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## MICROBIOLOGICAL QUALITY ASSESSMENT OF MARKETED BROILER MEAT IN DIFFERENT MARKETS OF DHAKA CITY

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### ABSTRACT

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The present research work was undertaken to assess the bacterial quality and to know the prevalence of zoonotic bacteria from broiler meat samples sold in Krishi market, Bihari camp market, Agargaon market, Taltola market and SAU (Sher-e-Bangla Agricultural University) mini bazar, Dhaka, Bangladesh from January to May 2018. After processing of samples primary culture was done in nutrient broth and nutrient agar media then pure culture was obtained from different selective media. Total Viable Count (TVC), Total Coliform Count (TCC) and Total Salmonella Count (TSC) in broiler meat of different broiler markets were determined. Mean of TVC, TCC and TSC for the Krishi market, Agargaon market, Taltola market, Bihari camp market and SAU Mini market were 5.67, 4.32, 2.96 log<sub>10</sub> CFU/g, 5.88, 4.64, 3.56 log<sub>10</sub> CFU/g, 6.10, 4.68, 3.78 log<sub>10</sub> CFU/g, 6.68, 4.87, 3.84 log<sub>10</sub> CFU/g and 5.84, 4.25, 3.13 log<sub>10</sub> CFU/g respectively. The prevalence of *Escherichia coli* and *Salmonella spp.* were 74% and 42% respectively. *E. coli* isolates were showed sensitive to Ciprofloxacin (91.6%), Gentamycin (87.5%), Azithromycin (66.66%), and Tetracycline (58%) and resistant to Penicillin (79.16%) then Amoxicillin (75%), Streptomycin (75%) and Ampicillin (58.3%). Highest resistant pattern was showed by Tetracycline (58%), Streptomycin (72.72%), Penicillin (72.72%) and Amoxicillin (63.63%). Highest resistant pattern showed by Amoxicillin (71.42%) and Penicillin (71.42%). This study revealed that broiler meat sold at some local markets of Dhaka city were contaminated with multiple species of multidrug resistant bacteria which may risk for human health.

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## INTRODUCTION

Food is considered as energy source for humans and animals. Most of the foods contain viable bacteria unless thoroughly heated or made sterile. Otherwise, it serves as an important medium for transmission of pathogenic organisms to the consumers. Meat is most perishable of all important foods since it contains sufficient nutrients needed to support the growth of microorganisms (Magnus, 1981). Meat contamination occur by a variety of ways, including bowel rupture during evisceration in direct contamination with tainted water and also handling and packaging of finished meat products. Apart from these factors, meat at the point of sale may also carry disease causing bacteria whose mere presence may be of concern because the meat then becomes the vehicle for food poisoning outbreaks (Jackson *et al.*, 2001).

Meat may be easily contaminated with different pathogens if not handled appropriately (Mead *et al.*, 1999). There are more than 200 known causative agents can cause food borne diseases; these include bacteria, parasites, viruses, prions, toxins and metals. In practice of slaughtering, the main sources of microorganisms are exterior of the animal and the intestinal tract. Meat carcasses may become contaminated from fecal material, paunch content, and from the hide (Maharjan, 2006). Contaminated raw or undercooked red meats are particularly important in transmitting these food borne pathogens (Meng *et al.*, 1998). The nature and level of microbial contamination in meat have important consequence in relation to public health, storage life and the type of spoilage of meat. The most important pathogens associated with meat include *Salmonella*, *Salmonella aureus*, *Escherichia coli*, *Clostridium perfringens*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophilia* (Koutsoumanis, 2004). The occurrence of antimicrobial-resistant bacteria is associated with the use of antimicrobial agents in food producing animals. Considering the above facts, the present study was conducted to investigate the prevalence and antibiogram assay of zoonotic bacteria in raw meat in selected areas of Dhaka city. On the above situation the present study was undertaken with the following objectives: bacterial quality assessment of fresh broiler meat sold in different markets of Dhaka City, to isolate and identify the *Salmonella* spp., *E. coli*, *Salmonella* spp. from raw meat samples, to study the prevalence of zoonotic infection in raw meat.

## MATERIALS AND METHODS

This study was conducted at the laboratory of the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University (SAU), Dhaka 1207, during the period of January to May, 2018.

### Sample collection (Source and Transportation)

This study was designed to investigate the bacterial quality and prevalence of bacteria in poultry meat at various markets in Dhaka city. A total of 45 raw broiler meat samples were collected from Krishi market, Bihari camp market, Agargaon market, Taltola market and SAU mini bazaar, Dhaka. Collected samples were immediately transported on ice to the Microbiology and Parasitology laboratory of the Sher-e-Bangla Agricultural University for analysis.

### Sample Preparation for Bacteriological Studies

All samples was macerated in a mechanical blender with a sterile diluents recommended by International Organisation of Standardisation (ISO,1995).Ten gm samples with 90 ml homogenized 0.1% peptone water was taken and suspension was made with the help of a sterile blender.

### Estimation of TVC

Total bacterial count was determined by transferring 0.1 ml of each ten-fold dilution and spread on triplicate plate count agar media using fresh pipette for each dilution. Samples were spread quickly on the surface of agar plate containing media with the help of a sterile glass spreader. Plates were kept in an incubator at temperature 37°C for 24-48 hours. Plates containing 30-300 colonies were counted after incubation and out the range were rejected. TVC was obtained by multiplying the average no of colonies along with the dilution factor. The TVC was calculated according to ISO (1995) and express as the number of organism or colony forming units per gram (CFU/g) of chicken meat sample.

**Estimation of TCC**

Mc-conkey agar (Himedia, India) was used for the determination of TCC. 0.1 ml of each ten-fold diluted sample was transferred on the agar plate containing media with the help of a sterile pipette. The samples were quickly spread on the plate surface using sterile spreader. Plates were kept in an incubator at temperature 37°C for 24-48 hours. Growth of the organism was assured by the outlook of turbidity on the plate and results were prepared from MPN tables.

**Estimation of TSC**

For TSC determination, procedure of TVC was followed in terms of dilution and streaking. For salmonella count xylose lysine deoxycholate agar (XLDA) was used. TSC value was calculated followed by the TVC calculation.

**Isolation of bacteria by culturing of sample into different bacteriological media**

Primary growth was performed in nutrient broth followed by inoculation at 37°C for overnight. Enriched culture from nutrient broth was streaked on to selective agar media and incubated at 37°C for 24 hours.

**Identification of isolated bacteria**

The cultural examination of meat samples for bacteriological analysis was done according to the standard method (ICMSF, 1985). Identification of bacteria was performed on the basis of colony morphology; Gram's staining reaction and biochemical test.

**Morphological identification of bacteria by Gram's staining**

Gram's staining of the pure culture was performed according to method described by Cheesbrough (2006). Briefly a single colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violet was then applied onto smear to stain for two minutes and then washed with running tap water. Few drops of Gram's iodine were then added for few seconds. After washing with water, Safranin was added as counter stain and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried in air and then examined under light microscope (400X) using immersion oil.

**Maintenance of stock culture**

Stock culture was mixed with a medium prepared by adding one ml of 50% sterilized glycerol in one ml of pure culture in nutrient broth and this was stored at -20°C for further use.

**Antibiogram study test**

The disc diffusion method was used to detect antimicrobial susceptibility assay according to the recommendation of Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards, CCLS: 2016). Antimicrobial drug susceptibility against nine commonly used antibiotics were performed by disc diffusion or Kirby-Bauer method (Bauer *et al.*, 1966).

**Interpretation of the results**

After the discs are placed on the plate, the plates were inverted and incubated at 35°C for 8 to 12 hours following which the diameter of the zones of complete inhibition (including the diameter of the disc) was measured and recorded in millimeters. The measurements were made with a ruler on the under surface of the plate without opening the lid. The zones of growth inhibition were compared with the zone-size interpretative table provided by Clinical and Laboratory Standards Institute (CLSI, 2016). Antimicrobial testing results were recorded as susceptible, intermediate and resistant according to zone diameter interpretive standards provided by CLSI (2016).

## RESULTS AND DISCUSSION

The mean value with standard deviation of Total Viable Count (TVC), Total Coliform Count (TCC), and Total Salmonella Count (TSC) in broiler meat of Krishi market, Agargoan market, Taltola market, Bihari camp market and SAU mini markets are presented in table 1 and Summary of prevalence of bacteria from chicken meat shown in table 2. Results of TVC, TCC and TSC of the collected meat samples in five different markets differed significantly ( $p < 0.05$ ). TVC (mean value) in five markets varies between log 5.67 to log 6.68 with highest at Bihari camp market and lowest at Krishi market; TCC (mean value) in five markets varies between log 4.87 to log 4.25 with highest at Bihari camp market and lowest at SAU mini market and TSC (mean value) in five markets varies between log 2.96 to log 3.84 with highest at Bihari camp market and lowest at Krishi market. The probable reason of this variation in TVC, TCC and TSC values might be due to variations in hygiene practice and overall management systems. Supervision noticed that Krishi markets the slaughtering system and procedure of broiler meat production was relative more hygienic than others. Here the consumers are more conscious about the hazardous elements and associated risk factors. On the other hand in Bihari camp markets the slaughtering system and procedure of broiler meat production are not so, rather the butchers are illiterate and unskilled and the consumers are mostly poor, interested to purchase comparatively poor quality meat if the price is low. The results of present investigation more or less similar with the findings of Hasan et al. (2015), Abu-Ruwaida et al. (1994), Adu-Gyamfi et al. (2012) and Anwar et al. (2004).

**Table 1.** Microbial load in marketed broiler meat at different market of Dhaka city

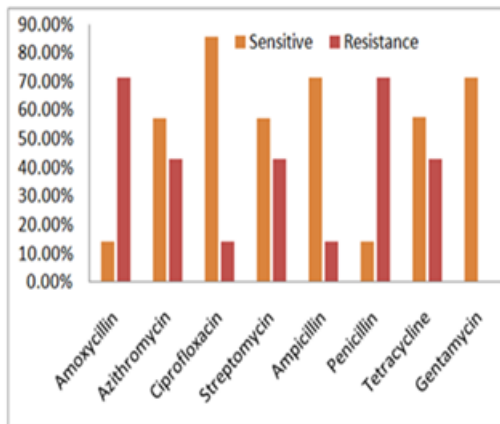
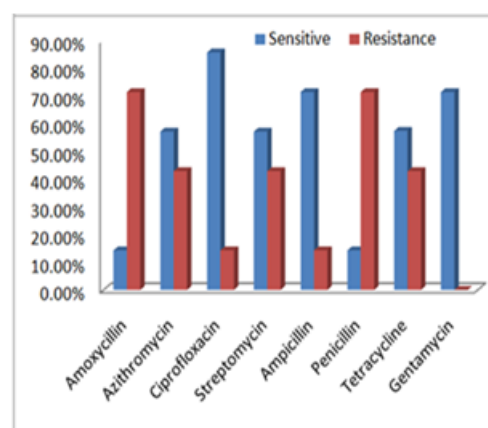
Place of Collection	Bacterial Count		
	TVC (CFU/g) Mean $\pm$ SD	TCC (CFU/g) Mean $\pm$ SD	TSC (CFU/g) Mean $\pm$ SD
Krishi Market	5.67 $\pm$ 0.49	4.32 $\pm$ 0.19	2.96 $\pm$ 0.39
Agargoan Market	5.88 $\pm$ 0.19	4.64 $\pm$ 0.35	3.56 $\pm$ 0.18
Taltola Market	6.10 $\pm$ 0.16	4.68 $\pm$ 0.27	3.78 $\pm$ 0.38
Bihari Camp Market	6.68 $\pm$ 0.21	4.87 $\pm$ 0.31	3.84 $\pm$ 0.67
SAU Mini Bazar	5.84 $\pm$ 0.33	4.25 $\pm$ 0.17	3.13 $\pm$ 0.53

\*\*Results are expressed in logarithms and CFU/g of meat

**Table 2.** Summary of prevalence of bacteria from chicken meat

Sources and Location	Total	Prevalence of <i>E. coli</i> (%)	Prevalence of <i>Salmonella</i> spp. (%)
Krishi Market	10	60	50
Bihari Camp	10	100	60
Agargaon Bazar	10	70s	50
Taltola Bazar	10	80	40
SAU Mini Bazar	5	60	10

Two isolates such as *E. coli* and *Salmonella* spp. were subjected to antibiogram assay. *E. coli* isolates were tested against eight different antibiotics. Among them Ciprofloxacin showed the highest susceptibility pattern followed by the gentamycin, azithromycin and tetracycline found sensitive in this study. Highest resistant pattern was showed by penicillin then amoxycillin, streptomycin and ampicillin. *Salmonella* isolates were tested against eight different antibiotics. Among them Ciprofloxacin showed the highest susceptibility pattern followed by the Gentamycin and Azithromycin. Highest resistant pattern was showed by Tetracycline, Streptomycin, Penicillin and Amoxycillin (Figure 2 and 3).

Figure 2. Antibiogram of *E. coli*Figure 2. Antibiogram of *Salmonella* spp.

In the present study, specific enriched media were used for the isolation and identification of *Salmonella* spp. which was also used by a number of researchers such as (Kabir *et al.*, 2017). The morphology of the isolated *Salmonella* spp. was Gram negative, very short plump rod arranged as single or paired and those properties of *Salmonella* spp. were supported by other authors (Musa *et al.*, (2017); Kamal *et al.*, (2018). Isolated *Salmonella* spp. were able to ferment dextrose, maltose and mannitol with the production of both acid and gas but did not ferment lactose and sucrose and those characteristics of *Salmonella* spp. were satisfied the statement of (Han *et al.*, 2011; Musa *et al.*, (2017).

Prevalence rate of *E. coli* in meat from different market in Dhaka city was 74% where the highest prevalence was 100% at Bihari Camp and lowest 60% at SAU Mini bazar & Krishi market. It might be due to the very unhygienic practice in Bihari Camp Market than Mini Bazar & Krishi Market. Besides, the above result is more or less similar to the results of Al-Salauddin *et al.*, (2015) who reported the prevalence of *E. coli* was 83.33% in broiler meat at various market of Mymensingh, Gazipur, and Sherpur districts. Prevalence rate of *Salmonella* in meat from different market in Dhaka city was 42% which is not agree with Al-Salauddin *et al.*, (2015) who found 31.66% prevalence of *Salmonella* species in various market of Mymensingh, Gazipur, and Sherpur districts. As *Salmonella* is waterborne pathogen, high water contamination in Dhaka city than other city could be the reason of higher prevalence rate in Dhaka city.

## CONCLUSION

Present study showed that the genera of bacteria are isolated is known as food borne bacteria which may cause food borne diseases and intoxication. The TVC, TCC and TSC of the collected meat samples in five different markets differed significantly and the probable reason of this variation in TVC, TCC and TSC values might be due to variations in hygiene practice and overall management systems. Supervision noticed that Krishi markets the slaughtering system and procedure of broiler meat production was relative more hygienic than others. Overall, the prevalence of *Escherichia coli*, *Salmonella* spp. and *Salmonella* spp. in broiler meat and their drug resistance is very alarming. Therefore, broiler meat industry should be provided with an immediate attention by the government to maintain strict bio-security and hygienic managements in farm and live bird markets all over the country. Future study needed to Pulsed Field Gel Electrophoresis (PFGE), PCR of other antibiotic gene and Characterization of toxin gene.

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