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Microbiological Evaluation of Poultry Meat Obtained from Different Retail Markets in Khulna District

Bidyut Matubber ^α, Joyanta Kumar Das ^ο, Md. Ahsan Habib ^ρ, Sabuj Kanti Nath ^ω & Md. Uzzal Hossain [¥]

Abstract- The research work conducted to evaluate microbial load in poultry meat from different retail markets in the Khulna district (Nirala, Dumuria, and Fultola). The objective of the present study was to get quality poultry products from commercial farms and retail markets. A total of 48 samples collected and brought to Quality Feed Lab. for laboratory assay. Samples were propagating in nutrient agar followed by culture on selective media, Eosin Methylene Blue Agar, Mac-Conkey agar, Brilliant Green Agar, Salmonella-Shigella Agar. Total numbers of 48 samples examined, and 30 samples were found positive to *E. coli*, and the prevalence of *E. coli* in this study was 62.20 %. 37.50% of thigh meat samples were positive for *E. coli* within 24 tested samples whereas 87.50% of breast meat samples were positive within 24 tested samples for this bacteria. On the other hand, 23 samples were found positive for *Salmonella* spp, and the prevalence of *Salmonella* spp in this study was 49.91 %. 29.16% of thigh samples were positive for *Salmonella* spp within 24 tested samples whereas 66.66% of litter samples were positive within 24 tested samples for this bacteria. Total Viable Count (TVC), Total Coliform Count (TCC), Total Salmonella Count (TSC), and Total Campylobacter Count (CPC) in meat samples of different broiler markets like Nirala market at Khulna city corporation, Dumuria, and Fultola were determined. Mean of TVC, TCC, TSC, and TCpC for the Nirala market at Khulna city corporation, Dumuria and Fultola markets were 5.61, 5.84, 6.29 log₁₀ CFU/g, 4.72, 4.50, 4.47 log₁₀ CFU/g, 3.19, 3.44, 3.49, log₁₀ CFU/g and 2.31, 2.50, 2.54 log₁₀ CFU/g, respectively. It observed that the mean values of TVC, TCC, TSC and TCpC in the case of Dumuria, and Fultola market exceeded the ICMSF recommendations, which may cause alarm to consumer's health.

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I. INTRODUCTION

Poultry meat is equally important as a microbiological safety and quality to producers, retailers, and consumers (Mead et al., 2004). Because of advantages such as easy digestibility and acceptance, poultry meat is becoming more popular in the consumer market by most people (Yashoda et al., 2001). However, chicken meat consists of high-quality protein and many other nutrients that are very important for body function (Kralik et al., 2017). Poultry meats to be optimally incorporated into the diet at all ages because of their high-biological-value protein, vitamin, and mineral content associated with a low-fat content (Marangoni et al., 2015). The consumption of poultry meat has increased worldwide as it's a highly nutritious and safe food (Gonzalez-Ortiz et al., 2013). About 90 percent of the rural families are consuming small numbers of chickens (Das et al., 2008). Poultry meat contaminated by different types of microorganisms during processing in the processing plants (Maharjan et al., 2019). During slaughtering, poultry meat contaminated because of the malpractices in handling and management with remains foodborne pathogens remains important health-hazardous issue (Javadi and Safarmashaei et al., 2011). Chicken meat products from retail markets contaminated with foodborne pathogens, namely, *Staphylococcus aureus*, *Salmonella*, *E. coli*, and *Listeria monocytogenes*, and contamination with mold and yeasts (Khalafalla et al., 2019). Pathogenic strains of salmonella, *S. aureus*, *S. epidermidis*, shigella, enterobacter, and Citrobacter are serious health threats for a human beings (Alam et al., 2015). Foodborne pathogens are causing many diseases with significant effects on human health and the economy (Bintsis, 2017). The food-borne pathogen causes various of illness and death that loses billions of dollars for medical care, medical and social costs (Fratmico et al., 2005). Food-borne illnesses are still public health issue in both developing and developed countries despite applying many control and preventive measures (Zhou et al., 2010). The aim of this study to detect food-borne pathogens in Poultry meat of different areas in the Khulna district and to know the potential risk factor of food-borne pathogens in Khulna district.

II. MATERIALS AND METHODS

a) Study Area and Sample Collection

The present research work was conducted at different retail markets in Khulna district from July 2016 to

November 2018 in Quality Feed Lab, Khulna. A total no. of 48 samples collected from the retail markets (Nirala market at Khulna city corporation, Dumuria, and Fultola market) in Khulna district.

Table 1: List of the retail market for sample collection

SL. No.	Name of the retail markets	No. of collected samples Meat (Thigh + Breast)	Total No. of collected samples
01.	Nirala	16(8+8)	48
02.	Dumuria	16(8+8)	
03.	Fultola	16(8+8)	

b) Preparation of sample for bacteriological studies

Each of the raw meat samples was macerated in a mechanical blender using a sterile diluent as per the recommendation of the International Organisation for Standardisation (ISO, 1995). Ten grams of the thigh meat sample was taken aseptically with sterile forceps and transferred into sterile containers containing 90 ml of 0.1% peptone water. A homogenized suspension made in a sterile blender. Thus 1:10 dilution of the samples were obtained. Later on, using a whirly mixture machine, different serial dilutions ranging from 10⁻² to 10⁻⁶ were prepared according to the standard method (ISO, 1995).

c) Enumeration of TVC

For total bacterial count, 0.1 ml of each ten-fold dilution transferred and spread on duplicate PCA using a fresh pipette for each dilution. Then the diluted samples spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader used for each plate. The plates then kept in an incubator at 37 °C for 24-48 hours. Plates exhibiting 30-300 colonies following incubation. The average number of colonies in a particular dilution multiplied by the dilution factor to obtain the total viable count. The TVC calculated according to ISO (1995). The results of the total bacterial count expressed as the number of organism or colony-forming units per gram (CFU/g) of meat sample.

d) Enumeration of TCC

For TCC, 0.1 ml of each ten-fold dilution transferred and spread on Mac Conkey agar using a sterile pipette for each dilution. Then the diluted samples spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader used for each plate. The plates then kept in an incubator at 37 °C for 24-48 hours. The growth of the organism confirmed by the appearance of turbidity. Results calculated from MPN tables.

e) Enumeration of TSC

For total salmonella count, the procedures of sampling, dilution and streaking were similar to those followed in total viable bacterial count. Xylose lysine deoxycholate agar (XLDA) used only in the case of

salmonella count. The calculation for TSC was similar to that of the total viable count.

f) Enumeration of TCpC

For TCpC, 0.1 ml of each ten-fold dilution transferred and spread on the selective blood base agar with 5% sheep or cattle blood. The diluted samples spread as quickly as possible on a 0.45 mm filter placed on blood agar base agar no 2 with a sterile glass spreader. The plates then kept in an incubator at 42° C for 24-48 hours. Plates exhibiting 30-300 colonies following incubation. The average number of colonies in a particular dilution multiplied by the dilution factor to obtain the total viable count. The total viable count calculated according to ISO (1995). The results of the total bacterial count expressed as the number of organism or colony-forming units per gram (CFU/g) of meat sample. In young culture, the organism is comma-shaped and S-shaped. In old culture, organisms cling together. Gram (-ve) colonies were round, smooth, and translucent with a dewdrop appearance.

g) Cultural and biochemical examination of samples

The cultural examination of chicken thigh meat samples for bacteriological analysis done according to the standard method (ICMSF, 1985). The examination followed a detail study of colony characteristics, including the morphological and biochemical properties. To find out different types of microorganisms in chicken thigh meat samples, different kinds of bacterial colonies isolated in pure culture from the plate count agar (PCA), Mac Conkey agar (MCA), blood agar (BA), and xylose lysine deoxycholate agar (XLDA) and subsequently identified according to the methods described by Krieg et al., 1994. The isolated organisms supporting growth characteristics on various media subjected to different biochemical tests such as sugar fermentation test, indole production test, catalase test, coagulase test, methyl-red, and Voges-Proskauer (VP) test. In all cases, standard methods as described by Cowan (1985) followed for conducting these tests.

h) Statistical analysis

The data on TVC TCC, TSC, and TCpC obtained from the bacteriological examination of meat samples of the poultry carcass collected from Nirala,

Dumuria, and Fultola markets of Khulna district were analyzed in a completely randomized design (CRD) using a computer package subjected to Analysis of Variance using SPSS Software (Version 16, 2007). The differences between means evaluated by Duncan's Multiple Range Test (Gomez and Gomez, 1984).

III. RESULTS AND DISCUSSION

The mean and standard deviation of the TVC in poultry meats of Nirala market, Dumuria, and Fultola markets are presented in (Tables 2, 3, and 4). The variation of TVC in meats of different poultry markets was significant ($P < 0.05$) a 5% level of probability, as shown in (Table 5). The result of TVC in three different retail markets was differed significantly ($P < 0.05$). The maximum and minimum range of TVC in poultry meat recorded at Nirala market, Dumuria market, and Fultola markets were log 6.5, log 6.59, log 6.8 and log 4.80, log 5.30, log 5.90, respectively (Table 6). However, the average value of TVC at three markets are log 5.65, log 5.94, and log 6.35, as shown in (Table 6). In the Nirala market the value of TVC was lower than the Dumuria market, but it is the highest in the Fultola market shown in (Tables 2, 3, and 4). The possible cause of this variation in microbial load might be thought to be due to differences in management and hygienic practices. Observation of the investigation revealed the fact that in the case of the Nirala market, the slaughter hygiene and process of poultry meat production were relatively more hygienic in respect of sanitation and handling systems. The butchers generally are skilled, and the consumers are well conscious about risk factors and hazardous elements associated with meat production and handling. On the contrary, in Fultola markets, these are not so, the butchers are unskilled and illiterate, and the consumers mostly are poor and do not hesitate to purchase poor quality meat. The results obtained were in close agreement with the findings of Mahami et al. (2019), Sultan et al. (2017), and Adu-Gyamfi et al. (2012), respectively. The mean and standard deviation of the TCC of Poultry meat processed at slaughter yards of Nirala, Dumuria, and Fultola markets are summarized in (Tables 2, 3 and 4). The result evaluated in (Table 5) revealed that the mean values of TCC in meats of Nirala market, Dumuria and Fultola market were not significant ($P > 0.05$). Nevertheless, no significant variation demonstrated between the interactions of the three markets. The interpretation of TCC in three different retail markets was not differed significantly (Table 5). The maximum and minimum range of TCC in thigh meat recorded at Nirala market, Dumuria, and Fultola markets was log 6.40, log 4.92, log 5.25 and log 3.90, log 4.20, log 4.10, respectively (Table 6). However, the average value of TCC at three markets were log 5.16, log 4.56, and logged 4.68, as shown in (Table 6). These findings are closely related to the observations of

Faruque et al. (2019), Bhandari et al. (2013), and Datta et al. (2012), respectively. The mean values of TSC in Poultry meat of three different areas like Nirala market, Dumuria market, and Fultola market are summarized in (Tables 2, 3, and 4). The mean values of TSC in Poultry meat of three different areas like Nirala market, Dumuria market, and Fultola market were logged 3.19 ± 0.55 , $\log 3.44 \pm 0.21$, and $\log 3.49 \pm 0.75$ CFU/g, respectively (Table 5). The variation of TSC in meats of the different market areas was not significant ($P > 0.05$) presented in (Table 5). The interpretation of TSC in three different retail markets was not differed significantly ($P > 0.05$). The maximum and minimum range of TSC in meat recorded at Nirala, Dumuria, and Fultola markets were log 3.8, log 3.78, log 4.00 and log 2.50, log 3.22, log 3.00, respectively (Table 6). However, the average value of TSC at three markets was log 3.15, log 3.50, and logged 3.50, as shown in (Table 6). The TSC value in the Nirala market was lower than the Dumuria market, but it is the highest in the Fultola market. This signifies the fact that all these meats are more or less handled in the same manner. The findings are also closely related to the findings of several other researchers (Sultan et al. 2017 and Bhandari et al., 2013).

The mean values of TCpC in broiler meat of three different markets like Nirala market, Dumuria, and Fultola markets are summarized in (Tables 2, 3, and 4). The mean values of TCpC in Poultry meat of three different markets like Nirala, Dumuria, and Fultola markets were logged 2.31 ± 0.16 , $\log 2.50 \pm 0.02$, and $\log 2.34 \pm 0.05$ CFU/g, respectively (Table 5). The result presented in Table 5 revealed that the mean values of TCpC in meats of Nirala, Dumuria, and Fultola market were highly significant with a 1% level of probability ($P < 0.01$). Similarly, this variation of TCpC is observed in meats of different Poultry carcass as significant ($P < 0.05$). The value of Total Campylobacter Count in three different retail markets were differed significantly ($P < 0.01$). The maximum and minimum range of TSC in thigh meat estimated at Nirala, Dumuria, and Fultola markets were logged 2.60, log 2.90; log 3.10 and log 2.00, log 2.20, log 2.10 respectively evaluated in (Table 6). The average value of TSC at three markets a log 2.30, log 2.55, and log 2.60 evaluated in (Table 6). The CPC value of the Nirala market is lower than the Dumuria market, but it is the highest in the Fultola market. These findings are more similar to the findings of Isohanni (2013). Bodhidatta et al. (2013) reported a higher TCpC value from fresh broiler meat and was log 2.5 to log 3.1.

The value of TCpC at the Nirala market of Khulna City Corporation is the lowest (log 2.31) and the highest in the Fultola market (log 2.54); the findings are closely related to Hasan et al. (2015), Bjorkroth et al. (2000), and Shane et al. (2000) respectively.

Table 2: Enumeration of microbial load in broiler meat obtained from the Nirala market of Khulna City Corporation.

Place of collection	Sample no.	Microbial load			
		TVC (CFU/g)	TCC (CFU/g)	TSC (CFU/g)	TCpC (CFU/gm)
Nirala Market	1	4.80	4.50	3.00	2.20
	2	6.00	5.20	3.80	2.40
	3	5.70	6.40	3.50	2.30
	4	5.00	3.90	3.40	2.40
	5	4.80	4.00	3.20	2.30
	6	5.60	4.50	3.60	2.60
	7	6.50	5.00	3.40	2.13
	8	6.00	4.00	2.60	2.31
	9	5.85	4.72	3.00	2.40
	10	6.40	5.80	3.20	2.30
	11	5.61	5.20	3.19	2.40
	12	5.40	4.50	3.20	2.50
	13	5.70	3.90	3.30	2.30
	14	5.20	4.00	2.50	2.40
	15	5.00	5.00	3.10	2.10
	16	6.20	5.00	3.00	2.00
Mean $\pm$ SD		5.61 $\pm$ 0.27	4.72 $\pm$ 0.15	3.19 $\pm$ 0.55	2.31 $\pm$ 0.17

All counts expressed in logarithms and CFU/g of meat.

Table 3: Enumeration of microbial load in Poultry meat obtained from the Dumuria market.

Place of collection	Sample no.	Microbial load			
		TVC (CFU/g)	TCC (CFU/g)	TSC (CFU/g)	TCpC (CFU/g)
Dumuria Market	1	5.80	4.70	3.50	2.50
	2	5.40	4.38	3.50	2.50
	3	5.40	4.92	3.60	2.40
	4	5.70	4.56	3.78	2.60
	5	6.30	4.85	3.29	2.90
	6	6.20	4.50	3.40	2.50
	7	5.80	4.40	3.53	2.45
	8	6.30	4.55	3.60	2.77
	9	6.20	4.88	3.35	2.45
	10	6.59	4.28	3.40	2.50
	11	6.10	4.20	3.50	2.60
	12	5.30	4.20	3.20	2.45
	13	5.84	4.30	3.24	2.35

	14	5.60	4.50	3.22	2.20
	15	5.50	4.30	3.44	2.50
	16	5.40	4.50	3.60	2.50
Mean ±SD		5.84± 0.48	4.50±0.28	3.44±0.21	2.50±0.02

All counts expressed in logarithms and CFU/g of meat.

Table 4: Enumeration of microbial load in Poultry meat obtained from the Fultola market.

Place of Collection	Sample no.	Microbial load			
		TVC (CFU/g)	TCC (CFU/g)	TSC (CFU/g)	TCpC (CFU/g)
Fultola Market	1	6.50	4.60	4.00	2.70
	2	6.45	5.00	3.90	3.10
	3	6.30	4.55	3.50	2.65
	4	5.90	4.70	3.60	2.70
	5	6.80	5.00	3.70	3.00
	6	5.70	4.20	3.29	2.10
	7	6.70	4.00	4.20	2.60
	8	5.98	4.30	3.59	2.40
	9	6.29	4.50	3.20	2.60
	10	6.50	4.47	3.33	2.34
	11	6.00	5.00	3.49	2.10
	12	6.25	4.10	3.45	2.20
	13	6.40	4.00	3.00	2.30
	14	6.38	4.33	3.25	2.50
	15	6.10	4.00	3.00	2.60
	16	6.48	5.25	3.45	2.30
Mean±SD		6.29±0.12	4.47±0.06	3.49±0.75	2.34±0.05

All counts expressed in logarithms and CFU/g of meat.

Table 5: Determination of mean and standard deviation for statistical analysis of microbiological quality of poultry meat from different retail markets in Khulna District.

Retail Market	TVC Mean ± SD	TCC Mean ± SD	TSC Mean ± SD	TCpC Mean ± SD
Nirala Market	5.61 ± 0.27 ^b	4.72 ± 0.15 ^a	3.19 ± 0.55 ^a	2.31 ± 0.17 ^b
Dumuria Market	5.84 ± 0.44 ^{ab}	4.50 ± 0.28 ^a	3.44 ± 0.21 ^a	2.50 ± 0.02 ^a
Fultola Market	6.29 ± 0.12 ^a	4.47 ± 0.06 ^a	3.49 ± 0.75 ^a	2.54 ± 0.05 ^a
LSD	0.36	0.28	0.27	0.19
Level of sig.	*	NS	NS	**

* = Single asterisk (*) means Significant at 5% level of probability; ** = Double asterisk (**) means Significant at 1% level of probability; NS = Not significant

In a column, figures with the same letters do not differ significantly ($p > 0.05$), whereas figures with dissimilar letters differ significantly (as per SPSS).

Table 6: Range of total viable bacteria, coliform, salmonella, and campylobacter count in poultry meats obtained from the Nirala at Khulna City Corporation, the Dumuria, and the Fultola markets.

Source	Examine	TVC			TCC			TSC			TCpC		
		Max	Min	Av.	Max	Min	Av.	Max	Min	Av.	Max	Min	Av.
Nirala Market	Meat	6.5	4.80	5.65	6.40	3.90	5.19	3.80	2.50	3.15	2.60	2.0	2.30
Dumuria Market	Meat	6.59	5.30	5.94	4.92	4.20	4.56	3.78	3.22	3.50	2.90	2.20	2.55
Fultola market	Meat	6.80	5.90	6.35	5.25	4.10	4.68	4.00	3.00	3.50	3.10	2.1	2.60

All counts expressed in logarithms and CFU/gm of meat; Av. = Average

Table 7: Cultural prevalence of *E. coli* in the selected retail market

No. of retail market	Type of sample	No. of samples	Positive for <i>E. coli</i>	Percentage
3	Thigh meat	24	9	37.50(n=24)
	Breast meat	24	21	87.50(n=24)
Total		48	30	62.20(n=48)

a) Isolation of *E. coli* from the selected retail market

E. coli isolated and identified from the samples after cultivation on NA, EMB agar, and MC agar. *E. coli*

detected from total of 48 samples. Among them, 30 samples were found positive for *E. coli*, and the prevalence of *E. coli* in that study was 62.20% (Table 7).

Table 9: Cultural prevalence of *Salmonella spp* in the selected retail market

No. of retail market	Type of sample	No. of samples	Positive for <i>Salmonella</i>	Percentage
3	Thigh	24	7	29.16(n=24)
	Breast	24	16	66.66(n=24)
Total		48	23	49.91(n=48)

b) Isolation of *Salmonella spp* from the selected retail market

Salmonella spp. isolated and identified from the samples after cultivation on NA, MC agar, EMB agar, SS agar, BGA medium. *Salmonella spp.* detected from total of 48 samples 23 were found positive for *Salmonella spp.*, and the prevalence of *salmonella spp* in that study was 49.91% (Table 9). The positive samples collected from the Fultola market.

IV. CONCLUSION

The findings of this study provide valuable data about the hygienic level for retail markets. The presence of *Escherichia coli*, *Salmonella spp.*, and *Campylobacter spp* in meat must receive particular attention. These organisms are food-borne pathogens and highly responsible for causing a hazard to public health. It also reflects the poor hygienic quality of poultry meat. So the need for microbial assessment of fresh meats for human consumption is emphasized and recommended to reduce the possible hazards. Also, use of antibiotics should be considered as many strains get resistant to common antibiotics. The evidence suggests that efforts to improve food safety in poultry production should start at the village level with simple regulations directed towards addressing the most prominent deficiencies in the food-safety system into the food chain.

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Conflict of Interest

None of the authors have a conflict of interest to declare.

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