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ABSTRACT

BACKGROUND: Meat safety is important for public health. As part of the meat chain abattoirs are required to give attention to meat hygiene and safety in order to minimize hazards. Therefore, the current study was conducted to evaluate the bacteriological quality of sheep carcasses, knowledge and hygienic practices of workers in a selected abattoir and to determine the effect level of 2.5% citric acid spray on total coliforms and aerobic bacteria load of raw sheep carcasses surfaces.

METHODS: A cross-sectional study design with structured questionnaire and observational checklists observation were used. A systematic random sampling technique was employed. A total of 50 sample swabs (25 swabs before citric acid spray and 25 after citric acid spray) were randomly taken from brisket, flank and rump of sheep's carcasses. Swabs were moistened with buffered peptone water (BPW) and samples were taken by rubbing 100 cm^2 (10 cm × 10 cm) area delineated by sterile aluminum template. In addition, we administered a structured questionnaire and an observational checklists to assess knowledge and hygienic practices of workers. Bacteriological quality of **sheep carcasses** were analyzed using the methods described by the US bacteriological analytical manual.

RESULTS: The mean count for aerobic bacteria of the sheep carcasses before and after citric acid spray were 7.2$\log_{10}$ CFU/ml and 6.4$\log_{10}$ CFU/ml, respectively. The test results also showed that 21 (84%) and 15 (60%) of the swab samples were positive before and after spraying citric acid, respectively. The mean counts for coliform bacterial of the sheep carcasses before and after citric acid spray were 3.5$\log_{10}$ CFU/ml and 2.9$\log_{10}$ CFU/ml, respectively. The mean total aerobic and coliform counts before and after citric acid spray were significantly different ($P < .05$). Regarding the hygiene condition of workers, all the respondents reported that they always washed their hands with soap before and after entering the slaughtering room and 23 (53.5%) of the workers reported that they used hot water. Thirty-one (72.1%) of the workers reported that they do not used soap to wash hands after visiting toilet. Thirty-five (81.4%) of the production workers did not wear mouth mask while handling and distribute meat/carcass. On the other hand, all of the workers wore capes, gowns and boots at the time of the observation and only 18 (18.6%) of the production workers wore gloves at the time of the survey.

CONCLUSION: The current study revealed that significant proportion of sheep carcasses were positive for **t**otal aerobic bacteria and total coliform. Moreover, the study also showed that spraying of sheep carcasses with 2.5% citric acid significantly reduced the total coliform and aerobic counts. However, we did not assessed how much spray results to this effect. Therefore, we recommended further studies to determine how much spray of 2.5% citric acid significantly reduce bacterial contamination of sheep carcasses. In addition, the abattoir has to follow the food hazard analysis critical control point (HACCP) system to minimize meat contamination during harvesting and processing. The abattoir has to also implement strict operation laws to improve hygiene conditions of the workers. In addition, the abattoir can minimize meat contamination using 2.5% citric acid as a decontaminant.

KEYWORDS: Abattoir, bacteriological quality, 2.5% citric acid, hygienic practices, sheep carcasses

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Background

Food borne diseases occur commonly in developing countries particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food-handlers.[1] Of all the foods intended for human consumption those of animal origin tend to be most hazardous unless the principles of food hygiene

are employed. Food of animal origins such as meat are generally regarded as high-risk commodity in respect of pathogen contents, and other possible contaminants and adulterants.[2]

Meat is considered as an important source of proteins, essential amino acids, B complex vitamins, and minerals. Unfortunately due to its rich compositions, it offers a highly favorable environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughterhouses, and retail establishments.[3] Fecal matter is a major source of contamination and can reach carcasses through direct deposition, as well as by indirect contact through contaminated carcasses, equipment, workers, installations, and air.[4]

Bacterial contamination of meat products is an unavoidable consequence of meat processing.[5] Data regarding meat borne diseases in Ethiopia are extremely scarce, with a few studies conducted in different parts of the country that have shown the public health importance of several bacterial pathogens associated with foods of animal origin.[6]

According to Gordon-Davis[7] one of the major risks of food contamination originates from the working practices of food handlers and disease-causing micro-organisms present in or on the food handler's body are subsequently transported from the food handler to the food during the handling process. Forsythe[8] mentioned that an estimated one in every 50-food handlers sheds around 10^9 pathogens per gram of feces without showing any clinical manifestations of the related illness. Subsequently, poor personal hygienic practices such as negligence to wash hands after visiting the bathroom may result in up to 10^7 pathogens under the fingernails of the food handler. Organisms originating from infected food handlers include *Salmonella* species, *Shigella* species, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and fecal *streptococci*.[9]

The abattoir is a labor intensive working environment, the knowledge and level of training of the food handlers regarding personal and general hygiene is of particular importance to ensure the health and safety of the consumer because meat is such a highly perishable foodstuff.[10,11] In view of this, Martínez-Tomé et al[12] highlighted the education of food handlers as a crucial line of defense in the prevention of most types of foodborne illnesses. In line with this basic medical fact, 2 issues must be considered to ensure that staff members conform to personal hygiene requirements: (1) the environment within which the staff operates and (2) the "quality" of the staff members. From a food hygiene point of view the quality of the working environment depends on the facilities or equipment provided, which includes toilets and protective clothing. The quality of staff depends upon their health, hygiene and habits.[13]

Meat safety is important to a public health as it plays a significant role in health development and consequently national economic development. Thus, great endeavors should be made to improve it at all levels of the meat chain. As part of the meat chain abattoirs are required to give detailed attention to meat hygiene and safety in order to minimize hazards, given that abattoirs serve a large part of the society. Therefore, the current research was conducted to evaluate the bacteriological quality of **sheep carcasses**, the knowledge and the hygienic practices of workers in a selected abattoir and to determine the effect level of 2.5% citric acid spray on total coliforms and aerobic bacteria load.

Methods

Study design and setting

A cross-sectional design with structured questionnaire and observational checklists were used to assess bacteriological quality of raw sheep meat and hygiene practices in selected abattoir house in Debrezeit town, central Ethiopia in June 2014. Debrezeit town is located 45 km far from Addis Ababa, the capital city of Ethiopia in the southeast direction. The town has a total human population size of 95 000.[14] The area has an altitude of 1850 m above sea level and experiences a bimodal rainfall pattern with a long rainy season that extends from June to October and a short rainy season from March to May. The average annual rainfall, average maximum, and minimum temperature for the area are 800 mm, 27.7°C, and 12.3°C, respectively.[14] In the town, there is an abattoir established in the year 1974 and later named elfora. Currently, the abattoir has a total of 106 production staffs out of this 73 (66.9%) of them are males and 33 (31.1%) are females. There are 5 veterinarians and 2 assistant veterinarians who are part of the staff at the abattoir.

Meat sample collection and transportation

A systematic random sampling technique was employed A total of 50 sample swabs (25 swabs before citric acid spray and 25 after citric acid spray) had been sampled. The swab samples were collected from the surface of sheep carcasses just before citric acid spray and again 30 minutes after citric acid was sprayed on the carcasses. Swabs were moistened with buffered peptone water (BPW) and samples were taken by rubbing 100°cm^2 (10 cm × 10 cm) area delineated by sterile aluminum template from different sites such as brisket, flank, and rump. After the cotton swab is rubbed on the marked site vertically, horizontally and diagonally, it was put in to a universal bottle container which contains a volume of 10 ml of 0.1% peptone water. The bottles were placed in insulated ice box at a temperature of 4°C and transported to the Microbiology Laboratory of Ethiopian Meat and Dairy Industry Development Technology Institute (EMDIDTI) located in the north east part of Debrezeit. The samples were processed within 24 hours of arrival.

Administration of questionnaire

In addition, we administered structured questionnaire and observational checklist to assess knowledge and hygienic practices of workers in the abattoir. We prepared the questionnaire and observational checklist from related published studies. The questionnaire was prepared in English language and then

translated to Amharic and Oromiffa languages and back translated to English. The questionnaire comprised socio-demographic information, personal hygiene conditions, and meat hygiene/safety knowledge of production workers. We pretested the questionnaire to check the extent to which items on a questionnaire are actually measuring the research objectives. Data collectors were trained on assessment of personal hygiene and ethical issues during inspection. We then interviewed a total of 43 volunteer production service staffs. On a daily basis, supervisors supervised the data collection process and checked the completeness of the data.

Bacteriological analysis of meat

Meat swabs were analyzed following the methods described in the US bacteriological analytical manual.[15] The principal method employed in this section was bacteriological analysis of carcass swab samples collected from slaughtered sheep. The sample taken by swabs was homogenized into 10 ml sterile 0.1% peptone water, up on arrival at the laboratory and were processed within 24 hours of collection.

A series of sterile test tubes were filled with 9 ml peptone water labeled as 10^{-1} to 10^{-5}. After thorough agitation of the swabs collected from the abattoir, 1 ml of the aliquot was transferred from properly homogenized initial test tube samples in to a test tube labeled as 10^{-1} by using sterile micro pipette. Again, it was transferred and thoroughly agitated by using new sterilize pipette from a 10^{-1} test tubes 1 ml was transferred to the test tube labeled 10^{-2}. This was repeated for the remaining bottles (10^{-3}-10^{-5}) from the 10-fold dilutions of the homogenates 1 ml of 10^{-1} to 10^{-5}, dilutions of the homogenates were plated in replicate on standard plate count agar using pour plate method for aerobic bacteria count.

The same procedure was followed for coliform bacteria except 10^{0} to 10^{-3} dilutions and red violet bile agar were used. The sample and the media were mixed by moving the petri dishes in circular motion and were left on a table until solidified. The plates were then incubated at 37°C for 24 to 48 hours and 18 to 24 hours for total aerobic and coliform bacteria, respectively. At the end of the incubation period bacterial colonies count were done using the illuminated colony counter. Counts were computed by the following formula.[16]

$$N = \frac{\sum \text{Colonies}}{\left[1xn1\right)+\left(0.1xn2\right)d]}$$, where N is number of colonies per milliliter of swab sample, Σ Colonies is the sum of colonies on plates counted, n_1 is number of plates on lower dilution counted, n_2 is number of plates in next higher dilution counted, and *d* is dilution from which the first counts are obtained.

Statistical analysis and interpretations

Data were entered, processed, coded, and analyzed using statistical package for social sciences (SPSS) statistical software. Descriptive statistics such as means, proportions, standard deviations and 95% confidence intervals were performed. Aerobic bacterial and coliform counts were counted as CFUs per ml; the values were transformed into $\log_{10}$ CFU/ml to normalize the data. The difference in bacterial load between the sheep carcass swab samples before and again 30 minutes after citric acid spray were analyzed using paired *t*-test. A *P*-value of $<.05$ was considered indicative of a statistically significant difference.

Results

We interviewed a total of 43 production service staffs and 1 operational manager. Thirteen (30.2%) of the workers were female. Of the 43 production service staffs, 5 (11.6%) were bleeders, 6 (14%) flayers, 16 (37.2%) eviscerates, 2 (4.7%) stunners, 2 (4.7%) citric acid sprayers, 3 (7%) cleaners of carcasses, and 12 (27.8%) were categorized under others which includes quality controller, inspector of the carcass, and trimmers. Nineteen (44.2%) of the workers attended elementary education (Table 1).

Respondents' Knowledge of Meat Hygiene and Pathogen Links With Meat

Majority of the meat production workers, 32 (74%) were not aware of the correct chilling temperature for carcass/meat. Thirty-five (86.4%) of the workers reported that they had no any information about food hazard analysis critical control point (HACCP). All of the workers in the abattoir knew that they were not supposed to work whenever they have had sore on the dorsal surface of their hands. Most respondents, 30 (68.9%) knew the zoonotic importance of animal tuberculosis. Out of 43 respondents, only 8 (18.6%) knew the importance of citric acid spray on meat. Moreover, 25 (58.1%) of the workers knew when to clean and sanitize the abattoir hall (Table 2).

Personal Hygiene of Production Service Workers

The abattoir checks the health condition of workers before employment and 2 times annually for the employees. All the respondents indicated that they always washed their hands with soap before and after entering the slaughtering room and 23 (53.5%) of the workers indicated that they used hot water. Thirty-one (72.1%) of the workers reported that they do not used soap to wash hands after visiting toilet. Half of the production workers reported that they attended food safety training 4 times per year. All of the respondents said that they reported illness to the management whenever they were ill. Thirty-five (81.4%) of the production workers did not wear mouth mask while handling and distribute meat/carcass. On the other hand, all of the workers wore capes, gowns, and boots at the time of the observation and only 18(18.6%) of the production workers wore gloves at the time of the survey. In addition, all the production workers indicated that they did not eat, smoke or wear jewelry inside the slaughter house (Table 3).

Table 1. Socio-demographic characteristics of production service staffs in the abattoir in Debrezeit town, central Ethiopia.

SOCIO DEMOGRAPHIC CHARACTERISTICS	FREQUENCY	PERCENTAGE
Sex		
Male	13	30.2
Female	30	69.8
Types of work		
Eviscerates	16	37.2
Flayers	6	14
Bleeders	5	11.6
Stunners	2	4.7
Citric acid sprayers	2	4.7
Cleaners of carcass	3	7
Others*	12	27
Level of education		
Elementary	19	44.2
Secondary school	11	25.6
Degree	1	2.3
Diploma	3	7
Illiterate	6	14

*Others include inspectors of carcass, trimmer, and quality control.

Bacteriological Quality of Carcasses

Results showed that all the samples collected before and after spraying citric acid were positive for the bacteria tested. The mean colony count for aerobic bacteria of the meat before and after citric acid spray were $7.2\log_{10}$ CFU/ml and $6.4\log_{10}$ CFU/ml, respectively. The total coliform count test result showed that 21 (84%) and 15 (60%) of the swab samples were positive before and after spraying carcasses with citric acid, respectively. The mean colony counts for coliform bacterial of the meat before and after citric acid spray were $3.5\log_{10}$ CFU/ml and $2.9\log_{10}$ CFU/ml, respectively (Table 4). A paired t-test statistical analysis showed that the mean total aerobic colony counts before and after citric acid spray was significantly different ($P<.05$). Similarly, the mean of coliform counts showed statistically significant difference before and after citric acid spray ($P<.05$).

Discussion

The study was carried out to assess bacteriological quality of raw sheep meat and hygienic practices of the selected abattoir. In the current study all of the production workers always wash their hands before handling carcasses. Washing hands with soap and hot running water can reduce the microbiological load on hands.[17]

Table 2. Knowledge of respondents' on meat hygiene and pathogen links with meat in the abattoir in Debrezeit town, central Ethiopia.

KNOWLEDGE STATEMENT	FREQUENCY	PERCENTAGE
Cleaning slaughter houses routinely plays a role to improve meat quality		
Yes	25	58.1
No	28	41.9
Do you know the correct chilling temperature for chilling meat/carcass?		
Yes	11	25.6
No	32	74.4
Do you know the importance of citric acid sprayed on meat?		
Yes	8	18.6
No	35	81.4
Freezing of meat eliminates germs		
Yes	22	51.2
No	21	48.8
Animal TB is related to food borne disease		
Yes	30	68.9
No	13	31.1
Animal liver fluke is related to food borne disease		
Yes	5	68.9
No	38	88.4
Animal *vibrio cholera* is related to food borne disease		
Yes	7	12.2
No	36	83.7
Do you aware that not handle meat/carcass if you have sore on the back of your hand		
Yes	43	100
No	0	0
Use of protective cloths reduce meat contamination		
Yes	38	88.4
No	5	11.6
Do you have information about HACCP		
Yes	9	20.9
No	34	79.1

This study showed that significant proportion of production workers wore protective equipment (PE) such as overalls, caps, gowns boots, mouth masks, and gloves. Wearing protective equipment protects the meat from microbial contamination as meat handlers are the main source for microbial contamination. Haileselassie et al[18] reported that meat handlers are probable sources of contamination for microorganisms and it is important that all possible measures should be taken to reduce or eliminate such contamination.[18] In addition, all the respondents indicated

that they did not eat, smoke, and wearing jewelry inside the slaughtering hall. Jewelry are a potential source of micro-organisms, because the skin under the jewelry provides a favorable habitat for microorganisms. Smoking may furthermore cause coughing, thus transferring aerosols containing micro-organisms to the food.[19]

In this study, half of the production workers received food safety training. Food safety training increases workers awareness on food quality measures and so that assists the workers to implement quality control measures. Adams and Moss[20] reported that training and education of food handlers with regard to the basic concepts and requirements of personal hygiene play an integral part in ensuring a safe product for the consumer.

In this study all of the respondents indicated that they reported illness to the management of the abattoir. Because meat handlers an act as a source of contamination due to commensal organisms. It is important that all possible measures be taken to reduce or eliminate such contamination. Trickett[21] suggests that whenever a food handler experiences diarrhea, sore throat, fever, cold, or open skin lesions, they should be obliged to report the condition to the supervisor or to management. All the respondents who indicated that they had reported illness were sent to health center for medical examination. In this study all the respondents were subjected to a medical examination before employment and 2 times per year after employment. This is consistent with the recommendation by Ziady et al[22] who are of the view that food handlers must undergo medical examinations before employment to assess their general health.

Table 3. Personal hygiene of production service workers in the abattoir in Debrezeit town, central Ethiopia.

PERSONAL HYGIENE PRACTICES	FREQUENCY	PERCENTAGE
What do use to wash hands in the slaughter room		
Hot water and soap	23	53.5
Cold water and soap	20	46.5
What do use to wash hands after visiting toilet		
Cold water and soap	12	27.9
Cold water only	31	72.1
Wear mouth mask during handling and contact meat at the time of the observation		
Yes	18	18.6
No	35	81.4
Wear gloves during handling and contact meat at the time of the observation		
Yes	18	18.6
No	35	81.4

The mean value for total aerobic bacteria before acid spray in this study is ranging from 7log$_{10}$ CFU/ml to 7.9log$_{10}$ CFU/ml; this is comparable to the findings by Tegegne and Ashenafi[23] in Ethiopia who reported a range of 7.3log$_{10}$ CFU/g to 8.3log$_{10}$ CFU/g for the same organisms. However, higher mean values were reported by Mukhopadhyay et al[24] in India who reported as high as 7log$_{10}$ CFU/g to 11log$_{10}$ CFU/g, while lower finding was mentioned by Arain et al[25] and Haileselassie et al[18] in Ethiopia who reported 5.2log$_{10}$ CFU/cm^2 and 6.6log$_{10}$ CFU/g, respectively. Similarly Obeng et al[26] reported 6.9log$_{10}$ CFU/g in Ghana and Haque et al[27] reported log 6.03log$_{10}$ CFU/g for Bangladesh.

The total aerobic bacteria examined from sheep carcass samples reduced by 0.8log$_{10}$ CFU/ml after spraying carcasses with 2.5% citric acid. This finding is similar to what was reported by Delmore et al[28] for total colony count reduction up to 1.2 to 2log$_{10}$CFU/cm^2, in Egypt and USA, respectively. Bell[29] also reported reduction of total aerobic count by 0.43log$_{10}$CFU/cm^2 in London.

The mean total coliform count on fresh sheep carcass sample reported in the current study ranged from 2log$_{10}$ CFU/ml to 2.7log$_{10}$ CFU/ml before citric acid spray. This finding is in agreement with that of Wudie et al[30] who reported a range of 2.2log$_{10}$ CFU/cm^2 to 2.9log$_{10}$ CFU/cm^2 and Tegegne and Ashenafi[23] who reported 3.1log$_{10}$CFU/cm^2 in Ethiopia.

The reduction in the number of coliform on sheep carcasses due to 2.5% citric acid which acts as an antimicrobial agent was 0.6log$_{10}$ CFU/ml. Similar finding was reported by Beyaz and Tayar[31] who demonstrate that citric is effective

Table 4. Results of swab samples taken from sheep carcasses for total aerobic bacteriological and total coliform bacteriological examinations.

TEST RESULTS OF SWAB SAMPLES TAKEN FROM SHEEP CARCASSES FOR TOTAL AEROBIC BACTERIOLOGICAL EXAMINATION							
BEFORE SPRAY A SOLUTION OF 2.5% CITRIC ACID				AFTER SPRAY A SOLUTION OF 2.5% CITRIC ACID			
NUMBER OF POSITIVE SAMPLES	MIN.	MAX.	MEAN	NUMBER OF POSITIVE SAMPLES	MIN.	MAX.	MEAN
25 (100%)	6.9log$_{10}$ CFU/ml	7.9log$_{10}$ CFU/ml	7.2log$_{10}$ CFU/ml	25 (100%)	3.1Log$_{10}$ CFU/ml	7.9Log$_{10}$ CFU/ml	6.4Log$_{10}$ CFU/ml
TEST RESULTS OF SWAB SAMPLES TAKEN FROM SHEEP CARCASSES FOR TOTAL COLIFORM BACTERIOLOGICAL EXAMINATION							
21 (84%)	1.7log$_{10}$ CFU/ml	4.1log$_{10}$ CFU/ml	3.5log$_{10}$ CFU/ml	15 (60%)	0.9log$_{10}$ CFU/ml	3.4log$_{10}$ CFU/ml	2.9log$_{10}$ CFU/ml

against *E. coli O157:H7* by reducing the pathogen count by $0.1\log_{10}$cfu/cm^2 to $4.67\log_{10}$cfu/cm^2. Likewise, Wudie et al[30] in Ethiopia and Ransom (2003) in Brazil reported that the number of coliform bacteria was reduced by $1.2\log_{10}$CFU/cm^2 and $2.6\log_{10}$CFU/cm^2, respectively due to the effect of citric acid.

Conclusion

The current study revealed that significant proportion of swab samples taken from sheep carcasses were positive for total aerobic bacteria and total coliform tests. Moreover, the study also showed that spraying of sheep carcasses with 2.5% citric acid significantly reduced the total coliform and aerobic counts. However, we did not assessed how much spray results to this effect. Therefore, we recommended further studies to determine how much spray of 2.5% citric acid significantly reduce bacterial contamination of sheep carcasses. In addition, the abattoir needs to pay attention to ways that minimize contamination during carcass harvesting by following modern hygienic procedures and implementing strict operation laws. In addition, the abattoir can minimize meat contamination using of 2.5% citric acid as a decontaminant.

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Authors' Contribution

HT and AHF actively participated during conception of the research issue, development of a research proposal, data collection, analysis, and interpretation, and writing various parts of the research report. ZG reviewed and controlled the quality issues of the research and prepared the manuscript. All the authors read and approved the final manuscript.

Availability of Data and Material

Data will be made available upon requesting from the primary author.

Consent Publication

This manuscript does not contain any personal identification.

Ethics Approval and Consent to Participate

The ethical and methodological aspects of this research was approved by College of Veterinary Medicine and Agriculture, Addis Ababa University. For the interview, verbal informed consent was obtained from production workers. There were no risks due to participation in this research project, and the collected data were used only for this research purpose. All information collected was treated with complete confidentiality.

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