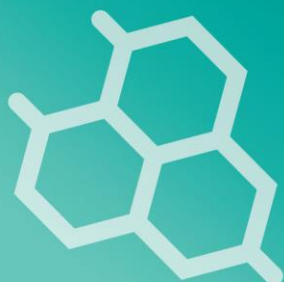


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## MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE OF *Escherichia coli* ISOLATED FROM RETAIL CHICKEN MEAT

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### ABSTRACT

This study was conducted to investigate the prevalence of *E. coli* in retail chicken meat and to determine the drug resistance profile of *E. coli* in Dinajpur district, Bangladesh. A total of 38 chicken meat samples were collected from different markets of Dinajpur city. *E. coli* were isolated and identified by colony characteristics on selective agar like Eosine-methylene blue (EMB) agar, Gram staining, biochemical test and Polymerase Chain Reaction (PCR). Universal Primers (16SrRNA) were used for molecular characterization of *E. coli* during PCR. The amplified size of PCR product was 1000 bp and after NCBI BLAST of the sequence which was obtained by Sanger sequencing method was mostly matched (98%) to *Escherichia coli* IAI39. The overall prevalence of *E. coli* in chicken meat was 60.5%. Antibiotic sensitivity test showed that *E. coli* isolated from chicken meat were resistant to amoxicillin (91.4%), erythromycin (73.9%), and susceptible to Ciprofloxacin(82.6%), Gentamicin (78.2%) and Azithromycin (60.8%). One of the major findings of the study was that 43.5% isolated *E. coli* were resistant against colistin (one of the last-resort antibiotics). The higher prevalence of *E. coli* in chicken meat indicated unhygienic production and processing of these meat samples. Presence of multi-drug resistant *E. coli* in these chicken meat samples might pose serious public health threats. The antibiogram profile of the isolated *E. coli* will help therapeutic decision making in the treatment of colibacillosis in Bangladesh.

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## 1. INTRODUCTION

*Escherichia coli* is one of the common microbial flora of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both (Jawetz *et al.*, 1984). Although most isolates of *E. coli* are nonpathogenic but they are considered as indicator of faecal contamination in food and about 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes (Barnes and Gross, 1997).

Antimicrobial resistance, which is caused mainly by the imprudent use of antimicrobial agents (Okeke *et al.*, 1999), (Zowalaty *et al.*, 2015), is becoming an increasing global concern in animals and humans. Due to the magnitude of the threat, the World Health Organization (WHO) recommended global surveillance programs in animal and human populations.

Due to enormous exploitation of antibiotics in the field of veterinary medicine, an increased number of resistant bacterial strains were developed in recent years. Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections caused by *E. coli*, which are a major source of illness, death, and increased healthcare costs (Gupta *et al.*, 2001).

Food is an important factor for the transfer of antimicrobial resistance. Such transfer can occur by means of residues of antibiotics in food like poultry meat (Jhonson *et al.*, 2007) because, in the process of food production many kinds of antimicrobials are used for preventing and controlling diseases, enhancing growth and increasing feed efficiency in food producing animals.

In Asian countries, live bird markets (LBMs) are considered as the most important terminal figure of the poultry industry, where people prefer to buy freshly slaughtered or live poultry (Li B *et al.*, 2017). Birds are introduced in LBMs from different sources and collected from other areas that caged at high densities. These conditions provoke an optimal environment for amplification of bacteria and persistence of environmental bacteria has been rendered as a reservoir of AMR (antimicrobial resistance) genes in different ecological niches (Dantas G *et al.*, 2008). Scientists, physicians, as well as politicians are anxious about the alarming problem of AMR as it leads to the treatment failure with antimicrobial drugs. Genes responsible for the AMR of bacteria can be transmitted horizontally and vertically to other bacteria and can enter the human food chain. The antimicrobial resistance patterns of indicator bacteria can be used in pathogenic bacteria to obtain the information on antimicrobial resistance trends (Koo HJ *et al.*, 2012). *Escherichia coli* attain the resistance genes due to selective pressure, induction, or mutation (Shaikh *et al.*, 2015).

The present study was designed to isolate *E. coli* from retail chicken meat for assessing their susceptibility and resistance patterns to some selected antimicrobials.

## 2. MATERIALS AND METHODS

A total of 38 poultry meat samples were collected from five (5) live bird markets of Dinajpur district (Basherhat bazar, Suihari bazar, Gopalgong bazar, Bot-tola bazar, Labur mor bazar) of Bangladesh and were carried in an ice box containing ice to the microbiology laboratory of the department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur and processed for the isolation and characterization of *E. coli*.

### 2.1 Isolation and identification of *E.coli*

The processed samples were cultured onto the nutrient agar and then sub-cultured on the MacConkey agar. The desired colonies were selected from MacConkey agar and further sub-cultured on Eosin Methylene Blue agar (EMB agar). Plates were incubated in the incubator for 18-24 hours at 37°C for

bacterial growth. *E.coli* were identified and confirmed according to colony characteristics on the agar plate (Carter, GR. 1979).

Gram's staining method was followed to study the morphological and staining characteristics bacteria and to provide information about the presumptive bacterial identification as per recommendation of Merchant and Packer (1967).

Isolated organisms with supporting growth characteristics were identified by biochemical test. Several types of biochemical tests were performed in this study to confirm the specific bacteria such as Sugar fermentation test, Oxidase test, Catalase test, Indole test, Methyl Red (MR) Test, Voges-proskauer (VP) test, Simmons's citrate, Triple Sugar Iron (TSI) agar, Motility Indole Urease (MIU) test.

## **2.2 Molecular characterization**

### **2.2.1 DNA Extraction**

The pure cultures were grown overnight in nutrient broth and then were used to isolate the DNA. The Wizard® Genomic DNA Purification Kit was used to obtain genomic DNA from the potential experimental bacterial isolate. 1 ml from the overnight broth culture was added to a 1.5 ml micro centrifuge tube. Then it was centrifuged at 13,500 RPM for 2 minutes to separate the cells. The bacterial pellet was resuspended, and DNA was extracted according to the protocol provided with the Wizard® Genomic DNA Purification Kit. The DNA was stored at -20°C until use.

### **2.2.2 Polymerase chain reaction**

PCR was performed to amplify 16S rRNA gene of *E. coli* according to the methods described by Schippa et al. (2010). Two different primers pairs were used for this purpose, 16S rRNA gene (27F: AGA GTT TGA TCM TGG CTC AG and the sequence of the reverse primer is 1492 R: CGG TTA CCT TGT TAC GAC TT). Each 20 µl reaction mixture consists of 3 µl genomic DNA, 10 µl PCR master mixtures (M7431), 1 µl of each of the two primers, 1 µl of TDNA with the final volume adjusted to 20 µl with 7µl of nuclease free water. Amplification was done by initial pre heating at 95°C for 3 minutes, followed by denaturation at 95°C for 30 sec, annealing temperature of primers was 48°C for 30 sec and extension at 72°C for 90 sec. The final extension was conducted at 72°C for 5 minutes and then holding 4°C overnight. The total reaction was performed at 35 cycles. The amplified PCR products were resolved by electrophoresis in 1% agarose gel at 80v for 40 minutes. For comparison, a known band size called ladder was also added to the gel base to interpret specific results. The PCR products were loaded into the gel with 6x loading dye before starting the run. The results were observed using a UV trans illuminator.

### **2.2.3 Sequencing and BLAST analysis**

For sequencing, the dideoxy chain termination method (Sanger and Coulson method) was used (Chen *et al.*, 2015) by Genetic Analyzer 3,130 (Applied Biosystem Inc). Amplified PCR product was used with the single 16S rRNA 27F Forward primer: 27F: AGA GTT TGA TCM TGG CTC AG). The obtained Sequence was analysed with Genbank database {<http://www.ncbi.nlm.nih.gov/>. 16S Ribosomal RNA Sequences (Bacteria and Archaea)}, (Chen *et al.*, 2015) by NCBI BLAST analysis to identify the organism.

## **2.3 Antibiotic sensitivity test**

Antibiotic sensitivity test was performed according to the procedure Kirby-bauer disk diffusion susceptibility test protocol. Drug resistant of *E. coli* were detected by disc diffusion method using 9 commonly used antibiotics on Mueller-Hinton agar plate (**Fig-5**). Isolates were classified as susceptible, intermediate and resistant categories based on the standard according to the Clinical and Laboratory Standards Institution (CLSI, 2018).

### 3. RESULTS

Out of 38 retail poultry meat samples *E. coli* were found from only 23 samples. The isolates were identified as *E. coli* on the basis of their cultural properties, morphological properties and biochemical characteristics. In EMB agar colonies produced by *E. coli* were smooth, circular, black color colonies with metallic sheen (**Fig- 1**). The microscopic examination of Gram's stained smears from MacConkey agar and EMB showed that the isolated bacteria were Gram negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain (**Fig-2**).

The overall prevalence of *E. coli* in retail chicken meat was 60.5% (n=23/38). Prevalence of *E. coli* at five markets of Dinajpur namely Basherhat, Suihari, Gopalgong, Bot-tola and Laburmor was 50.5%, 62.5%, 71.4%, 57.4% and 62.5% respectively. The highest percentage of *E. coli* (71.4%) was observed at Gopalgong bazar and the lowest percentage of *E. coli* (50.5%) was observed at Basherhat bazar in Dinajpur.

Molecular characterization of *E. coli* isolated from chicken meat sample was performed by polymerase chain reaction (PCR), nucleotide sequencing and BLAST analysis. The PCR assay was able to amplify 1000 bp fragment of the targeted gene from the genomic DNA of *E. coli* successfully (**fig-3**). Sequence obtained from Sanger sequencing method was blasted in NCBI to identify the organism (**Fig-4**). After BLAST obtained sequence was mostly matched (98%) to *Escherichia coli* IAI39.

A total of 23 *E. coli* isolates were subjected to antimicrobial susceptibility testing. All of the isolates of *E. coli* were resistant in varying degrees to the used 8 different antimicrobial agents (Amoxicillin, Erythromycin, Azithromycin, Gentamicin, Doxycycline, Streptomycin, Colistin, Tetracycline). Most of the isolates were resistant to Amoxicillin (91.4%), Erythromycin (73.9%), Colistin (43.5%) and Doxycycline. (47.8%). Almost all of the isolates of *E. coli* showed their highest sensitivity to Ciprofloxacin (82.6%), Gentamycin (78.2%), Azithromycin (60.8%) (**Table-1**).

### 4. DISCUSSIONS

In this study, the overall prevalence of *E. coli* in retail chicken meat was 60.5%. The result indicated a high prevalence of *E. coli* in chicken meat which suggests that the production and processing of these foods were not hygienic. The farmers and people involved in every stage of food production and processing should be educated about food hygiene. Almost similar finding was also reported by Rahman *et al.* (2020) who reported 63.5% prevalence and Samun *et al.* (2019) who reported 61.67% prevalence. Whereas Ranjbar *et al.* (2017), Moawad *et al.* (2017) and Younis *et al.* (2017) showed lower prevalence of *E. coli* in raw chicken. In contrast other research groups detected high frequency of *E. coli* in poultry meat (Rashid, M. *et al.* 2013), (Park *et al.* 2015). Hussain *et al.* (2017) in India found that 78% of broiler chicken meat specimens from retail shops were contaminated with *E. coli*.

The colony characteristics of *E. coli* observed in MacConkey, EMB agar were similar to the findings of Sharada *et al.* (1999). In gram staining isolated bacteria showed pink color small rod shaped organism which was also reported by others Sharada *et al.* (1999) and Merchant and Packer *et al.* (1967). The *E. coli* isolates revealed a complete fermentation of 5 basic sugars by producing both acid and gas which was supported by Thomas (1998). The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test (Honda *et al.*, 1982).

Universal Primers (16SrRNA) were used for molecular characterization of *E. coli* during PCR. The amplified size of PCR product was 1000 bp and after NCBI BLAST the sequence which was obtained by Sanger sequencing method mostly matched to *Escherichia coli* IAI39.

According to our study, all isolates of *E. coli* were resistant in varying degrees to the used 8 different antimicrobial agents (Amoxicillin, Erythromycin, Azithromycin, Gentamicin, Doxycycline, Streptomycin, Colistin, Tetracycline). Most of the isolates were sensitive to Ciprofloxacin ((82.6%), Gentamicin (78.2%), Azithromycin (60.8%) and were resistant to Amoxicillin ((91.4%), Erythromycin (73.9%) and Doxycycline (47.8%). Some of the isolates were multidrug resistant. The results strengthen the earlier observations of Akond *et al.* (2009) and Islam *et al.* (2004). Our findings were more less similar to the findings of Hossain *et al.* (2008) and Azad *et al.* (2019).

One of the major findings of the study was bacterial resistance against Colistin (43.5%), which is striking and worrying because it is one of the last resort antibiotics. However, From the present study Ciprofloxacin were proved to be the best antibiotics to treat *E. coli* infection since they were highly effective. The results agreed with the investigations of Islam *et al.* (2004) and Ozaki *et al.* (2011) who also obtained similar resistant patterns of *E. coli* isolated from broiler.

## 5. CONCLUSIONS

The study revealed the fact that raw chicken meat from retails outlets was heavily contaminated with *E. coli* pathogens. It was also found that the raw chicken meat could act as a reservoir of antibiotic resistant *E. coli*, which can be transferred to humans there by causing gastrointestinal disorders and food borne illness which can be life threatening. It is imperative that basic hygienic practice should be incorporated in abattoirs and retail meat outlets to ensure food safety. Training should be given to meat handlers and butchers regarding food safety practice and proper inspection procedures should strictly be adhered to minimize the contamination of raw chicken meat and meat products sold at the market places.

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None to disclose.

## Conflict of interests

The authors declare no conflict of interest.

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TABLES

Table no-1: Result of Antibiotic sensitivity test of *E. coli* isolated from retail chicken meat.

Antimicrobial agent	No. (%) of isolates (23)		
	S	I	R
Amoxicillin	0 (0.0%)	2 (8.6%)	21(91.4%)
Erythromycin	0 (0.0%)	6 (26.1%)	17 (73.9%)
Azithromycin	14 (60.8%)	6 (26.1%)	3 (13.1%)
Gentamicin	18 (78.2%)	4 (17.3%)	1 (4.3%)
Doxycycline	2 (8.6%)	9 (39.1%)	11(47.8%)
Streptomycin	6 (26.1%)	13 (56.5%)	4 (17.3%)
Ciprofloxacin	19 (82.6%)	4 (17.3%)	0 (0.0%)
Colistin	2 (8.6%)	11(47.8%)	10 (43.5%)
Tetracycline	12 (52.1%)	7 (30.4%)	4 (17.3%)

Legends: S=susceptible; I=intermediate; R=Resistant.

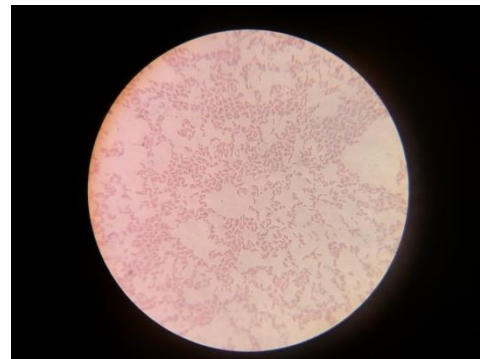


Fig-1: Growth of *E. coli* in EMB Agar

Fig-2: Microscopic examination of *E. coli* in gram staining

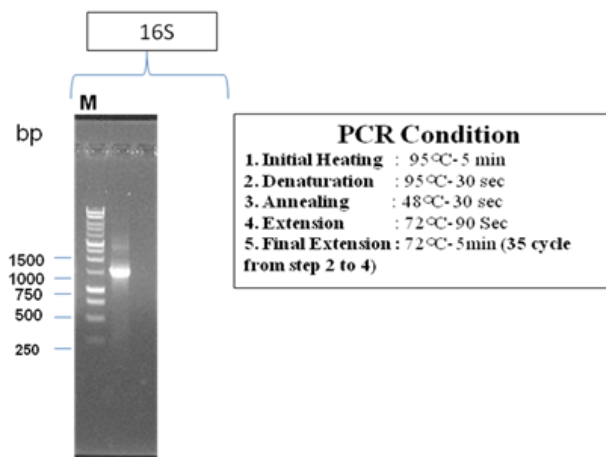


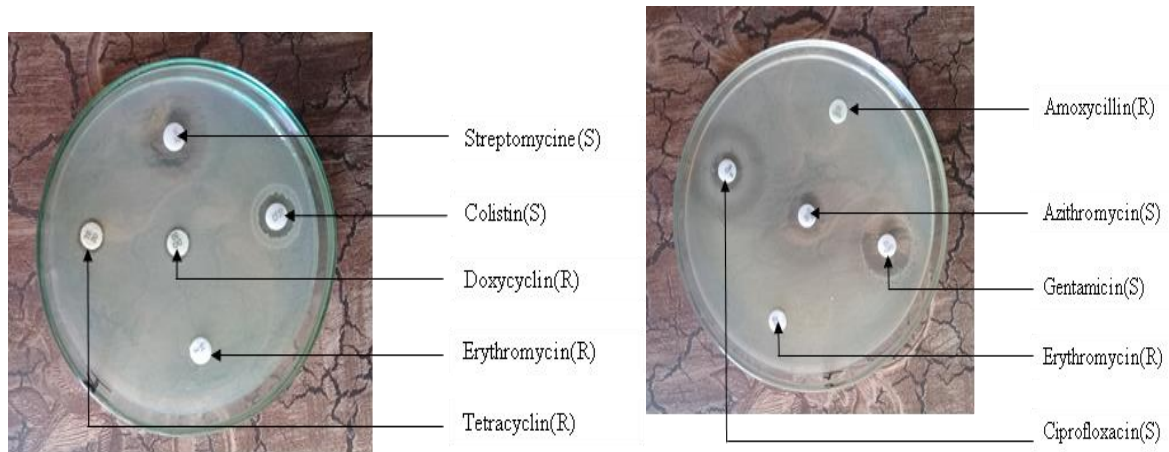
Fig-3: Test samples of *E. coli* showed band at 1000 bp (agarose gel electrophoresis).

AAGGTACCAGTCTAATAATAGCAAGCTCGAACGTGTAACACGGAATAAGCTTGCTTCTTTGCTGACGAGTGGCG  
 GACGGGTGAGTAATGTCTGGGAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATA  
 ACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCCTTTGCCATCGGATGTGCCAGATGGGATTAGCTAGTAG

GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAGTGA  
CACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC  
CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTG  
CTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAA  
GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTAAAGTCAGATGTGAAATCCCCGGGCTCA  
ACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGA  
AATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCATGGACGAAGACTGACGCTCAGGTGCGAA  
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCGACTTGGAGGTTGTGCC  
CTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCTAGGTTAAAACTCA  
AATGAATTGACGGGGGCCAGCACAAAGCG

**Fig- 4: Nucleotide sequence data**

(Contig- 1465bp, blast: 98% similar Identified strain: Escherichia coli IAI39)



**Fig-5: Antibiotic sensitivity test for E. coli on Mueller-Hinton agar media**



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