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SHORT COMMUNICATION

Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh

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ABSTRACT

Objective: The present study was carried out to appraise the antibiotic resistance and to detect some of the target resistant genes in *Escherichia coli* (*E. coli*) isolated from apparently healthy broilers.

Materials and Methods: Cloacal swab samples (n = 60) were collected from apparently healthy broilers (n = 60) sold at two different live bird markets (LBMs) of Chattogram, Bangladesh. Isolation and identification of the *Escherichia coli* was done by the following standard bacteriological techniques followed by biochemical tests. The antibiotic susceptibility of *E. coli* isolates was determined by the disk diffusion method. The antibiotic resistant genes were detected by polymerase chain reaction (PCR) using specific primers.

Results: The overall prevalence of *E. coli* in broilers was 61.67% (n = 37/60) (95% CI = 49–72.93). The antibiogram study showed that the isolates were 100% resistant to ampicillin and tetracycline followed by sulfomethoxazole-trimethoprim (94.59%, n = 35/37) and nalidixic acid (91.89%, n = 34/37). To the contrary, 56.76% (n = 21/37) isolates were sensitive to both ceftriaxone and gentamicin followed by colistin (48.65%, n = 18/37). All of *E. coli* isolates were multidrug resistant (MDR) and carried bla_{TEM}, *tet*A, and *Sul*2 genes.

Conclusion: The presence of MDR genes in *E. coli* isolates in broilers could pose a serious public health threat.

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Introduction

Bangladesh, a developing country, having an area of 147,570 square km. is struggling to improve its infrastructure, especially in agricultural sector. The poultry industry in Bangladesh is considered as an important sub-sector in economic growth and creating employment opportunities [1]. However, common bacterial pathogen like *Escherichia coli* in poultry can exert detrimental effect on human health owing to specific toxicity, drug allergy, and evolvement of multidrug resistant (MDR) strains of bacteria. Most of the *E. coli* isolates are nonpathogenic, considered as a fecal contamination indicator in foods and only 10%–15% serotypes are pathogenic [2].

Food-producing animals and its associated products have been recognized as a reservoir of foodborne bacteria having resistant genes [3]. However, isolation rate of antimicrobial resistant bacteria in food animals has been increased worldwide [4,5].

The antimicrobial resistance (AMR) patterns of indicator bacteria can be used in pathogenic bacteria to obtain

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the information on antimicrobial resistance trends [6]. The emergence of MDR strains have been increased alarmingly in the past 20 years [7]. *Escherichia coli* attain the resistance genes due to selective pressure, induction, or mutation [8]. Genes responsible for the AMR of bacteria can be transmitted horizontally and vertically to other bacteria and can enter the human food chain.

In Asian countries, live bird markets (LBMs) are considered as the most important terminal figure of the poultry industry, as where people prefer to buy freshly slaughtered or live poultry [9]. Birds are continuingly introduced in LBMs from different sources and collected from other areas that caged at high densities. These conditions provoke an optimal environment for amplification and persistence of environmental bacteria has been rendered as a reservoir of AMR genes in different ecological niches [10]. Moreover, consumers at LBMs are in direct and close contact with live or freshly slaughtered poultry. Unitedly, these conditions of LBMs are exacerbated the risk of transmission of AMR bacteria in human food chain. AMR in low-income and middle-income countries like Bangladesh and all over the world is becoming a major health threat day by day. Scientists, physicians, as well as politicians are anxious about the alarming problem of AMR as it leads to the treatment failure with antimicrobial drugs. Therefore, in this study, we have looked into the prevalence and AMR with some corresponding resistant genes of *E. coli* isolates collected from broilers sold at two LBMs in Chattogram, Bangladesh.

Materials and Methods

Samples collection

Swab samples were collected aseptically from cloaca of apparently healthy broiler chicken from two live bird markets (LBMs) of Chattogram, Bangladesh, namely, Jhawtola LBM (n = 30) and Pahartoli LBM (n = 30) during the period January to February 2016. Sample was placed into a sterile screw capped falcon tube containing buffered peptone water (BPW) (Oxoid, UK). The samples were carried into an ice box to the Poultry Research and Training Centre, Chattogram Veterinary and Animal Sciences University (CVASU).

Isolation and Identification of E. coli

The BPW containing samples were overnight incubated at 37°C for enrichment. The culture was then streaked onto MacConkey agar (Oxoid, UK), and incubated at 37°C for 18–24 h. Large Target colonies were transferred to Eosin Methylene Blue (EMB) agar (Merck, India) media and incubated at 37°C for 24 h. The biochemical tests including Voges-Proskauer (VP), Methy Red (MR), and indole production tests were performed to confirm *E. coli* [11]. The bacteria were preserved with 15% glycerol at –80°C until use.

Antimicrobial susceptibility testing

Disk diffusion method was performed to assess the antibiotic susceptibility of all the *E. coli* isolates using Mueller-Hinton agar (Oxoid, UK) plate according to the guidelines and recommendations of CLSI [12]. Ampicillin (10 µg), Tetracycline (30 µg), Ceftriaxone (30 µg), Trimethoprim-sulfomathoxazole (25 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Colistin (10 µg), Nalidixic acid (30 µg), Ciprofloxacin (5 µg), and Erythromycin (15 µg) (HiMedia, India) were used for antibiotic susceptibility test. The results were interpreted using the guideline of CLSI [12].

Extraction of chromosomal DNA

Total DNA from the bacteria was extracted by simple boiling method [13] with slight modifications. In brief, two to three freshly cultured bacterial colonies were taken into 1.5-ml sterile Eppendorf tube having 200 µl of deionized water. The mixture was thoroughly vortexed, boiled for 15 min, centrifuged at 15,000 rpm for 2 min. The collected supernatant was used as a DNA template.

Amplification of antibiotic resistant genes

To amplify antibiotic resistant genes in *E. coli* isolates, PCR reactions were performed in a Thermocycler (2720 Thermal cycler, Applied Biosystems, USA) using total volume of 25 µl containing 12.5 µl dream Taq PCR master mix (Thermo Scientific, USA), 0.5 µl of each primer, 1 µl template DNA, and 10.5 µl deionized water. The primers used in the study are listed in Table 1. PCR conditions for bla_{TEM} gene: an initial denaturation of 94°C for 4 min followed by 30

Table 1. Primers used to identify antibiotic resistant genes, bla_{TEM}, *tet*A, and *Sul* 2.

Target genes	Primers sequence (5′-3′)	Amplicon size	References
bla_{TEM}	F: TACGATACGGGAGGGCTTAC R: TTCCTGTTTTTGCTCACCCA	716-bp	Belaaouaj et al. [14]
*tet*A	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210-bp	Karczmarczyk et al. [15]
Sul 2	F: CGGCATCGTCAACATAACCT R: TGTGCGGATGAAGTCAGCTC	721-bp	Lanz et al. [16]

cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, elongation at 72°C for 1 min with a final step of 72°C for 7 min; for *tet*A gene: 35 cycles with initial temperature at 95°C for 4 min, denaturation at 95°C for 1 min, annealing at 64°C for 1 min, elongation at 72°C for 1 min, and final extension at 72°C for 7 min; and for *Sul*2: initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 59°C and elongation for 1 min at 72°C, and 7 min of extension at 72°C. PCR products were then electrophoresed in 1.5% agarose gels stained with ethidium bromide (0.5 µg/ml) (Sigma-Aldrich, USA) and visualized under an ultraviolet transilluminator (BDA digital, Biometra GmbH, Germany).

Data analysis

Data were managed and analyzed into a spreadsheet of Microsoft Excel 2010 and transferred to QuickCalcs Graphpad software (https://www.graphpad.com/quickcalcs/) for data summary and descriptive statistics.

Results

A total of 60 cloacal swab samples were examined. The overall prevalence of *E. coli* was 61.67% (*n* = 37/60) (95% CI 49–72.93) (Table 2). *Escherichia coli* produced bright pink colonies on MacConkey agar and characteristic green colonies with metallic sheen on EMB agar. The isolated bacteria were positive to MR and indole production but negative to VP test.

All of the tested isolates of *E. coli* showed 100% (*n* = 37/37) resistance to ampicillin and tetracycline. Resistance to trimethoprim-sulfomathoxazole and nalidixic acid were 94.59% (*n* = 35/37) and 91.89% (*n* = 34/37), respectively. Results also dictated 56.76% (*n* = 21/37) of *E. coli* isolates were sensitive to both Ceftriaxone and gentamicin followed by colistin (48.65%, *n* = 18/21). Antimicrobial susceptibility to different antibiotics is illustrated in Figure 1. Antibiogram study of *E. coli* isolates unveiled that all were MDR. There were 20 different MDR patterns were identified in *E. coli* isolates (Table 3).

We have amplified some of the corresponding antibiotic resistance genes, namely, bla_{TEM} (Ampicillin resistance genes), *tet*A (Tetracycline resistance genes), and *Sul*2 (sulfur drug resistance gene). Of the 37 ampicillin and tetracycline resistant isolates, 28 gave positive amplicons for the bla_{TEM} gene (Fig. 2), whereas 15 isolates revealed *tet*A gene

Table 2. Prevalence of *E. coli* in two different LBMs.

Name of LBM	No. of sample examined	No. of positive	Prevalence of *E. coli* (%)	95% CI
Jhawtola	30	17	56.67	39.18–72.64
Pahartoli	30	20	66.67	48.68–80.87
Total	60	37	61.67	49–72.93

CI: Confidence Interval.

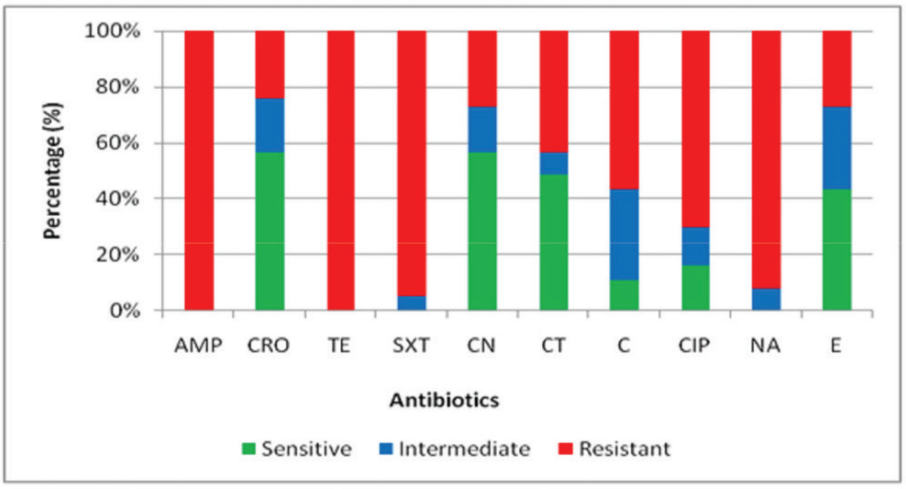

Figure 1. Antibiogram profile of *E. coli* isolates against different antibiotics.
AMP=Ampicillin, CRO=Ceftriaxone, TE=Tetracycline, SXT= Sulfomethoxazole-trimethoprim, CN=Gentamycin, CT=Colistin, C=Chloramphenicol, CIP=Ciprofloxacin, NA=Nalidixic acid, E=Erythromycin.

Table 3. Antibiotic resistance patterns in *E. coli* isolates (*n* = 37).

Antibiotic resistant patterns	No. of isolates
AMP-TE-SXT-NA	4
AMP-TE-SXT-CIP	1
AMP-TE-C-CIP	1
AMP-TE-SXT-CIP-NA	4
AMP-TE-SXT-C-CIP	1
AMP-TE-SXT-C-NA	2
AMP-TE-SXT-NA-E	1
AMP-TE-SXT-C-CIP-NA	4
AMP-TE-SXT-CT-C-NA	1
AMP-TE-SXT-CT-CIP-NA	3
AMP-TE-SXT-CN-CT-C-NA	1
AMP-TE-SXT-CN-C-CIP-NA	1
AMP-TE-SXT-CT-CIP-NA-E	1
AMP-TE-SXT-CT-C-CIP-NA	3
AMP-CRO-TE-SXT-CN-C-CIP-NA	1
AMP-CRO-TE-CN-C-CIP-NA-E	1
AMP-CRO-TE-SXT-CN-CT-C-NA-E	2
AMP-CRO-TE-SXT-CT-C-CIP-NA-E	1
AMP-CRO-TE-SXT-CN-CT-CIP-NA-E	2
AMP-CRO-TE-SXT-CN-CT-C-CIP-NA-E	2

AMP=Ampicillin, CRO=Ceftriaxone, TE=Tetracycline, SXT= Sulfomethoxazole-trimethoprim, CN=Gentamycin, CT=Colistin, C=Chloramphenicol, CIP=Ciprofloxacin, NA=Nalidixic acid, E=Erythromycin

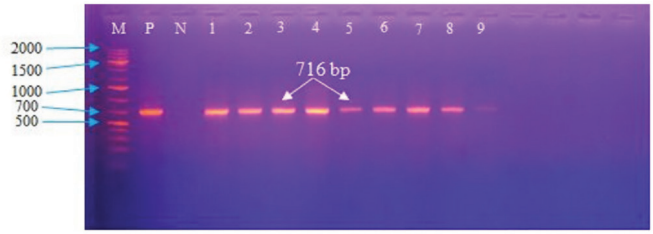

Figure 2. Amplification of bla_{TEM} gene (716-bp). (Lane M: 2 kb ladder; lane P: positive control; lane N: negative control; lane 1-8: positive for bla_{TEM} gene).

(Fig. 3). Out of 35 trimethoprim-sulfomathoxazole resistant isolates, 13 isolates exposed *Sul*2 gene (Fig. 4).

Discussion

In this study, the overall prevalence of *E. coli* in broilers from two LBMs were recorded as 61.67%, which is almost similar with the finding of Hossain et al. [17] who reported the prevalence was 63.6%, whereas Jakaria et al. [18] found 82% prevalence and Bashar et al. [19] found 100% prevalence of *E. coli* in poultry. In our study, all the

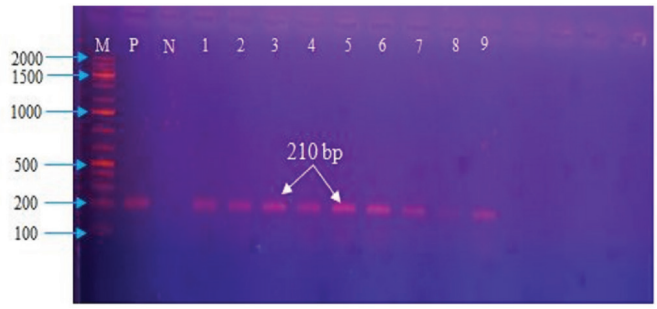

Figure 3. Amplification of *tet*A gene (210-bp). (Lane M: 2 kb ladder; lane P: positive control; lane N: negative control; lane 1-7, 9: positive for *tet*A gene).

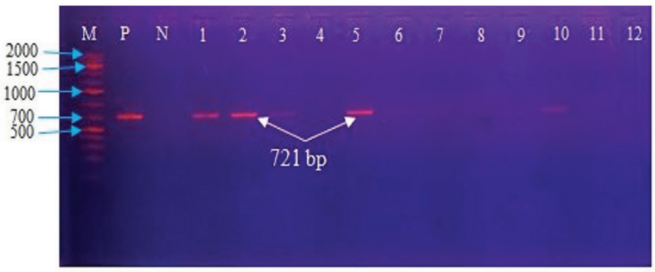

Figure 4. Amplification of *Sul* 2 gene (721-bp). (Lane M: 2 kb ladder; lane P: positive control; lane N: negative control; lane 1, 2, 5: positive for *Sul* 2 gene).

tested isolates were 100% resistant to ampicillin and tetracycline followed by trimethoprim-sulfomathoxazole (94.59%) and nalidixic acid (91.89%). A similar result was reported by Azad et al. [20], who observed 100% resistance in *E. coli* isolates to ampicillin, tetracycline and trimethoprim-sulfamethoxazole isolated from broiler colacal swab samples in Rajshahi area, Bangladesh. In contrast to our study, Hossain et al. [17] reported 100% resistant to nalidixic acid and 62.85% to ampicillin from Bangladesh. Although, these findings are extremely high but not surprising due to the use of these antimicrobials for long term in veterinary practice. Additionally, antibiotics from the same class are responsible for the cross resistance, also contribute to the high resistance rates. Tetracycline and sulfamethoxazole are common antibiotics prescribed for poultry treatment and commonly used with feed additives and sometimes as a growth promoter. Most of the time, poultry farmers use these medicines without concern of the veterinarian.

Development of bacterial resistance to fluoroquinolone like ciprofloxacin is a global issue. Most of the cases, this development of resistance is related to the extensive use of this antibiotic in food animal practice. Currently, ciprofloxacin are extensively used in poultry industries which have been introduced in poultry for the treatment during

the last decade in Bangladesh. This widespread occurrence of this resistance is striking since it could complicate the therapy in clinical infections both in poultry and humans.

One of the major findings of the study is to bacterial resistance against colistin (43.24%), which is striking and worrying. Colistin, the last resort drug, is being used extensively in agriculture and veterinary medicine [21], but its use in human medicine is restricted because of nephrotoxicity and neurotoxicity. The present study revealed that ceftriaxone, gentamicin, and erythromycin were sensitive to 56.76%, 56.76%, and 43.24% isolates, respectively, while Azad et al. [20] reported 36% to gentamicin and 100% to erythromycin.

Our study represented that 100% of the isolates were MDR. Similar findings were reported on MDR in *E. coli* isolates from Bangladesh and different parts of the world [19,20,22]. Due to the indiscriminate victimization of antimicrobial agents, MDR strains may apparently be occurred with high incidence [23]. However, the findings of the MDR patterns of this study will help for the choosing of drugs for the veterinarians to practice in the poultry farms level.

A number of different resistance genes, namely, bla_{TEM}, *tet*A, and *Sul*2 were detected among *E. coli* resistant isolates. Adelowo et al. [24] and Messaili et al. [25] reported presence of bla_{TEM}, *tet*A and *Sul*2 genes in *E. coli* isolates from their respective study. Compared to the previous report, the antimicrobial resistance genes in *E. coli* from poultry is quite variable. It may depend on the geographical distribution and usage of antimicrobials in each area.

Conclusion

The study disclosed the presence of MDR and existence of bla_{TEM}, *tet*A, and *Sul*2 resistant genes in cloacal samples of broilers at LBMs, Chattogram in Bangladesh. The AMR surveillance in animals, knowledge on AMR of veterinarian and livestock practitioner, implementation of authoritative guidelines and regulations are urgently needed to control the antimicrobial use to prevent the AMR progress in near future.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contribution

MSS and AA designed the study. MSS and MB collected samples from LBMs. MSS and ZBB were involved in laboratory works, data analysis, and manuscript writing. AA, MSM, and MYA critically reviewed the manuscript. All the authors read and approved for publication.

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