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Bacteriological Assessment of Beef and Surfaces of Equipment and Apparel Worn in the Kotokuraba Slaughterhouse

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Abstract

This study was conducted to determine the bacteriological quality of beef and contact surfaces of equipment and apparel worn in the Kotokuraba slaughterhouse in the Cape Coast Metropolis. Because of the high demand for the product among residents of the Cape Coast Metropolis, as well as economic crises in developing countries, including Ghana, beef producers enter the industry with little knowledge of hygienic and sanitation practices, resulting in bacteriological contamination from cow slaughter to beef handling and storage. Some of these pollutants have the potential to harm humans by creating diseases. This study, therefore, aids in the isolation and identification of harmful bacteria found in beef as well as other surfaces of equipment and apparel that come into contact with beef. Results revealed that, *Staphylococcus* spp. recorded the highest means in all the samples and the number of bacteria identified expressed in percentages had *Staphylococcus* spp. being the highest identified species in the samples taken. *Bacillus subtilis* had the least number with a percentage of about 21.05 %. The percentage occurrences of the various bacteria species were 31.58 %, 21.05 %, 23.68 %, and 23.68 % for *Staphylococcus* spp., *Bacillus* spp., *Micrococcus* spp., and *Escherichia* spp. respectively. This shows that *Staphylococcus* spp. was the most frequently occurring species with *Bacillus* spp. being the least. That is, the dominant species isolated was *Staphylococcus* spp. followed closely by *Micrococcus* spp. and *Escherichia* spp. The presence of these bacteria in the beef and other contact surfaces is a clear indication of contamination of the beef and regular consumption of the beef is harmful to the health of the consumers.

Keywords: Isolation, Identification, Plate Count, Enumeration

1. Introduction

Meat is one of the most perishable meals, with a composition that encourages the growth of a variety of microorganisms [1]. The microbiological quality of meat and meat products is important since contaminated meat has been connected to not just epidemics of a variety of human health problems, but also financial losses for producers owing to market recalls and mortality [2]. According to the World Health Organization, 30 % of people in industrialized countries contract foodborne infections each year, with meat eating accounting for the majority of the causes.

In the United States, foodborne infections caused by recognized bacteria cause an estimated 9.4 million illnesses each year. According to the Centers for Disease Control and Prevention, 839 foodborne disease

outbreaks were reported in 2016, resulting in 1,425 illnesses, 875 hospitalizations, 17 deaths, and 18 food product recalls (CDC, 2016). In addition, the World Health Organization reported that 600 million people worldwide died from foodborne diseases in 2010 [3].

Shigellosis, caused by *Shigella* spp., and Salmonellosis, caused by *Salmonella* spp. are examples of foodborne infections [2]. Bacteria commonly discovered on or in beef include *Escherichia coli 0157:H7, Staphylococcus aureus, and Salmonella* spp., as well as *Pseudomonas* spp., *Acinetobacter* spp., *Brochothrix thermosphacta, Lactobacillus* spp., *Bacillus subtilis, Micrococcus* spp., *Listeria monocytogenes, Yersinia enterocolitica, Clostridium botulinum* [1]. The majority of these pathogens are capable of causing not just meat degradation but also foodborne illnesses.

In many countries, the demand for and consumption of raw beef has expanded, and legislation defining raw beef's microbiological standards has been established to protect customers' health and promote its commercialization [4]. Meat from healthy animals is thought to be devoid of microbes, however, processing and selling procedures make it susceptible to microbial contamination [2]. Causes that are directly associated with the animal, such as its skin or feces, are potential sources of meat contamination [5].

Microorganisms have been shown to contaminate beef during the production, processing, shipping, and distribution phases, according to the literature [6]. According to reports, animal butchering in rural villages is frequently done in unsanitary settings [4]. Because portable water is rarely available, butchers must rely on unsafe water sources. These factors, as well as the high ambient warmth and humidity, are to blame.

The slaughterhouse environment, the retail outlet environment, the vehicle used to transport the meat from the slaughterhouse, and, of course, the meat handlers are all external sources of meat contamination [5]. Because of a lack of essential instruments, crude meat processing techniques could be a source of meat contamination [4]. The first stage in ensuring the microbiological quality of beef is to assess the initial levels of contamination and identify the microorganisms involved. The cleaning of surfaces where beef is processed is another key stage in the management of the bacteriological quality of beef [7].

The absence of a modern slaughterhouse facility, the presence of tiny retail shops, and non-compliance with hygienic production norms have all been identified as key obstacles to hygienic beef production [8]. The first stage in ensuring the microbiological quality of beef is to assess the initial levels of contamination and identify the microorganisms involved. The cleaning of surfaces where beef is processed is another key stage in the management of the bacteriological quality of beef [7].

This study, therefore, assessed the bacteriological quality of fresh beef sold in Kotokuraba Market, Cape Coast Metropolis.

2. Materials and Methods

All culture media and chemicals were purchased from Nesco chemical company in Cape Coast, Ghana. All materials and chemicals which were used were of analytical grade. Experiments were performed carefully with appropriate controlled room temperature, humidity, and other environmental conditions.

2.1 Study Area

This study was confined to Cape Coast Metropolis, Ghana. Cape Coast or Cabo Corso was originally named by the Portuguese as the capital of Cape Coast Metropolitan District and Central region of Ghana. Cape Coast is situated south of the Gulf of Guinea. The Metropolis is an urban center with various occupations with natives mostly fishermen and fishmongers. It has a population of about 169,894

according to the Ghana Statistical Service (GSS) in the 2010 Population and Housing Census (PHC) (GSS, 2012), with the youth and children forming the majority of the population. The treated samples were taken from the Kotokuraba slaughterhouse.

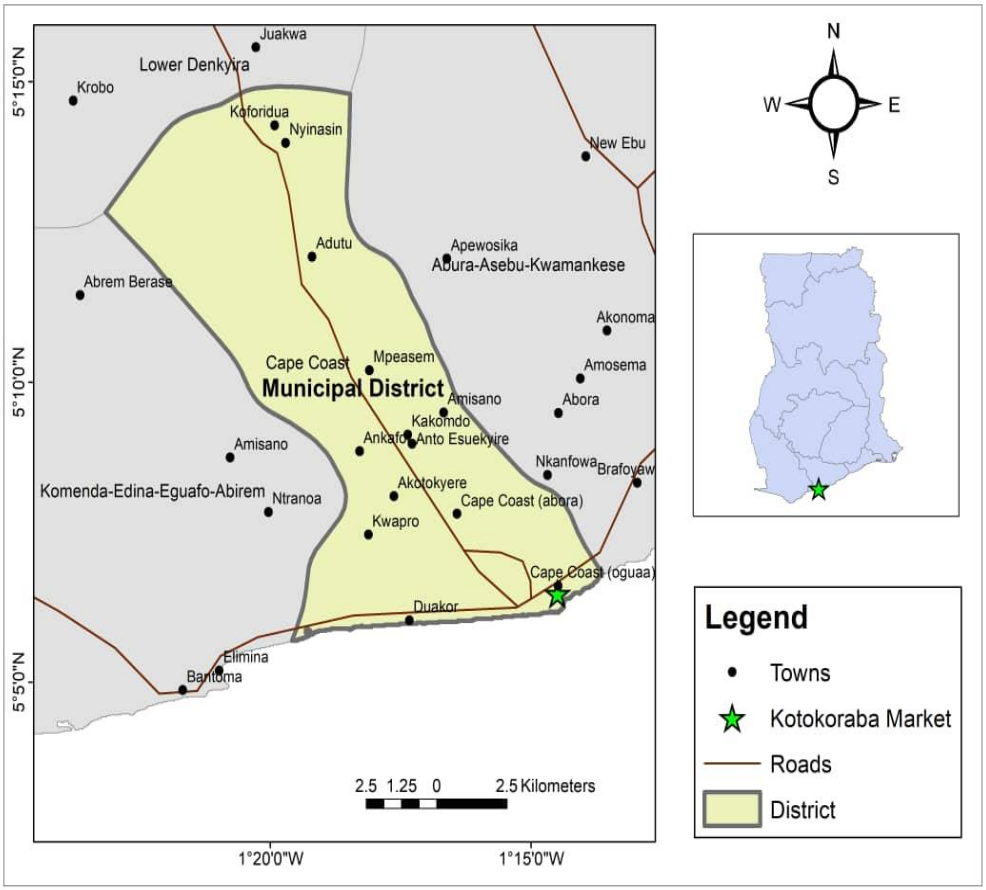


Figure 1: A map of the study area

2.2 Study Population

A total of four (4) samples were obtained. These were all taken from the Kotokuraba slaughterhouse. On a market day, the samples were taken. This was done because it was expected that beef would have a higher bacterial load. After all, there would be a large number of customers present, prohibiting vendors from taking all of the essential precautions to prevent beef contamination. Microbial alterations due to ambient temperatures and post-slaughter timings were minimized by collecting the samples early in the morning. The shop owners' cooperation was required for collection.

2.3 Sample Collection and Processing

Using sterile cotton-tipped swabs, four (4) separate samples were taken from the slaughterhouse at Kotokuraba. The samples were appropriately labeled and stored in an ice chest with ice packs before being delivered to the Department of Laboratory Technology's research laboratory for bacteriological analysis. To avoid contamination from other sources, collectors' hands and other objects were not allowed to come into contact with the meat after it had been packaged by the seller.

2.4 Media Preparation and Sterilization

All media and solutions were prepared and Petri dishes were sterilized by an autoclave at 121 ^{o}C for 15 minutes at a pressure of 15 psi. Glass wares were also sterilized sometimes by the hot flame method or in

the autoclave. Workbenches/surfaces were disinfected with detergent and then 70 % ethanol. Inoculating loops were flamed to red hot using the Bunsen burner before use.

Commercially prepared media (eosin methylene blue agar and nutrient agar powder) are expensive and as such were prepared and used in a manner that ensured little or no wastage. It is known that about 15 ml of liquefied media (agar) is required to be poured into each petri dish. The total number of plates or Petri dishes that were required for this research work was counted and the corresponding total volume or mass of the media powder was calculated.

Commercially prepared media were used and these media had a standard mass that corresponded to a particular volume that must be prepared according to its manufacturer. With the predetermined volume of liquefied media that was needed for the plating already known, its corresponding gram of media (powder) that must be used for the media preparations was also determined using ratio and proportion. All the different media that were used for this research study were prepared using this principle and their manufacturer's instructions as guidelines or standards.

2.5 Preparation of Nutrient Agar

11.20 g of dehydrated media was weighed and suspended in 400 ml of distilled water. The suspension was mixed until it was uniform and boiled to dissolve the medium completely. It was sterilized by autoclaving at 15psi. pressure (121°C) for 15 minutes. It was allowed to cool to 45-50 °C, and with frequent gentle swirling, poured into sterile Petri plates. It was labeled with the initials of the name of the medium and the date of preparation.

2.6 Preparation of Peptone Water

3.75 g of peptone water was transferred into a conical flask. It was then diluted with 250 mL of distilled water. McCartney bottles were washed and dried in the oven. 10 mL of peptone water was transferred into each McCartney bottle and then autoclaved to kill any unwanted microorganisms.

After about an hour the McCartney bottles were removed from the autoclave and allowed to cool down. The bottles were opened and their necks passed through the flame to sterilize them. The swab sticks were broken into the peptone water and the microbes were allowed to grow overnight. The bottle was labeled 10^{-1}.

Samples were serially diluted to the fourth power by pipetting 9ml of peptone water into sterile McCartney bottles labeled 10^{-2}, 10^{-3},10^{-4}. A 10^{-2} dilution was prepared by adding 1ml of the 10^{-1} solution to 9ml of the peptone water and carefully agitated for a thorough mixture. Subsequent serial dilutions for 10^{-3} and 10^{-4} were also prepared following the same procedure as 10^{-2}. The purpose of performing serial dilution was to estimate the concentration (number of organisms or colonies) of an unknown sample by enumeration the number of colonies cultured from serial dilution of the sample.

2.7 Inoculation and Incubation

From appropriate dilutions, 1ml of the suspension was inoculated into labeled sterilized Petri dish in duplicate plates, and about 20 ml of melted nutrient agar at (40-50 ^{0}C) was poured into each plate and mixed by swirling clockwise and counterclockwise. The agar was allowed to completely gel without disturbance. The plates were inverted and incubated at 37 ^{o}C for 24-48 hours.

2.8 Bacterial Enumeration

2.8.1 Plate Count Method

The plate count method relies on bacterial growing colonies on a nutrient medium so that the colony becomes visible to the naked eye and the number of colonies on a plate can be counted. For efficiency, the dilution of the original sample must be arranged so that on average, it ranges from 0 to 300 of the target bacteria grown. Fewer than 30 colonies make the interpretation statically unsound (too few to count) whilst greater than 300 colonies often result in overlapping colonies and imprecision in count (too many to count). To ensure that an appropriate number of colonies will be generated several dilutions are normally cultured.

2.8.2 Viable Bacterial Count

The colony forming unit (CFU) which determines the number of live bacteria was calculated using the formula:

$$\text{CFU/ml} = \frac{\text{Number of colonies per ml plated}}{\text{Total dilution factor}}$$

2.9 Bacterial Identification

2.9.1 Gram Staining

Pure cultures of bacteria isolates were Gram stained to identify the bacteria type based on cell shapes and Gram reaction. During gram stain, a drop of distilled water was placed on a clean flamed and cooled microscopic slide. Distinct colonies on the agar plates were picked using a sterilized inoculating loop which was then smeared firmly on the slide. The smears were allowed to air dry and heat-fixed by passing the slide over the Bunsen burner flame. The heat-fixed smear was then allowed to cool and was gram stained.

The slides were first of all flooded with crystal violet for a minute. The slides were then washed gently under tap water for about 5 seconds and excess water was drained off the slide as well. The slides were again flooded with Gram's iodine for about 1 minute and washed gently under tap water and excess water drained off. 70 % ethanol (decolorizer) was used to wash the slides again for about 5 seconds and also washed gently under tap water to stop decolorization. The water was drained and counter-stained with Safranin for a minute and washed, drained, and examined under oil immersion with an X100 objective lens of the microscope. The cells showing morphology, grouping, color (purple or pink), and relative sizes were drawn (American Society for Microbiology, 2019).

2.9.2 Biochemical Test

2.9.2.1 Citrate Utilization Test

Citrate utilization employed Simmon's citrate agar. The citrate agar was inoculated with a light inoculum taken from the center of a well-isolated colony on the nutrient agar and streaked back and forth on the slant. It was then incubated aerobically for up to 18 hours at 35 to 37 degrees Celsius. The presence of green butt and blue slant indicated a positive test for water.

2.9.2.2 Triple Sugar Iron Test

Triple sugar iron (TSI) agar was used for the differentiation of Enterobacteriaceae. Using a sterile straight wire, the TSI was stabbed deep to the bottom and the surface of the agar slant was streaked with the test organism. By the different three sugar fermentation, gas accumulation, and hydrogen sulfide production abilities, the enterobacteria were identified as water.

2.9.2.3 Indole Test

The indole test was performed by inoculating peptone and incubating it overnight. The detection of indole was by the addition of Kovac's reagent. E. coli is positive (forms a red ring on the surface) in this test, so was used to distinguish it from Klebsiella water.

2.9.2.4 Catalase Test

A microscope slide was placed in a petri dish. A sterile inoculating loop was used to pick a small number of microorganisms from a well-isolated colony and placed them on the microscope slide. A drop of 3% H_2O_2 was added to the microorganism on the slide. The petri dish was immediately covered and observed for immediate bubble formation.

2.9.2.5 Coagulase Test

This was used to distinguish between pathogenic and non-pathogenic members of the genus Staphylococcus. All pathogenic strains of *S. aureus* are coagulase positive and non-pathogenic *(Staphylococcus epidermis*) are coagulase negative.

0.5 ml of the diluted rabbit plasma was pipetted into a test tube. 5 drops (0.1 ml) of the test organism were added to the tube. After mixing, the test tube was incubated at 35-37 ^{0}C. The tube was then examined for clotting after 1 hour.

2.9.2.6 Oxidase Test

A strip of filter paper was immersed in a freshly prepared 1 % Kovac's oxidase reagent solution. With a platinum loop, a speck of culture was rubbed on it. An intense deep-purple hue that appeared within 5-10 seconds marked the bacteria as oxidase positive.

2.10 Data Analysis

Results obtained were presented in tables and figures for the various isolates obtained and also expressed as mean values of the various bacteria isolated during the laboratory analysis. The statistical package used for the data analysis was Minitab version 19. One-way ANOVA was used to analyze the means of the values obtained. Tukey HSD post hoc test was also conducted to check statistical differences.

3. Results and Discussion

Unless the principles of food-borne hygienic standards are implemented, abattoirs are one of the food sectors that contribute to the problem of probable food-borne diseases and health dangers linked with food. The current investigation discovered that the slaughtering procedure was not divided into stunning, bleeding, skinning, evisceration, hanging, and cutting/deboning and that the same room was used to carry out all the processes. Furthermore, at the abattoir, there was no preventive device for insects and rodents.

This is in line with the deductions made by Koffi-Nevry, Koussemon & Coulibaly [2] and Tarwate, Sherikar & Murugkar [9].

The sanitary conditions of abattoir workers have the potential to contribute to meat contamination. Wearing protective clothing can assist to limit the number of pollutants in meat. As a result, when handling meat, protective overalls or a hair cover should be worn at all times to safeguard meat and meat handling facilities from contamination caused by personal apparel [10]. Unfortunately, in this study just like in the studies of Zerabruk et al. [11] and Gitahi, Wangoh & Njage [12], less than half of the butchers wore their aprons or overcoats albeit they had them. None of them wore hair nets, similar to the findings of Bersisa, Tulu & Negera [13].

Table 1: A table showing the mean and standard deviation of bacteria isolates on beef and contact surfaces of equipment and apparel in the slaughterhouse

	Isolates			
Samples	*Staphylococcus* spp.	*Bacillus* spp.	*Micrococcus* spp.	*Escherichia* spp.
Hand	61.00 ± 14.53^{b}	10.33 ± 3.06^{d}	17.67 ± 3.79^{d}	0.00 ± 0.00^{d}
Cloth	121.67 ± 14.19^{a}	11.00 ± 19.10^{d}	56.67 ± 1662^{bc}	7.33 ± 1.16^{d}
Knife	80.33 ± 7.51^{b}	17.33 ± 16.56^{d}	21.70 ± 18.80^{cd}	5.67 ± 4.16^{d}
Beef	76.00 ± 3.61^{b}	20.70 ± 18.30^{cd}	8.00 ± 13.86^{d}	9.67 ± 3.06^{d}
p-value	0.001	0.820	0.0016	0.011

The results from Table 1 represent the means and standard deviations of the bacteria isolates on the beef and contact surfaces. The means in a column that does not share a letter are significantly different from each other. The p values obtained are also recorded in this table. The means in a column that does not share a letter as a superscript are significantly different from each other.

From Table 1, the *Staphylococcus* spp. identified were significantly different among the samples with a p-value of 0.001. There was no statistical difference between the *Staphylococcus* spp. on the hand, knife, and beef. Comparing each of these against the *Staphylococcus* spp. on the cloth showed statistical differences. The *Staphylococcus* spp. found on the sample taken from the cloth recorded the highest mean of 121.67, and the sample from the hand recorded the lowest mean of 61.00. *Bacillus* species identified from each of the samples showed no statistical difference with a p-value of 0.820. Hand, cloth, and knife had no statistical differences but differences existed when each was analyzed against the beef sample. *Bacillus* species identified on the beef recorded the highest mean of 20.70, and the hand recorded the lowest mean of 10.33. Statistical differences existed between the *Micrococcus* spp. identified in the samples. The p-value obtained statistically confirmed the differences. There were no differences between the hand and beef samples but there were statistical differences between the cloth and knife samples. The *Micrococcus* spp. identified on the cloth had the highest mean of 56.67 with beef recording the lowest mean of 8.00. There were no statistical differences between the *Escherichia* spp. identified from the

samples. Statistical analysis confirmed this with the p-value obtained being 0.011. The *Escherichia* spp. relatively recorded the least means in all the isolates with samples from the hand recording the lowest mean of 0.00.

Table 2: Frequency of occurrence of bacteria isolates in the meat and other contact surfaces.

	Contact Surfaces					
Isolates	Beef	Hand	Cloth	Knife	Total	Frequency (%)
Staphylococcus spp.	3	3	3	3	12	31.58
Bacillus spp.	2	3	1	2	8	21.05
Micrococcus spp.	1	3	3	2	9	23.68
Escherichia spp.	0	3	3	3	9	23.68
Total	9	10	10	9	38	100

The frequency of occurrence (in %) of bacteria isolates on the beef and other contact surfaces is what is represented in Table 2. From this table, it was observed that *Staphylococcus* spp. had the highest frequency of occurrence with 31.58%, followed by *Micrococcus* spp. and *E. coli* with a percentage of 23.68 % each and *Bacillus subtilis* occurred the least with a percentage of 21.05 %.

The frequency of occurrence depicts how often these species appear on the beef and contact surfaces. The higher the frequency of occurrence, the more dominant the species and the higher their distribution. The percentage occurrences of the various bacteria species were 31.58 %, 21.05 %, 23.68 %, and 23.68 % for *Staphylococcus* spp., *Bacillus* spp., *Micrococcus* spp., and *Escherichia* spp. respectively. This shows that *Staphylococcus* spp. was the most frequently occurring species with *Bacillus* spp. being the least. That is, the dominant species isolated was *Staphylococcus* spp. followed closely by *Micrococcus* spp. and *Escherichia* spp. This finding is in accord with the findings made by Atlabachew & Mamo [14] as well as a host of other researchers such as Bersisa, Tulu & Negera [13].

Table 3: Distribution of bacteria in beef and contact surfaces

Isolates	Hand	Cloth	Knife	Beef	Percentage (%)	Distribution
Staphylococcus spp.	+	+	+	+	100	
Bacillus spp.	+	+	+	+	100	

Micrococcus spp.	+	+	+	+	100
Escherichia spp.	-	+	+	+	75

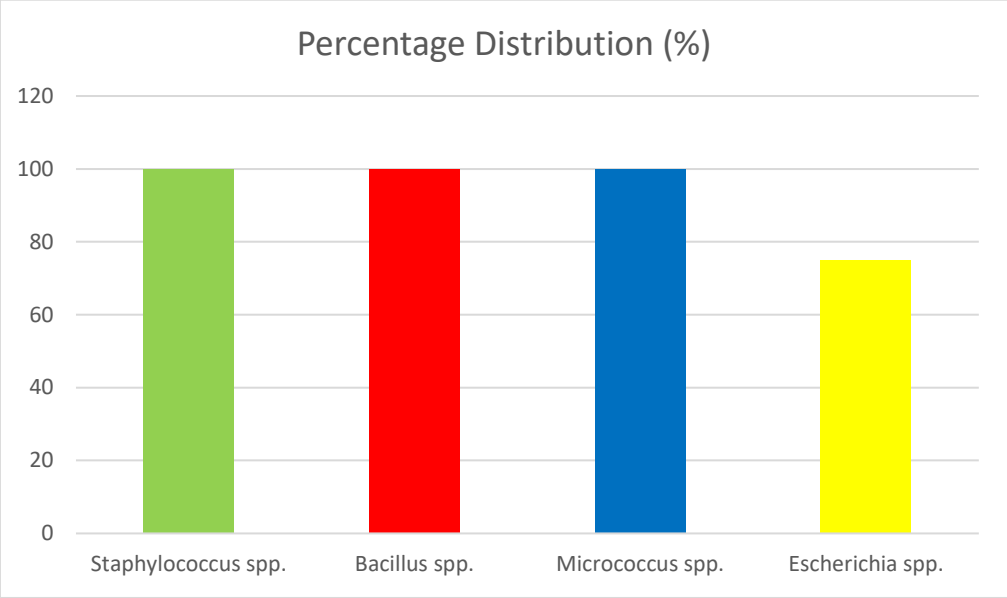


Figure 2: A graph showing the percentage distribution of bacteria isolates on beef and other contact surfaces of equipment

Table 3 and Figure 2 show the distribution of the bacteria isolates on beef and contact surfaces. A (+) sign indicates the presence of bacteria isolates on the surfaces or beef while a (-) sign indicates the absence of bacteria isolates on the surfaces or beef. All isolates were present on the beef and each surface swabbed except for *E. coli* which was absent on the hands. From Table 3 it could be seen that all isolates were present on the beef and all the contact surfaces except for *Escherichia* spp. which was absent in the sample taken from the hand.

The presence of these bacteria in the beef and other contact surfaces is a clear indication of contamination of the beef. Their presence in and on the beef should not be underestimated. The reason is that some of the bacteria are pathogenic; that's posing a threat to humans in terms of causing diseases and allergies. The most important source of *Staphylococcus* especially the genera aureus is in the human body with the principal reservoir being the skin. Between thirty to forty percent of healthy people carry the organism in the nasal cavity, on their hands, and on other parts of the body.

Hygiene issues are not just confined to slaughterhouses and linked to poor processing and marketing techniques. During the investigation, it was discovered that the majority of butcher shop employees handle money while serving beef. In countries all around the world, paper currency is commonly used to exchange products and services. It is utilized in many types of business. All of these transactions are conducted in hard currency, with lesser value notes garnering the most attention because they are exchanged often, causing them to last only a few years in circulation and providing a vast surface area for viruses to thrive [15]. Handling beef and money with the same unwashed hands could be good sources of contamination as was deduced by Birhanu et al. [1] report, handling of foods with bare hands may also result in cross-

contamination. Because meat handlers are likely sources of microbial contamination, all available efforts must be taken to limit or eliminate such contamination, as this study supports.

To decrease the risk of contamination, butcher shops should use protective clothing such as aprons and hair nets. Workers at abattoirs and butcher shops should wear protective clothing while working to prevent both food products and meat handlers from cross-contamination [16].

The water used in slaughterhouses had the potential to contaminate the meat while it was being washed since Ng et al. [17] found in their study that the water used in the abattoir was contaminated with bacteria of fecal origin. The water used in the abattoir for washing and meat processing must fulfill drinking water regulations [18]. As a result, an adequate supply of potable water should be provided to satisfy operational and cleanup needs, and its quality should be checked regularly [19].

The high microbiological load on the knife indicates that it was not properly cleaned. Knives are usually merely rinsed with water, and there is insufficient sanitation and the usage of a single knife despite contact with unclean or contaminated surfaces. These findings were in contrast with those of Haileselassie et al. [20] where the knife had the lowest bacterial load.

Even though the goal of this investigation was to isolate *Escherichia* spp. and *Staphylococcus* spp. alone, *Micrococcus* spp. and *Bacillus* spp. were also discovered. Different researchers have found similar bacterial contamination in food, water, and environmental samples. *Staphylococcus* spp. was the most common organism among isolated bacteria, followed by Micrococcus spp. and *Escherichia* spp., with *Escherichia* spp. having the lowest load from objectively isolated and identified bacteria in this investigation. Other researchers such as Ajao and Atere [10] reported similar outcomes when they isolated these germs from meat and other environmental samples. The increased percentage of contamination of meat with these organisms indicates a dismal condition of poor hygienic and sanitary measures used from the slaughterhouse through transportation to butcher shops to butcher shop processing as was also discussed by Datta et al. [21] in his study.

4. Conclusion and Recommendation

The results obtained from this study showed that there was a high microbial load in abattoir and butcher shops with *Staphylococcus* spp. recording the highest means in all the samples. The high microbial load from the samples tested is an indication of poor meat quality, making it a potential source of food poisoning caused by *E. coli*, *S. aureus*. This was due to many factors such as the low level of sophistication, poor hygienic and sanitation procedures conducted at the abattoir and butcher shops, lack of training, and low educational level of the workers. From these results, it can be figured out that contamination was present right from the abattoir and is contaminated before it gets into the hands of consumers. Therefore, it is important to create awareness about the hygiene and sanitation of meat both in abattoirs and butcher shops, and appropriate control methods for the problems should be designed and implemented. Moreover, further investigation should be carried out to isolate and characterize the bacterial load of meat in different study areas.

Since this study was conducted solely to assess the bacteriological quality of beef and contact surfaces of equipment and apparel worn in the slaughterhouse, further studies can be conducted to determine the fungal and viral quality of the beef and contact surfaces of equipment and apparel worn in the slaughterhouse because these viruses or fungi can also cause consumers to fall sick when contaminated beef is consumed.

Appendix

Table 4: Results for colony morphology

Sample	Isolate number	Form	Elevation	Margin	Color
Hands	1	Circular	Raised	Entire	White
	2	Irregular	Flat	Undulate	White
	3	Circular	Flat	Entire	Yellow
Beef	1	Circular	Raised	Entire	White
	2	Irregular	Flat	Undulate	White
Knife	1	Circular	Raised	Entire	White
	2	Irregular	Flat	Undulate	White
	4	Irregular	Raised	Entire	White
Clothes	1	Circular	Raised	Entire	White
	2	Irregular	Flat	Undulate	White

Table 5: Results for Gram staining of bacteria isolates

Isolate number	Shape	Arrangement	Gram Reaction
1	Rod	Dispersed	Pink (gram-negative)
2	Spherical	Clustered	Purple (gram-positive)
3	Spherical	Tetrad	Purple (gram-positive)
4	Rod	Clustered	Purple (gram-positive)

Table 6: Biochemical test results for all bacteria isolates

Isolate number	Indole test	Oxidase test	Catalase test	Citrate test	Isolate name
1	Positive	Negative	Positive	Negative	*Escherichia* spp.
2	Negative	Negative	Positive	Positive	*Staphylococcus* spp.
3	Negative	Positive	Positive	Negative	*Micrococcus* spp.
4	Negative	Positive	Positive	Positive	*Bacillus* spp.

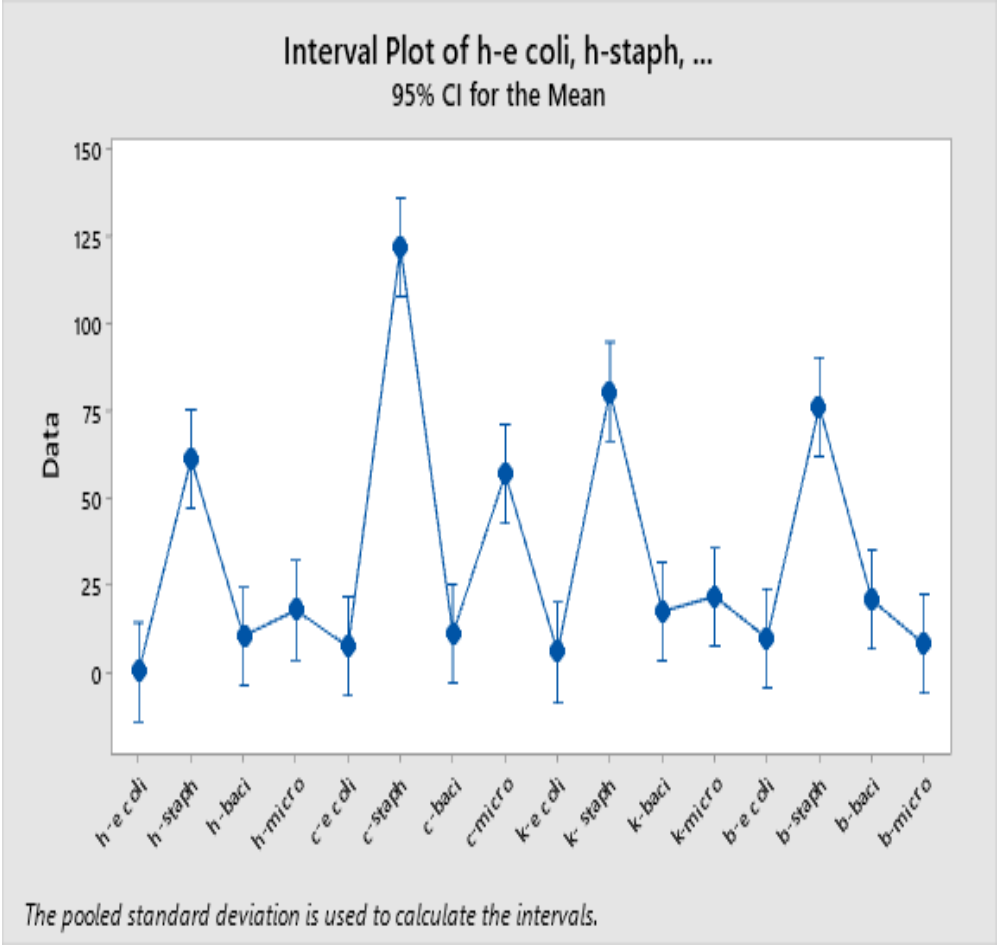
Table 7: Microbial loads of bacteria isolates on the beef and contact surfaces of equipment

		Isolates			
Contact Surfaces	Replicates	*Escherichia* spp.	*Staphylococcus* spp.	*Bacillus* spp.	*Micrococcus* spp.

Hand	R1	0	60	7	15
	R2	0	47	13	16
	R3	0	76	11	22
Cloth	R1	8	109	0	72
	R2	6	137	0	59
	R3	8	119	33	39
Knife	R1	1	73	0	33
	R2	7	80	33	0
	R3	9	88	19	32
Beef	R1	13	77	35	0
	R2	9	72	27	0
	R3	7	79	0	24

R1= Replicate 1, R2= Replicate 2, R3= Replicate 3

Figure 2: A graph comparing the bacteria loads of bacteria isolates on beef and other contact surfaces of equipment



h- e. coli =*Escherichia* spp. found on the hand, h- staph = *Staphylococcus* spp. found on the hand, h- baci = *Bacillus* spp. found on the hand, h- micro = *Micrococcus* spp. found on the hand, c- e. coli = *Escherichia* spp. found on the cloth, c- staph = *Staphylococcus* spp. found on the cloth, c- baci = *Bacillus* spp. found on the cloth, c- micro = *Micrococcus* spp. found on the cloth, k- e. coli = *Escherichia* spp. found on the

knife, h- staph = *Staphylococcus* spp. found on the knife, h- baci = *Bacillus* spp. found on the knife, h-micro = *Micrococcus* spp. found on the knife.

References

1. Birhanu, W., Weldegebriel, S., Bassazin, G., Mitku, F., Birku, L., & Tadesse, M. (2017). Assessment of microbiological quality and meat handling practices in butcher shops and abattoirs found in Gondar town, Ethiopia. *International Journal of Microbiological Research*, *8*(2), 59-68.
2. Koffi-Nevry, R., Koussemon, M., & Coulibaly, S. O. (2011). Bacteriological quality of beef offered for retail sale in Cote d'Ivoire. *American Journal of Food Technology*, *6*(9), 835-842.
3. WHO, *Food Safety and Food Borne Illness*, World Health Organization, Geneva, Switzerland, 2015, Fact sheet No. 237.
4. Nouichi, S., & Hamdi, T. M. (2009). Superficial bacterial contamination of ovine and bovine carcasses at El-Harrach slaughterhouse (Algeria). *Eur. J. Sci. Res*, *38*(3), 474-485.
5. Bhandare, S. G., Paturkar, A. M., Waskar, V. S., & Zende, R. J. (2009). Bacteriological screening of environmental sources of contamination in an abattoir and the meat shops in Mumbai, India. *Asian Journal of Food and Agro-Industry*, *2*(3), 280-290.
6. Nekouei, B. G., Sherikar, A. T. & Murugkar, H. V. (2018). Microbiological analysis of environmental sources of contamination in deonar abattoir. *Journal of Food Science and Technology (Mysore)*, *30*(2), 127-129.
7. Omoloya, B. O., & Adeleke, O. (2013). Comparative study of bacteriological qualities of meat pies sold in some standard eateries and local kiosks in Ogun State, Nigeria. *Applied Science Reports*, *2*(2), 39-45.
8. Kumar, P., Rao, J., & Haribabu, Y. (2014). Microbiological quality of meat collected from municipal slaughterhouses and retail meat shops from Hyderabad Karnataka region, India. *APCBEE procedia*, *8*, 364-369.
9. Tarwate, B. G., Sherikar, A. T., & Murugkar, H. V. (1993). Microbiological analysis of environmental sources of contamination in deonar abattoir. *Journal of Food Science and Technology (Mysore)*, *30*(2), 127-129.
10. Ajao, A. T., & Atere, T. G. (2009). Bacteriological assessment and hygienic standard of food canteens in Kwara State Polytechnic, Ilorin, Nigeria. *African scientist journal*, *3*(10), 173-180.
11. Zerabruk, K., Retta, N., Muleta, D., & Tefera, A. T. (2019). Assessment of microbiological safety and quality of minced meat and meat contact surfaces in selected butcher shops of Addis Ababa, Ethiopia. *Journal of Food Quality*, *2019*.
12. Gitahi, M. G., Wangoh, J., & Njage, P. M. K. (2012). Microbial safety of street foods in Industrial Area, Nairobi. *Research Journal of Microbiology*, *7*(6), 297.
13. Bersisa, A., Tulu, D., & Negera, C. (2019). Investigation of bacteriological quality of meat from abattoir and butcher shops in Bishoftu, Central Ethiopia. *International Journal of Microbiology*, *2019*.
14. Atlabachew, T., & Mamo, J. (2021). Microbiological quality of meat and swabs from contact surface in butcher shops in Debre Berhan, Ethiopia. *Journal of Food Quality*, *2021*, 1-11.
15. Gadsby, P. (1998). Filthy lucre-Money contaminated with bacteria. *Discover*, *19*, 76.
16. Nel, S., Lues, J. F. R., Buys, E. M., & Venter, P. (2004). The personal and general hygiene practices in the deboning room of a high throughput red meat abattoir. *Food Control*, *15*(7), 571-578.

17. Ng, Y. F., Wong, S. L., Cheng, H. L., Yu, P. H. F., & Chan, S. W. (2013). The microbiological quality of ready-to-eat food in Siu Mei and Lo Mei shops in Hong Kong. *Food Control*, *34*(2), 547-553.
18. Adebowale, O. O., Alonge, D. O., Agbede, S. A., & Adeyemo, O. (2010). Bacteriological assessment of the quality of water used at the Bodija municipal abattoir, Ibadan, Nigeria. *Sahel J. Vet. Sci*, *9*(2), 63-67.
19. Canadian Food Inspection Agency, *Guideline for Food Safety*, Canadian Food Inspection Agency, Kitchener, Canada, 2015.
20. Haileselassie, M., Taddele, H., Adhana, K., & Kalayou, S. (2013). Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. *Asian Pacific Journal of tropical biomedicine*, *3*(5), 407-412.
21. Datta, S., Akter, A., Shah, I. G., Fatema, K., Islam, T. H., Bandyopadhyay, A., ... & Biswas, D. (2012). Microbiological quality assessment of raw meat and meat products, and antibiotic susceptibility of isolated Staphylococcus aureus. *Agriculture, Food and Analytical Bacteriology*, *2*(3), 187-194.