



## Original Article

# Microbiological quality of beef and beef products in Dhaka city

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## ABSTRACT

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### Keywords

Beef, Bacteria, Meat safety, Public health, Ready-to-eat.

The aim of the study was to observe the overall microbiological quality and occurrence of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. in beef and beef products in Dhaka city, Bangladesh. Total 9 types of beef including 2 raw type beef, 3 ready-to-cook (RTC) and 4 ready-to-eat (RTE) beef samples were considered for microbiological analyses. Total Aerobic Count (TAC), Total Yeast Mold Count (TYMC), Total Coliform Count (TCC), *Listeria* spp., *Salmonella* spp. and *E. coli* count were measured in these beef and beef products to assess the microbial safety level. API Listeria Kit analysis was done to ensure the presence or absence of *Listeria monocytogenes*. Results showed that in raw, ready-to-cook (RTC) and ready-to-eat (RTE) beef items, the mean TAC ranges 6.2 to 6.5, 4.2 to 4.5 and 5.6 to 5.75 log CFU/g respectively. Similarly, the mean TCC ranges 3.3 to 3.6, 3.2 to 3.5, 3.3 to 3.8 log CFU/g respectively. TYMC ranges 3.3 to 4.2, 2.4 and 2.5 to 3.7 log CFU/g respectively for raw beef, RTC and RTE beef items. The presence and load of pathogenic microorganisms such as *Salmonella* spp., *Escherichia (E.) coli*, and *Listeria (L.)* spp. were examined. The occurrence of *Listeria* spp. *E. coli* and *Salmonella* in the beef and beef products has great significance in public health. According to the results, recommendation such as implementation hygienic rules, routine inspection, and training in the production chain may be suggested to increase safety in terms of microbiology as well as to minimize the risk of foodborne outbreak from the raw beef and RTC and RTE beef items.

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## Introduction

Food safety and public health has become the significant part of human life now-a-days. Different types of meat borne pathogen and pathogenic microorganisms are the possible threat for the food safety issue (Niyonzima *et al.*, 2017; Meisel *et al.*, 2014; Kotzekidou, 2013). Therefore counter action against these pathogenic microorganisms in meat as well as food items is immense need for this nation. But this will also be very difficult task for developing countries like Bangladesh (Atter *et al.*, 2015). Therefore it is high time to make policy and take immediate action by the government to establish rules and regulations for maintenance of meat safety by reducing the microbial contamination in different sectors of meat production from farm to fork. According to (DLS, 2018), in Bangladesh the annual demand of meat is 7.21 million metric ton (120 g/day/head) where the annual

meat production is 7.26 million metric ton. Therefore, the demand has been fully managed but the meat quality is a question for the consumers in our country. In Bangladesh, meat is cooked at higher temperature, mostly above the boiling point. That's why, it is considered of less risky but during preparation or processing, that meat can be the source of pathogenic microbes. For example, cutting or slicing of contaminated meat will contaminate the cutting board, knife, floor, the handle's hands etc. Next time preparation of salad items might be contaminated from these previous contamination which is not cooked before consumption. Now the seriousness about food safety among the consumers is increasing day by day. Therefore, it is important to maintain the food quality at best level. All the sections of food preparation from farm to fork need to be careful. For instance, during meat production and preparation, it will start

from slaughtering of animal then hiding, evisceration, thus going on and finally the hygienic meat will be reached to the consumer's hand. Faulty or wrong of preparation may contaminate the meat by various pathogenic organisms like *E. coli*, *Salmonella* etc. (Sudhakar et al., 2009). In some cases the ready-to-eat (RTE) and ready-to-cook (RTC) meat also contain these types of microbes which indicate the post processing contamination of meat.

Among the types of food, meat has good nutritional value for not only human but also acts as good growth media for different types of microorganisms. According to Mayr et al. (2003), meat composition provides the essential nutrients for microbial growth. Raw beef may be contaminated by pathogenic microbes which may cause different types of food-borne diseases for human (Mukopadhyay et al., 2009). This is because gastro-intestinal tract of cattle or other farm animal is the harbor of pathogenic and non-pathogenic microorganisms which can contaminate meat if wrong techniques are used for processing. Types of Ready-to-eat meat products are mostly harbor the common pathogenic microorganisms like *Salmonella* spp. *E. coli*, *Listeria* spp. etc. (Magwedere et al., 2013). Different disease like diarrhea, typhoid, listeriosis, gut problem, are found closely related with the various food borne pathogen (Asiegbu et al., 2016).

Among these different pathogenic microorganisms in meat, *L. monocytogenes* is mostly found and it is psychotropic which survives at freezing temperature. So meat preservation at lower temperature may also contain this organism which is alarming issue for consumption of frozen meat items (EFSA, 2015). ICMSF (1996) reported that this microorganism could last and multiply at larger range of temperature, different salt concentration and adverse pH level. Since the significance of *L. monocytogenes* in public health, a "zero tolerance" policy was developed by US controlling authority for different ready-to-eat food items (Gombas et al., 2003).

In spite of being good nutrient source for human, ready-to-eat meat products may be contaminated by different foodborne pathogenic microorganisms which may cause human diseases outbreak (Niyonzima et al., 2017; Kotzekidou, 2013; Rane, 2011). Unhygienic environment, meat processing equipment, lack of cleaning facilities, raw meat cross-contamination, unskilled butcher, personnel and lack of hygienic knowledge mostly lead to contamination of ready-to-eat meat products (Niyonzima et al., 2017; WHO, 2011; Manguiat & Fang, 2013). Paudyal et al. (2017) reported that *Enterobacteriaceae*, *L. monocytogenes*, *E. coli*, *Salmonella*, *Staphylococcus aureus* are the dominating bacteria which is pathogenic in nature and found in types of food items. It was also found that among the different foodborne pathogenic microorganisms *E. coli*, *Salmonella*, *L. monocytogenes*, *Bacillus cereus*, *Shigella*, *Staphylococcus aureus* are mostly found in meat and meat products (Magwedere et al., 2013). As a food safety issue, microbiological contamination of meat has important impact (Wong et al., 2000; Dey et al., 2013) on human health. The negative impact of *L. monocytogenes* is considered very severe in case of food safety because this organism is related to the possible cause of miscarriage, gut problem, septicemia, meningitis etc. (Latorre et al., 2007). From the survey in Sweden at 2010, it was showed that the occurrence of listeriosis from 2005 to 2009 was increasing day by day due to the consumption of ready to eat meat (Lambertz et al., 2012).

The information for the incidence of pathogenic microbes specially *L. monocytogenes* in beef and beef products (RTC, RTE) marketed in Dhaka city of Bangladesh is not enough for growing food safety issue. Therefore, the study was conducted to know the occurrence of *E. coli*, *Salmonella* spp., *L. monocytogenes*, TAC (total aerobic count), TYMC (total yeast mold count), TCC (total coliform count) in beef (raw type) and beef products, marketed in the super shop in Dhaka city as well as API Listeria kit was used to assure the presence or absence of *L. monocytogenes* among the samples.

## Materials and Methods

### Sample preparation

Total 9 various types of beef (raw) and beef products (ready-to-cook and ready-to-eat) samples were collected from super shop and local butchers of Dhaka city, Bangladesh. All the samples were collected at aseptic condition. The microbiological analyses were done in "Food analysis and research laboratory, Centre for Advanced Research in Science, University of Dhaka, Dhaka 1000".

Among these samples, 2 sample were retail cut raw beef and raw ground beef, 3 sample were ready-to-cook type- beef ball, beef sausage and beef burger petty and 4 sample were as ready-to-eat type- beef pepperoni, beef salami, beef loaf, beef bacon. Replication of the samples were considered to avoid error. Retail cut raw beef samples were taken from different local butchers of Dhaka city and other types of beef products were purchased from "Agora super shop", Mohammadpur, Dhaka.

At the time of beef products purchasing, the preservation temperature, expire date, brand name all these products details were recorded. After purchasing and before taking these sample to the laboratory sterile poly bag and cool chain were maintained to avoid product spoilage as well as prevent further contamination. At the laboratory, analyses were done soon to maintain the sample quality.

### Microbiological analyses

In case of microbiological analyses, all the necessary tools, equipment were sterilized and prepared. The chopping board, knife, scissors, weight machine, stomacher bag and the sample were taken to the laminar air flow. Then 10 g of the sample and 90 ml normal saline were taken to the sterile stomacher bag as 1:9 ratio. The stomacher content was blended by using the "stomacher machine (Stomacher® 400 Circulator Seward, UK)" at 200-250 rpm for 1 minutes. After that  $10^{-1}$  to  $10^{-4}$  serial dilution of the sample were also prepared.

For microbiological culture, different culture media was used. To detect total aerobic count (TAC) Plate count agar (PCA) was used. Similarly for *E. coli* and total coliform count (TCC) Chromocult agar media was used as well as for *Salmonella* spp., Total yeast mold count (TYMC), *Listeria* spp. determination Bismuth Sulfite Agar (BSA), PDA (Potato Dextrose Agar), *Listeria* PALCAM agar were used respectively. The microbiological culture media were prepared by following the manufacture guidelines. After preparation of sterile culture media, the sample of different diluent were inoculated and spread over the media. Then all the sample inoculated media were incubated at 37°C for 1-2 days except PDA media where incubation temperature and time was 30°C and 48-72 hours respectively. After this step counting of the microbial colony from the plate were completed. In case of Chromocult agar media, the violet blue

colony was counted as *E. coli* and pink colony was counted as coliform. The deep black centered colony of BSA media were counted as *Salmonella* spp.

#### API *Listeria* kit analysis

This API kit (species specific) was specifically for checking whether there was *L. monocytogenes* or not in these samples. This rapid test was mainly done on the basis of biochemical and morphological properties of *Listeria* organism. *Listeria* spp. were determined from the culture of sample at *Listeria* PALCOM agar media.

At first *Listeria* spp. colony were dissolved into the kit supplied solution. After incubation of these kit strips different color were found where number against the strip color were noted. The recorded number from the kit was input into the API web software and then the *Listeria* species name was found from the software. Manufacturing guidelines were followed to prepare samples and kit as well.

#### Statistical analysis

The results and related calculation were performed in MS Excel software 2010 (Microsoft Corporation). Each of the value was represented as mean  $\pm$  SD (Standard Deviation) and each sample was triplicated to avoid the error.

### Results and Discussion

#### Microbiological quality of Raw beef samples

Figure 1 represents the microbiological quality of retail cut raw beef and ground beef samples. Between raw and ground beef, TAC, TCC, *Salmonella* spp., *Listeria* spp. counts were almost similar and their range was 6-6.8, 3.2-3.8, 3-3.2, 2.2-2.6 log CFU/g. But for *E. coli* and total yeast mold, the count was different where *E. coli* count for raw beef was 2.3-2.6 log CFU/g and for ground beef, it was 2 log CFU/g. Also for the total yeast mold count, the count for raw beef was higher than ground beef. These findings interpret that in both cases the TAC, *Salmonella* spp. and *Listeria* spp. were at an unacceptable level according to ICMSF (2001) where there should be less than 5 log CFU/g of sample for TAC and less than 2 log CFU/g for *Salmonella* spp. and *Listeria* spp. respectively as well. A similar kind of experiment of Dutta *et al.* (2012) had shown higher TAC value for raw type meat. Svobodova *et al.*, (2012) reported such like unacceptable raw meat in terms of microbial contamination. The results of Anowar *et al.*, (2004) was almost similar to our results for raw meat microbiological results. The unhygienic slaughter floor, fecal contamination can be the possible reason for higher TAC in raw type meat (Adu-Gyamfi *et al.*, 2012; Haque *et al.*, 2008). Improper handling facilities and lack of hygienic structure of the slaughter area are mostly responsible for higher level of microbiological contamination for raw meat in Bangladesh (Bolton *et al.*, 1996; Hassall, 1995). The TCC of our results for raw meat were not so alarming as these were less than 4 log CFU/g according to ICMSF (2001). Afrin *et al.*, 2017 found less TCC than our findings from raw beef samples in Mymensingh. Dutta *et al.* (2012) found Highest TCC at 5.5 log CFU/g in raw type of meat which was more than our findings and at unacceptable level. Murshed *et al.* (2016) found TCC of raw beef at 3.3 log CFU/g which was almost similar like this findings. *Salmonella* spp., *E. coli* and *Listeria* spp. level of raw beef in this experiment were not at satisfactory level as the acceptable level is considered at  $\leq 2$  log CFU/g of meat (ICMSF, 2001). The incidence of *E. coli*, *Salmonella* spp. and *Listeria* spp. reflected that the beef sample was

contaminated by fecal materials by the animal itself or from outer sources (Akhtar *et al.*, 2014). Among the *Listeria* spp., *L. monocytogenes* was not found after API *Listeria* diagnostic test. So in this cases, *Listeria* spp. is not pathogenic. The TCC and *E. coli* of raw beef indicated that it was not prepared hygienically (Harhoura *et al.*, 2012). Total yeast mold count was also very high for both but raw beef had higher count. Afrin *et al.* (2017) found 1.8 log CFU/g yeast mold count from raw meat which lower than our findings. Sometimes the contaminated water can be the *Salmonella* spp. source which may contaminate the carcass and meat as well. Simasiku, 2016 found 14% *Salmonella* contaminated beef sample in Namibia. Gill and McGinnis (2000) reported that sometimes the meat chopper, grinders or such like of instruments for meat preparation could also be the source for raw meat contamination. Fenlon *et al.* (1996) found raw meat contamination by *L. monocytogenes* due to slaughtering and wrong of carcass processing. Faulty ways of meat preparation and unhygienic practice are mostly cause these various undesirable raw beef microbial contamination (WHO, 2011).

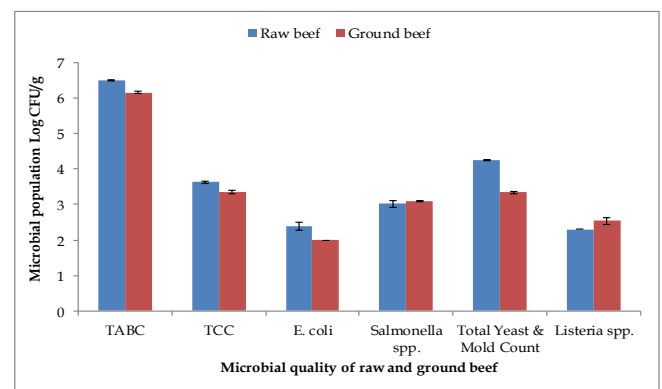
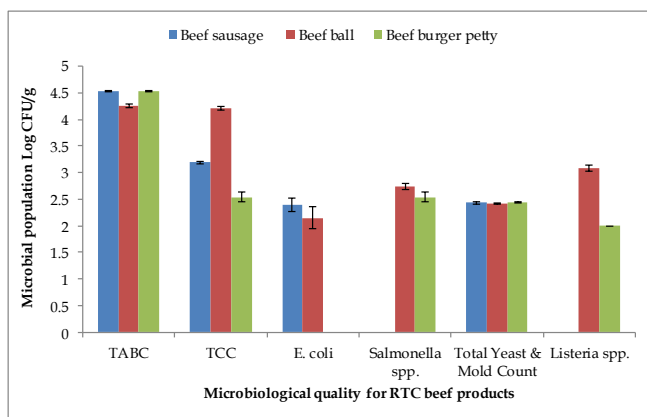


Figure 1. Microbiological quality of raw and ground beef.

#### Microbiological quality of Ready-to-cook (RTC) beef products samples

The microbiological quality of three different ready-to-cook beef products (beef ball, beef sausage and beef burger petty) had been shown in figure 2. Among the ready-to-cook beef products, TAC and Total yeast mold count (TYMC) were almost similar in condition and their range was 4.2- 4.6 and 2.3-2.4 log CFU/g respectively. TCC varied from different products where highest count was for beef ball and it was 4.2 log CFU/g and lowest for beef burger petty and it was 2.5 log CFU/g. The TCC of beef sausage was 3.2 log CFU/g. For *E. coli* count, beef sausage and beef ball had *E. coli* and it was 2.2- 2.5 log CFU/g where the beef burger petty had no *E. coli*. For *Salmonella* spp., beef sausage had no *Salmonella* but beef ball and beef burger petty had *Salmonella* spp. and it was within the range of 2.5- 2.8 log CFU/g. For *Listeria* spp., no *Listeria* spp. was found in beef sausage but in beef ball and beef burger petty had the *Listeria* spp. and the count was 3 and 2 log CFU/g. The TAC and total yeast mold count were not too much high when compared with raw beef types. TAC of all RTC were in acceptable range ( $\leq 5$  log CFU/g) (ICMSF, 2001). Cooked meat samples of Dutta *et al.* (2012) had unsatisfactory TAC level which didn't match with our results. Similarly Murshed *et al.* (2016) found higher level of TAC in chicken toast, sharma samples of Mymensingh city which was higher than 6 log CFU/g. According to ICMSF (2001), the TCC and TYMC results of the experiment were at satisfactory level ( $\leq 4$  log CFU/g). Murshed *et al.* (2016)

and Mousa *et al.* (2014) found similar kind of results after analyzing TCC and TYMC of different cooked meat products like hot dog, beef burger, beef sausage, luncheon, pastirma etc. The *E. coli*, *Salmonella* spp., *Listeria* spp. were at satisfactory level in these RTC samples ( $\leq 2$  log CFU/g) (ICMSF, 2001). Unhygienic environment and lack of processing skill may also be the source for meat contamination by different pathogenic microorganisms in RTC meat products (Akhtar *et al.*, 2014). In Mexico, 11.6% grilled chicken sample was reported of *E. coli* contamination (Diaz-Lopez *et al.*, 2011). Among the *Listeria* spp., *L. monocytogenes* were not found from the API *Listeria* kit analyses. Therefore these RTC products beef ball, beef burger petty, beef sausage may be considered at a safe level for consumption and it can be realized that these were prepared at hygienic production and processing area.

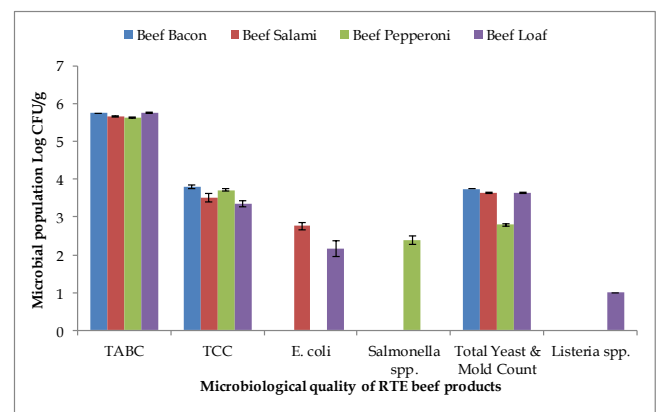


**Figure 2. Microbiological quality of ready-to-cook (RTC) beef products.**

### Microbiological quality of ready-to-eat (RTE) beef products

Figure 3 showed the microbiological qualities of RTE beef products. Among the different ready to eat beef products (beef bacon, beef salami, beef pepperoni, beef loaf) TAC, TCC and total yeast mold count were in similar condition and their range was 5.5-6, 3.5-4 and 2.8-3.8 log CFU/g respectively. *E. coli* presence in beef salami and beef loaf was within the range of 2.2-2.7 log CFU/g but absent in beef bacon and beef pepperoni. The TAC of these RTE beef products were at unsatisfactory level according to ICMSF (2001) ( $\geq 5$  log CFU/g) which were above 5 log CFU/g. In Korea TAC level was found for RTE meat at satisfactory level in an experiment which was 4.71 log CFU/g (Cho *et al.*, 2011). In other hand, Ologhobo *et al.* (2010) found TAC value at 6 log CFU/g of RTE meat products in Nigeria. Similarly higher TAC value like 8.2 log CFU/g was also reported by Manguiat & Fang (2013) for RTE pork head meat. The higher range of TAC indicated that the raw materials used for the preparation of these products were in poor microbial quality. There may have higher contamination sources of contamination during production and preparation of these products (Olanyinka *et al.*, 2008). The TCC and TYMC value for our RTE beef was considered to satisfactory level in terms of food safety which was  $\leq 4$  log CFU/g (ICMSF, 2001). In chicken sandwich sample, the TCC were at 5 log CFU/g recorded from Bangladesh which is not considered for safe consumption (Dutta *et al.*, 2012). The higher coliform count than the normal range ( $\geq 4$  log CFU/g) is the indicator of poor quality raw meat as well as unhygienic processing environment (ICMSF, 2001;

Harhoura *et al.*, 2012). In Bangladesh, no fungal count was reported for chicken sandwich, chicken cutlet and chicken fry (Dutta *et al.*, 2012). This experiment found acceptable TYMC value which was below 4 log CFU/g of meat (ICMSF, 2001) but high contamination indicated the lengthy preservation of RTE beef products in the sales center. *Salmonella* spp. was only present in beef pepperoni and it was 2.4 log CFU/g which is not safe for consumption ( $\leq 2$  log CFU/g) (ICMSF, 2001). So it might be also contaminated by fecal materials but beef bacon, beef salami and beef loaf had no *Salmonella* spp. *Listeria* spp. was only present in beef loaf sample. *E. coli* level among the positive sample were slightly above the safety level which was 2.2-2.7 log CFU/g ( $\leq 2$  log CFU/g) (ICMSF, 2001). 72% RTE chicken meat found contaminated by *E. coli* which was reported by Manguiat & Fang (2013). In Taiwan, the RTE meat was found highly contaminated by *Salmonella* (Manguiat & Fang, 2013). Djoulde *et al.* (2015) reported *Salmonella* contamination in 16% RTE meat and meat products at Cameroon. Different RTE meat were reported as contaminated by *L. monocytogenes* as per 28% among the sample (Manios *et al.*, 2014). Gonzalez-Fandos *et al.* (2021) found no effect of *L. monocytogenes* reduction in modified stored chicken meat. 1.5% and 2.7% RTE beef and chicken respectively were found contaminated by *L. monocytogenes* (Osaili *et al.*, 2014). *L. monocytogenes* contaminated beef and pork were reported in Baltic region (Berzin *et al.*, 2009; Berzin *et al.*, 2007). Higher pathogenic bacteria incidence in meat and meat products indicate outer contamination source as well as unhygienic preparation method (Harhoura *et al.*, 2012). Other ready-to-eat beef products were free from *Listeria* spp. contamination. After API *Listeria* confirmation test, no *L. monocytogenes* was found in this experiment.



**Figure 3. Microbiological quality of ready-to-eat (RTE) beef products.**

### Conclusion

The higher incidence and load of pathogenic microorganisms in raw and RTE beef products had an adverse impact on public health significance. Although the raw beef is cooked before consumption, it may contaminate the other regular items which are consumed without further processing. Now, maintaining the safe microbiological quality of beef and beef products is very urgent. Therefore, regular hygienic management and strict rules for production, preparation, processing, transportation and preservation should be implemented. Training and basic hygienic knowledge should be developed for the personnel related to raw beef and beef products production and handling. Along with, it is also needed to periodic inspection of these products to check the

property and increase the microbial safety as well as to minimize the risk of foodborne outbreak considering the significance of public health.

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