

## Isolation, identification, and antibiogram studies of *Salmonella* species and *Escherichia coli* from boiler meat in some selected areas of Bangladesh

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### ABSTRACT

**Background:** The present study was carried out for the isolation, identification of *Salmonella* and *Escherichia coli* from broiler meat samples (leg muscle, breast muscle and drumstick) which were collected from different upazilla markets of Mymensingh, Gazipur, and Sherpur districts during the period of January 2015 to May 2015.

**Methods:** A total of 60 samples were subjected to bacterial isolation and identification by using cultural, biochemical, and polymerase chain reaction assays.

**Results:** Using standard bacteriological techniques *E. coli* was isolated from 50 (83.33%) samples and *Salmonella* spp. from 18 (31.66%) samples. Furthermore, the isolates were subjected to antibiogram studies by disk diffusion method using eight commonly used antibiotics. Antibiogram studies revealed that gentamicin, ciprofloxacin, and norfloxacin were highly sensitive against all the isolated bacteria, whereas most of the isolates were resistant to amoxicillin, erythromycin, and tetracycline. Out of all the isolates, 5 isolates of *E. coli* and 3 isolates of *Salmonella* were found multidrug resistant.

**Conclusions:** The study revealed the presence of multidrug resistant *Salmonella* and *E. coli* in broiler meat sold in live bird market of different upazilla.

**Keywords:** Broiler meat, *Salmonella* spp., *Escherichia coli*, Antibiogram

### INTRODUCTION

Foodborne diseases and poisoning are the widespread and great public health concerns of the modern world. Both developed and developing countries are largely affected by foodborne infections. Foodborne diseases not only affect people's health and well-being but also have an economic impact on individuals and the countries<sup>1</sup> while the impact

in case of developing countries like Bangladesh is higher. It reduces markedly social and economic productivity of the countries.<sup>2</sup> Because of the relatively high frequency of contamination of poultry with pathogenic bacteria, raw products are responsible for a significant number of cases of human food poisoning. Contamination of poultry meat during processing, handling, marketing, and storage prior to cooking, can lead to food poisoning illness in humans.

Bangladesh is an agriculture based country. As such poultry rearing is considered superior to the others in the agricultural sector because of an almost assured in a relatively short period of time. Poultry meat substantially contributes to the human diet.<sup>3</sup> In Bangladesh, broiler meat is an important and low-cost source of animal protein. This encourages the consumption of broiler meat by the large of consumers. Various pathogenic microbes such as *Salmonella* and *Escherichia coli* have been implicated to reduce the growth of broiler. The modern poultry industry can produce market ready broiler chickens in <6 weeks through genetic selection, improved feeding and keen health management practices including usage of antibiotics as therapeutic agents to treat bacterial diseases in intensive farming systems.<sup>4</sup> Resistance against frequently used antibiotics has been observed in bacteria present in poultry since the introduction of these antimicrobial agents in poultry. The rise in antibiotic resistance has been reported in the past two decades in many countries including Bangladesh.<sup>5</sup> Therefore, this study was designed to isolate and identify the associated bacteria prevalent in broiler meat and to find out the effective antibiotics against the bacteria through antibiogram studies.

## METHODS

### Collection and transportation of samples

A total of 60 dressed broiler carcasses were collected during the period of January 2015 to May 2015 and immediately brought to Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh through maintaining cool chain using ice box. After that samples were processed immediately for the isolation and identification of *Salmonella* and *E. coli*.

### Isolation of associated bacteria

For the isolation of bacteria 10 g of meat (thigh muscle, breast muscle, and drumstick) were taken in a sterile pack and allowed to prepare meat homogenates by adding 90 ml of 0.1% peptone water using stomacher blender. The primary culture was performed in nutrient agar and nutrient broth media. For sub-culturing, suspected bacteria were inoculated separately onto different bacteriological media under the aseptic condition and incubated at 37°C for 24 hrs. Pure cultures were achieved by further sub culturing on selective agar.<sup>6,7</sup>

### Identification of associated bacteria

Cultural, morphological, and biochemical characteristics were studied to identify the bacterial flora. The cultural characteristics or colonial morphology of the bacteria grown on the eosin methylene blue (EMB) and xylose lysine deoxycholate (XLD) agar were recorded. Gram staining was performed to study the morphology and staining characteristics of the bacteria.<sup>8</sup> Biochemical tests, such as sugar fermentation, methyl red (MR), voges-proskauer (VP), and indole tests, were performed to identify the bacteria tentatively.<sup>9</sup>

### Molecular characterization by polymerase chain reaction (PCR)

Bacterial DNA template was prepared by using boiling method.<sup>10</sup> All the samples were examined by two pairs of primers (Table 1) to detect 16S rRNA gene of *E. coli* and histidine transport operon gene of *Salmonella* spp. Thermal profiles used in PCR are discussed (Tables 2 and 3). PCR products were separated on 2% agarose gel, stained with ethidium bromide and photographed using a Gel documentation system (BioRad).

### Antibiotic sensitivity test

Antibiotic susceptibility test was performed by disk diffusion method using the commercial antibiotic disk on Mullar-Hinton agar to assess the susceptibility and resistance pattern of the isolates. For this purpose, eight different antibiotic discs were obtained from commercial sources (Himedia, India and Oxoid Ltd. England). The selected antibiotics used were ciprofloxacin (5 µg/disc), azithromycin (30 µg/disc), amoxicillin (30 µg/disc), gentamicin (10 µg/disc), norfloxacin (10 µg/disc), erythromycin (30 µg/disc), streptomycin (10 µg/disc), and tetracycline (30 µg/disc). The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2012) formerly known as NCCLS.

## RESULTS

After 2 hrs culture in nutrient broth, the clear transparent broth were changed to turbid, which indicates bacterial growth. EMB agar plates streaked with the organism and incubated at 37°C for 24 hrs. The growth of *E. coli* was indicated smooth circular, greenish black color colonies with

**Table 1: Primers used in this study.**

Primers	Sequence (5'-3')	Target gene	Amplicon size (bp)	Reference
ECO-1	GACCTCGGTTTAGTTCACAGA	16S rRNA	585	30
ECO-2	CACACGCTGACGCTGACCA			
Upper strand	ACTGGCGTTATCCCTTTCTCTGGTG	Histidine transport	496	19
Lower strand	ATGTTGTCCTGCCCTGGTAAGAGA	Operon gene		

**Table 2: Thermal profiles used to amplify 16S rRNA gene in *E. coli*.**

PCR condition	Temperature (°C)	Time	Cycle
Initial denaturation	95	5 mins	1
Denaturation	94	45 sec	
Annealing	52	45 sec	30
Extension	72	1 min	
Final extension	72	5 mins	1

*E. coli*: *Escherichia coli*, PCR: Polymerase chain reaction

**Table 3: Thermal profiles used to amplify genus-specific *Salmonella*.**

PCR condition	Temperature (°C)	Time	Cycle
Initial denaturation	94	5 mins	1
Denaturation	94	30 sec	
Annealing	56	30 sec	30
Extension	72	45 sec	
Final extension	72	5 mins	1

PCR: Polymerase chain reaction

**Table 4: Summary of isolated bacteria from broiler meat of different upazilla markets.**

District (number of samples)	Number of isolates	
Mymensingh (30)	<i>E. coli</i> (25)	<i>Salmonella</i> spp.(9)
Gazipur (15)	<i>E. coli</i> (12)	<i>Salmonella</i> spp.(5)
Sherpur (15)	<i>E. coli</i> (13)	<i>Salmonella</i> spp.(4)

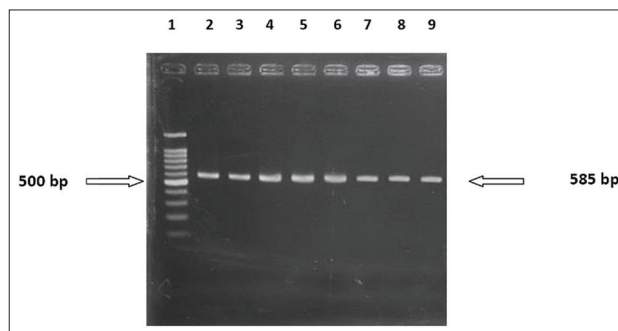
*E. coli*: *Escherichia coli*

a metallic sheen. XLD agar plates streaked with the organism and incubated at 37°C for 24 hrs aerobically, and the growth of *Salmonella* was indicated by smooth, circular, and black centered colonies. A series of biochemical test especially selective for *Salmonella* and *E. coli* were performed. *E. coli* can ferment all the five basic sugars (dextrose, sucrose, lactose, maltose, and mannitol) and produce acid and gas. *E. coli* also showed the positive reaction in MR and Indole test but negative to VP reaction. Furthermore *Salmonella* ferment three basic sugars (dextrose, maltose, and mannitol) but does not ferment sucrose and lactose and *Salmonella* showed a positive reaction to MR and negative to Indole, VP reaction.

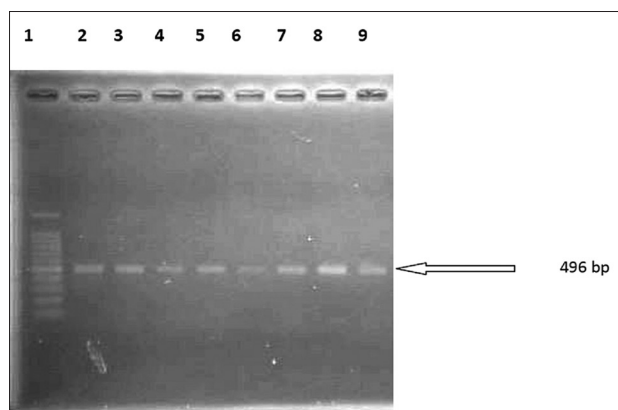
All the isolates of *E. coli* were positive to 16S rRNA gene amplification, and histidine transport operon gene amplification were positive for all *Salmonella* isolates (Figures 1 and 2). *E. coli* was most prevalent organism in all samples comparing to *Salmonella* (Table 4).

**Antibiogram studies**

Based on the susceptibility to antibiotics, the bacteria were categorized into three group's viz. sensitive, intermediate,



**Figure 1: Polymerase chain reaction of *E. coli*: Lane 1: 100 bp DNA ladder, Lane 2: positive control. Lane 3-9: tested sample, positive for 16S rRNA gene.**



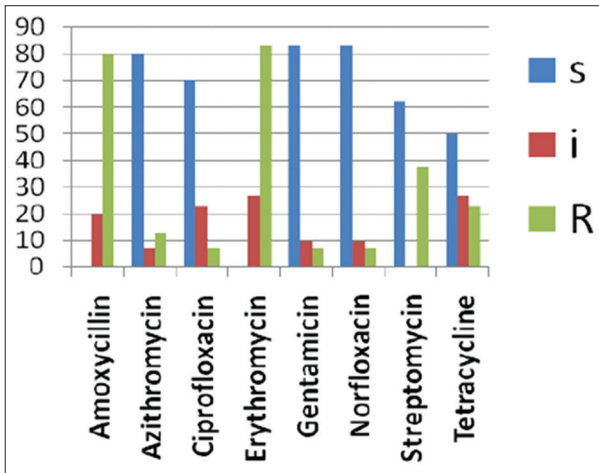
**Figure 2: Polymerase chain reaction of *Salmonella* Lane 1: 100 bp DNA ladder, Lane 2: positive control, Lane 3-9: Tested sample, Positive for histidine transport operon gene.**

and resistance. Out of eight antibiotics used this study, ciprofloxacin, gentamicin, streptomycin, and norfloxacin were found to be sensitive to all isolates of *Salmonella* and *E. coli*, whereas amoxicillin and erythromycin were resistant against most of the isolates. The antibiotic sensitivity patterns have been summarized in Figures 3 and 4.

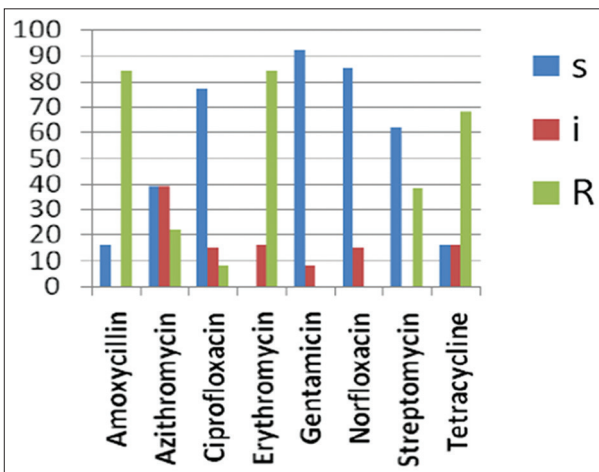
**DISCUSSION**

In the recent study, *E. coli* and *Salmonella* were isolated from broiler.<sup>11,12</sup> Considering all the 60 samples *E. coli* was isolated from 50 (83.33%) samples. These findings is supported with some of the previous study<sup>13,14</sup> where described the prevalence of 51% in the broiler. In this study, the prevalence of *Salmonella* was 30% in broiler.<sup>15-17</sup>

Isolates of *E. coli* observed in EMB agar revealed smooth, circular, greenish black color colonies with metallic sheen and pink color colonies on McConkey agar.<sup>18-21</sup> In Gram's staining, the morphology of the isolated bacteria exhibited Gram-negative, short rod arranged in single or paired.<sup>22,23</sup> The *Salmonella* revealed smooth, circular, black centered colonies on XLD agar.<sup>24,25</sup> Morphology of the *Salmonella* exhibited Gram-negative, arranged in single or pair and motile.<sup>26,27</sup>



**Figure 3: Antibigram profiles of *Escherichia coli* (S: Sensitive, I: Intermediate, R: Resistance). X axis: Name of antibiotics, Y axis: (%).**



**Figure 4: Antibigram profiles of *Salmonella* (S: Sensitive, I: Intermediate, R: Resistance). X axis: Name of antibiotics, Y axis: (%).**

For the confirmation of the presence of *E. coli* in the samples ECO-1 and ECO-2 primer was used in the PCR to amplify the 16S rRNA gene of *E. coli*<sup>30</sup> and for the amplification of histidine transport operon gene to confirm *Salmonella* spp. Upper strand and Lower strand primer was used.<sup>19</sup>

In the present study, it was found that the *E. coli* isolated from broiler were sensitive to ciprofloxacin and gentamicin. The results strengthen the earlier observations of some study.<sup>5,34,43</sup> Resistance of *E. coli* was observed against erythromycin, amoxicillin.<sup>5,32,34,43</sup> The possession of such factors by the *E. coli* isolates signify the fact that the organisms might have gained the resistance property due to the indiscriminate use of antibiotics. The occurrence of isolated bacteria should be considered as hazardous to health and advocate the preventing risk factors. However, in the present study, ciprofloxacin were proved to be the best antibiotics to treat *E. coli* infection since they were highly effective.<sup>43,44</sup>

It was revealed that *Salmonella* spp. were sensitive to ciprofloxacin, gentamicin and azithromycin<sup>28,29</sup> and resistant to erythromycin, and amoxicillin.<sup>30-32</sup>

**CONCLUSION**

*E. coli* and *Salmonella* spp. were isolated from the meat samples of broilers collected from different upazilla live bird market of Mymensingh, Gazipur, and Sherpur districts in Bangladesh. Prudent use of antibiotics should be considered in broiler production (where permissible) since many strains are resistant to common antibiotics, and some were multidrug resistant as described in this study. Potential drug resistant pathogens in otherwise normal broilers may be a serious public health concern. Current findings warrants further studies with the isolated strains of bacteria.

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