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Coliform Contamination of Raw Beef at the Slaughterhouse and Butchery Levels in Herat City, Afghanistan

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ABSTRACT

Background: Contaminated food products are responsible for causing various food borne diseases all over the world. Microbial contaminants are present in various food products, particularly raw meat. Among the most common microbial contaminants in meat and meat products are coliform bacteria. This study aimed to determine the coliform quality of raw beef with special emphasis on *Escherichia coli* in Herat city, Afghanistan and to compare the coliform contamination levels in the slaughterhouse and butchery levels.

Materials and Methods: In this study, 150 samples of raw beef were randomly selected from butcheries and slaughterhouses of Herat city. Coliform identification was performed by standard bacteriological methods.

Findings: The findings of this study revealed that coliform bacteria were detected in 70% and 75 % of beef samples from slaughterhouses and butcheries respectively. A total of 58 % of positive samples were upon the standard accepted ratio of coliforms contaminant per gram. The *E. coli* contamination ratio of samples in slaughterhouses and butcheries were 52% and 72% respectively. Our study demonstrates a significant difference in coliform bacteria contamination between slaughterhouse samples and butcheries.

Conclusion: It was concluded that more than half of the coliform contamination in raw beef from the slaughterhouse and butcheries was exceeded the maximum limit which highlights the need to focus on effective monitoring of the slaughterhouse and butcheries in Herat city for prevention of meat borne intoxication and infection.

Keywords: Butchery, Coliform, *E. coli*, Herat city, Slaughterhouse.

INTRODUCTION

Food borne diseases (FBDs) are a global public health problem, that cause significant morbidity and mortality in all age groups (He et al., 2023). It is estimated that approximately 600 million FBDs caused by contaminated food resulted in 33 million disability-adjusted life years (DALYs) in 2010 (Faour-Klingbeil & C. D. Todd, 2019). Apart from this, the World Bank has estimated that the economic cost of FBDs in middle and low-income countries is US$95.2 billion annually (Havelaar et al., 2022). Basically, there are three types of food contamination: physical, chemical and biological; thus the introduction of any biological, chemical or foreign substances into food (raw or cooked) at any stage of the food process endangers food safety and leads to food contamination (Habib et al., 2023).

Bacterial pathogens are primarily responsible for FBDs, which are a leading cause of hospitalization and death (Asrar & Aleem, 2023). FBDs associated with meat represent a significant global public health concern because the high risk of bacterial contamination of meat by several types of pathogens (Osemwowa et al., 2021).

Meat is a good medium for bacterial growth due to its high nutrient content (Harlia et al., 2017). The most significant of foodborne bacterial pathogens associated with meat are *Salmonella spp*., *Escherichia coli*, *Campylobacter jejuni Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringes*, *Yersinia enterocolitica* and *Aeromonas hydrophila* (Bantawa et al., 2018). *Escherichia coli* and *Salmonella spp.* are common findings worldwide in livestock including cattle which contaminate the carcass and spread to the cut or raw beef (Soepranianondo et al., 2019). Generally, the contamination of food products may occur by specific pathogens, such as the bacteria, viruses, parasites, fungi, and mycotoxins, and prions at any step from ‘‘farm to fork’’ (He et al., 2023).

However, one of these contaminants involves coliforms, the presence of which in beef indicates that the carcasses of animals or beef were contaminated with animal feces or potentially by pathogenic microorganisms (Zafar et al., 2016). The presence of coliforms bacteria in beef indicates the possibility of toxigenic or enteropathogenic microorganisms which are harmful to human health (Harlia et al., 2017). The meat of healthy animals contains less or nil microorganisms (Bantawa et al., 2018). Thus, the contamination of raw beef most likely occurred during the slaughtering and processing phases as a result of extremely poor health and safety measures (Zafar et al., 2016). The microorganism contamination of raw beef commonly occurs from external sources during bleeding, handling, and processing via hands, clothes, knives, tools and air (Bantawa et al., 2018). Thus, food safety is necessary to ensure that food is safe at every stage of the food chain, from production to consumption (Habib et al., 2023).

The Precise and quick detection of food boor pathogens is significant to ensure public health (Habib et al., 2023). In Herat city, meat and its products may contain various microbial contaminations due to the absence of a standardized slaughterhouse and a lack of hygienic operations during and after animals are slaughtered. However, hygiene practices remain poor along the beef production chain in Herat city, and there is lack of information on the microbial contamination of raw beef in Herat city. In view of the above explanations, the purpose of this study was to evaluate the coliform quality of raw beef with special emphasis on *Escherichia coli* in Herat city, Afghanistan and to compare the coliform contamination levels in the slaughterhouse and butchery levels in order to provide useful information to inform relevant organizations to implement sanitary measures to protect public health.

MATERIALS AND METHODS

Study Design and Sample Collection

A descriptive cross-sectional study was carried out to evaluate coliform contamination of raw beef in Herat city, Afghanistan. A total of 150 samples (N = n1 + n2) of raw beef from the butchers in different areas of Herat city and Herat slaughterhouses were randomly purchased between 23/04/2018 to 23/08/2019. One hundred samples (n1) of raw beef from carcasses ‘superficial areas from butcheries along with fifty samples (n2) from slaughterhouses (100 g meat per sample) were taken in sterile conditions and delivered to the Microbiology Laboratory of Herat University maintained cold chain. In the laboratory, the samples were processed immediately as soon as possible otherwise preserved at 4 °C. The samples were first mixed by FDA methods using a blender. The whole processes of culturing bacteria and obtaining pure cultures were performed inside the laminar flow where sterile conditions were maintained.

Bacteriological Analysis of Samples

Coliforms were counted in each meat sample as previously described by Ebner *et al.* (2015). Initially, buffer-mixed samples (10 g of meat in 90 ml buffer) in sterile plastic containers were separately diluted in 5 test tubes containing PBS with 0.1, 0.01. 0.001, 0.0001 and 0.00001 dilutions and were labeled accordingly. Following this, 1 mL from each dilution was delivered to two plates of Violet Red Bile Agar (VRBA) for sub-culture using the Pour Plate technique where they were incubated at 37°C for 24-48 hours. Subsequently, the colonies present on VRBA were counted and calculated in accordance with the method previously described by Harley and Prescott (Harley and Prescott, 2002). For accuracy and to evaluate whether the colonies are coliforms or not, a minimum of 5 colonies from each sample were selected and delivered to Brilliant Green Bile Lactose Broth (BGLB) for sub-culturing.

E. coli Isolation in pure culture: Lactose fermenting colonies were selected from VRBA plates of each sample and sub-cultured on the Eosin methylene blue (EMB) media using the streaking method and incubated at 37°C for 24 hours. The isolated metallic shining single colonies of *E. coli* were utilized for biochemical tests (IMViC). Bacterial identification for *E. coli* was completed based on colony morphology, gram-staining, and biochemical reactions which were previously described by Cheesbrough, (1985).

Biochemical identification

For biochemical identification purposes, IMVIC (Indole, Methyl red, Voges-Proskauer, and Citrate) tests were performed on isolated E. coli, which included:

Indole test: To perform this test, 5 mL of bacterial culture was added to 2 mL of peptone water and incubated at 37°C for 48 hours. Then, 0.5 mL of Kovac's reagent was added to the culture and mixed well. The formation of a red ring on top indicates positive reaction.

MR test: The methyl red test was performed by inoculating a single colony of a pure bacterial culture into 0.5 mL of glucose phosphate broth. Following incubation at 37°C for 24 hours, one drop of methyl red solution was added. A resulting red color indicated a positive reaction, while a yellow color signified a negative reaction.

VP test: The test was performed by inoculating 5 mL of bacterial culture into 2 mL of glucose phosphate broth and incubating at 37°C for 48 hours. A small amount of Creatine was added and mixed well. Then Sodium hydroxide solution was added and mixed again. The tube was placed at room temperature for one hour and then the result was observed. A positive result is indicated by pink color, while a negative result is indicated by the absence of pink color.

Citrate test: This test was performed using culture of Simmon Citrate Agar and incubation of the tubes at 37°C for 24 hours. The Bromothymol Blue indicator in the culture medium, which is green in acidic environments, changes to a deep blue color due to the alkalinization of the environment by the activity of the bacteria, which indicates a positive citrate reaction.

Statistical analysis:

Data obtained from this study were analyzed using Microsoft Excel version 2013 (Microsoft, Redmond, WA, USA) and GraphPad Prism 8.3.0 (GraphPad Software, Inc., 2019). Descriptive statistics and t-tests were performed to determine if there were significant differences between the means of the samples, with $p < 0.05$ considered statistically significant.

RESULTS

Of the 50 samples taken from the slaughterhouse, 35 (70%) were positive coliform contamination, with an average contamination level of 3.05 Log10 CFU/gr. Also, out of 100 samples taken from butcher shops**,** 75 samples (75%) with an average Log10 CFU/gr of 3.66 were positive for coliforms (Table 1, 2 and 3).

Table 1 Bacterial frequencies on the meat samples

Bacteria	Coliform	*E. coli*
Positive	110 (73.3%)	107 (71.3%)
Negative	40 (26.7%)	43 (24.7%)
Total	150	150

Table 2 Mean bacterial load at slaughterhouse and butchery levels

Locations	Log10 CFU/gr	p value	Confidence interval, 95%
Slaughterhouse	3.05	0.0121	0.1366 to 1.0906
Butcheries	3.66		

Table 3 Coliform and E. Coli contamination of raw beef samples from butchery levels and slaughterhouse of Herat city, Afghanistan

District	**Total Simple**	**Positive Simple Coliform**	**Negative Simple Coliform**	**Average***	**Mean log $_{10}$ CFU/gr**	**SD**	**Present E.coli/Sample**
First	5	4	1	126984.25	3.335	±1.83	**4**
Second	5	2	3	202466	3.305	±3.26	**1**
Third	4	4	0	88412.5	3.528	±2.06	**3**
Fourth	4	4	0	2370.25	2.777	±1.12	**4**
Fifth	6	2	4	198546.80	3.145	±2.60	**2**
Sixth	5	3	2	11207432.6	4.833	±3.12	**3**
Seventh	7	5	0	84205.6	5.511	±4.03	**5**
Eighth	6	6	0	519677.67	5.246	±3.78	**6**
Ninth	5	3	2	4130701.33	5.240	±1.25	**3**
Tenth	6	6	0	88.3333333	1.592	±0.48	**6**
Eleventh	8	7	1	2607.14285	2.577	±0.95	**6**
Twelfth	10	6	4	1969506.16	4.435	±1.89	**6**
Thirteenth	11	7	4	8012158.57	4.183	±4.27	**7**
Fourteenth	9	8	1	839773.5	3.051	±1.70	**8**
Fifteenth	9	9	0	1831.1111	2.218	±1.66	**9**
Total	**100**	**75**	**25**	**27386761.82**	**3.66373**	**±2.212**	**72**
Slaughter House	50	35	15	10506.96	3.05012	± 0.6783	26

Contamination of coliform was observed in both slaughterhouse and butchery samples. The study shows that the samples collected from butcheries had higher contamination ratio when compared to the samples that were collected from slaughterhouses ($p < 0.05$) as compared to the standard ratio (Figure 1).

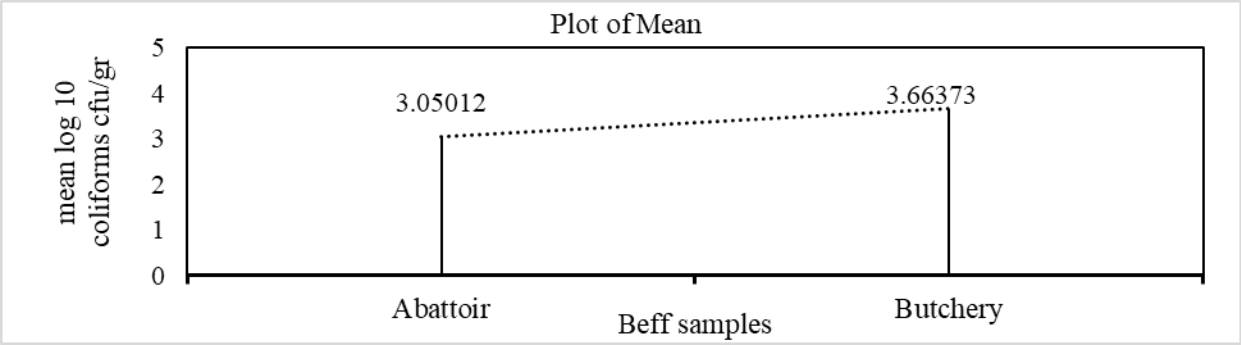

Figure 1. Mean Coliform counts at the slaughterhouse and butchery levels.

The results revealed 26 positive samples of *E. coli* contamination from a total of 35 samples in slaughterhouses and 72 positive samples from a total of 75 samples in butcheries within different districts of Herat city. Therefore, the *E. coli* contamination was 52% and 72% in slaughterhouses and butcheries respectively (Figure 2).

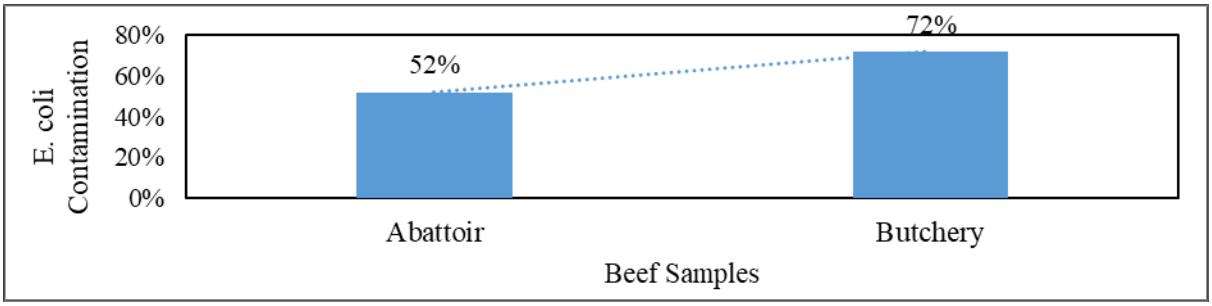

Figure 2 Percentage of *E. coli* contamination at the slaughterhouse and butchery levels.

DISCUSSION

The presence of coliform in raw beef did not always indicate fecal contamination. It could be a sign of improper processing or poor hygiene. it is crucial to have accurate and regular evaluation of microbial contamination of meat to ensure public health. Although many studies have been conducted elsewhere (Bantawa et al., 2018; Bintoro et al., 2012; Harlia et al., 2017; He et al., 2023; Mouafo et al., 2020; Muhammad Bilal Habib , Umer Anayyat , Faiza Usmani , Ali Raza Jafri , Aleena Ramzan , Numrah Safdar, 2023; Olu-Taiwo et al., 2021; Osemwowa et al., 2021; Soepranianondo et al., 2019), this was the first study that assessed the bacteriological quality of raw beef in Herat city, Afghanistan.

In this study around 73.3% of beef samples were contaminated with coliform bacteria which is higher compared to findings from some other parts of the world, such as 54%, 58% have been reported by Mouafo et al., (2020) and Sadiqi et al., (2012). This is probably meat sellers and processors in slaughterhouses and butchers cause cross-contamination of meat, which explains why a high microbial load has been observed throughout this study. It was also determined that 58% of these positive samples were contaminated with coliform which is more than the acceptable standard (10^2 CFU/gr). The results of this study showed that there was a significant difference in the bacterial load between slaughterhouse and butchery samples ($p < .05$). The

average coliform in meat samples collected from butchers was slightly higher than that of slaughterhouses. Meat contamination during transportation from slaughterhouse to butcheries and poor hygiene practices in the butcheries due to the bacterial growth curve, lack of hand washing facilities and cold chain facilities can be effective factors stimulating bacterial growth on beef.

Coliforms were found to be present in greater quantities in the butcheries 'samples (3.66 log10 CFU/gr) in comparison to the slaughterhouse samples (3.05 log10 CFU/gr). The results indicate a high percentage of *E. coli* contamination in butchery samples than that of slaughterhouses. These findings correspond with what was reported by Bogere and Baluka, (2014) and Fazlina et al. (2012) that found lower meat contamination with coliforms at the slaughterhouse level.

In this study, the rate of *E. coli* contamination in slaughterhouse and butcheries samples was determined to be 52% and 72% respectively. Our finding nearly consistent and comparable with 59% and 55% that have been reported by Rahimi et al. (2006) and Tavakoli et al. (2006) in Iran respectively. Also Iroha et al. (2011) reported that *E. coli* was the most prevalent microorganism in meat sold at retail markets in Abakaliki, Ebonyi state in Nigeria. in contrast a research by Soepranianondo et al., (2019) indicates that 32.5% of *E.coli* contamination in beef sample. However, Presence of *E. coli* and other coliforms in meat is an indicator/ suggestive of fecal contamination. Appropriate evisceration techniques at the slaughterhouse could explain the low levels of *E. coli* since these organisms are found abundantly within the viscera of cattle.

CONCLUSION

The findings of this study demonstrated that raw beef in slaughterhouses and butcheries was highly contaminated with bacteria such as *E. coli* and other coliforms that can cause foodborne illnesses to consumers unless the cooking process is effective enough to kill these bacteria. However, since consumers can also get these contaminants when slicing and washing meat, it poses a serious risk to public health. The study butcheries possessed poor hygiene and working conditions, explaining why the average coliform contamination in meat samples was uniformly high. This study recommends stricter inspection, regular supervision and monitoring of hygiene practices in slaughterhouses and butcheries as well as in other meat processing facilities in Herat city via both the veterinary and public health inspectors. Further research should be undertaken to assess other bacterial contamination of meat, the meat safety and hygiene knowledge levels of meat handlers, bacterial load on meat processing equipment and hand microbiology of meat handlers at the slaughterhouse and butchery levels. Also, continuous monitoring of coliform contamination at slaughterhouses and butcheries should be implemented to prevent infection and intoxication due to meat.

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