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**Pathogenic Bacteria Contamination along the Beef line at Asella Municipal Abattoir,
Arsi Zone Oromia Regional State, Ethiopia**

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Abstract

Background: Foodborne bacterial pathogens and spoilage microbes leads to economic loss as a result of foods deterioration and public health risk. In areas where there is habit of raw meat consumption, various bacteria may result in acquire food-borne diseases and intoxication.

Methods: A cross-sectional study was conducted to assess microbial contamination along Asella Municipal Beef Abattoir line. A total of 470 samples consisting of 400 beef carcasses swab and 70 environmental samples (apron, carcass splitting axil, personnel hand, hooks, knife, meat wrapping plastics, cleaning water) were collected and examined for presences of microbial. One colony type was characterized per positive samples, with no mixed microbial contamination report in this work. The isolation and identification of bacterial contaminates was performed according to the standard microbiological procedures. For *E. coli*, the hemolytic nature and appearance of metallic sheen on eosin methylene blue agar were used to define the pathogenicity. Additionally, rabbit plasma-based coagulase test was used to differentiate coagulase positive *Staphylococcus* (CPS) and coagulase negative *Staphylococcus* (CNS) as indication of pathogenicity.

Results: Out of the total samples examined, 99.1% (95% CI= 97.8-99.8) were contaminated with bacteria. The observed proportion of beef abattoir line contamination was; *Staphylococcus aureus* (36.4%), CNS (16.2%), other grams positive isolates (14.0%), *Staphylococcus hyicus* (13.8%), and *E. coli* (10.9%), and *Staphylococcus intermedius* (7.9%). The total proportion of samples positive for coagulase positive (pathogenic) *Staphylococcus* species were 58.1% (95% CI= 53.5-62.6), with a 61.4% and 57.5% occurrence in abattoir environment and carcass samples, respectively. All sampling locations were found contaminated with *S. aureus* (34.3-60.0%) and *E. coli* (10.0-30.0%). All locations were contaminated with CNS (10.0-20.0%), except for carcass splitting axil. *S. hyicus* contaminated all location at rate of 10.0-30.0%, except the hooks and cleaning water. *S.*

intermedius was observed only at carcass splitting axil (10.0%), cleaning water (10.0%) and carcass (8.0%). Other grams positive isolates were observed at 10.0-30.0%, but not on carcass splitting axil and hooks. The observed isolation rate of CPS (40-90%), *S. aureus* (30-60%), *S. hyicus* (0-30%), *S. intermedius* (0-10%), *E. coli* (10-30), and CNS (0-20) in different abattoir environment was higher than the isolation rate from carcass samples at proportion of 57.5%, 34.3%, 14.5%, 8.8%, 10.5, and 16.8%, respectively. Based on chi-square analysis *S. aureus* was significantly ($p < 0.05$) higher in environmental (48.6%) than in meat (43.3%) samples. But the proportion of distribution with other isolates was insignificant ($p > 0.05$) between environmental (2.9-12.9%) and meat (8.0-16.8%) samples.

Conclusion: The finding indicates the presence of one or more zoonotic and spoilage bacterial pathogens in the beef abattoir which might lead to mixed foodborne infection and intoxication. Therefore, hygienic operations in the beef line; proper handling of working equipment's and carcass; as well as the application of modern working facilities could reduce the risk of such microbial hazards associated with beef production in the abattoirs.

Keywords: *Abattoir environment, Beef, Personnel, Coagulase positive Staphylococcus, E. coli.*

Background

The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughterhouses, and also during handling at retail establishments (Abdalla *et al.*, 2009) where each step may pose a risk of microbial contamination (Abdalla *et al.*, 2009; Komba *et al.*, 2012). Other potential sources of contaminations are contaminated personnel hands and the physical facilities themselves are also all implicated sources of contaminates (Goja *et al.*, 2013; Nouichi and Hamdi, 2009). The sanitary conditions of the processing surrounding environment are another major factors contributing in bacterial contamination of the product (Gill *et al.*, 2000).

Although tissue from healthy animals are an excellent source of protein for human (Nouichi and Hamdi, 2009, Komba *et al.*, 2012), they are highly susceptible to microbial contaminations, which can cause spoilage and food borne infections (Zadoks *et al.*, 2000; Howard *et al.*, 1990), which in turn resulting economic losses (Zadoks *et al.*, 2000). Spoilage microbes includes bacteria that will cause deterioration of foods through breakdown of the food constituents and/or accumulation of undesirable end products of bacterial metabolism (Bergh, 2007).

Some microbes such as *S. aureus* are prevalent along beef line and causes systemic infections globally (Kwon *et al.*, 2006; Zadoks *et al.*, 2000) as well as foodborne infections in different countries (Kwon *et al.*, 2006; Pereira *et al.*, 2009). Other coagulase positive *Staphylococcus* species (*S. intermedius*) can also contaminate meat, and produce enterotoxins which resulted in foodborne disease (Goja *et al.*, 2013; HPA, 2009; Nouichi and Hamdi, 2009). Food borne grams positive bacilli including *Bacillus subtilis* group, *C. perfringens*, *B. cereus* and *L. monocytogenes* can also occur in meat processing line at variable frequency, in which some might lead to gastroenteritis syndrome (HPA, 2009; Nel *et al.*, 2004). Besides its zoonotic importance, *E. coli* were considered to be one of indicators of fecal contamination of the food and facilities (HPA, 2009; Gill *et al.*, 2000). The presence of *B. cereus*, *Staphylococcus aureus*, *Pseudomonas spp.*, *L. monocytogenes*, *E. coli*, *Salmonella spp.*, and *Enterobacteriaceae* on meat from the deboning room were reported as a potential spoilage bacteria and public health risk pathogens (Nel *et al.*, 2004). On the other hand, consumption of raw beef meat is a common habit in Ethiopia (Tewodros and Gedebe, 1984; Gizachew *et al.*, 2015). This may result in acquire food-borne diseases and intoxication (Eshetie *et al.*, 2016; Gizachew *et al.*, 2015; Gizaw, 2014; Adugna, 2014; Tewodros and Gedebe, 1984). To the best of researcher's knowledge, survey on microbial contamination along Asella Municipal Beef Abattoir line were not yet conducted. Thus, a study on the occurrences or contamination rate of pathogenic/potentially pathogenic bacteria in the abattoir environment and meat could help to monitor hygienic practices in abattoirs. Therefore, the study was aimed to assess significances of microbial contamination along Asella Municipal Beef Abattoir line in Ethiopia.

Materials and Methods

Study Area Description

The study was conducted at Asella town located in Arsi Zone, Oromia Regional State, Ethiopia. Asella town, the capital of Arsi Zone, is located at about 175 km Southeast of Addis Ababa at 6° 59' to 8° 49' N and 38° 41' to 40° 44' E with an altitude of the area ranges from 2500 to 3000 meter above sea level. The maximum and minimum temperatures of the area are 25°C and 10° C respectively with an estimated human population of 67, 269 (CSA, 2007).

Study Design and Sample Size Determination

A cross-sectional study was employed to isolate possible bacterial contaminants of beef and its environment at Asella Municipal Abattoir in Asella town from November 2017 to May 2018. The

sample size was calculated using 50% expected risk of contamination of the carcasses from slaughtered animal within 95% Confidence Intervals (CI) at 5% desired accuracy, according to the Thrusfield (Thrusfield, 2005) formula.

$$\text{Thus, } n = \frac{1.96^2 * P_{\text{exp}} (1 - P_{\text{exp}})}{d^2}$$

Where: n= required sample size, d= desired absolute precision, P_{exp}= expected prevalence (50%)

However, to avoid sampling error a minimum of 5% sample was added to the original sample size. Thus, a total of 400 beef animals presented for slaughter were identified during antemortem examination and traced for carcass swab samples. An average of 2-3 environmental samples were also collected from each sampling location during every sampling occasions. Generally, the total number of samples were 470, which comprises 400 carcass swabs, and 10 swab samples from each of the knives, splitting axe, carcasses hanging hooks, abattoir personnel hand, meat wrapping plastic, apron, and cleaning water.

Sample Sources and Sample Collection

The samples were taken from beef carcass and its processing operations such as, the beef processing environments, working facilities, and cleaning water at Asella Municipal Abattoir. Sampling was done twice per week for about 6 months (25 weeks). On average 16 carcasses were sampled on a weekly basis and other samples were collected alongside the carcass samples. The selected carcasses were swabbed according to the method described by ISO6888-2 (ISO, 2005) by placing sterile template (10 x 10 cm) on specific sites of a carcass. Swabbing was done using a sterile cotton tipped swab (2*3 cm) fitted with shaft soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England). Rubbing carcasses surfaces was preformed horizontally and then vertically several times. The abdomen (flank), thorax (lateral), crutch and breast (lateral) sites were sampled according to ISO6888-2 (ISO, 2005). On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube disposing the cotton swab in the test tube.

In the similar manner, swab samples were also taken from knives, splitting axe, personal hands, apron, meat wrapping plastic and hooks while a about 10 ml sample of cleaning water were taken directly from the pipe line. For convenience, the swabs acquired from the equipment's and the working environment were considered as samples from the immediate slaughter environment.

Finally, all samples were kept in the cold chain (+4°C) using icebox and shipped to Asella Regional Veterinary Laboratory for bacterial isolation and identification.

Bacterial Culture and Identification

The swab samples which has been dipped into 10 ml BPW during collection were directly incubated for 24 h at 37 °C. The water samples were also enriched using 1: 10 proportion (i.e. 1ml water to 9ml of BPW) and incubated for 24 h at 37°C. On the next day, the broth culture was vortexed and a loop full of enrichment was streaked on 7% heparinized sheep blood-based agar plate (BAP) and incubated at 37 °C for 24 hrs under aerobic condition. All microbes grown on the blood agar were characterized for colony features (transparency, color, size) and hemolysis patterns (Quinn *et al.*, 2002; Jay *et al.*, 2005). The selection of isolates for subculture was based on the observance of colonies which are dominant in the primary inoculation. Thus, only one colony type was considered for further identification. Then each of characteristically similar colonies (2-3 colonies) were transferred on to nutrient agar (Oxoid, UK) and incubated at 37 °C for 24 hrs under aerobic condition to use for further tests. Each of the isolates were subjected to gram staining procedures to examine grams' reaction and microbial morphology.

Identification of *Staphylococcus* Species: Those gram-positive, grape like colonies, suspected to be *Staphylococcus species* were transferred on to mannitol salt agar and incubated at 37°C for 24 hrs to get characteristic colony. Characteristic colonies were further incubated on nutrient agar at 37°C for 24hrs for identification based on biochemical tests. Tests such as catalase test, oxidase test, DNase test agar to detection of DNase activity (for detection of deoxyribonuclease enzyme), coagulase test by using rabbit plasma (Quinn *et al.*, 2002; ISO, 2005; Jay *et al.*, 2005) were employed. Those Staphylococci isolates were differentiated into *S. aureus*, coagulase negative *Staphylococcus* (CNS), *S. intermedius* and *S. hyicus* according to the test results indicated in Table 1.

Table 1. Differential tests used and their reactions on presumptive *Staphylococcus* colonies

<i>Staphylococcus</i> spp.	Hemolysis	Grams stain	Catalase test	Coagulases test	OF test	Mannitol Fermentation	Clear zone on DNase agar
<i>S. aureus</i>	+	+	+	+	F	+	+
CN <i>Staphylococcus</i>	-	+	+	-	F	+	-
<i>S. intermedius</i>	+	+	+	+	F	(+)	-
<i>S. hyicus</i>	-	+	+	(+)	F	-	-

CN = Coagulase negative; + = positive; - = Negative, F = Fermentative, OF = Oxidation-fermentation; (+) = weak reaction (Quinn *et al.*, 2002; ISO, 2005; Jay *et al.*, 2005).

Other grams Positive Isolates: All none-cocci gram's positive isolates were grouped under "other gram's positive isolates". They were rod shaped, coccobacilli or filamentous. Further tests were not employed due to shortage of laboratory biochemical test reagents.

Identification of Pathogenic Escherichia coli: Characteristic gram's negative isolates from nutrient agar were subjected to tests such as indole, methyl red, Voges-Proskauer, and citrate utilization according to Quinn *et al.* (2002). Furthermore, the isolates were cultured on eosin methylene blue (EMB) agar to confirm for presumptive *E. coli* colony with metallic sheen. This characteristic along with the hemolytic nature of the isolates was considered as pathogenic *E. coli* as described by Quinn *et al.* (2011).

Data Analysis

The data was entered in Microsoft Excel 2013[®] and filtered for completeness. Descriptive statistics such as frequency distributions, proportions, and percentages were applied to compute some of the data. Pearson's chi-square and odds ratio were used to present the association of factors with rate of bacterial contamination and STATA 11 version and SPSS version 20.0, 2011 software were used for analysis. Values < 0.05 were considered significant at 95% confidence interval.

Results

Proportion of Bacterial Isolates from the Beef Line

From a total of 470 samples examined, 99.1% (95% CI= 97.8-99.8) were contaminated with different species and groups of bacteria with only 4 (0.85%) to be free from bacterial contaminants. Based on species of the isolated bacteria, *S. aureus* was the dominant one at a proportion of 36.4% followed by *Staphylococcus hyicus* (13.3%), *Escherichia coli* (10.9%), and *Staphylococcus intermedius* (5.9%) (Table 2).

Table 2. Microbial contamination along Asella Municipal Beef Abattoir line, Arsi-Oromia-Ethiopia

Isolated bacterial contaminants	No. (%) observed (n=470)	95% CI
<i>CP Staphylococcus:</i>		
<i>Staphylococcus aureus</i>	171 (36.4)	32.0-40.9
<i>Staphylococcus hyicus</i>	65 (13.8)	10.8-17.3
<i>Staphylococcus intermedius</i>	37 (7.9)	5.6-10.7
Subtotal	273 (58.1)	53.5-62.6
<i>CN Staphylococcus</i>	76 (16.2)	12.9-19.8
Other grams positive isolates	66 (14.0)	11.0-17.5
<i>Escherichia coli</i>	51 (10.9)	8.1-14.0
Samples free of bacteria	4 (0.85)	0.23-2.2

n= Number of samples examined; *CP*= Coagulase positive; *CN*= Coagulases negative.

Comparison of Bacterial Isolates among Different Sample Sources

Regardless of number of samples (Table 3), all sampling locations were found contaminated with *S. aureus* (34.3% to 60.0%) and *E. coli* (10.0-30.0%). Again, all locations were contaminated with *CNS* (10.0%-20.0%), except for carcass splitting axil (0%). *S. hyicus* contaminated all location at rate of 10.0%-30.0%, except the hooks and cleaning water. Contamination of the beef line with *S. intermedius* was observed at carcass splitting axil (10.0%), cleaning water (10.0%) and carcass (8.0%). Furthermore, except for carcass splitting axil and the hooks, contamination of the beef line with other grams positive isolates were observed at 10.0-30.0%.

Table 3. Distribution of microbial contaminants along Asella Municipal Beef Abattoir line, Arsi-Oromia-Ethiopia

Sample sources		No. of tested samples	No. (%) of samples positive to:						
			CNS	CP <i>Staphylococcus</i>				OGPB [¶]	<i>E. coli</i>
				<i>S. aureus</i>	<i>S. hyicus</i>	<i>S. intermedius</i>	Overall		
Environmental sample	Apron	10	2 (20.0)	4 (40.0)	1 (10.0)	0	5 (50)	2 (20.0)	1 (10.0)
	Carcass splitting axil	10	0	5 (50.0)	3 (30.0)	1 (10.0)	9 (90)	0	1 (10.0)
	Personnel hand	10	1 (10.0)	6 (60.0)	1 (10.0)	0	7 (70)	1 (10.0)	1 (10.0)
	Hooks	10	1 (10.0)	6 (60.0)	0	0	6 (60)	0	3 (30.0)
	Knife	10	2 (20.0)	5 (50.0)	1 (10.0)	0	6 (60)	1 (10.0)	1 (10.0)
	MWP	10	1 (10.0)	5 (50.0)	1 (10.0)	0	6 (60)	1 (10.0)	1 (10.0)
	Cleaning water	10	2 (20.0)	3 (30.0)	0	1 (10.0)	4 (40)	3 (30.0)	1 (10.0)
	Sub total	70	9 (12.9)	34 (48.6)	7 (10)	2 (2.9)	43 (61.4)	8 (11.4)	9 (12.9)
Meat	Carcass	400	67 (16.8)	137 (34.3)	58 (14.5)	35 (8.8)	230 (57.5)	58 (14.5)	42 (10.5)
Total		470	76 (16.2)	171 (36.4)	65 (13.3)	37 (7.9)	273 (58.1)	66 (14.1)	51 (10.9)

CN= Coagulases negative *Staphylococcus*; CP= Coagulase positive; OGPB[¶]= Other grams positive bacteria; MWP= Meat wrapping plastic

As shown in Table 4, prevalence of *S. aureus* was significantly higher (48.6%) in environmental samples than in meat samples (43.3%) ($p < 0.05$), whereas the distribution of other bacteria species or groups between carcass and its environment didn't vary significantly ($p > 0.05$). However, with the exception of *E. coli*, the distribution of other isolates varied among the sources with relatively higher values in meat samples (8.0-16.8%) than environmental samples (2.9-12.9%) (Table 4).

Table 4. Occurrence of microbial contamination at the abattoir environment and on the carcass along Asella Municipal Beef Abattoir line at Arsi-Oromia-Ethiopia

Isolated Microbial contaminants	No. (%) positive by sample origin		χ^2	P-value
	Environmental sample (n=70)	Meat sample (n=400)		
<i>Staphylococcus aureus</i>	34 (48.6)	137 (34.3)	5.27	0.022
<i>Coagulase Negative Staphylococcus</i>	9 (12.9)	67 (16.8)	0.66	0.414
<i>Staphylococcus hyicus</i>	7 (10.0)	58 (14.5)	1.01	0.314
<i>Staphylococcus intermedius</i>	2 (2.9)	35 (8.0)	2.85	0.091
Other grams positive isolates	8 (11.4)	58 (14.5)	0.46	0.495
<i>Escherichia coli</i>	9 (12.9)	42 (10.5)	0.34	0.559

n= Number of samples examined.

Discussion

The contamination of raw meat with *Staphylococcus aureus* was principally from skin of humans and animals (Adams and Moss, 2008), and for animals which are killed for meat, the hide may be one of the most important sources of spoilage organisms. The present finding where the risk of the abattoir beef line contamination with *Staphylococcus aureus* (36.4%) indicated the risk of consumers acquiring infection from the meat. The present finding is higher than the 26 % reported from food and food environments in Italy (Marino *et al.*, 2010), 9.3% from abattoir, 19.5% from retail shop and 17.5% from equipment of retail shop and abattoir (Adugna, 2014) in Addis Ababa Abattoir and retail shop, (16.1%) from abattoir (Beyene *et al.*, 2017) Ethiopia. Those variations might be due to difference in degrees of environmental hygiene and food safety tool application among areas. The present 34.3% in beef samples were lower than 68% meat samples collected from retainer in Thailand (Bunnoeng *et al.*, 2014) due to higher probability of contamination at retail shop than at abattoir. Regardless of sample size, the present 60.0% *S. aureus* from personnel hand were lower than the 16% (Dagnew *et al.*, 2013) from finger nail bed of cafeteria food handlers. In the present study, the observed *S. aureus* prevalence of 34.3-60.0% from the sampling locations justify significant presence of *S. aureus* as a zoonotic contaminant along the abattoir line, which could be due to poor sanitation and poor handling process. Moreover, the 30.0% occurrence of *S. aureus* in cleaning water indicated that it can act as source for further contamination of working facilities and the meat. The occurrences of *S. aureus* at 40.0-60.0% in the abattoir working facilities with significantly ($p<0.05$) higher rate than on that of carcass (34.3%) indicates highly contamination of the beef line with *S. aureus* intern, frequent contamination of the facilities during slaughtering, dressing and evisceration.

The rate of *Staphylococcus hyicus* (13.3%) in the present findings is similar with prevalence report of 8.3% by Beyene *et al.* (2017) from various sample sources in Addis Ababa, Ethiopia. The distribution of *S. intermedius* along the studied abattoir line ranged from 8.0-10% is similar with 10.9% report (Beyene *et al.*, 2017) from various sample sources in Addis Ababa, Ethiopia. Coagulase-positive staphylococci other than *S. aureus*, such as *Staphylococcus intermedius* and *Staphylococcus hyicus* have been isolated from the commensal flora of skin and nasopharynx, as well as from infection sites of animals (Quinn *et al.*, 2011) and can be responsible for sepsis (Casanova *et al.*, 2011), nail bed infection (Pottumarthy *et al.*, 2004), and wound infection (Osterlund *et al.*, 1997; Kelesidis and Tsiodras, 2010) in humans following exposure to animals or the pathogens. More importantly, it was observed that coagulase positive *Staphylococcus* are found in larger proportion (58.1%) of sampled sources, particularly in 61.4% of samples from abattoir environment and 57.5% carcass. This indicates a high risk for human health, because coagulase is considered as a virulence factor for Staphylococcal infections (Kayser, 2005; Quinn *et al.*, 2011).

The present findings of coagulase-negative staphylococci (16.2%) and with a range of 10.0-20.0% across all locations of beef line (except on carcass splitting axil) indicated the wider distribution of the agent on the working facilities. Furthermore, the carcass contamination rate (16.8%) could be linked with the wider distribution in the abattoir working facilities. The present 10.0% CNS observed in personal hand swab samples were similar with the 12.4% (Beyene *et al.*, 2017) from abattoir, but lower the predominant bacteria species (33%) isolated from finger nail bed from food handlers at certain cafeteria (Dagnew *et al.*, 2013). Coagulase-negative staphylococci comprise a large group of related species commonly found on the surface of healthy persons, in whom they are opportunistic pathogens that cause infection in debilitated or compromised patients (Kayser, 2005). The pathogenic potential in food poisoning, as well as reservoirs for drug resistant genes (Bora *et al.*, 2018) in the meat of slaughtered animal needs a consideration in monitoring the contamination rate along the beef chain.

The present study indicated contamination of the majorities of facilities (except for carcass splitting axil and the hooks) and the carcass along the line at 10.0-30.0% with some other non-cocci gram positive bacteria. Regardless of absences of further biochemical, the present finding indicated the presence of other microbial contaminants. Based on the Gram's reaction, the rod shaped/filamentous, gram positive isolates might coincide with the possibility of occurrences of food borne gram positive bacilli such as *Bacillus subtilis* group such as *B. subtilis*, *B. licheniformis*, *B. pumilis* and *B. amyloliquifaciens* were reported (HPA, 2009).

The present high rate of *E. coli* (10.9%) in the studied abattoir line, in which all sampling locations were found contaminated with *E. coli* at rate ranging from 10.0-30.0% indicated the possible presence of faulty evisceration, contaminated working surfaces and/or handling of the meat contaminated handlers. *E. coli* were frequently isolated from meat and the handling environment in Ethiopia (Tassew *et al.*, 2010; Dagnew *et al.*, 2013; Beyene *et al.*, 2017). It was also suggested that *E. coli* may be used as an indicator microorganism for fecal contamination and poor sanitation during processing (Gill *et al.*, 2000; Goja *et al.*, 2013; Nouichi and Hamdi, 2009). The present over all 10.9% *E. coli* from abattoir was lower than the 26.6% report from food (Tassew *et al.*, 2010), whereas it was higher than the 2.67% report by Dagnew *et al.* (2013) from finger nail bed of cafeteria food handlers. Therefore, it is of the most important to implement hygienic operation along the abattoir line via application of care at evisceration process, carcass handling and transportation so as reduces contamination by enteric pathogens.

Conclusion

The observed various bacterial contaminants including zoonotic bacteria along the studied beef line indicated poor personal hygiene and working practices. The lack of sterilization of equipment and working surfaces, insufficient water supply for abattoir for washing carcass, lack of safety rule in abattoir and lack of hygienic awareness of meat handlers are the major challenges along the studied beef abattoir line. These were demonstrated by contamination of the line with potentially pathogenic bacteria such as *S. aureus*, other coagulase positive *Staphylococcus* species, *E. coli*, and other opportunistic bacterial pathogens whereby the raw meat can be spoiled and also act as a reservoir of zoonotic pathogen, leading to health problem in consumers as well as those in close contact with the beef.

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Availability of data and materials

All necessary data supporting our findings can be found in the repository.

Authors' contributions

AH, BM, AH, and AA developed the research concept and designed the methodology, data analysis and interpretation and preparation of the manuscript for publication. AH carried out the sample collection, laboratory work. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

There was no involvement of animals or humans for sample taking. As this study was conducted on slaughtered animals and contact surfaces of abattoir environments, there is no any harm or risk for animal or human.

Consent for publication

In our study, we don't have any images or videos, etc. of individual participants.

Competing interests

The authors declare that there is no financial or non-financial competing interest from any person or institute. We did not receive any technical assistance for developing the research concept or preparing the manuscript.

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