Bacteriological assessments of foodborne pathogens in poultry meat at different super shops in Dhaka, Bangladesh

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Abstract

Poultry is now considered as a major fast-growing source of meat in the world. The consumers demand safe and hygienic products without contamination with pathogenic microorganisms when the production and consumption of poultry meat is gradually increasing. The present study was conducted to assess the bacterial contamination of dressed chicken collected from different supershops in Dhaka, Bangladesh. The chicken samples from S_1, S_2, M1, M_2 and A supershops were analyzed to determine the enteropathogenic bacteria in poultry meat. Three genera of bacteria were isolated from all of the chicken meat samples. These enteropathogens from various organs of dressing chickens were also enumerated. The isolates were presumptively identified as *E. coli, Salmonella* spp*., and Shigella* spp. by conventional culture method. The three enteropathogens were subjected to PCR assay for their confirmation as virulent enteropathogens. Only *E. coli* isolates were confirmed as pathogenic *E. coli* (Enterotoxigenic), other isolates were not confirmed as virulent *Salmonella* spp*., Shigella* spp.. Results of this study demonstrated that more cautions are recommended for personnel hygiene in processing and handling of poultry and poultry products to prevent occurrence of enterotoxigenic *E. coli* in dressed poultry meat sold by the supershops in Bangladesh.

Introduction

During 1980s, poultry industry was started as an excellent agribusiness in Bangladesh (Haque, 2001) and a tremendous development of this sector has been occurred since last decades (1996-2006) in the country (Rahman, 2003). In the meantime, the sector has been a means of potential income generation and poverty alleviation, as well as improving human nutrition through the supply of meat and eggs to their daily life (BBS, 2008).

Though poultry meat and eggs provide nutritionally beneficial food containing protein of high quality, contamination of poultry meat and eggs can lead to food poisoning in humans through processing, handling, marketing and storage prior to cooking. The main causative agents of human intestinal infections from this source are bacteria, mainly *Salmonella* spp., *E. coli*, *Staphylococcus* spp. and *Campylobacter* spp. (FAO, 2013).

Escherichia coli is one of the common microbial flora that is found in the gastrointestinal tract of poultry and human being including other animals. It may become pathogenic to both poultry and human (Akond *et al*., 2009; Levine, 1987) although most isolates of *E. coli* are nonpathogenic. About 10 to 15% of intestinal coliforms are opportunistic, pathogenic serospecies (Akond *et al*., 2009) and responsible for a variety of lesions in immune-compromised hosts. They are associated with often severe diseases and sometimes with lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children (Akond *et al*., 2009). In addition, yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis are caused by *Escherichia coli* (Gross, 1994).

Poultry and poultry products are also an important reservoir of intestinal and foodborn pathogen like *Salmonella.* They are recognized as vital sources of *Salmonella* infection in human (Limawongpranee *et al*., 1999; Ocheni, 2015). Mostly, salmonellosis in human is caused by the consumption of contaminated poultry, pork, beef and eggs children (Akond *et al*., 2013).

According to the CDC Emerging Infections Program (CDC 2003), *Shigella* spp. was considered the third most reported food-borne bacterial pathogen in 2002 (Mokhtari *et al*., 2012) which are common especially with foods requiring processing or prepared by hand. These food-borne bacterial pathogens are found in foods when the foods are exposed to a limited heat treatment or served raw to the consumer (Wu *et al*., 2000). There is limited data on the prevalence of *Shigella* spp. amongst food handlers or on food products, though they cause shigellosis at high incident rate (Kapperud *et al*., 1995).

Generally, the people living in urban community of Bangladesh rely on the supershops for poultry meat. Alam *et al*., 2015 carried out an investigation on preprocessed raw chicken meat from different supershops of Dhaka city, Bangladesh where they identified *Shigella*, *Salmonella* in the chicken meat (Alam *et al*., 2015). Still, there are relatively few reports on food-borne microorganisms in chicken meat from the major supershops of Dhaka city. Therefore, this study was focused to determine the bacterial contamination in dressed poultry meat sold by main supershops in Bangladesh.

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Key words: Poultry meat, Prevalence, Foodborne pathogens, Super Shop.

Acknowledgements: the auhtors would like to thanks all laboratory staffs, Department of Microbiology, Jahangirnagar University to provide kind support during this research work.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Received for publication: 22 March 2018.
Revision received: 16 January 2019.
Accepted for publication: 17 January 2019.

Italian Journal of Food Safety 2019; 8:6720
doi:10.4081/ijfs.2019.6720

Materials and Methods

Sample collection and transportation

Dressed chicken samples (*Gallus gallus*) were collected from five renowned super shops including S_1, S_2, M_1, M_2, A of Dhaka, Bangladesh. The super shops were visited two times during the investigation. All the chicken samples were kept at -20ºC for a maximum of 7 days in the shop. Five chicken samples were taken, two of which were from the supershop S_1, S_2 and other two were from M_1, M_2 respectively. The other sample was taken from supershop A. The different kind of samples (skin, wings,

leg and chest) were collected from all the chicken carcasses to determine the presence of three enteric pathogens such as *Escherichia coli, Salmonella* spp., and S*higella* spp. Samplings were carried out aseptically during the collection of chicken sample. After collection, all the samples were transported to the laboratory immediately in an insulated box with ice to avoid any change in the quality of sample due to microbial action.

Sample processing

Skin, wings, leg and chest portion of dressed chicken was cut using separate knife and gloves. Skin parts were basically selected from the muscle surfaces. Chopping board cover, gloves were changed every time and knife was cleaned with 70% ethanol and burnt in order to prevent transferring of bacteria from one part to another. Then, 1 gram of each sample was weighed and transferred into a sterile falcon tube containing 9 mL of sterile normal saline (0.85% NaCl). The contents of falcon tube were mixed properly using a vortex machine and serial dilution was performed up to 10^{-3} dilution.

Enumeration of *Escherichia coli, Salmonella* spp. and *Shigella* spp.

An aliquot of 50 µL was spread on Eosin Methylene Blue (EMB) agar for the enumeration of *E. coli* and Salmonella-Shigella (SS) agar for the enumeration of *Salmonella* spp., *Shigella* spp. respectively. All plates were incubated at 37ºC for 24 to 48 hours. The colonies of *E. coli*, *Salmonella* spp. and *Shigella* spp. appeared to be green metallic sheen, black centered and transparent in the medium after incubation. Following incubation, number of colony was counted in CFU/g units.

Molecular characterization of *E. coli*, *Salmonella* spp. and *Shigella* spp.

Isolation of pure bacterial colonies

To isolate pure colonies of bacteria, green metallic sheen colonies on EMB agar, black centered and transparent colony on SS agar were sub-cultured onto nutrient agar (NA) agar plates. The plates were incubated at 37ºC for 24h.

DNA extraction

After isolation of pure colony from each bacterium, DNA was extracted by heat shock method to ensure the presence of *E.coli*, *Salmonella* spp. and *Shigella* spp. by Polymerase Chain Reaction (PCR). A loop full (2 or 3 numbers of colonies) of overnight bacterial culture was suspended in a 1.5 mL Eppendorf tube containing 500 µL of sterile distilled water and mixed thoroughly by using vortex machine. Then it was boiled for 100ºC for 10 minutes and immediately cooled at 0ºC for 10 minutes. The tube was then placed in a centrifuge (Eppendorf, Germany) and centrifuged for 13000 rpm (Rotation per minutes) for 8 minutes. The supernatant was withdrawn (70 µL) from the tube and used as the DNA template for PCR amplification of the specific bacteria. The template DNA was then stored at -20°C until analysis.

Polymerase chain reaction assay

Monoplex Polymerase chain reaction (PCR) was used for the identification of *E. coli*, *Salmonella* spp. and *Shigella* spp. present in the meat samples. Three set of primers (LT-F and LT-R targeting LT virulence gene of *E. coli*; Sal-201f and sal-597r targeting 16S rRNA gene of *Salmonella* spp.; IpaH-F and IpaH-R targeting IpaH virulence gene of *Shigella* spp.) were used for the detection of these specified bacteria.

Molecular detection of enterotoxigenic *E. coli*

The PCR assay for detection of Enterotoxigenic *E. coli* was carried out in 25 µL reaction mixture using two primers (Nguyen *et al*., 2009) LT-F (5'-TAGAGACCGGTATTACAGAAATCTGA-3'), LT-R (3'–TCATCCCGAATTCTGTTATATATGTC-5'). The reaction mixture consisted of 1 µL each of reverse and forward primer, 12.5 µL of master mixture and 6.5 µL of distilled water and 4 µL of template DNA. The PCR reaction was performed with a total of 32 cycles: 94ºC for 3min, followed by 94ºC for 30sec, 55ºC for 60sec, 72º C for 60sec and then finally extended at 72ºC for 10 min and held at 4ºC. The PCR products were electrophoresed in a 1.5% agarose gel, followed by staining with ethidium bromide for 30 minutes. It was then visualized under ultraviolet (UV) light. The bands were recorded by photography.

PCR amplification of 16s r-RNA gene of *Salmonella* spp.

The 16s r-RNA gene of *Salmonella* spp. was detected using Sal-201f (5'-CGGGCCTCTTGCCATCAGGTG-3') and sal-597r (3'-CACATCCGACTTGACAGACCG-5') primers (Amit-Romach *et al*., 2004). For PCR amplification of *Salmonella* spp., 4 µL of DNA extract was added to 21 µL of PCR mixture containing 12.5 µL of nuclease-free water, 1 µL of each primer, 6.5 µL of distilled water. After initial denaturation at 94ºC for3min,the reaction mixture was run through 35 cycles of denaturation at 94ºC for 30s,60ºC for 1min and 68°C for 2 min, and finally 1 cycle of 68°C for 7 min. Products of PCR were visualized by agarose gel (2%) electrophoresis containing ethidium bromide.

Detection of IpaH virulence gene of *Shigella* spp. by PCR

The PCR reaction for amplification of IpaH gene of *Shigella* spp. was carried out in standard 25 µL reaction in 0.2 mL PCR tube (Eppendorf, Germany) using IpaH F (5'-GCTGGAAAAACTCAGTGCCT-3') and IpaH R (5'-CCAGTCCGTAAATTCATTCT-3') primers (Sharma *et al*., 2010). 4 µL of DNA was used as a template and 12.5 µL of nuclease-free water, 1 µL of each primer, 6.5 µL of distilled water was added in the reaction mixture. The mixture containing PCR tubes were placed in thermal cycler (Eppendorf, Germany). The cycling conditions for amplification included 94ºC for 1 min (initial denaturation), 94ºC for 2 min (denaturation), annealing at 55ºC for 2 min, 72ºC for 3 min (polymerization) followed by 72ºC for 10 min. The amplicon was visualized by electrophoresis of the product in 1.5% agarose gel stained with ethidium bromide.

Results

Three genera of bacteria were isolated from all of the chicken meat samples. The isolates were presumptively identified as *E. coli, Salmonella* spp., *and Shigella* spp. by conventional culture method whereas only *E. coli* was confirmed by polymerase chain reaction. These enteropathogens from various organs of dressing chickens were also enumerated. The load of *E. coli, Shigella* spp. were higher than that of *Salmonella* spp. in dressed chickens.

E. coli count in dressed chickens

The load of *E. coli* in different parts of dressed chickens from different supermarket shops ranged between 0 log CFU/g to 3.38 log CFU/g respectively. The highest number of *E. coli* was found in M_2 (3.05 log CFU/g in average of chest, wings, leg, skin) followed by M_1 (3.04 log CFU/g in average), S_1 (2.46 log CFU/g in average), A (0.60 log CFU/g in average). No count of *E. coli* was recorded in S_2 sample. The highest count of *E. coli* was in wings (3.38 log CFU/g) rather than leg (3.37 log CFU/g), chest (3.31 log CFU/g) or skin (0 log CFU/g) for the chicken sample collected from S_1. *E. coli* was never detected from wings, leg, chest and skin samples of second chicken collected from S_2. The count of *E. coli* was also higher, mostly in the part of the chest (3.36 log, 3.31 log CFU/g) rather than leg (3.17 log, 3.06 log CFU/g), skin (3 log, 2.92 log CFU/g) or wings (2.69 log, 2.87 log CFU/g) for the dressed chicken of M (M_2 & M_1). Mean levels of *E. coli* were

very low (1.36 log CFU/g in skin, 1.07 log CFU/g in wings, 0 log CFU/g in leg and in skin respectively) in the chicken collected from A (Figure 1).

Salmonella spp. in dressed chickens

Salmonella spp. load in dressed chickens of five supershops was relatively lower than *E. coli* found in chicken samples of these supershops. The number of *Salmonella* spp. was higher in wings part (1.82 log CFU/g) of chicken samples of S_1 than other parts such as chest (1.54 log CFU/g), leg and skin (0 log CFU/g). The skin parts of chicken samples collected from S_2 was also found contaminated with high number of *Salmonella* spp. (3.36 log CFU/g) compared to wings (1.69 log CFU/g), legs (1.67 log CFU/g) and chest parts (0.47 log CFU/g). Again, the highest count of *Salmonella* spp. was recorded in chest of chicken samples from M_2 and M_1 supershops. The load of *Salmonella* spp. was 2.67 log CFU/g in chest followed by 2.07 log CFU/g, 1.47 log CFU/g and 1.3 log CFU/g in skin, wings and leg respectively in chicken sample of M_2. Similarly, the highest count of *Salmonella* was found in chest (2.64 log CFU/g) compared to wings (1.84 CFU/g), skin (1.3 CFU/g) and leg (1.07 CFU/g) of chicken collected from M_1. In case of the chicken which was collected from A, *Salmonella* spp. was never detected from chest, leg, wings and skin samples (Figure 2).

Shigella spp. count in dressed chickens

The chicken samples from five supershops were also contaminated with high number of *Shigella* spp. The count of *Shigella* was higher in wings part (3.42 log CFU/g) than any other parts such as chest (3.29 log CFU/g), skin (3.25 CFU/g) and leg (3.02 CFU/g) in dressed chicken of S_1. *Shigella* spp. was also found in high numbers in skin and leg parts (3.47 log CFU/g, 3.47 log CFU/g) than wings, chest (2.95 log CFU/g, 2.47 log CFU/g) parts of chicken from S_2.

Again, the highest count of *Shigella* spp. was recorded in skin parts of chicken collected from M_1 and M_2 (3.45 log CFU/g, 3.39 log CFU/g). The load of *Shigella* spp. in wings, leg and chest parts of chicken from M_1 was 3.25, 3.02 and 2.97 log CFU/g respectively. The leg, wings and chest of chicken sample of M_2 was also recorded with high number of *Shigella* spp. (3.3 log CFU/g, 3.17 log CFU/g, 3 log CFU/g). *Shigella* spp. was never detected in skin and leg of chicken sample from A except in wings (1.23 log CFU/g), chest (1 log CFU/g) part (Figure 3).

Confirmation of enteropathogens as virulent by PCR assay

Three enteropathogens (*E. coli, Salmonella* spp., *Shigella* spp.) presumptively isolated from chicken samples by conventional culture methods were further tested for their confirmation as virulent enteropathogens by PCR assay. Only *E coli* isolates were confirmed as enterotoxigenic *E coli*. The enterotoxigenic *E coli* was identified on the basis of the 282 bp PCR product corresponding to the sequence of LT virulent gene on 1.5% agarose gel whereas other isolates were not confirmed as virulent *Salmonella* spp., *Shigella* spp. When they were analyzed with virulent 16S rRNA (*Salmonella* spp.) and IpaH gene (*Shigella* spp.) specific PCR amplification, they did

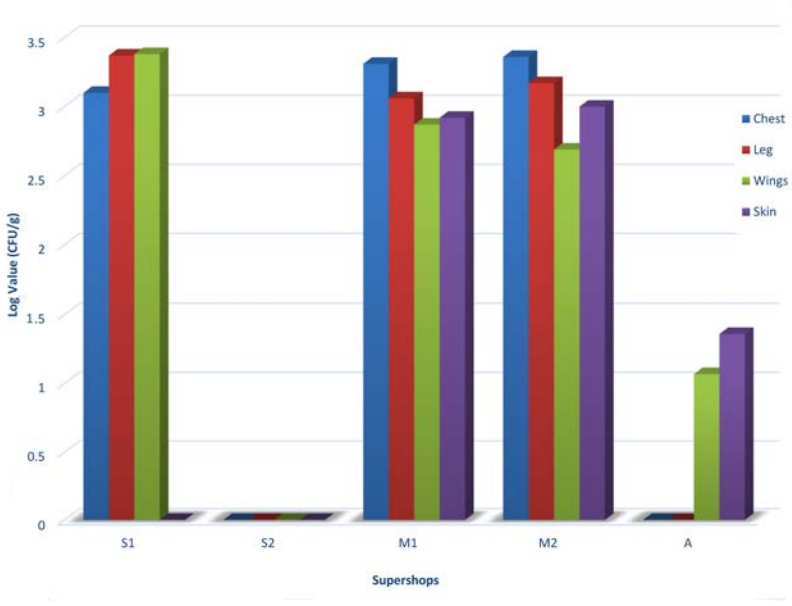

Figure 1. Enumeration of *E. coli* in various parts of dressed chicken samples.

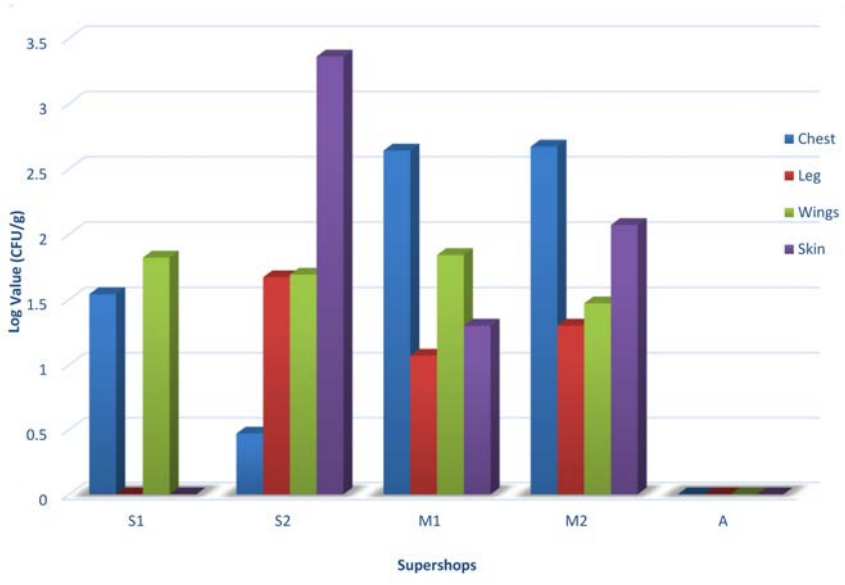

Figure 2. *Salmonella* spp. count in different parts of dressed chicken.

not show any virulent 16S rRNA and IpaH gene (Table 1).

Discussion

Poultry meat is found with *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. (Malmuthuge *et al*., 2012; Sudershan *et al*., 2012; Voidarou *et al*., 2011; Torok *et al*., 2011; Petrović *et al*., 2011; Awad-Alla *et al*., 2010; Ahmed et al., 2009). Several studies had been carried out in Bangladesh by Akond *et al*, 2009 and Islam *et al*., 2014 where they reported the presence of *E. coli, Salmonella* spp., *Staphylococcus aureus* in poultry meat and chicken rinse samples (Akond *et al*, 2009; Islam *et al*., 2014). In the present study, several microorganisms like *E. coli, Salmonella* spp., *Shigella* spp. were found from various parts of chicken samples collected from five supershops of Dhaka city, Bangladesh. These various parts of chicken samples are often bought separately by the consumers. The pathogenic bacteria usually absent in the muscle tissue and body fluids of healthy living animals. But, the pathogens can be introduced into the meat during slaughtering or at the time of processing where the source of these pathogens may be endogenous from the gastrointestinal tract or from surrounding environment in farm and/ or slaughterhouse (Samaha *et al*., 2012).

During the present investigation, pathogens like *E coli, Salmonella* spp., *Shigella* spp. were found from dressed chicken samples of five supershops where the count of *E. coli* and *Shigella* spp. were higher than that of *Salmonella* spp. in dressed chickens. Two study by Frazier and Westhoff (1983) and Hashim (2003) showed that *E. coli* is present in examined chicken meat and chicken meat products due to improper handling or unhygienic conditions (Frazier *et al.,* 1983; Hashim, 2003).

Shah *et al*., 2012 reported that the high prevalence of *Salmonella* in chicken meat may be due to cross-contamination from intestines during processing and cutting or from cages, floor and workers during retailing or marketing. They also concluded that water used for washing of carcasses may be responsible for this and the meat could be contaminated with *Salmonella* from feces or from the butcher's hands during washing (Shah *et al.*, 2012). Another study by Cason *et al*., 1999 and James *et al*., 1992 revealed that contamination of poultry by *Salmonella* may be occurred during poultry meat production and processing. The contamination may occur during transportation to the poultry-processing plant or during the steps involved in slaughtering, scalding, defeathering, plucking and chilling of the poultry carcasses, (Cason *et al*., 1999; James *et al*., 1992). The *Shigella* spp. in the dressed chicken may be attributed to the unhygienic practices of workers while handling and processing of meat.

The high incidence of contamination of poultry meat with enteric pathogens appears to have two major causes. The causes may be the practice of intensive rearing that encourages rapid transmission of pathogens through flocks and the very high rates of throughput at large processing plants which enhance the spread of microorganisms among carcasses during processing (Robinson, 1985).

The dressed chicken samples were contaminated with *E. coli, Salmonella* spp. *and Shigella* spp., but when they were analyzed by virulence gene specific PCR, Only *E. coli* were found as enterotoxigenic. The other strain *Salmonella* spp. and *Shigella* spp. were not confirmed as pathogenic. It is well understood that that the pathogenic form of *E. coli* is a public health threat by which bloody diarrhea, hemorrhagic colitis and a life-threatening hemolytic-uremic syndrome (HUS) can be occurred (Magwedere *et al*., 2013; Parma *et al*., 2012; Liu *et al*., 2011; Käppeli *et al*., 2011; Fratamico *et al*., 2011. When this *E. coli* is enterotoxigenic, the infection caused by the entrotoxigenic *E. coli* due to ingestion of contaminated food or water produces

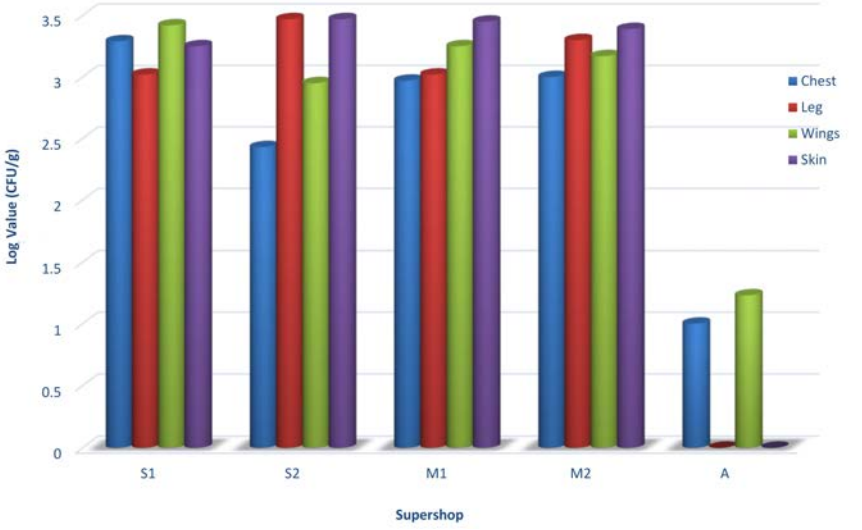

Figure 3. Enumeration of *Shigella* spp. in chest, leg, wings and skin parts of dressed chicken.

Table 1. Molecular confirmation of enteropathogens isolated from different parts of chicken samples by polymerase chain reaction assay.

Different parts of chicken samples	Name of supershops	Bacterial genera *E. coli* (ETEC) LT gene	*Salmonella* spp. 16s-rRNA gene	*Shigella* spp. IpaH gene
Chest	S_1 - S_2 - M_1 - M_2 - A	+	-	-
Leg	S_1 - S_2 - M_1 - M_2 - A	+	-	-
Wings	S_1 - S_2 - M_1- M_2 - A	+	-	-
Skin	S_1 - S_2- M_1 - M_2 - A	+	-	-

abdominal cramps, low-grade, fever, watery diarrhea and nausea (Nweze, 2009).

The increasing prevalence of pathogens in foods owing to poor hygienic practices is increasing the risk of food borne disease for consumers (Sivapalasingam *et al*., 2004). The present study demonstrates that dressed chicken meat is contaminated with enterotoxigenic *E. coli* (ETEC) and this may be a potential hazard to the consumers. The government should adopt regulation to enforce the application of the handling, marketing and storage of poultry meat as a means to identify and control this potential hazard in poultry slaughter houses. These measures may be helpful to prevent ETEC infection in dressed chicken meat sold by main supershops in Bangladesh.

Conclusions

The result demonstrated that dressed chicken samples possess virulent LT gene of *E. coli* could be a potential hazard to the consumers. Further exploration of other virulent strains like enteropathogenic *E coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC) is needed to figure out the particular situation of *E. coli* contaminated poultry meat at different supershops of Dhaka as well as other cities in Bangladesh.

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