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Microbial assessment of beef in selected areas of Mymensingh district in Bangladesh

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Abstract

This study was conducted to evaluate microbial load of beef meat during handling and selling in market. Total 12 samples were collected from *K.R* market, *Sheshmore* market, *Kewatkhali bazaar* and Mymensingh *Sadar* market. These samples were subjected to determine Total Viable Count (TVC), Total Coliform Count (TCC) and Total Yeast and Mould Count (TYMC) by using standard protocol at 0 hr, 2hrs and 5hrs time intarvel. The microbial counts of beef at different markets were high but there were no significant differences ($p>0.05$) among the markets. In every cases with the increased of time interval all types of microbial count increased significantly ($p<o.o1$) than the initial time. Microbial contamination of beef occurs as plant workers and machinery repeatedly touch contaminated surfaces and the carcass. Thoughtful design of operating procedures, especially for flaying and evisceration, can greatly reduce this problem. Frequent washing and sanitizing of hands, tools, and machinery is required in order to remove bacteria before they reach the exposed surface of the carcass.

Key words: beef, microbial assessment, contamination, storage quality, local market

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Introduction

The highest percentage of slaughtering in Bangladesh is cattle (Ali *et al,* 2013). Despite the contribution to the daily protein intake, beef can be a source of food-borne illnesses especially under the condition in which animals are handled, slaughtered, transported and sold on markets. Prescott *et al,* 2002 have shown that food items especially meat, are not only of high nutritional value to those who consume them but often are ideal culture media for microbial growth. Meat is one of the most perishable foods, and its composition is ideal for the growth of a wide range of spoilage bacteria (Mayr *et al*, 2003). Fresh raw meat like beef have been implicated for a number of meat borne infections and intoxications in several countries (Mukhopadhyay *et al*, 2009). This is because both pathogenic and non-pathogenic organisms live in the gastro-intestinal tract of cattle which can be transferred into the meat under faulty and poor processing conditions. Microbial food poisoning or infections are a matter of grave public health concern. Beef is a high protein food which is widely consumed by majority of the populace and it is a very delicate product which is susceptible to microbial invasion and subsequent deterioration.

However, raw retail meats have been identified as potential vehicles for transmitting food-borne diseases, and hence the need for increased implementation of hazard analysis of critical control point (HACCP) and consumer food safety education efforts. Bacterial food poisoning is widely spread and occurs when our environments are untidy and the foods are not hygienically maintained. Fresh meats are sometimes contaminated with bacteria, which can be harmful to the human body. The major bacterial pathogens include: Bacillus cereus, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Coliforms, *Bacillus cereus* and *Escherichia coli*. The sources of these microbes in meat could be inherent micro-flora in normal tissues of animals, air, environment, or contamination due to unhygienic slaughtering, handling and processing conditions.

At each stage of beef processing after slaughtering, different microbes get introduced and these tend to contaminate the meat. The microbes cause biochemical and microbiological changes in the meat which may lead to the production of noxious substances resulting in the incidence of illnesses such as cholera, typhoid fever and other fatal diseases. Besides the chemical composition, meat culinary and technological value is determined by its

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physicochemical properties and one such main indicator is pH. The pH of food is critical because at low levels, it favors the growth of moulds and yeasts. In neutral or alkaline pH foods such as meat and meat products, bacteria are more dominant in spoilage process. The high protein content of meat makes the pH approximately neutral and it leads to a high level of spoilage in the meat and this is further explained by the breakdown of muscle glycogen leading to the production of lactic acid in the muscle fibres. The implementation of appropriate risk mitigation strategies which includes the design of public health policies and appropriate food safety measures is urgently needed. This will require food borne disease surveillance data from food monitoring systems in mandated institutions as well as independent institutions that have no personal interests at stake. To date, there is no literature on the level of contamination of fresh meat sold in the market. Majority of the slaughter houses have no HACCP systems in place. Hence poorly designed facilities for the slaughtering and processing of beef can easily result in contamination of food products and lead to food-poisoning incidents. Sanitation of slaughter house is very poor. A number of abattoirs and meat processing units are under standard and operated without adequate quality control systems in Bangladesh. Meats are normally transported to the markets either in meat vans, taxis, motor cycle and bicycles. Furthermore meats are sold in the open markets sometimes in sieves or without sieves, and on tables that are not well maintained or cleaned after work. This exposes the meat to a number of pathogens some of which may be pathogenic or non-pathogenic.

In order to ascertain this, the present study was undertaken to evaluate the microbiology of beef sold in some selected market of Mymensingh municipal area and to identify the possible sources of contamination with the following objectives:

i. To examine the microbial load of beef at different hours of interval from slaughtering to selling.

ii. To measure the microbial load of beef in different market during handling and selling.

Materials and Methods

Experimental site and period

The experiment was conducted in the Animal Science laboratory under the department of Animal Science, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. The experiment was conducted from July to December, 2014.

Sample collection

The samples of beef were collected from the Bangladesh Agricultural University *sheshmore* market , *K.R* market , *Kewatkhali* market and *Mymensingh sadar* market. A quantity of 1000 ±30 g of beef samples were collected from each market. The samples were collected from thigh region of bull without the fat, ligaments, bone and tendons. Meat sample were collected in sterilized bags, level the bags and stored in -20^{0}C for the pending analysis.

Experimental design

There were three treatments in this study. These were T_0= 0 hr, T_1= 2 hrs, T_2= 5 hrs that is the bacterial counts were taken at 0, 2, and 5 hours after the collection of the sample from each location. For microbial assessment total viable count (TVC) , total coliform count (TCC) , and total yeast-mould count (TYMC) were undertaken. The experimental lay out have shown in Table 1.

Table1. Experimental lay out

Samples	Treatments (Time interval)	No. of Replications
Place 1: *K.R Market*	T_0-0 hr (control), T_1-2hr, T_2-5hr	3
Place 2: *Sheshmore*	T_0, T_1, T_2	3
Place 3: *Kewatkhali*	T_0, T_1, T_2	3
Place 4: Mymensingh *sadar*	T_0, T_1, T_2	3

Media and reagents

For the bacteriological analysis the three used media were plate count agar (PCA), MacConkey agar (MA) and potato dextrose agar (PDA).To prepare the PCA and MA 13.13g PCA and 33.49g MA agar were dissolved in 750 ml and 650 ml of cold distilled water respectively in two separate conical flasks and heated to boiling for dissolving the ingredients completely. The media was sterilized at 121^{0}C for 15 minutes in an autoclave. The final reaction was adjusted to pH 7.0±0.1. The agar was then ready for pouring. In case of PDA, 25.35 g of previously peeled and sliced potato were

taken in 650 ml of distilled water in a conical flask and boiling for dissolving the ingredients completely. After boiling, sieving had done through clean cheesecloth. 5.76 g peptone agar was dissolved in 576 ml distilled water and heated up to boiling to dissolve the ingredients pouring, the media was kept in boiling water bath at 45 ^{0}C.

Experimental equipments

Different types of glassware and equipments were used during the period of the experiment. These were: test tubes, petrideshes, conical flask, pipette (1 ml, 5ml, 10ml and 25ml capacities), glass rod spreader, test tube stand, mortar and pestle, whirly mixture machine, blender machine, water bath, incubator, refrigerator, sterilizing instruments, hot air oven, ice boxes, electronic balance.

Preparation of sample for microbial studies

Each of the beef samples were thoroughly and uniformly macerated in a mechanical blender using a sterile diluents (0.1% peptone water) as per recommendation of International Organization for Standardization (ISO ,1995). A quantity of 30g of the minced meat sample were taken aseptically transferred into a sterile container containing 90 ml of 0.1% peptone water. Homogenized suspensions were 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} and 10^{-6} will be prepared according to the instruction of the standard method (ISO, 1995).

Enumeration of Total Viable Count (TVC)

For the determination of TVC, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate PCA 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 35^{0}C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. Colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution were multiplied by the dilution factor to obtain the Total Viable Count. The TVC was calculated according to ISO (1995).

Enumeration of Total Coliform Count (TCC)

For the determination of TCC, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate MA 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. The plates were kept in an incubator at 35^{0}C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted with the aid of a colony counter. The average numbers of colonies in a particular dilution were multiplied by the dilution factor to obtain the TCC. The TCC were calculated according to ISO (1995).

Enumeration of Total Yeast and Mould Count (TYMC)

For the determination of TYMC, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate PDA 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. The plates were kept in an incubator at 25^{0}C for 48-72 hours. Following incubation, plates exhibiting 30-300 colonies were counted by colony counter. The average numbers of colonies in a particular dilution were multiplied by the dilution factor to obtain the TYMC. The TYMC were calculated according to ISO (1995).

All the results of microbial count were expressed as the number of organisms or colony forming units per gram (cfu/g) of meat sample. Then results were calculated into log value.

Table 2. Total Viable Count (TVC)

Sources of meat	Microbial Count (log cfu/g)±SD			Significance level
	T_0	T_1	T_2	
K.R Market	4.794±0.01	4.931±0.03	5.173±0.05	
Sheshmore	4.783 ±0.02	4.884±0.01	5.287±0.01	**
Kewatkhali	4.710±0.01	4.995±0.01	5.349±0.03	
Mymensingh *sadar*	4.750±0.02	4.966±0.01	5.548±0.01	
Significance level		NS		

T_0, 0 hr; T_1, 2 hrs; T_2, 5 hrs; SD, Standard deviation, ** at 1% level of significance, NS, Non significant

Statistical analysis

All the average, means and standard deviations were calculated through Microsoft Excel 2010 Data analysis tool pack. SPSS 17.0 is used to determine the correlation among different microbial counts. One way ANOVA from Microsoft Excel 2010 Data analysis tool pack was used to calculate *P* value. Means were considered significantly different for $P<0.05$. Data are shown as mean ± SD.

Result and Discussion

Total Viable Count (TVC)

The TVC values of different treatment levels are shown in Table 2. The highest values for TVC were found at 5 hrs in Mymensingh *Sadar*, but there were no significant differences in TVC among the markets assessed ($p>0.05$). All the results of TVC at 5 hrs showed a gradual increase in TVC in all markets than 0 hrs. Significant differences among different hours in every cases ($p<0.01$) were found, which is alike as found in goat meat by Parvin *et al*, (2017). TVC values found in the meat samples of the present study were a slightly lower at 0 hour and 2 hours but higher at 5 hours in all markets than the range of Haque *et al,* (2008). TVC found in meat samples of the present study is a slightly higher than results of Bhandare *et al*, (2007) .The possible cause of this variation in microbial load might think to be due to differences in management and hygienic practices. Observation of the investigation revealed the fact that timing is important factor in case of meat hygiene. Similarly Hassall (1995) lead the opinion that meat production in Bangladesh took place in a much disorganized way for which TVC increases with time passes.

Total Coliform Count (TCC)

The results of TCC are showed in Table 3. Coliform present on the intestinal content and can multiply rapidly in the meat. The highest values for TCC were found at 5 hrs in Mymensingh *Sadar*, but there were no significant differences in TCC among the markets assessed ($p>0.05$). The TCC in Mymensingh *Sadar* might be reflected the opinion the carcass was more subjected to exposure and contamination with the fecal materials during handling, and processing of the carcass. Like TVC all the results of TCC at 5 hrs showed a gradual increase in TCC in all markets than 0 hrs. In all cases significant differences ($p<0.01$) in TCC levels were found with increases of time. Haque *et al,* (2008) also found higher TCC in late hours meat. The TCC levels of this study were in close agreement with the findings of Datta *et al,* (2012), Eze *et al, (*2012), Kumar *et al,* (2010) and Arian *et al,* (2010).

Table 3. Total Coliform Count (TCC)

Sources of meat	Microbial Count (log cfu/g)±SD			Significance level
	T_0	T_1	T_2	
K.R Market	1.547±0.0 2	1.647 ±0.02	1.717±0.01	**
Sheshmore	1.531 ±0.01	1.617±0.02	1.727±0.03	
Kewatkhali	1.549±0.02	1.646±0.02	1.706±0.03	
Mymensingh *sadar*	1.505±0.01	1.696±0.01	1.771±0.03	
Significance level		NS		

T_0, 0 hr; T_1, 2 hrs; T_2, 5 hrs; SD, Standard deviation, ** at 1% level of significance, NS, Non significant

Table 4. Total Yeast and Mould Count (TYMC)

Sources of meat	Microbial Count(log CFU/g)±SD			Significance level
	T_1	T_2	T_3	
K.R Market	1.850±0.02	1.901 ±0.01	1.978±0.01	**
Sheshmore	1.900 ±0.03	1.923±0.01	1.996±0.02	
Kewatkhali	1.830±0.01	1.934±0.02	2.061±0.03	
Mymensingh *sadar*	1.877±0.01	1.933±0.02	2.037±0.03	
Significance level		NS		

T_0, 0 hr; T_1, 2 hrs; T_2, 5 hrs; SD, Standard deviation, ** at 1% level of significance, NS, Non significant

Total Yeast and Mould Count (TYMC)

The TYMC value of different treatment levels with different market places is shown in Table 4. The highest values for TYMC were found at 5 hrs in Kewatkhali market, but there were no significant differences in TYMC among the markets assessed ($p>0.05$). Among all treatments, the TYMC at 5 hour after slaughter were significantly ($p<0.01$) higher than the samples at 0 and 2 hours. TYMC found in meat samples of the present study were higher than the range of the experiment of Arian *et al* (2010), probably due to the time factor.

Conclusion

From this study it has been found that the microbial count of beef of different markets was high and there were no differences among the markets. In every cases with the increased of time interval microbial count also increased significantly. Thus, it is important to further protect the meat by slowing bacterial growth or destroying the bacteria after slaughter. Even with the best of procedures, avoidance or removal of all bacteria is not practical in the dressing and manufacturing of fresh meat, but hygiene practices, microbial risk evaluation, and quality management may augment the safe meat production. Slowing bacterial growth is usually achieved by chilling the carcass or meat to 4^{o}C or less.

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