ASSESSMENT OF MICROBIAL LOAD IN MARKETED BROILER MEAT AT MYMENSINGH DISTRICT OF BANGLADESH AND ITS PUBLIC HEALTH IMPLICATIONS

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ABSTRACT

An investigation was conducted to assess the bacteriological quality of 30 samples of fresh broiler thigh meat samples sold in different retail markets in Mymensingh. Total viable count (TVC), total coliform count (TCC), total salmonella count (TSC) and total campylobacter count (TCpC) in meat samples of different broiler markets like K.R. at BAU campus, Boyra and Kewatkhali were determined. Mean of TVC, TCC, TSC and TCpC for the K.R. at BAU campus, Boyra and Kewatkhali markets were 5.69, 6.03, 6.17 $\log_{10}$ CFU/g, 4.52, 4.66, 4.69 $\log_{10}$ CFU/g, 3.35, 3.51, 3.61, $\log_{10}$ CFU/g and 2.31, 2.56, 2.66 $\log_{10}$ CFU/g, respectively. It was observed that the mean values of TVC, TCC, TSC and TCpC in case of Boyra and Kewatkhali market exceeded the ICMSF recommendations which may cause alarm to consumer's health. The variation of TVC in meats of different broiler market was significant ($P<0.05$) at 5% level of probability whereas TCC and TSC obtained from meat samples of different markets were not showed significant ($P>0.05$).The mean values of TCpC in meats of three different market were highly significant with 1% level of probability ($P<0.01$).There was no significant correlation found between TVC and TCC ($P>0.05$), but a significant correlation found between TVC with TSC and TVC with TCpC in meats of three different markets respectively. Presence of *Escherichia coli*, *Campylobacter* and *Salmonella* spp. in meats must receive particular attention, as these organisms are responsible for causing harm to public health. Suggestion has been given to improve the present sanitary condition of meat processing to minimize bacterial load.

INTRUDUCTION

Chicken is one of the most widely used delicious and versatile meats in the world largely because its protein is of excellent quality and contains all the essential amino acids needed by man. At present broiler is the cheapest meat source in Bangladesh and it contributes about 30% to the total animal protein for human consumption (Huque, 1996). Although small scale commercial boiler farms are gradually rising due to their production yield in shortest period of time and low investment. But contamination of poultry meat with food borne pathogens remains an important health hazardous issue, because of the practices of handling and management during in slaughtering, cooking or post cooking storage of the product (Javadi and Safarmashaei, 2011). Meat was the first important food that met up the hunger of ancient people living in cave (Johanson et al., 1983). It plays a very vital role in keeping the human body strong in order to provide energy, health and vigour (Rahman, 2000).

Meat obtained from chicken does not only undergo spoilage, but may also frequently been found implicated in the spread of food-borne illnesses. If hygienic care is not maintained during the various stages of slaughter operations and processing, the potential edible tissues get subjected to contamination from a variety of sources within and outside the animal and also from the environment, equipment and operators. Morethan 30 genera of micro-organisms including seven pathogens (*E. coli* O157:H7, *Campylobacter*, *Salmonella*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, are known to contaminate poultry products. Since poultry meat itself offers an excellent medium for the multiplication of most bacteria, including those that are not inhibited by low temperatures, storage of processed poultry meat is vital and therefore considered only under circumstances which inhibit the multiplication of the initial load of bacteria (Adu-Gyamfi et al., 2012). In recent years, food borne infections and intoxications have assumed significance as a health hazard. Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning (Mulder, 1999). Poultry meat is more popular in the consumer market because of advantages such as easy digestibility and acceptance by the majority of people (Yashoda et al. 2001). However, the presence of pathogenic and spoilage microorganisms in poultry meat and its by-products remains a significant concern for suppliers, consumers and public health officials worldwide. Bacterial contamination of these foods depends on the bacterial level of the poultry carcasses used as the raw product, the hygienic practices during manipulation and on the time and temperature of storage (El-Leithy and Rashad, 1989). However, the control and inspection during production, storage and distribution are generally rare. It is important to prevent the hazards and to provide a safe and wholesome product for human consumption (Singh et al., 1984). Therefore, an investigation was conducted to assess the bacteriological quality of fresh broiler thigh meat samples sold in different retail markets in Mymensingh.

MATERIALS AND METHODS

Sources, collection and transportation of samples

This experiment was carried out in the Microbiology Laboratory of Bangladesh Agricultural University, Mymensingh from 1 January to 1 April, 2014. All samples were obtained from local retail markets situated in K.R. market at BAU campus, Boyra and Kewatkhali market. The attendant first immersed the slaughtered broilers in a special tank containing hot water for some time. The immersed birds were defeathered traditionally by hand plucking and subsequently evisceration was done using special tricks or techniques. Then cut the thigh region muscle and put into a sterilized container. Samples were collected aseptically in sterile containers and brought to the laboratory within 30 minutes to determine the TVC and occurrences of different microflora gaining access to meat. During transportation the sterile containers were kept cool in iceboxes containing fragments of ice.

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Preparation of sample for bacteriological studies

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

Enumeration of TVC

For the determination of total bacterial count, 0.1 ml of each ten-fold dilution was transferred and spread on duplicate PCA using a fresh pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 37^0 C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The TVC was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organism or colony forming units per gram (CFU/g) of meat sample.

Enumeration of TCC

For the determination of TCC, 0.1 ml of each ten-fold dilution was transferred and spread on Mac Conkey agar using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 37^0C for 24-48 hours. Growth of the organism was confirmed by the appearance of turbidity. Results were calculated from MPN tables.

Enumeration of TSC

For the determination of total salmonella count the procedures of sampling, dilution and streaking were similar to those followed in total viable bacterial count. Only in case of salmonella count, xylose lysine deoxycholate agar (XLDA) was used. The calculation for TSC was similar to that of total viable count.

Enumeration of TCpC

For the determination of TCpC, 0.1 ml of each ten-fold dilution was transferred and spread on the selective blood base agar with 5% sheep or cattle blood. The diluted samples were spread as quickly as possible on 0.45 mm filter placed on blood agar base agar no 2 with a sterile glass spreader. The plates were then kept in an incubator at 42^0C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995). The results of the total bacterial count were 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-negative colonies were round, smooth and translucent with a dewdrop appearance.

Cultural and biochemical examination of samples

The cultural examination of chicken thigh meat samples for bacteriological analysis was done according to the standard method (ICMSF, 1985). The examination followed detail study of colony characteristics including the morphological and biochemical properties. In order to find out different types of microorganisms in chicken thigh meat samples, different kinds of bacterial colonies were 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 with supporting growth characteristics on various media were 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) were followed for conducting these tests.

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Statistical analysis of experimental data

The data on TVC, TCC, TSC and TCpC obtained from the bacteriological examination of meat samples of the poultry carcass collected from different area of Mymensingh district were analysed in completely randomised design (CRD) using computer package subjected to Analysis of Variance using SPSS Software (Version 16, 2007) . The differences between means were evaluated by Duncan's Multiple Range Test (Gomez and Gomez, 1984). Correlation between TVC, TCC, TSC and TCpC were also evaluated.

RESULTS AND DISCUSSION

The mean and standard deviation of the TVC in broiler thigh meats of K.R. market, Boyra and Kewatkhali markets are presented in Tables 1, 2 and 3. The variation of TVC in meats of different broiler market was significant (P<0.05) at 5% level of probability as shown in Table 4. The result of TVC in three different retail markets were differed significantly (P<0.05). The maximum and minimum range of TVC in thigh meat recorded at K.R. market, Boyra and Kewatkhali markets were log 6.2, log 6.69, log 6.6 and log 5.25, log 5.28, log 5.77, respectively (Table 5). However the average value of TVC at three markets are log 5.72, log5.93 and log 6.18 as shown in Table 5. In K.R. market the value of TVC was lower than Boyra market but it is highest in Kewatkhali market shown in Tables 1, 2 and 3. 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 case of K.R. market, the slaughter hygiene and process of broiler meat production was 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 Kewatkhali markets these are not so, rather 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 Adu-Gyamfi et al. (2012), Anwar et al. (2004) and Abu-Ruwaida et al. (1994).

The mean and standard deviation of the TCC of broiler meat processed at slaughter yards of K.R, Boyra, and Kewatkhali markets are summarized in Tables 1, 2 and 3. The result evaluated in Table 4 revealed that the mean values of TCC in meats of K.R. market, Boyra and Kewatkhali market were not significant (P>0.05). Nevertheless no significant variation was demonstrated between the interactions of the three markets. The interpretation of TCC in three different retail markets were not differed significantly (Table 4).The maximum and minimum range of TCC in thigh meat recorded at K.R. market, Boyra and Kewatkhali markets were log 5.1, log 4.96, log 5.15 and log 4.25, log 4.28, log 4.28, respectively (Table 5). However the average value of TCC at three markets were log 4.67, log 4.62 and log 4.71 as shown in Table 5. These findings were agreement with the observations of Datta et al. (2012) and Abu-Ruwaida et al. (1994), respectively. In a study Altabari and Al-Dughaym (2002) and Ahmad et al. (2013) identified lower coliform count of log 3.8 and log 2.94 CFU/ gm, respectively.

The mean values of TSC in broiler meat of three different area like K.R. market at BAU campus Market, Boyra Market and Kewatkhali Market are summarized in Tables 1, 2 and 3. The mean values of TSC in broiler meat of three different area like K.R. market at BAU campus Market, Boyra Market and Kewatkhali Market were log 3.35 ± 0.57, log3.51 ± 0.22 and log3.61 ± 0.76 CFU/g respectively (Table 4). The variation of TSC in meats of different market area was not significant (P>0.05) presented in Table 4. The interpretation of TSC in three different retail markets were not differed significantly (P>0.05). The maximum and minimum range of TSC in thigh meat recorded at K.R., Boyra and Kewatkhali markets were log 3.9 , log 3.8 , log 4.3 and log 2.75, log 3.25, log 3.1 respectively (Table 5). However the average value of TSC at three markets were log 3.32, log 3.47 and log 3.7 as shown in Table 5. The TSC value in K.R market was lower than Boyra market but it is highest in Kewatkhali 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 (Mead et al.1994 and Boonmar et al. 1998).

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The mean values of TCpC in broiler meat of three different markets like K.R. market, Boyra and Kewatkhali Markets are summarized in Tables 1, 2 and 3. The mean values of TCpC in broiler meat of three different markets like K.R, Boyra and Kewatkhali Markets were log 2.31±0.16, log 2.56 ± 0.03 and log 2.66 ± 0.07 CFU/g respectively (Table 4). The result presented in Table 4 revealed that the mean values of TCpC in meats of K.R. market, Boyra and Kewatkhali market were highly significant with 1% level of probability (P<0.01) .Similarly this variation of TCpC is observed in meats of different broiler 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 K.R. market, Boyra and Kewatkhali markets were log 2.56 , log 2.77 , log 3.2 and log 2, log 2.5, log 2.1 respectively evaluated in Table 5. Whatever the average value of TSC at three markets a log 2.28, log 2.62 and log 2.65 evaluated in Table 5. The TCpC value of K.R. market is lower than Boyra market but it is highest in Kewatkhali market. These findings have proximal relationship with the findings of Isohanni (2013) and Park et al. (1981).On the other hand Bodhidatta *et al.* (2013) found higher TCpC value from fresh broiler meat and was log 2.5 to log 3.1 .The value of TCpC at K.R market of BAU campus is lowest (log 2.31) and highest in Kewatkhali market (log 2.66), the findings are also very much close to Federighi et al. (1995), Bjorkroth et al.(2000) and Shane *et al.* (2000) respectively. A significant and positive correlation was found in TCpC at different retail markets with different broiler meat. A highest result 5.33 log 10 c.f.u. per carcass, respectively noted by Cason et al. (1997).

The result estimated in Figure 1 showed weakly correlated between the TVC and TCC. In this study, viable counts were did not significantly correlated with total coliform count in three market area. The regression equation and correlation coefficient values were, y = -0.1323x + 6.5798., and R^2=0.0088 as shown in Fig. 1. The result shown in figure 2 revealed that the regression was positively correlated with TVC and TSC in different market, where correlation coefficient was R^2=0.0377 and regression equation was y = 0.2419x + 5.1227, respectively. Hence the increase of TVC will enhance by the increase of TSC abruptly the decrease of TVC will be decrease with the TSC count (Figure 2). The result evaluated in figure 3 showed positively and significantly correlated between the viable count and total Campylobacter count in three market place. The regression equation and correlation coefficient values were y = 0.5741x +4.5265 and R^2 = 0.1468, respectively (Figure 3). It seems that a significant relation were found between TVC and TCpC, respectively.

Table 1. Enumeration of microbial load in broiler meat obtained from K.R market of BAU Campus.

Place of collection	Sample no.	Microbial load			
		TVC (CFU/g)	TCC (CFU/g)	TSC (CFU/g)	TCpC (CFU/gm)
	1	5.8	4.63	3.9	2.1
	2	5.57	5.0	3.83	2.0
	3	5.4	4.25	3.26	2.34
	4	5.25	4.55	3.38	2.44
	5	5.3	5.1	3.15	2.2
K.R. Market	6	5.8.	4.35	3.36	2.5
	7	6.0	3.9	3.65	2.13
	8	6.1	4.5	2.75	2.47
	9	5.55	4.56	3.2	2.56
	10	6.2	4.4	3.1	2.33
Mean ± SD		**5.69± 0.28**	**4.52± 0.16**	**3.35± 0.57**	**2.31± 0.16**

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

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Table 2. Enumeration of microbial load in broiler meat obtained from Boyra market.

Place of collection	Sample no.	Microbial load			
		TVC (CFU/g)	TCC (CFU/g)	TSC (CFU/g)	TCpC (CFU/g)
	1	5.9	4.7	3.56	2.6
	2	5.28	4.68	3.35	2.56
	3	5.39	4.89	3.65	2.36
	4	5.8	4.77	3.70	2.77
Boyra Market	5	6.45	4.95	3.68	2.8
	6	6.29	4.45	3.29	2.45
	7	5.93	4.35	3.45	2.5
	8	6.37	4.65	3.43	2.66
	9	6.28	4.88	3.7	2.4
	10	6.59	4.28	3.25	2.53
Mean ±SD		**6.03± 0.49**	**4.66±0.29**	**3.51±0.22**	**2.56±0.03**

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

Table 3. Enumeration of microbial load in broiler meat obtained from Kewatkhali market.

Place of collection	Sample no.	Microbial load			
		TVC (CFU/g)	TCC (CFU/g)	TSC (CFU/g)	TCpC (CFU/g)
	1	6.32	4.66	4.3	2.66
	2	6.45	5.15	3.8	3.1
	3	6.2	4.78	3.4	2.55
	4	5.8	4.65	3.67	2.8
Kewatkhali Market	5	6.15	4.8	3.56	3.2
	6	5.77	5.0	3.29	2.1
	7	6.6	4.36	4.1	2.44
	8	5.98	4.28	3.69	2.5
	9	6.05	4.5	3.10	2.7
	10	6.46	4.76	3.22	2.56
Mean±SD		**6.17±0.09**	**4.69±0.07**	**3.61±0.76**	**2.66±0.07**

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

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Table 4. Determination of mean and standard deviation for statistical analysis of microbiological quality of chicken thighs at different retail markets in Mymensingh.

Retail Market	TVC Mean ± SD	TCC Mean ± SD	TSC Mean ± SD	TCpC Mean ± SD
K.R. Market	5.69 ± 0.28^b	4.52 ± 0.16^a	3.35 ± 0.57^a	2.31 ± 0.16^b
Boyra Market	6.03 ± 0.49^{ab}	4.66 ± 0.29^a	3.51 ± 0.22^a	2.56 ± 0.03^a
Kewatkhali Market	6.17 ± 0.09^a	4.69 ± 0.07^a	3.61 ± 0.76^a	2.66 ± 0.07^a
LSD	0.35	0.28	0.31	0.22
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 same letter do not differ significantly (p>0.05) whereas figures with dissimilar letter differ significantly (as per DMRT)
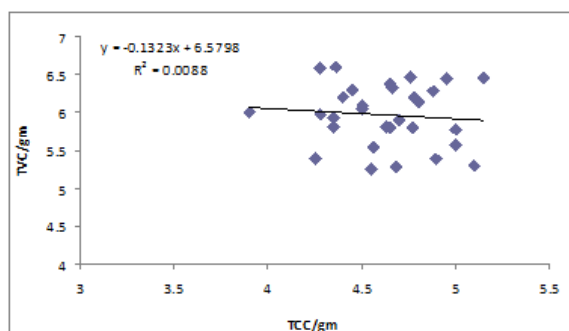

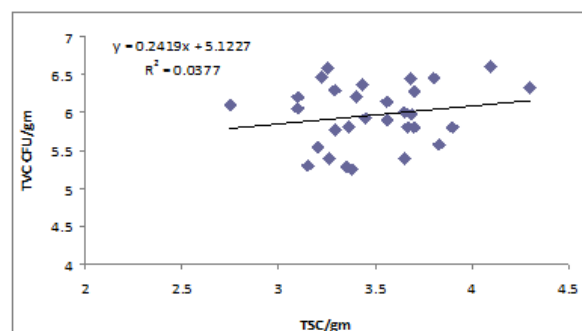**Figure 1.** Correlation between TVC and TCC in CFU/g meat of three different retail market

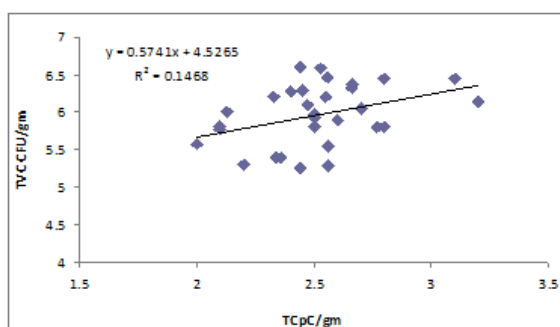**Figure 2.** Relationship between TVC and TSC in CFU/g meat of different market area

Figure 3. Relationship between TVC and TCpC in CFU/gm meat of different retail market

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Table 5. Range of total viable bacteria, coliform, salmonella and campylobacter count in broiler meats obtained from K.R. at BAU, Boyra and Kewatkhali markets.

Source	Examine	TVC			TCC			TSC			TCpC		
		Max	Min	Av.	Max	Min	Av.	Max	Min	Av.	Max	Min	Av.
K.R. Market	Thigh	6.2	5.25	5.72	5.1	4.25	4.67	3.9	2.75	3.32	2.56	2.0	2.28
Boyra Market	Thigh	6.59	5.28	5.93	4.95	4.28	4.61	3.70	3.25	3.47	2.77	2.5	2.62
Kewatk-hali Market	Thigh	6.6	5.77	6.18	5.15	4.28	4.71	4.3	3.1	3.70	3.2	2.1	2.65

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

CONCLUSIONS

Bacterial genera identified in this study are known as food borne bacteria which may cause food borne infection and intoxication. The study revealed that the mean values of TVC, TCC, TSC and TCpC of K.R. market was lower than the other two related markets. On the basis of bacterial load K.R. market at BAU campus, Boyra and Kewatkhali markets were graded as A, B and C respectively. Presence of *Escherichia coli*, *Salmonella* and *Campylobacter* in meat must receive particular attention, as these organisms are responsible for causing hazard to public health. These high levels of microbial contamination reflect the poor hygienic quality of poultry meat.

ACKNOWLEDGEMENTS

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