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# ASSESSMENT OF BACTERIAL CONTAMINATION LEVELS ON THE SURFACE OF THE BOVINE CARCASSES AT SLAUGHTERHOUSES OF BARISHAL CITY IN BANGLADESH

A. Das,[1] F. I. Rume,[1] W. K. Ansari,[1] M. N. Alam,[2] M. R. Islam,[3] P. K. Dutta[4] and A. K. M. M. Anower[1]*

[1]Department of Microbiology and Public Health, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Barishal, Bangladesh. [2]District Livestock Office, Barishal, Bangladesh. [3]Veterinary Section, Barishal City Corporation, Barishal, Bangladesh. [4]Department of Livestock Services, Dhaka, Bangladesh *Corresponding address: anower@pstu.ac.bd

**ABSTRACT**

**Background:** Meat industries in developing countries including Bangladesh are found to be challenged by severe hygienic and sanitation problems which are associated with heavy bacterial load on the different sites of carcasses and meat.

**Objectives:** The major objectives of this study were to determine the bacterial load at the different risk sites of bovine carcasses and identification of pathogenic bacteria as well as to assess the associated hygienic and sanitation practices with public health significance of the isolated bacteria.

**Materials and Methods:** The study on bacterial load of bovine carcasses was conducted on 200 swab samples of different risk sites of 20 bovine carcasses in the different slaughterhouses of Barishal City Corporation during the period from April to October 2017. The aseptically collected swab samples from different sites of bovine carcasses were processed and analyzed individually by standard bacteriological procedures for Total viable count (TVC), Total coliform count (TCC), Total Staphylococcus count (TSC) and Total Salmonella count (TSAC) and the bacterial species were identified by the conventional aerobic cultural, morphological and biochemical tests. The hygienic status and practices of slaughterhouses and workers were evaluated through a structured questionnaire survey and also a visual inspection.

**Results:** *Staphylococcus aureus* (78.5%), *Salmonella* spp. (64.5%) and *Escherichia coli* (64.0%) were found widely prevalent bacteria on the surface of freshly slaughtered bovine carcasses. The mean TVC of bacteria at pre-and post-washing of the different risk sites of carcasses including neck, shoulder, rump and their used knife and workers' hand was calculated and highest one was recorded for neck at both pre (8.17 ± 1.22) and post (8.41 ± 0.92) washings. These TVC were found higher compared to standards set by WHO. The TSC was found highest at shoulder site at both pre (7.26 ± 0.94) and post (7.43 ± 1.02) washing, whereas the neck site was found highest contaminated with *E. coli* (6.69 ± 1.06 and 6.87 ± 1.04) and *Salmonella* spp.(5.73 ± 1.05 and 6.07 ± 0.92), respectively. The questionnaire survey and visual inspection of slaughter houses revealed that none of the slaughterhouse workers received any training on slaughterhouses management and hygiene, not used any head and hair covering, protective clothes and hand gloves whereas only 25.33% workers washed their hands and 34.67% used clean water at slaughterhouses during processing of carcasses and meat.

**Conclusions:** The slaughtering of the animals on the ground and then skinning and evisceration in the same place under poor hygienic conditions are the major risk factors for heavy bacterial contamination of carcasses which has been recognized as a threat to food safety and consumers health to foodborne illness. Therefore, it requires a serious attention from all relevant authorities to apply and maintain the basic hygienic slaughterhouse practices to prevent the bacterial contamination of carcasses to prevent food borne illness. However, these data should serve as a baseline for future comparisons in measuring the bacteriological status of beef carcasses in other districts and further research works.

**Keywords**: Bacterial contamination, Slaughterhouses, Barishal city, Beef carcass, Risk sites, Total viable count, Total coliform count, Total salmonella count, Total staphylococcus count, Public health

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## INTRODUCTION

The chemical composition of meat is so ideal because of rich with proteins, essential amino acids, vitamin B complex and minerals which is highly favorable for the growth of pathogenic bacteria involved in spoilage and food borne illness in humans.[1,2] The majority of the bacterial contamination of carcasses occurs mainly during processing and manipulation of carcasses at the slaughterhouses, where conventional veterinary inspection cannot detect the presence of bacteria on apparently healthy carcasses.[3,4] Carcass and meat quality defects such as pale soft exudative, dark firm dry meat, skin blemish, bruising, cyanosis, high microbial load, spoilage of meat, broken bones and death may occur from improper animal handling.[5] A study results revealed that after evisceration the bacterial count is high due to fecal contamination and the neck is most contaminated site.[6] Unhygienic practices in abattoirs and post-process handling are associated with potential health risk to consumers due to presence of pathogens in meat and contaminated equipments.[7] Most of the food-borne diseases from meat are caused by bacteria such as *Salmonella* species., *Staphylococcus aureus*, *Listeria monocytogenes*, Campylobacter species and *Escherichia coli* O157: H7.[8,9] Bacterial counts of the different sites of carcasses and meat are used as an acceptable indicator of its hygienic quality of slaughterhouses and meat shop.[9] Several inland research reports have indiscriminately addressed on slaughtered animals, slaughterhouses and bacterial contamination of the bovine carcasses and beef meat chain from slaughterhouses to consumers which include the prevalence of diseases and disorders of slaughtered animals at ante-mortem examination,[10] quality of beef wholesale cuts,[11] assessment of hygiene and sanitary quality of beef carcasses,[12] bacteriological contamination of freshly slaughtered meat and town market meat,[13] bacteriological quality assessment of raw beef,[14] coliform in market beef,[15] public health impact of post-harvest contamination of beef carcasses,[16] isolation of Shiga-toxin producing *E. coli* (STEC) from beef slaughterhouses,[17] multi-drug resistance *E. coli*,[18] multi-drug resistance *Staphylococcus aureus* from beef,[19] multi-drug resistant *Salmonella* spp.,[20] quality and safety of meat and meat products.[21] However, literature on bovine carcasses and meat contamination with sources and risk factors and possible control measures considering the entire meat chain is still limited in Bangladesh. This paper describes the contamination status at the different risk sites of bovine carcasses at slaughterhouses of Barishal city and their public health significance.

## MATERIALS AND METHODS

**Study locations:** The study was carried out at 100 different slaughterhouses of Barishal City Corporation under Barishal district of Bangladesh from April to October 2017.

**Collection and transportation of sample** A total of 200 samples were collected from superficial different risk sites (neck, shoulder, rump, knife and worker's hand) of 20 beef carcasses under two conditions (pre-washing and post-washing). Samples were collected aseptically with sterile cotton swab. The surface area was selected and then cotton was rubbed to the selected area for several times. After that the cotton swab was kept in a sterile polythene bag containing 10 ml normal saline. The swabs were agitated up and down in the polythene bag to aid in rinsing the bacteria from the surface of the swabs. Then the sample was brought to bacteriological laboratory of the Field Diseases Investigation Laboratory (FDIL), Barisal in a cooling box.

**Bacterial cultural identification**

MacConkey, Mannital salt and Salmonella-Shigella (SS) agar plates were streaked separately with the organism and incubated aerobically at 37°C for 24 hours for the growth of bacteria.[22-24]

**Bacterial biochemical identification**

The isolated pure cultures were subjected to conventional biochemical tests using Dextrose fermentation, Indole test, TSI agar test and catalase tests.[22,25,26]

**Determination of bacterial counts**

One (1.0) ml of normal saline of swab sample was transferred into a sterile test tube containing 9.0 ml of sterile (0.1%) peptone water to provide the original dilution ($10^{-1}$) and mixed properly with a sterile glass stirrer. From which further 10-fold decimal dilutions were prepared up to $10^6$ and by using whirly mixture machine different serial dilutions ranging from $10^{-2}$ to $10^{-6}$ were prepared according to the standard method.[27]

**Total Viable (Aerobic) Count (TVC)**

Spread 0.1 ml of each ten-fold dilution onto the surface of duplicated nutrient agar plates enriched with 5% blood and then the petri-dishes were kept in an incubator at 37 $^0$C for 24 hours. Following incubation, petri-dishes exhibiting colonies ranging 30 to 300 were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the TVC. The TVC was calculated.[27] The results of the TVC were expressed as the number of organism or colony forming units per gram (cfu / $cm^2$) of beef sample. Then results were calculated into log value.

**Total Coliform, *Staphylococcus and Salmonella* Count:** Same procedure like TVC was applied but media used for them MacConkey, Mannital salt, SS agar, respectively. All counts were normalized to colony forming units per square centimeter (cfu / $cm^2$) and converted into $\log_{10}$ values.

**Statistical analysis of experimental data:** All the raw data were arranged in a excel sheet using Microsoft Office Excel 2010. Then Chi-square test was done to analysis the data.[28] P-value was set less than 0.05 ($p < 0.05$) being considered statistically significant.

## RESULTS AND DISCUSSION

The different stages of the conversion from live animals into meat make the microbial contamination of carcasses and meat can occur at any stage of the meat chain that causes an unavoidable and undesirable result.[8,29,30] Bovine carcasses are usually contaminated with pathogenic bacteria that are present naturally in the digestive tract (feces) and on the hides of the slaughtered animals, especially when perform on the floor with the absence of a carcass suspension system onto the meat surface (Photo 1), slaughterhouse workers with their hands and clothes (Photo 2), contaminated slaughtering and processing equipment and materials and to a lesser degree, contamination from air via aerosols and carcass dressing water.[9,31-33]

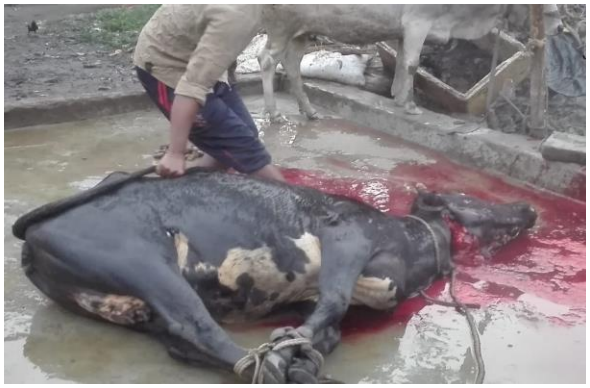

Photo 1.A slaughtered cow is lying on the widely spread blood and a cow is standing at pre-slaughter stage on the same dirty unhygienic ground

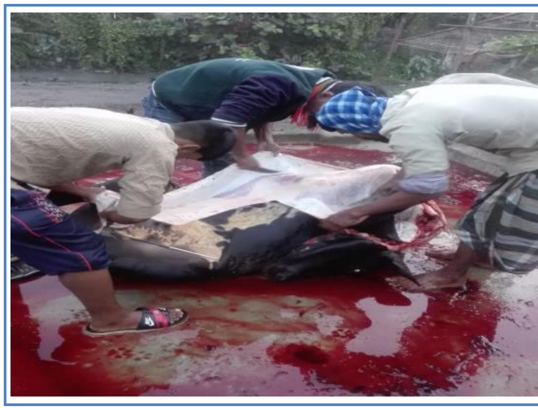

Photo 2. Three men are skinning and evisceration the carcasses of the cow on the same slaughtered ground with widely spread blood

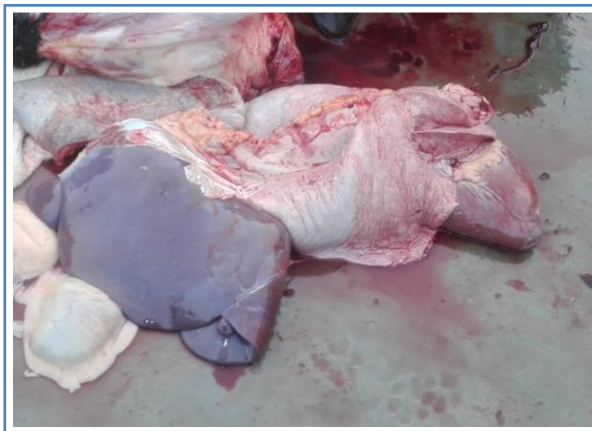

Photo 3. Heart, liver, spleen and legs the cows are kept on the same dirty unhygienic ground

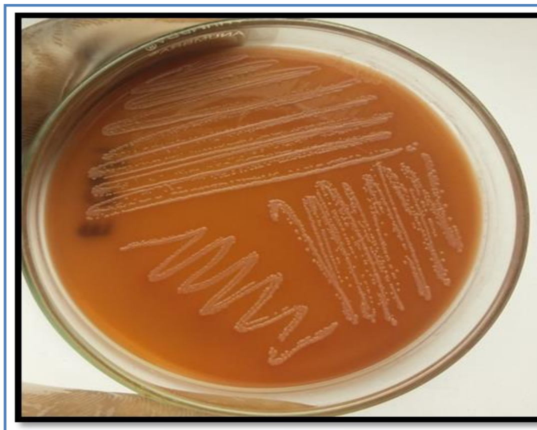

Photo 4. Smooth pinkish colony of *E. coli* on MacConkey agar

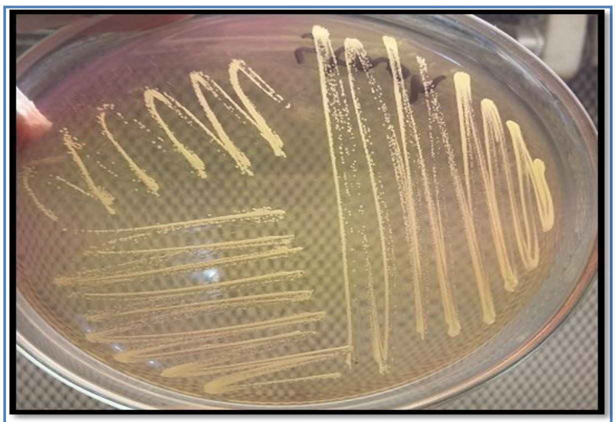

Photo 5. Yellow color colony of *Staphylococcus aureus* on mannitol salt agar.

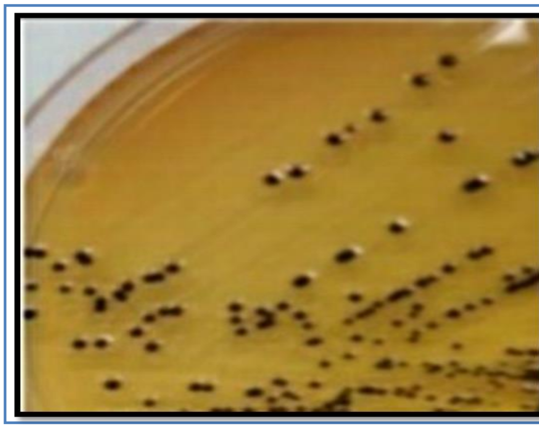

Photo 6.  Transparent colorless colony with black color in the center of *Salmonella* spp. on SS agar

The cultural and biochemical tests of 200 swabs samples collected from the different sites of bovine carcasses were conducted to isolate and identify the contaminated bacteria. On MacConkey agar plates after streaked with organisms and incubated aerobically at 37°C for 24 hours growth was indicated by the development of bright pink colored colony.[21] On mannitol salt agar plates after streaked with organisms and incubated aerobically at 37 °C for 24 hours growth was indicated by the development of yellow color colony and this also has similar finding with earlier report.[25] The SS agar plates were streaked separately with the organism and incubated aerobically at 37 °C for 24 hours for the growth of bacteria. The growth was indicated by the development of transparent or transparent colorless colony with black color in the center as reported earlier.[34] Colonies of isolated bacteria from different culture media were examined under compound microscope after Gram's staining, where *E. coli* and *Salmonella* spp. were found Gram negatives but *S. aureus* Gram positive. All the three types of bacteria were identified through conventional biochemical tests. Dextrose was fermented by *E. coli* and *Salmonella* spp. but not by *S. aureus* and the dextrose fermentation test profile was closely related with previous research reports.[22,23,25] Also Triple Sugar Iron (TSI) slant test, iodole test and catalase test were conducted for those bacteria and found *E. coli* TSI yellow slant and butt with no gas, indole positive and catalase negative. The *S. aureus* caused same for TSI with no gas but indole negative and catalase positive, whereas *Salmonella* spp. caused black slant and butt with $H_2S$ gas and both indole and catalase negative.

The predominant bacteria isolated and identified from different sites of bovine carcasses were *E. coli*, *Salmonella* spp. and *S. aureus* (Table 1). Overall the *S. aureus* (78.5%) was found predominant, followed by *E. coli* (64.0%) and *Salmonella* spp. (64.5%) and most of the carcasses were contaminated concurrently with all the three types of bacteria (Table 1). These findings support the earlier reports on the contamination of Coliform, Staphylococci, Lactobacillus, Micrococcus, Streptococci, Pseudomonas, Enterobacter, Bacillus, Proteus and Salmonella bacteria in freshly slaughtered meat and town market meat in Bangladesh.[13,16] In addition, the slaughtered bovine carcasses have been reported to be only 44.98% approved and passed, whereas 18.98% local, 23.5% partial and 12.99% total condemned due to diseases and disorders in the slaughterhouses in Rajshahi district.[10] Comparatively lower contamination of *E. coli* (10.0%), *Salmonella* spp. (13.33%) and *Staphylococcus* spp. (26.67%) have been reported in the fresh raw beef from Sylhet district,[35] and *E. coli* (10.28%), *Staphylococcus* spp. (36.12%) and *Salmonella* spp. (1.0%) from bovine carcasses of meat stalls in Mymensingh.[16]

The individual and overall post-washing bacterial contamination of all the recorded three bacteria were significantly ($p < 0.05$) higher at post-washing samples of carcasses, knife and workers' hands than pre-washing samples (Table 1). It appears from Table 1 and Table 2 that all the selected sites of carcasses, knife and workers' hands were highly contaminated with these three bacteria. These findings support the findings of log TVC 5.71 in washing water of carcass surface, 5.86 in workers' hand washing and 4.43 in knife at slaughterhouses in Bangladesh.[36]

Table 1. Bacteria isolated from the surface of the different sites of bovine carcasses (n = 20)

| SN | Sample collection site | Washing status | No. of swabs tested | *Escherichia coli* Positive No. (%) | *Salmonella* spp. Positive No. (%) | *Staphylococcus* spp. Positive No. (%) |
|---|---|---|---|---|---|---|
| 1. | Neck | Pre- | 20 | 14 (70.0) | 13 (65.0) | 18 (90.0) |
| | | Post- | 20 | 18 (90.0) | 14 (70.0) | 19 (95.0) |
| 2. | Shoulder | Pre- | 20 | 12 (60.0) | 12 (60.0) | 17 (85.0) |
| | | Post- | 20 | 13 (65.0) | 13 (65.0) | 19 (95.0) |
| 3. | Rump | Pre- | 20 | 11(55.0) | 11 (55.0) | 16 (80.0) |
| | | Post- | 20 | 14 (70.0) | 15 (75.0) | 17 (85.0) |
| 4. | Knife | Pre- | 20 | 09 (45.0) | 10 (50.0) | 12 (60.0) |
| | | Post- | 20 | 10 (50.0) | 13 (65.0) | 14 (70.0) |
| 5. | Worker' hands | Pre- | 20 | 13 (65.0) | 12 (60.0) | 17 (85.0) |
| | | Post- | 20 | 14 (70.0) | 16 (80.0) | 18 (90.0) |
| | Total | Pre | 100 | 59 (59.0) | 58 (58.0) | 70 (70.0) |
| | | Post- | 100 | 69 (69.0) | 71 (71.0) | 87 (87.0) |
| | | Overall | 200 | 128 (64.0) | 129 (64.5) | 157 (78.5) |

Table 2 shows the TVC of bacteria at different sites of carcasses, knife and worker' hands of slaughterhouses.

Table 2. Total bacterial count at the different sites of carcasses of slaughtered cattle

| SN | Sample collection site | Washing status | No. of swabs tested | Bacterial counts ($log_{10}$ cfu/cm) | | | |
|---|---|---|---|---|---|---|---|
| | | | | TVC | TCC | TSAC | TSC |
| 1. | Neck | Pre- | 20 | 8.17 ± 1.22 | 6.69 ± 1.06 | 5.73 ± 1.05 | 7.12 ± 1.08 |
| | | Post- | 20 | 8.41 ± 0.92** | 6.87 ± 1.04** | 6.07 ± 0.92** | 7.29 ± 1.28** |
| 2. | Shoulder | Pre- | 20 | 8.09 ± 1.10 | 6.63 ± 1.15 | 5.86 ± 0.77 | 7.26 ± 0.94 |
| | | Post- | 20 | 8.25 ±0.82** | 6.75 ± 1.08** | 5.86 ± 0.77* | 7.43 ± 1.02** |
| 3. | Rump | Pre- | 20 | 8.05 ± 1.04 | 6.51 ± 1.04 | 5.59 ± 0.81 | 7.10 ± 1.07 |
| | | Post- | 20 | 8.30 ± 1.06** | 6.82 ± 0.83*** | 5.88 ± 0.49 | 7.24 ± 0.83 |
| 4. | Knife | Pre- | 20 | 7.78 ± 1.08 | 5.43 ± 1.01 | 5.54 ± 1.01 | 5.63 ± 1.08 |
| | | Post- | 20 | 7.93 ± 0.79 | 5.66 ± 0.88** | 5.71 ± 0.77* | 5.82 ± 1.12** |
| 5. | Workers' hands | Pre- | 20 | 8.15 ± 1.02 | 6.55 ± 1.03 | 5.64 ± 0.86 | 7.08 ± 1.02 |
| | | Post- | 20 | 8.37 ± 1.10* | 6.78 ± 0.92** | 5.91 ± 0.94** | 7.27 ± 0.72* |

TVC = Total viable count
TCC = Total Coliform count
TSAC = Total Salmonella count
TSC = Total Staphylococcus count
*Significant at ($p < 0.05$)
**Significant at ($p < 0.01$)

**Total viable count (TVC)**

The TVC of bacteria of all the sample collection sites at pre-washing varied from 8.05 to 8.17 and post-washing varied from 8.25 to 8.41 $\log_{10}$ cfu / cm$^2$ bacterial which exceeding the limit ($10^5$ cfu / cm$^2$ or 5.0 $\log_{10}$ cfu / cm$^2$) of total plate count on meat set by the WHO.[37] If the TVC exceeds the above standard in fresh carcass and meat, then the meat is not acceptable and this indicates alarm signals on meat hygiene along meat chain from slaughterhouses to butcher shops.[38] This study recorded comparatively higher TVC (pre-washing 8.05 to 8.17 and post-washing 8.25 to 8.41 $\log_{10}$ cfu / cm) on the surface of carcasses in comparison to 5.01 to 6.00 cfu / cm$^2$ and $2.5 \times 10^5$ to $2.25 \times 10^8$ cfu / g in earlier reports from Bangladesh,[16,35] 5.04 cfu / cm$^2$ from Tanzania,[39] 4.48 ± 0.63 log cfu / cm$^2$ from Algeria[40] and even 5.80 ± 0.17 log cfu /cm$^2$ from India.[41] Difference in the bacterial counts between meat from neck and rump region could be due to high risk of neck being spilled or spread of gastro-intestinal contents if good processing practices and good handling practices of meat are not consistent. However, the differences of bacterial loads depending on the anatomic sampling sites have also been reported.[42,43] The collar and the brisket have also been reported to be most contaminated sites, and also reported that the differences in levels of contamination between different sites are more significant in the bovine species.[44] These differences of TVC in different anatomic sites in different slaughterhouses and countries could be due the differences of hygienic condition used by the slaughterhouse management.

**Total coliform count (TCC)**

The TCC on the neck region was found significantly ($p < 0.01$) highest at both the two points of operation which were 6.69 ± 1.06 and 6.87±1.04 $\log_{10}$ cfu / cm$^2$ (Table 2). This finding could be compared with the earlier inland report with highest TCC on thigh (log 3.27/g) and lowest on brisket (log 2.64/g) sites.[15] In addition, the Shiga-toxin producing *E. coli* (STEC) has been identified in 3 beef samples of slaughter houses by PCR which had multi-drug resistance in Mymensingh.[17] Contaminated hides have also been identified as one of the major sources of *E. coli* 0157 carcass contamination.[45] The highest mean values recorded on the swab samples of neck region followed by shoulder, rump, worker's hand and lowest mean value was on knife washing samples (Table 1). These findings are in accord with the earlier reports in which they have reported the highest bacterial contamination at the neck site with coliform bacteria.[39,40] However, the coliform contamination has mostly been reported on the brisket and shoulder and least on thigh.[43]

**Total Salmonella count (TSAC)**

Salmonella is one of the most important causes of gastro-enteritis in humans worldwide. Meats are frequently contaminated with Salmonella species and are thought to be major sources of the pathogen for human gastro-enteritis. Of the 200 carcasses examined, 129 (64.5%) were found positive with Salmonella (Table 1). The significantly ($p < 0.01$) highest TSAC was recorded at the neck region with two operational points of 5.73 ± 1.05 and 6.07 ± 0.92 $\log_{10}$ cfu/cm$^2$ respectively, followed by shoulder, workers' hand and knife (Table 2). These results support the TSAC (4.35 ± 1.17) in beef carcasses, knife before (3.64 ± 1.53) and after processing (4.99 ± 2.23) $\log_{10}$ cfu/cm$^2$ from Mayasia.[46] Salmonella is known to colonize the gastro-intestinal tract of animals as carrier state and carcasses can become contaminated with Salmonella at the time of slaughter.[47] Cattle feces and hides might be considered as important sources of Salmonella for carcass contamination and the presence of potentially pathogenic Salmonella on carcass contamination could increase consumers' risks of infection if hygienic handling and processing of carcass and meat are not followed.[48]

### Total Staphylococcus count (TSC)

The highest TSC was recorded on the shoulder site at both the pre- ($7.26 \pm 0.94$ $log_{10}$ cfu/cm$^2$) and post- ($7.43 \pm 1.02$ $log_{10}$ cfu/cm$^2$) washing stages in comparison to other sites of carcasses (Table 2). These results could be compared with TSC of $6.19 \pm 0.30$ log cfu/g meat[28] and 1.75-3.29 with an average of 2.52 TSC/g meat reported from Bangladesh.[16] The TSC in knife and workers' hand at two operational points was $5.63 \pm 1.08$, $5.82 \pm 1.12$ log CFU/cm$^2$ and $7.08 \pm 1.02$, $7.27 \pm 0.72$ log cfu/cm$^2$ respectively (Table 2). These higher values of TSC recorded in this study in comparison to the reported from workers' hands of $3.00 \pm 0.47$ log cfu / hand at the start of the work to $4.00 \pm 0.53$ cfu / hand at the end of work.[49] These differences may be due to differences of hygienic practices of the slaughterhouse workers.

The level of the TVC is generally accepted as a criterion for bacterial contamination of carcasses and a useful indicator of hygiene.[44] The major sources of contamination are multiple contacts with contaminated tools and operators' hands, however, the severe contamination is explained by contact with the soil, bad evisceration practices, often resulting in rupture of the gastro-intestinal tract, and especially hide-to-carcass contamination transfer.[32]

### Effects of washing water

Table 1 shows that the TVC at the sites of neck, shoulder, rumps and workers' hands at post-washing counts were significantly ($p < 0.01$) higher in comparison to pre-washing bacterial counts. These findings are in conformity with log TVC 5.51 to 5.81 with an average of 5.71/ ml of washed water of carcass surface reported from Bangladesh.[36] The coliform counts log 5.7 / ml in beef carcass washing water and 3.73 / ml in butchers' hand washing water in the slaughterhouse have also been reported from Bangladesh.[15] This indicates that the water used in slaughterhouses contaminated the surface of the carcasses and slaughtering and meat processing instruments. Therefore, the water used for cleaning procedures and meat processing in the slaughter houses must meet drinking standards.[50] In addition, water supply of the slaughterhouses should be analyzed frequently to confirm its quality.

### Knives

The post-washing knives of the slaughterhouses showed comparatively higher levels of TVC and significantly ($p < 0.01$) higher *E. coli*, *S. aureus* and *Salmonella* spp. contamination than pre-washing knives (Table 2). These results support the earlier report of 4.63 TVC and 2.47 TCC in the slaughterhouse equipment washings.[12] The high bacterial load on the knives is an indication of inadequate hygienic practices in the slaughterhouses. The butchers' knives are usually washed with water without any sterilization and same knife is used for slaughtering of multiple animals and even in meat processing in Bangladesh. The variations of bacterial load reported in different studies might be due to differences of the hygienic and sanitary practices in slaughterhouses.

**Workers' hands**

All the three recorded bacteria were isolated and identified from the slaughterhouse workers' hands with 8.15 ± 1.02 TVC, 6.55 ± 1.03 TCC, 5.64 ± 0.86 TSAC and 7.08 ± 1.02 TSC at pre-washing stage (Table 2). These findings support the earlier report of 6.34 TVC/ ml and 3.73 TCC / ml of hand washing of slaughterhouse workers' hands.[12] The variation of bacterial count in different study may be due to spread out gastro-intestinal fecal contents and even the surrounding environmental contamination also plays an important role to contaminate beef carcass.

**Hygienic practices**

The slaughterhouses' workers were interviewed concerning their educational status, training, hygiene and sanitation, use of protective clothing and other hygienic measures (Table 3). Out of 100 interviewed slaughterhouses' workers, none of the workers had any training on bovine carcass and meat processing, none used hair covering, protective clothing and hand gloves during meat processing. However, only 25.33% workers washed hands, 62.67% removed extended nails, 4.0% maintained clean environment and 34.67% used clean water during meat processing (Table 3).

**Table 3:** Summary on the various hygienic management related to public health recorded during sample collection at the slaughter houses (n = 100)

| SN Hygienic management | Percent |
|---|---|
| 1. Washing hands frequently before processing meat | 25.33 |
| 2. Using hair covering during meat processing. | 0 |
| 3. Cutting nails regularly. | 62.67 |
| 4. Using different protective clothes during meat processing. | 0 |
| 5. Maintaining clean environment during meat processing. | 04.00 |
| 6. Using clean water. | 34.67 |
| 7. Using hand gloves during meat processing. | 0 |
| 8. Having any training on meat processing. | 0 |

n = No. of observations

The hygienic conditions of the slaughterhouses and workers have potential to contribute for contamination of carcasses and during processing of meat. Table 3 shows that all the slaughterhouses' workers did not cover their head and hair, did not use protective clothing (apron), did not use hand gloves and they didn't have any carcass and meat processing training. However, the hygienic practices in slaughterhouses in Ethiopia reported better than Bangladesh where only 11.3% slaughterhouse workers did not use protective clothes, 50.7% did not cover their hair and 47.9% of the butchers handled money while serving food which may result into cross contamination of meat with microbes.[38]

This study recorded that only 25.33% workers washed their hands frequently before processing meat, only 4.0% workers maintained clean environment during meat processing and 34.67% slaughterhouses used clean water which indicates very poor hygienic practices (Table 3). These observations are in accord with the report of bacterial contamination of red meat during butchering and skinning.[51]

The poor infrastructural facilities in slaughterhouses, unhygienic animals and poor handling of carcasses attribute to the high bacterial load in carcasses and meat. Thus, by assessing the bacterial counts, the threat posed to human health can be ascertained. Food-borne diseases occur commonly in developing countries like Bangladesh because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest safer equipment and lack of education for food handlers. In addition to protective devices, new workers should be examined clinically and bacteriologically especially stool and urine before they are employed and at regular intervals afterwards. However, workers with any history of diarrhea, vomiting, discharging wounds and sores should refrain from work until they are known not to be harboring dangerous pathogens.[9] The level of education and training of food handlers about the basic concept and requirements of personal hygiene and its environment plays an important part in safeguarding the safety of products to consumers.

E. *coli* is a Gram-negative, rod-shaped major pathogen causing colibacillosis in both the humans and animals all over the world. *E. coli* is present in the intestinal tract of human and warm-blooded animals. Retail foods, especially meat and meat products, have been believed to be important vehicles for spreading antimicrobial resistance and pathogenic *E. coli*.[52,53] *S. aureus* is a Gram positive bacteria with spherical to ovoid cell. It represents the major causal agent of food intoxication through its enterotoxins. Reports on street food epidemiological studies showed *S. aureus* is the most predominant virulent bacteria responsible for a wide range of human diseases.[54] *Salmonella* spp. is Gram-negative, small rod-shaped, non-spore forming organism and are potentially responsible for various pathogenic processes in man and animal.[34] Bacterial load of beef meat during handling and selling in market have been reported from Bangladesh.[55]

Foodborne diseases (FBD) are universal public health problems and the implications are great including health and economic losses. Contaminated raw meat is one of the main sources of foodborne illnesses. Epidemiological reports suggest that meat product is one of the major causes of diarrheal illness which account for 36% of mortality cases in developing countries.[56] For effective control of bacterial contamination, a microbiological test of meat products is required.[51] Implementation of good hygiene practice and procedures based on hazard analysis and critical control point (HACCP) principles at slaughterhouses are essential to minimize carcass contamination.[57,58]

## CONCLUSIONS

It appears from this study that the bacterial load of bovine carcasses at slaughterhouses in Barishal City Corporation was found very high in comparison to inland published reports and elsewhere. The neck was found highly contaminated with all the three bacteria (*S. aureus*, *E. coli* & *Salmonella* spp.) than shoulder and rump regions of carcasses. This may be due to low level of sanitation activities, carcass dressed on dirty floor and poor hygienic practices at the slaughterhouses. Most of the slaughterhouse workers are illiterate having no knowledge of food safety and professional training on slaughtering and processing of carcasses and meat. In addition, the washing water, equipment especially knife and workers' hands are also found contaminated with all these bacteria that had significant role to contaminate carcasses and meat. However, these contaminated meats finally reach to consumer's hand for human consumption which is highly risk with a view to public health significance. The levels of bacterial contamination in slaughterhouses in Bangladesh may reflect the hygienic status of meat production and public health. This study shows that the hygienic status of the slaughterhouses in Barisal City was found very poor and accordingly the bacterial load was found higher than the acceptable limit of the standard. Therefore, the Barisal City Corporation should take necessary strategies for the implement of animal slaughter act and improve hygiene and sanitation at the slaughterhouses and appropriate control method of the problems should be designed and implemented. However, further studies should be carried out to isolate and characterized the bacterial load of washing water, hides, feces, slaughterhouse environment, meat and meat shops to detect the associated risk factors for implementation of appropriate hygienic practices in the bovine slaughterhouses in Bangladesh.

## ETHICAL APPROVAL

All animal-related methods and procedures were carried out in accordance with the Animal Ethical Committee of the University

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest of this article

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