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Molecular detection and characterization of *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. isolated from broiler meat in Jamalpur, Tangail, Netrokona and Kishoreganj districts of Bangladesh

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The study was conducted to isolate, identify and characterize bacterial samples from broiler meat collected from 20 different upazilla markets of Jamalpur, Tangail, Netrokona and Kishoreganj districts of Bangladesh. A total of 20 samples were subjected to bacteriological isolation and identification, and the isolated bacteria were subjected to antimicrobial susceptibility testing using disc diffusion method. Among the samples, 75% (n=15) were contaminated with *Campylobacter* spp., 70% (n=14) were with *Salmonella* species and 85% (n=17) were contaminated with *Escherichia coli*. The *Campylobacter* spp., *Salmonella* spp. and *E. coli* were isolated and identified by culturing on Blood agar, Xylose-Lysine Deoxycholate (XLD) agar, and MacConky and eosin methylene blue (EMB) agar respectively. Isolates of *Campylobacter* spp., *Salmonella* spp. and *E. coli* were biochemically analyzed. *Campylobacter* specific *16S rRNA genes* were amplified from the isolates. *Campylobacter* spp. and *E. coli* isolates were positive to *16S rRNA gene* based polymerase chain reaction (PCR). Almost all isolates of *Campylobacter* spp., *Salmonella* spp. and *E. coli* showed highest susceptibility to ciprofloxacin, norfloxacin and gentamicin. However, most isolates were resistant to amoxicillin and erythromycin. Some isolates showed susceptibility to tetracycline, streptomycin and azithromycin. The findings of this study revealed that there is presence of multidrug resistant isolates of *Campylobacter* spp., *Salmonella* spp. and *E. coli* in broiler meat. Results of this research project demonstrated the high level of microbial contamination and occurrence of pathogenic bacteria in broiler meat sold in markets of Bangladesh.

Key words: Broiler meat, *Escherichia coli*, *Salmonella, Campylobacter*, molecular detection, characterization.

INTRODUCTION

Poultry industry which has started during 1980s is an excellent agribusiness (Haque, 2001) now in Bangladesh. Over the last decades surprising development has occurred in this sector (Rahman, 2003). It has become a

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vital sector for generating employment, creating additional income and improving the nutritional level of the country. Broiler meat is more popular to the consumers because of its easy digestibility and acceptance by the majority of people, although it could be contaminated with various potential food borne pathogens such as *Salmonella, Campylobacter, Escherichia coli* (Mulder et al., 1999). Broiler entering slaughter processing is highly contaminated by microorganisms such as *Salmonella* and *Campylobacter* and tends to be disseminated during processing (Mead et al., 1994).

Particular concern for human health is the inappropriate use of antibiotics in poultry production and the development of antibiotic resistant strains of bacteria (Sarker et al., 2018). Effective control systems are critical in ensuring product safety, and considerable information is available on how to minimize the risks (FAO, 2013). Various pathogenic microbes, such as *E. coli, Salmonella* spp. and *Staphylococcus* have been implicated to reduce the growth of poultry including duck.

Campylobacter is one of the most important pathogen and is regarded major bacterial cause of human gastroenteritis worldwide. Food animals, mainly poultry, cattle, sheep and pigs, may act as asymptomatic intestinal carriers of *Campylobacter* and animal food products can become contaminated by this pathogen during slaughter and carcass dressing (Berndtson et al., 1996). The abusive use of antimicrobials in food animals has resulted in the emergence and dissemination of antimicrobial resistant bacteria, including antimicrobial resistant *Campylobacter*, which has potentially serious impact on human health. Moreover, *Campylobacter* infections pose a serious public health problem for which many countries have monitored their infection and antimicrobial resistance patterns (Kabir et al., 2014). *E. coli* frequently cause bacterial infections including urinary tract infection, cholangitis, bacterimia and traveler's diarrhea. Enteropathogenic *E. coli* (EPEC) are an important cause of diarrhea in humans (Savkovic et al*.,* 2005). Haemolytic uramic syndrome caused by Shiga toxin producing *E. coli* (STEC) is dependent on release of Shiga toxin during intestinal infection and subsequent absorption into the blood stream. Poultry meat can be contaminated with *E. coli* during processing. Any food that has been in contact with raw meat can also be contaminated. The bacteria also spread from person to person, usually when infected person does not wash his hands well after using a toilet. *E. coli* cause different types of public health hazards including cholangitis, bacteremia, traveler's diarrhea, Shiga toxicity etc. (Savkovic et al*.,* 2005).

Salmonella spp. is potentially responsible for various pathogenic processes in man and animal including poultry (Freeman, 1985). It can cause diarrhea, vomition, fever, abdominal cramps in human. Sometimes severe diarrhea requires medical interventions such as intravenous fluid therapy. In cases, where bacteria enter into the bloodstream, symptoms include high fever, malaise, pain in the thorax and abdomen, chills and anorexia (Bell, 2002).

Antibiotics are extensively used in poultry industry either as growth promoters or to control infectious diseases (Sarker et al., 2018). Concern about antibiotic resistance and its transmission to human is important because these resistant bacteria may colonize the human gastrointestinal tract and may contribute to the development of resistance genes to human through R-factor, conjugative plasmids or chromosomal elements as reviewed by Kabir (2010). Therefore, the disease causing microbes that have become resistant to antibiotic drug therapy are increasing public health importance.

Undoubtedly, the poultry slaughtered and dressed under Bangladesh conditions carry extremely high initial contamination loading from the point of slaughtering process to the point at which the consumers are offered the product. There occurs biomagnifications at all levels of handling, poor transport and retailing conditions. Therefore, considering the present perspective, the present research project was designed to detect and characterize the bacteria specifically *Campylobacter* spp*. Salmonella* spp. and *E. coli* and their antimicrobial resistance patterns in broiler meat.

MATERIALS AND METHODS

Collection and transportation of samples

A total of 20 apparently healthy dressed broilers were collected randomly from 20 different upazilla live bird markets of Jamalpur, Tangail, Kishoreganj and Netrokona district of Bangladesh. Five upazilla were selected randomly from each district. After collection, samples were immediately brought to Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh through maintaining cool chain using ice box.

Bacterial culture media

Solid and liquid culture media were used to isolate the bacteria. Blood agar (BA), MacConkey (MC), Salmonella-Shigella (SS), Eosin Methylene Blue (EMB), Xylose-Lysine Deoxycholate (XLD), Mueller Hinton agars were used as solid culture media for this study. The liquid media used in the study were nutrient broth (NB), peptone broth, methyl-red and voges-proskauer broth (MR-VP), and sugar media, 1% hippurate solution, 3.5% ninhydrin solution, oxidase solution and sugar media.

Isolation and identification of bacteria

Pure culture of *E. coli* and *Salmonella* spp. were obtained as per the methods described by Krieg et al. (1994). Briefly; 10 g of samples were homogenized with 90 ml of 0.1% peptone water and 50 µl of homogenized sample was poured on to selective agar media and spread with glass spreader and incubated at 37°C for 24 h. Isolation of *Campylobacter* spp. was carried out by filtration method (0.45 µm filter) according to Shiramaru et al. (2012). The

Table 1. List of primers used in this study.

Primer	Sequence (5'-3')	Target	Amplicon size (bp)	References
16S9F 16S154OR	GAGTTTGATCCTGGCTC AAGGAGGTGATCCAGCC	*Campylobacter* 16S rRNA gene	1530	Samosornsuk et al. (2007)
Upper strand Lower strand	ACTGGCGTTATCCCTCTGGTG ATGTTGTCCTGCCCCTGGTAAGAGA	Histidine transport Operon gene	496	Cohen et al. (1993)
ECO-1 ECO-2	GACCATCGGTTTAGTTCACAGA CACACGCTGACGCTGACCA	*E. coli* 16S rRNA gene	585	Schippa et al. (2010)

collected samples were allowed to prepare meat homogenates and then 50 µl of meat homogenates were spread on the filter papers that were placed on the surface of Blood base agar no.2 and allowed to stand for 30 min at room temperature, after 30 min filter papers were removed from the BA and the plates were incubated at 37°C for 48 h in microaerobic condition (5% O_2, 10% CO_2 and 85% N_2). The colonies of primary cultures were repeatedly sub-cultured by streak plate method (Cheesbrough, 1985) until the pure cultures with homogenous colonies appeared. The representative bacterial colonies were characterized morphologically using Gram's stain according to the method describe by Merchant and Packer (1967). Biochemical characterizations of the *E. coli* and *Salmonella* isolates were performed with Sugar fermentation test, Methyl Red test (MR), Voges-Proskauer test (V-P) and indole test (Cheesbrough, 1985). Differentiation of isolated *Campylobacter* spp. with supporting growth characteristics were subjected to various biochemical tests such as catalase, oxidase and hippurate hydrolysis test according to the procedures followed by Nachamkin (2003) and Foster et al. (2004).

Molecular identification by polymerase chain reaction (PCR)

DNA template was prepared by boiling method as described by Rawool et al. (2007). All the samples were examined by two pairs of primers (Table 1) to detect 16S rRNA gene of *Campylobacter* spp*., E. coli* and Histidine transport operon gene of *Salmonella* spp*.* For *Campylobacter* spp*.* the PCR reactions were carried out using a thermocycler (ASTEC, Japan) with the following programme: initial denaturation for 5 minutes at 94°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 47°C for 30 s and extension at 72°C for 1 min and 30 s. The final extension was conducted at 72°C for 10 min. For *E. coli*, the PCR reactions were carried out using a thermocycler (ASTEC, Japan) with the following programme: Initial denaturation for 5 min at 95°C followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s and extension at 72°C for 1 min. The final extension was conducted at 72°C for 5 min. For Histidine transport operon gene identification in *Salmonella* spp*.* initial denaturation for 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 45 s. The final extension was conducted at 72°C for 5 min. 1 and 2% agarose (Invitrogen, USA) gel was used for electrophoresis of the PCR products.

Antibiotic sensitivity test

All isolates randomly selected from the three genera were tested for antimicrobial drug susceptibility against eight commonly used antibiotics by disc diffusion method according to the guidelines of Clinical and Laboratory Standard Institute (CLSI), 2012*.* 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, 2007) (Table 1).

RESULTS

Isolation of bacteria from poultry meat

A total of 20 samples were collected for isolation of bacteria from broiler meat of different upazilla markets of four districts where three types of bacteria were isolated from the collected meat samples. The isolates were identified as *E. coli, Salmonella* spp. and *Campylobacter* spp. on the basis of their morphological, cultural properties, biochemical characteristics with standard reference organisms and molecular methods. Among the isolated bacteria, *E. coli* were detected in 85% (n=17), *Salmonella* and *Campylobacter* in 70% (n=14) and 75% (n=15), respectively of the samples. The highest percentage of *E. coli* (100%) isolates was observed in Jamalpur and Tangail district. *Salmonella* isolates were higher (80%) in samples of Jamalpur, Tangail and Kishoreganj. Furthermore, *Campylobacter* isolates were 100% in samples of Jamalpur District.

Molecular detection of bacteria

Screening of *E. coli* isolates from meat sample in this study was performed by genus specific (16S rRNA gene) polymerase chain reaction (PCR). The PCR assay was able to amplify 585 bp fragment of the targeted gene from the genomic DNA of *E. coli* successfully (Figure 1). Specific 496 bp fragment of targeted histidine transport operon gene of *Salmonella* was amplified successfully (Figure 2). Genus specific (*16S rRNA* gene) PCR was performed for *Campylobacter* spp*.* 1530 bp fragment of

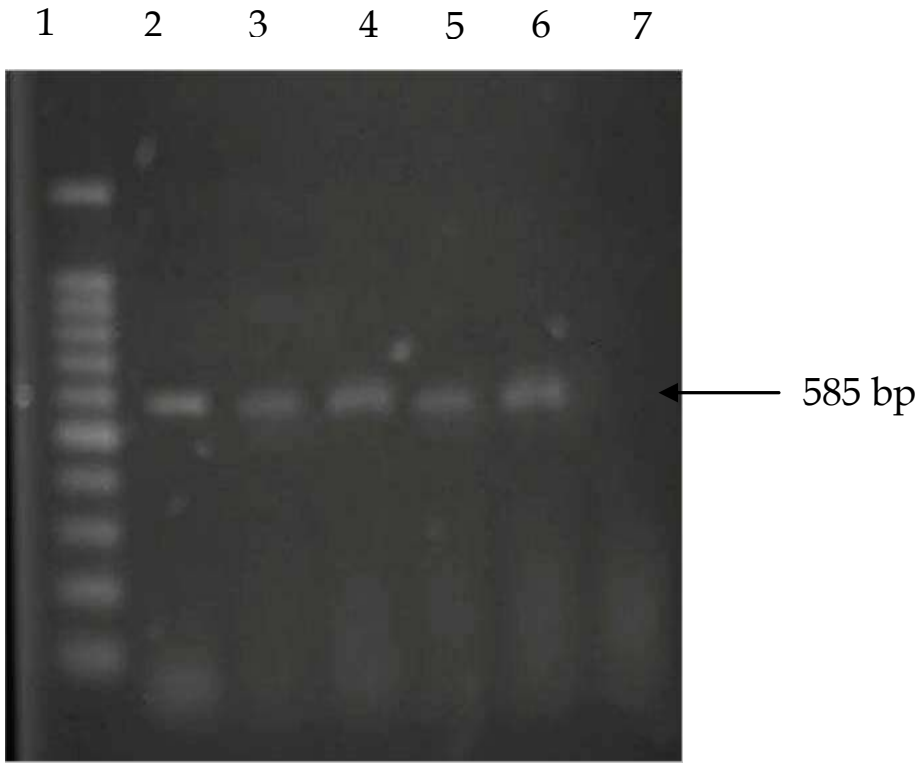

Figure 1. PCR targeting *16s rRNA* gene for identification of *E. coli.* Lane 1: 100 bp DNA marker, lane 2, 3, 4, 5, 6: DNA of *E. coli,* lane 7: negative control without DNA.

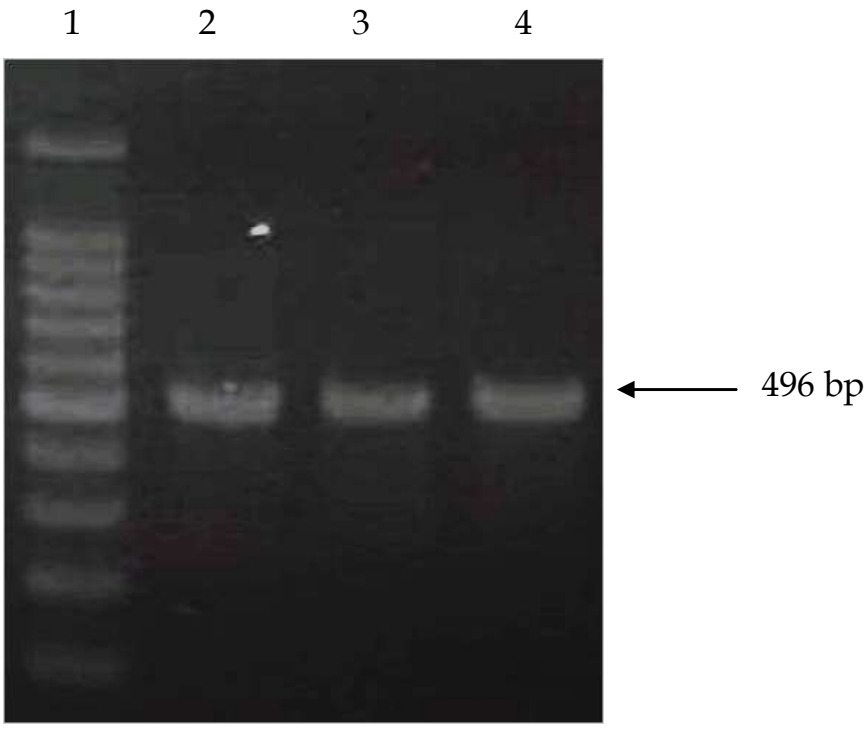

Figure 2. Detection of *Salmonella* spp. by histidine transport operon gene based PCR. Lane 1: 100 bp DNA marker, lane 2, 3, 4: DNA of *Salmonella.*

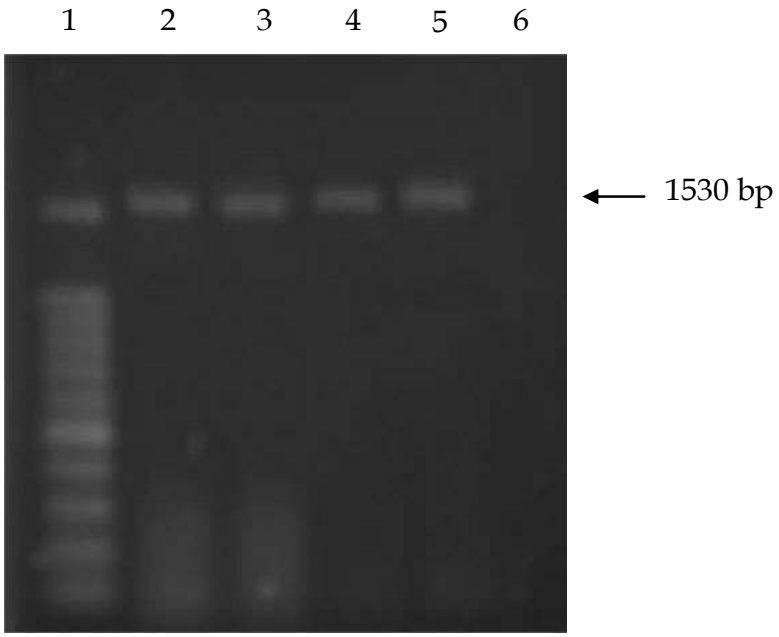

Figure 3. Detection of *Campylobacter* spp. by *16S rRNA* gene based PCR. Lane 1: 100 bp DNA marker, lane 2, 3, 4, 5: DNA of *Campylobacter* spp. and lane 6: negative control.

targeted gene was amplified successfully. The result of PCR is presented in Figure 3.

Antimicrobial susceptibility of isolated *E. coli*, *Salmonella* and *Campylobacter* spp.

A total of 17 *E. coli* isolates were subjected to antimicrobial susceptibility testing. Most of the isolates were resistant to erythromycin (70.58%) and amoxicillin (88.24%) and few isolates were intermediate. Almost all the isolates of *E. coli* showed their highest sensitivity to gentamicin (76.47%), norfloxacin (82.35%), ciprofloxacin (82.35%) and azithromycin (64.70%). Some isolates of *E. coli* were resistant and some were sensitive to tetracycline. The result is presented in Table 2. A total of 14 *Salmonella* isolates were subjected to antimicrobial susceptibility testing against eight selected antibiotics. Almost all isolates of *Salmonella* spp. were resistant to tetracycline (85.71%) and erythromycin (64.28%) whereas most of the isolates of *Salmonella* spp. were susceptible to gentamicin (92.85%), norfloxacin (78.57%) and ciprofloxacin (78.57%) respectively. 5 (35.71%) were sensitive to streptomycin and 10 (71.42%) isolates were intermediate to amoxicillin. Results are presented in Table 2.

The result of antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* are summarized in Table 2. Out of 11 *C. jejuni* isolates, 10 (90.90%) isolates were susceptible to gentamicin, 9 (81.81%) were sensitive to norfloxacin, 8 (72.72%) were sensitive to ciprofloxacin and 7 (63.63%) were susceptible to streptomycin. Most of the isolates were resistant to amoxicillin (81.81%) and 9 (81.81%) isolates were intermediate resistant to erythromycin. On the other hand, out of four *C. coli* isolates, all (100%) were sensitive to both ciprofloxacin and norfloxacin. 3 (75%) were sensitive to gentamicin, and 4 (100%) were resistant to amoxicillin, 2 were resistant to streptomycin.

Antimicrobial resistance pattern

Antimicrobial resistance pattern of E. coli

The results of the antimicrobial resistance pattern by disk diffusion method with 8 chosen antimicrobial agents are presented in Table 3. Out of 17 isolates, 6 (35.29%) were resistant to 1 antimicrobial agent. Each 1 (5.88%) was resistant to each of 2 antibiotics. Moreover, 2 (11.76%) were resistant to 2 antibiotics. Furthermore, each 1 (5.88%) was resistant to each of 3 antibiotics.

Antimicrobial resistance pattern of *Salmonella* spp.

The results of the antimicrobial resistance pattern by disk diffusion method with 8 chosen antimicrobial agents are presented in Table 4. Out of 14 isolates no resistance observed in 1 isolate. 1 (7.14%) was resistant to 1 antibiotics. Furthermore, each 2 (14.28%) were resistant to each of 2 antibiotics. Moreover, 1 (7.14%), 2 (14.28%),

Table 2. Antimicrobial susceptibility pattern of *E. coli*, *Salmonella* spp., *C. jejuni* and *C. coli* identified by disk diffusion method.

Antimicrobial agents		No. (%) of isolates		
		S	I	R
E. coli	Amoxicillin	0 (0.0%)	2 (11.76%)	15 (88.24%)
	Azithromycin	11(64.70%)	4 (23.52%)	2 (11.76%)
	Ciprofloxacin	14 (82.35%)	3 (17.64%)	0 (0.0%)
	Erythromycin	0 (0.0%)	5 (29.41%)	12 (70.58%)
	Gentamicin	13 (76.47%)	2 (11.76%)	2 (11.76%)
	Norfloxacin	14 (82.35%)	2 (11.76%)	1 (5.88%)
	Streptomycin	4 (23.52%)	10 (58.82%)	3 (17.64%)
	Tetracycline	9 (52.94%)	5 (29.41%)	3 (17.64%)
***Salmonella* spp.**	Amoxicillin	0 (0.0%)	10 (71.42%)	4 (28.57%)
	Azithromycin	3 (21.42%)	7 (50%)	4 (28.57%)
	Ciprofloxacin	11 (78.57%)	2 (14.28%)	1 (7.14%)
	Erythromycin	0 (0.0%)	5 (35.71%)	9 (64.28%)
	Gentamicin	13 (92.85%)	1 (7.14%)	0 (0.0%)
	Norfloxacin	11 (78.57%)	3 (21.42%)	0 (0.0%)
	Streptomycin	5 (35.71%)	2 (14.28%)	7 (50%)
	Tetracycline	0 (0.0%)	2 (14.28%)	12 (85.71%)
C. jejuni	Amoxicillin	0 (0.0%)	2 (18.18%)	9 (81.81%)
	Azithromycin	6 (54.54%)	3 (27.27%)	2 (18.18%)
	Ciprofloxacin	8 (72.72%)	2 (18.18%)	1 (9.09%)
	Erythromycin	0 (0.0%)	9 (81.81%)	2 (18.18%)
	Gentamicin	10 (90.90%)	1 (9.09%)	0 (0.0%)
	Norfloxacin	9 (81.81%)	1 (9.09%)	1 (9.09%)
	Streptomycin	7 (63.63%)	0 (0.00%)	4 (36.36%)
	Tetracycline	3 (27.27%)	2 (18.18%)	6 (54.54%)
C. coli	Amoxicillin	0 (0.0%)	0 (0.0%)	4 (100%)
	Azithromycin	2 (50.00%)	2 (50.00%)	0 (0.0%)
	Ciprofloxacin	4 (100%)	0 (0.0%)	0 (0.0%)
	Erythromycin	0 (0.0%)	3 (75.00%)	1 (25.00%)
	Gentamicin	3 (75.00%)	1 (25.00%)	0 (0.0%)
	Norfloxacin	4 (100%)	0 (0.0%)	0 (0.0%)
	Streptomycin	2 (50.00%)	0 (0.00%)	2 (50.00%)
	Tetracycline	0 (0.0%)	1 (25.00%)	3 (75.00%)

S= Susceptible; I= Intermediate; R= Resistant.

1 (7.14%) and 1 (7.14) were resistant to each of 3 antibiotics respectively. Furthermore, 1 (7.14%) and 1 (7.14%) were resistant to each of 4 antimicrobial agents. On the other hand, 1 was resistant to 5 antimicrobial agents.

Antimicrobial resistance pattern of *Campylobacter* spp.

The result of antimicrobial resistance patterns of *C. jejuni* and *C. coli* are summarized in Table 5. Out of 11 *C. jejuni* isolates, each 1 (9.09%) was resistant to each 1 antimicrobial agent. Furthermore, 3 (27.27%) and 1 (9.09%) were resistant to each of 2 antibiotics respectively. Moreover, 1 (9.09%) and 1 (9.09%) were resistant to each of 3 antibiotics. On the other hand, 1 (9.09%) and 1 (9.09%) were resistant to each of 3 and 4 antibiotics respectively. Out of 4 *C. coli* isolates, each 1 (25%) were resistant to each of 1 antibiotic. Furthermore, 2 (50%) were resistant to 3 antibiotics.

DISCUSSION

Meat products are important not only from nutritional

Table 3. Results of antimicrobial resistance pattern of *E. coli.*

Isolates	Resistance profiles	No. of isolates (%)
***E. coli* (n=17)**	No resistance demonstrated	-
	Resistant to 1 agent AMX	6 (35.29%)
	Resistant to 2 agent (AMX-NOR)	1 (5.88%)
	Resistant to 2 agent (S-TE)	1 (5.88%)
	Resistant to 2 agents (AMX- S)	1 (5.88%)
	Resistant to 2 agents (AMX-E)	2 (11.76%)
	Resistant to 3 agents (AMX-E-S)	1 (5.88%)
	Resistant to 3 agents (AMX-E-AZM)	1 (5.88%)
	Resistant to 3 agents (E-GEN-TE)	1 (5.88%)
	Resistant to 3 agents (AMX-E-TE)	1 (5.88%)
	Resistant to 3 agents (E-AMX-GEN)	1 (5.88%)
	Resistant to 3 agents (AMX-S-AZM)	1 (5.88%)
	Total resistant isolates	17 (100%)

Table 4. Results of antimicrobial resistance pattern of *Salmonella* spp.

Isolates	Resistance profiles	No. of isolates (%)
***Salmonella* spp. (n=14)**	No resistance demonstrated	1 (7.14%)
	Resistant to 1 agent TE	1 (7.14%)
	Resistant to 2 agent (E-TE)	2 (14.28%)
	Resistant to 2 agent (S-TE)	2 (14.28%)
	Resistant to 3 agents (E- AMX-TE)	1 (7.14%)
	Resistant to 3 agents (S-E-TE)	2 (14.28%)
	Resistant to 3 agents (AMX-AZM-TE)	1 (7.14%)
	Resistant to 3 agents (E-AZM-TE)	1 (7.14%)
	Resistant to 4 agents (AMX-E-S-TE)	1 (7.14%)
	Resistant to 4 agents (E-AZM-S-TE)	1 (7.14%)
	Resistant to 5 agents (AMX-S-E-CIP-AZM)	1 (7.14%)
	Total resistant isolates	14 (100%)

Table 5. Results of antimicrobial resistance pattern of *Campylobacter* spp.

Isolates	Resistance profiles	No. of isolates (%)
***C. jejuni* (n=11)**	No resistance demonstrated	-
	Resistant to 1 agent AMX	1 (9.09%)
	Resistant to 1 agent (E)	1(9.09%)
	Resistant to 1 agent (AZM)	1(9.09%)
	Resistant to 2 agents (AMX- TE)	3 (27.27%)
	Resistant to 2 agents (AMX-S)	1(9.09%)
	Resistant to 3 agents (AMX-S-TE)	1 (9.09%)
	Resistant to 3 agents (E-S-CIP)	1 (9.09%)
	Resistant to 4 agents (AMX-NOR-AZM-TE)	1 (9.09%)
	Resistant to 5 agents (AMX-S-E-AZM-TE)	1 (9.09%)
	Total resistant isolates	11 (100%)
***C. coli* (n=4)**	Resistant to 2 agent (AMX-TE)	1 (25%)
	Resistant to 2 agent (AMX-E)	1 (25%)
	Resistant to 3 agent (AMX-S-TE)	2 (50%)
	Total resistant isolates	4 (100%)

point of view, but also as an item of international trade and foreign exchange for a number of countries. However, they can also function as carriers of several microbial and other health hazards. The greatest risk to human health is due to the consumption of raw or contaminated meat and meat products.

In this study, *E. coli, Salmonella* and *Campylobacter* were found in the poultry meat, which was similar to the reports by Zhao et al. (2001), Ahmed et al. (2009), Awad-Alla et al. (2010), Torok et al. (2011), Voidarou et al. (2011), Sudershan et al. (2012), Malmuthuge et al. (2012) and Hossain et al. (2015). Several selective culture media were used simultaneously in this study to culture the organism. The media used in this study were selected considering the experience of the past researcher worked in various fields relevant to the present study by Kabir et al. (2014). The colony characteristics of *Campylobacter* spp. were gray color spreading colony which was supported by (Doyle, 1990; Rowe and Madden, 2000). In Gram's staining, the morphology of the isolated *Campylobacter* spp. was also supported by Doyle (1990). The colony characteristics of *E. coli* observed in Mac conkey, EMB agar were similar to the findings of Sharada et al. (1999). And in gram staining isolated bacteria showed pink color small rod shaped organism which was reported by others previously Sharada et al. (1999) and Merchant and Packer (1967). The colony morphology of *Salmonella* spp. was similar to that of Sarker et al. (2009) and Khan et al. (2005). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative small rod arranged in single or paired and motile which was supported by Sharada et al. (1999).

In catalase test, all the isolates (n = 15) produced bubbles those indicated positive for *Campylobacter.* In oxidase test, a purple color change was observed within ten seconds in all the isolates (n=15). In hippurate hydrolysis test, some of the isolates (n=4) develop a faint purple to no color change that indicated the isolates were *C. coli* and some of the test isolates (n=11) developed deep purple color that indicated the isolates were *C. jejuni.* These findings are similar to the findings of Kabir et al. (2014) and Jamshidi et al. (2008).

The *E. coli* isolates revealed a complete fermentation of 5 basic sugars by producing both acid and gas which was supported by Thomas (1998) and Beutin et al. (1991). The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test (Honda et al., 1982). The antimicrobial susceptibility of most of the isolates was sensitive to ciprofloxacin; gentamicin and all the isolates were resistant to amoxycillin. Some of the isolates were multidrug resistant which was similar to the result of Kabir et al. (2014). In this study, it was observed that the *E. coli* isolates were sensitive to ciprofloxacin, norfloxacin and gentamicin. The results strengthen the earlier observations of Akond et al. (2009) and Islam et al. (2004). Resistance of *E. coli* was observed against erythromycin, amoxycillin. The result was supported by Akond et al. (2009). The cause of such resistance by the *E. coli* might be for the fact that the organisms might have gained the resistance property due to the indiscriminate use of antibiotics.

The occurrence of isolation of bacterial pathogens from broiler 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. The results agreed with those reported by several investigations of Islam et al. (2004) and Ozaki et al. (2011) who also obtained similar resistant patterns of *E. coli* isolated from broiler. It was revealed that *Salmonella* spp. were sensitive to ciprofloxacin, gentamicin and norfloxacin. This result was supported by Jahan et al. (2013) and Khan et al. (2005) where isolates were sensitive to ciprofloxacin and chloramphenicol. The result is also consistent with Wouafo et al. (2010). The isolates were resistant to erythromycin, and amoxycillin which is similar to report of Hyeon et al. (2011) and Khan et al. (2005).

Conclusion

In this study, bacteria were isolated from only 20 samples of 20 different upazila of Bangladesh. The findings of the present study revealed the presence of multidrug resistant bacteria in broiler meat sold in markets of Bangladesh. Most of the isolates showed resistance to amoxycillin but sensitive to ciprofloxacin and gentamicin. Some isolates showed multidrug resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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