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 S1, S2, M1, M2 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 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 (Enterootoxigenic), 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 food-borne 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 pre-processed 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.

Materials and Methods

Sample collection and transportation

Dressed chicken samples (Gallus gallus) were collected from five renowned super shops including S1, S2, M1, M2, A of Dhaka, Bangladesh. The super shops were visited two times during the investigation. All the chicken samples were kept at -20°C for a maximum of 7 days in the shop. Five chicken samples were taken, two of which were from the supershop S1, S2 and other two were from M1, M2 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 *Shigella* 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 10⁶ 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*, *Salmonella* spp. and *Shigella* (SS) agar for the enumeration of *Salmonella* spp., *Shigella* spp. respectively. All plates were incubated at 37°C 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 37°C for 24 h.

**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 100°C for 10 minutes and immediately cooled at 0°C 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-211F 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’-TAGAGACCGTATTACAGAAATCTGA-3’), LT-R (5’-TCATCCGAATTCTGTTATATATGTC-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: 94°C for 3min, followed by 94°C for 30sec, 55°C for 60sec, 72°C for 60sec and then finally extended at 72°C for 10 min and held at 4°C. 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’-CGGGCCTCTTTGCCATCGAGGT-3’) and sal-597r (3’-CACATCCGAATTCTGTTATATTGTC-5’) primers (Amrit-Romich 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 nuclelease-free water, 1 µL of each primer, 6.5 µL of distilled water. After initial denaturation at 94°C for3min, the reaction mixture was run through 35 cycles of denaturation at 94°C for 30s,60°C 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’-CCAGTCCGTAATTCACTTCT-3’) primers (Sharma et al., 2010). 4 µL of DNA was used as a template and 12.5 µL of nuclelease-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 94°C for 1 min (initial denaturation), 94°C for 2 min (denaturation), annealing at 55°C for 2 min, 72°C for 3 min (polymerization) followed by 72°C 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 M₂ (3.05 log CFU/g in average of chest, wings, leg, skin) followed by M₁ (3.04 log CFU/g in average), S₁ (2.46 log CFU/g in average), A (0.60 log CFU/g in average). No count of *E. coli* was recorded in S₂ 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 S₁. *E. coli* was never detected from wings, leg, chest and skin samples of second chicken collected from S₂. 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 M₁ and M₂. 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 S1 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 S2 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 M1 and M2 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 M2. 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 M1. 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 S1. 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 S2.

Again, the highest count of Shigella spp. was recorded in skin parts of chicken collected from M1 and M2 (3.45 log CFU/g, 3.39 log CFU/g). The load of Shigella spp. in wings, leg and chest parts of chicken from M1 was 3.25, 3.02 and 2.97 log CFU/g respectively. The leg, wings and chest of chicken sample of M2 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
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-Allo 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, scaling, 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 entero- toxicogenic *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.**

| Different parts of chicken samples | Name of supershops | *E. coli* (ETEC) LT gene | Bacterial genera | *Salmonella* spp. 16S-rRNA gene | *Shigella* spp. IpaH gene |
|-----------------------------------|--------------------|--------------------------|-----------------|---------------------------------|--------------------------|
| Chest                             | S1 · S2 · M1 · M2 · A | +                        | -               | -                              | -                        |
| Leg                               | S1 · S2 · M1 · M2 · A | +                        | -               | -                              | -                        |
| Wings                             | S1 · S2 · M1 · M2 · A | +                        | -               | -                              | -                        |
| Skin                              | S1 · S2 · M1 · M2 · A | +                        | -               | -                              | -                        |

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