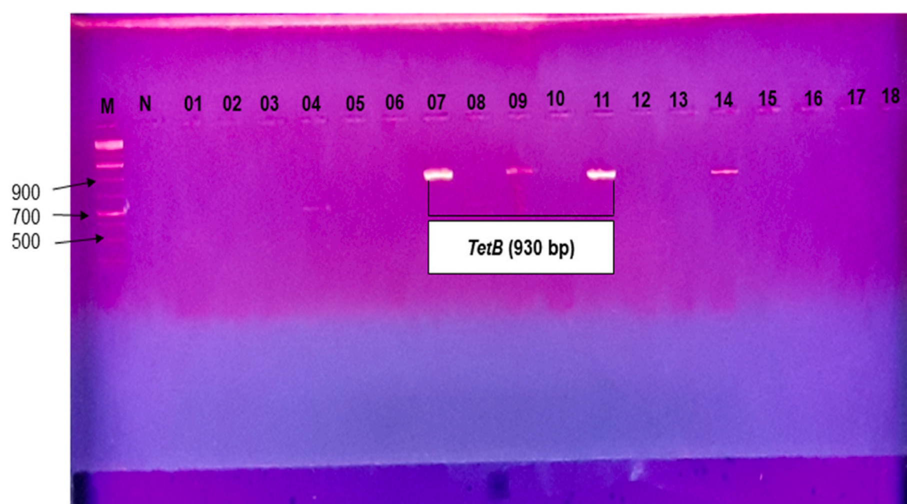

Fig. 2. PCR products-band showing of the *tetB* gene-sized amplicon (930 bp) of a representative number of isolates resistant to oxytetracycline (Legends: M = DNA marker; N=Negative control without DNA; 01–18 = Test samples).

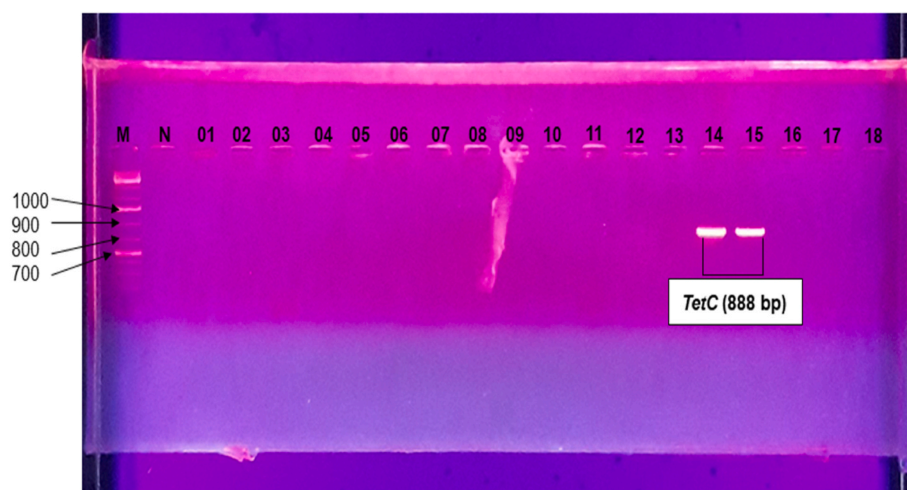

Fig. 3. PCR products-band showing of the *tetC* gene-sized amplicon (888 bp) of two isolates resistant to oxytetracycline (Legends: M = DNA marker; N=Negative control without DNA; 01–18 = Test samples).

univariable logistic regression analysis was performed. If a variable had $p < 0.05$, then an association was considered statistically significant.

3. Results

3.1. Isolation of E. coli

Among 100 swab samples for both oropharyngeal and tracheal origins collected from the same 100 farms, 51 from oropharynx and 41 from trachea were positive for *E. coli* respectively. For both oropharyngeal and tracheal swab samples, a total of 34 were *E. coli* positive. However, no significant difference ($p = 0.202$) between the frequency of *E. coli* isolation from oropharyngeal swab samples, and tracheal swab samples ($p = 0.202$), although proportionately, the isolation frequency was higher from the oropharyngeal swab samples than tracheal samples.

3.2. Antibiotic resistance

All the 41 isolates obtained from the tracheal samples were subjected to sensitivity testing to a panel of six antibiotics by Bauer – Kirby disk diffusion method (Table 1). According to Ref. [21], every isolate was seen to be resistant to oxytetracycline (TE), and sulphamethoxazole-trimethoprim (SXT). Not all but 90% of isolates were resistant to ciprofloxacin (CIP) and more than 80% of isolates were resistant to amoxicillin (AML). Around 39% of the *E. coli* isolates showed resistance against colistin (CT) and 63% against gentamycin (CN).

3.3. Resistance genes for tetracycline

To detect the presence of the tetA, tetB, and tetC genes, all the 41 isolates of tracheal origin were also tested where the amplicon sizes were 502bp, 930bp and 888bp respectively. According to the results obtained, 85.4% (95% CI 71.2%–93.5%) of them harboured the tetA gene, while 29.3% (95% CI 7.5%–44.6%) had the tetB gene, and 7.3% (95% CI 1.8% - 20.1) had the tetC gene. Notably, 82.9% isolates (95% CI 68.4%–91.8%) showed resistance to $\geq$3 groups of antimicrobials, and those were multi-drug resistant. Measurement of the typical amplicon sizes of the gene products, PCR technique was applied where the isolates having the tetA, tetB, and tetC genes were recorded to be resistant to oxytetracycline, and were displayed in Figs. 1–3, respectively. 34 (82.9%) out of the 41 *E. coli* isolates were MDR (Multi-drug resistance).

Factors related with the frequency of *E. coli* isolation from oropharyngeal, tracheal, and both kinds of swab samples.

The results of analysis of univariable logistic regression to identify

Table 2
Unconditional associations between isolation of *E. coli* from tracheal samples of poultry suffering from different clinical illness and the variables recorded during the collection of samples.

Variable	No. birds	No. positive for *E. coli* (%)	OR (95% CI)	*P*-value
Bird type (TOB):				
Broiler	59	27 (45.8%)	1.0	Reference
Layer	31	10 (32.3%)	0.6(0.2–1.4)	0.218
Sonali	10	4 (40%)	0.8(0.2–3.1)	0.735
Age				
1st week	0	0	1.0	Reference
2nd week	15	6 (40%)	2.2(0.6–7.9)	0.235
3rd week	27	16 (59.3%)	0.7(0.2–2.4)	0.599
Feeding system				
Commercial feed	85	34 (40%)	1.0	Reference
Handmade	15	7 (46.7%)	1.3 (0.4–4.0)	0.629
Litter				
Use of saw dust	66	26 (39.4%)	1.0	Reference
Use of rice husk	18	9 (50%)	1.5(0.5–4.4)	0.420
Cage system	16	6 (37.5%)	0.9(0.3–2.8)	0.889
Vaccination				
Yes	90	37 (41.1%)	1.0	Reference
No	10	4 (40%)	1.0 (0.3–4.0)	0.946
Antibiotic used			Omitted	–
Kind of antibiotics used				
Fluoroquinolone	27	16 (59%)	1.0	Reference
Tetracycline	12	8 (66.7%)	1.4(0.3–5.7)	0.667
Tylosin	14	9 (64.3%)	1.2 (0.3–4.7)	0.755
Colistin + Amoxycillin	13	6 (46.2%)	0.6(0.2–2.2)	0.437
Amynoglycoside	2	1 (50%)	0.7 (0.03–12.2)	0.798
Cephalosporin	2	1 (50%)	0.7 (0.03–12.2)	0.798

Table 3
Unconditional associations between isolation of *E. coli* from oropharyngeal samples of poultry suffering from different clinical illness and the variables recorded during the collection of samples.

Variable	No. birds	No. positive for *E. coli* (%)	OR (95% CI)	*P*-value
Bird type (TOB):				
Broiler	59	32 (54.2%)	1.0	Reference
Layer	31	14 (45.2%)	0.7(0.3–1.7)	0.414
Sonali	10	5 (50%)	0.8(0.2–3.2)	0.804
Age				
1st week	0	0	1.0	Reference
2nd week	15	6 (40%)	2.6(0.7–9.3)	0.157
3rd week	27	17 (63%)	1.4(0.4–4.4)	0.568
Feeding system				
Commercial feed	85	41 (48.2%)	1.0	Reference
Handmade	15	10 (66.7%)	2.1 (0.7–6.8)	0.195
Litter				
Use of saw dust	66	32 (48.5%)	1.0	Reference
Use of rice husk	18	12 (66.7%)	2.1(0.7–6.3)	0.176
Case system	16	7 (43.8%)	0.8(0.3–2.5)	0.734
Vaccination				
Yes	90	47 (52.2%)	1.0	Reference
No	10	4 (40%)	1.6 (0.4–6.2)	0.467
Antibiotic used				
Yes	69	43 (62.3%)	Reference	–
No	31	8 (25.8%)	4.8 (1.9–12.2)	0.001
Kind of antibiotics used				
Fluoroquinolone	27	15 (55.6%)	1.0	Reference
Tetracycline	12	8 (66.7%)	1.6(0.4–6.6)	0.517
Tylosin	14	11 (78.6%)	2.9 (0.7–13.0)	0.156
Colistin + Amoxycillin	13	8 (61.5%)	1.3(0.3–4.9)	0.720
Amynoglycoside	2	1 (50%)	0.8 (0.05–14.2)	0.879
Cephalosporin	2	1 (50%)	0.8 (0.05–14.2)	0.879

Table 4
Unconditional associations between isolation of *E. coli* from both tracheal and oropharyngeal samples of poultry suffering from different clinical illness and the variables recorded during the collection of samples.

Variable	No. birds	No. positive for *E. coli* (%)	OR (95% CI)	P-value
Bird type:				
Broiler	59	23 (39%)	1.0	Reference
Layer	31	7 (22.6%)	0.5(0.1–1.2)	0.121
Sonali	10	4 (40%)	1.0(0.3–4.1)	0.951
Age				
1st week	0		1.0	Reference
2 nd week	15	4 (26.7%)	3.0 (0.8–11.7)	0.121
3rd week	27	14 (51.9%)	1.0(0.3–3.8)	0.943
Feeding system				
Commercial feed	85	28 (33%)	1.0	Reference
Handmade	15	6 (40%)	1.4 (0.4–4.2)	0.596
Litter				
Use of saw dust	66	21 (31.9%)	1.0	Reference
Use of rice husk	18	8 (44.4%)	1.7 (0.0.6–5.0)	0.321
Case system	16	5 (31.3%)	0.9(0.3–3.2)	0.965
Vaccination				
Yes	90	31 (34.4%)	1.0	Reference
No	10	3 (30%)	1.2 (0.3–5.1)	0.28
Antibiotic used			Omitted	
Kind of antibiotics used				
Fluoroquinolone	27	12 (44.4%)	1.0	Reference
Tetracycline	12	6 (50%)	1.3(0.3–4.9)	0.748
Tylosin	14	8 (57.1%)	1.7 (0.-5-6.1)	0.442
Colistin + Amoxycillin	13	6 (46.1%)	1.1(0.3–4.0)	0.919
Amynoglycoside	2	1 (50%)	1.3 (0.07–22.1)	0.879
Cephalosporin	2	1 (50%)	1.3 (0.07–22.1)	0.879

the unconditional associations between isolation frequency of *E. coli* from tracheal, oropharyngeal, and both kinds of samples, and the variables recorded during the collection of samples were given in Tables 2–4 respectively. None of the variables had a significant association ($p \leq 0.05$) with the isolation frequency of *E. coli* from the tracheal or from both kinds of samples.

On the other hand, only one variable was unconditionally significant, which existed ''use of no antibiotics", for a higher isolation frequency of *E. coli* from oropharyngeal samples of birds suffering from clinical illness. This variable along with four others like 2 nd week of age, use of handmade feed, use of rice husk and use of tylosin having $p < 0.20$ were considered for multivariable logistic regression, and the results revealed that one variable "use of no antibiotics" was independently associated with a higher frequency of *E. coli* isolation from the birds investigated (Odds ratio 4.0 (95% CI: 1.5 – 11.2), $p = 0.007$) whereas the results of others four variables were (Odds ratio 2.5 (95% CI: 0.9 – 6.9), $p = 0.076$), (Odds ratio 2.7 (95% CI: 0.7– 9.8), $p = 0.135$), (Odds ratio 2.5 (95% CI: 0.7 – 8.6), $p = 0.147$) and (Odds ratio 3.9 (95% CI: 0.9– 16.0), $p = 0.061$) respectively.

Author's point of views

The magnitude of *E. coli* infection to the respiratory tract is around 40%, which seems to be a major disease problem in commercial poultry. When the same birds were investigated for the organisms by collecting the oropharyngeal swab samples the frequency of its isolation is about 10% higher compared with the tracheal swab samples, although such higher frequency was not proven to be statistically significant. Isolated *E. coli* strains from clinically sick poultry had acquired resistance against multiple antimicrobial agents including fluoroquinolones, indicating the widespread circulation of multi-drug resistant *E. coli* in poultry and poultry farm environment. The average prevalence of *E. coli* was 42% in

Table 5
Primers used to test tetracycline resistance genes.

Gene	Primer name	Primer sequence (5´– 3′)	Annealing (°C)	Fragment Size (bp)	Referen-ces
*tet*A	TetA-L	GGCGGTCTTCTTCATCATGC	64	502	Boerlin et al., 2005
	TetA-R	CGGCAGGCAGAGCAAGTAGA			
*tet*B	TetB-L	CATTAATAGGCGCATCGCTG	64	930	Boerlin et al., 2005
	TetB-R	TGAAGGTCATCGATAGCAGG			
*tet*C	TetC-L	GCTGTAGGCATAGGCTTGGT	64	888	Boerlin et al., 2005
	TetC-R	GCCGGAAGCGAGAAGAATCA			

trachea and oropharynx of poultry suffering from different clinical illnesses was almost similar to the earlier reports of [22] who reported a prevalence of 43.50%. The huge use of antibiotics by farmers occurs for therapeutic use to treat sick flocks; prophylactic use to prevent infections in the flock, and growth boosters to enhance feed utilization and production in poultry farms [23].

E coli isolates also exhibited as resistant to colistin (63.4%). Moreover, 31.7% of *E. coli* isolates from trachea of clinically sick poultry were sensitive to gentamicin. 43% *E. coli* isolates were resistant to ampicillin but no isolate was found to be resistant to gentamicin [13]. But in this study, 63.4% isolates were found to be resistant to gentamicin. So, the results of resistance to gentamicin in case of poultry isolates were not similar to Tricia study. Gentamicin is still a choice of medication in both human and veterinary practice in Bangladesh. But, to prevent the emergence of resistance in case of human pathogenic *E. coli*, the use of gentamicin should be restricted from the view of public health concerns. 80% strains which were resistant to tetracycline were also resistant to amoxycillin, and ciprofloxacin. Those findings were almost similar to this study as in this study 86.5% tetracycline-resistant strains were resistant to amoxycillin and ciprofloxacin [24]. The present research work demonstrated high frequency of resistance against tetracycline which was similar to another study, where high frequency of resistance was found not only against tetracycline but also against kanamycin, and pefloxacin [25]. In this study, we detected 82.9% resistance of the *E. coli* isolates against amoxycillin. Reports on resistance of *E. coli* of poultry origin to amoxycillin seem scanty in Bangladesh but indiscriminate use of it is the reason for creating this problem.

We recorded a high resistance pattern of *E. coli* isolates against ciprofloxacin (90%), which was dissimilar to the study conducted on broiler chickens [26]. More use of ciprofloxacin in poultry sector in Bangladesh may be the reason.

The detection of tetA gene in all the *E. coli* isolates exhibiting resistance to oxytetracycline suggests that tetA might be responsible for developing resistance to oxytetracycline where the contributions of other two genes, tetB, and tetC might be little. However, this result was almost similar to Das [27] who showed that 35% *E. coli* isolates harbored the tetA gene showing resistance to oxytetracycline. There were 16 reported tet genes. The possibility of other tet genes acquired by the strains investigated could not be ruled out, but it seems, most avian pathogenic *E. coli* (APEC) that are circulating in Bangladesh and are causing respiratory tract infections are resistant to oxytetracycline because of the commonest possession of the tetA gene and sometimes in combination with few other tet genes. Only one variable "use of no antibiotics" appeared to be a risk factor in finding a higher frequency of *E. coli* isolation from oropharyngeal swab samples.

Implications

Antimicrobial resistance is the global threat of the twenty-first century. The study reveals the isolation of *Escherichia coli* from tracheal and oropharyngeal swabs of clinically sick chickens and the antimicrobial resistance pattern of the strains Isolated. People and doctors can be aware of choosing and using antibiotics against *E. coli*.

Ethics approval

Our experimental animals (broiler chickens) were collected after the post-mortem of diseased birds from the hospital. In our entire research, there was no use of any live birds. So, there was no need for any approval by the institute's ethics committee before conducting the research. However, This study complies with all regulations.

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Author contributions

PKB conceived and planned the experiments. TF carried out the experiments, MSH and YNFM planned and carried out the simulations. SF contributed to sample preparation. PKB and TF contributed to the interpretation of the results. TF, SKN, and MS took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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