Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 10 ~ Issue 1 (2023) pp: 01-09
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Effect of Time of Purchase on Bacterial Contamination of Beef from Different Abattoirs and Butcher Shops in Bayelsa State

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ABSTRACT:*The study was aimed to examine the effect of time of purchase on bacterial contamination of beef from different abattoirs and butcher shops in Yenagoa, Bayelsa State. The experiment was carried out at the Department of Animal Science, Faculty of Agriculture, Niger Delta University, Wilberforce Island, Bayelsa State. A total of twenty-four beef samples were obtained for the experiment from two markets in Yenagoa, Bayelsa state. Six beef samples were purchased from each abattoir and six beef samples were purchased from selected butcher shops in both of the market. Results were analyzed using descriptive statistics (bar chart) and SPSS version 25, means were separated using Duncan multiple range test at P<0.05. The results were express for the level of bacterial contamination with respect to time of purchase. The bacterial species isolated from the meat samples include Staphylococcus epidermis, Salmonella paratyphi A, Staphylococcus aureus, Pseudomonas fragi and Escherichia coli with Staphylococcus epidermis having the highest occurrence and count. The results revealed that the bacteria count (CFU/g) of the evening samples were significantly higher than that of the morning samples. Similarly, the evening samples had more bacteria isolates than the morning samples. From the results, it can be deduced that meat purchased at the early hours of the day is safer for consumption compared to that which had been exposed for a long time. This is as a result of the observed increase in bacterial loads in the evening samples which predisposes to meat-borne infections in humans.*

KEYWORDS: *Abattoir, Butcher shops, Bacteria count, Beef, Isolates, Contamination, Markets, Meat samples*

R*eceived 20 Dec., 2022;* ***R****evised 01 Jan., 2023;* ***A****ccepted 02 Jan., 2023 © The author(s) 2023.*
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I. INTRODUCTION

"Meat is a freshly dressed or treated tissue, mainly skeletal muscles from warm blooded animals, suitable for use as food" [1]. It is the flesh that is obtained from poultry, sheep, cattle, goat, swine. etc., which serve as food to humans [2].

According to [3], "the composition of meat is ideal for growth of wide ranges of spoilage and pathogenic bacteria making it one of the most perishable foodstuffs". According to [4] and [5], "contaminated meat can be responsible for numerous meat-borne diseases among humans which result from causative agents that enter the body through ingestion of the meat. It is recognized that the most significant food-borne hazards from fresh meat involve pathogenic bacteria, such as Campylobacter species, *Staphylococcus aureus, Salmonellae species, Listeria monocytogenes,* and *Escherichia coli*. Some of these, particularly *E. coli,* will require only few bacteria to cause food poisoning in humans".

The main sources of meat contamination are from animal source [6], workers and their working environment, air contamination through aerosols and the water from carcass dressing [3]; [7]. Other sources of meat contamination include food contact surfaces which involves utensils, tables, and equipments used during slaughtering and butchering [8] as well as the environmental conditions where the animals are processed [9]. Moreover, "the contaminating organisms are derived mainly from the hide of the animals and comprise organisms that originate from stomachs and intestines, which are excreted in their faeces" [10].

Poor infrastructural facilities in slaughter houses, unhygienic animals, and poor handling of carcasses could attribute to the high bacterial load in meat. Thus, by assessing the bacterial counts, the threat posed to human health can be determined[3]. "Food-borne diseases occur commonly in developing countries 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"[11].

According to [12], "unhygienic practices like poor handling, the use of contaminated water, use of contaminated tables to display meat to be sold, and the use of contaminated knives and other equipment in cutting operations are major reasons for contaminated meats in abattoirs and butcher shops". However, knives, wooden boards, and weighing scales from butcher shops are also another source of bacterial contamination in fresh meats [13]. "Contaminated meat products from abattoirs and butcher shops associated with microbial and especially bacterial pathogens have been recalled for concerns of public health in meat safety with regards to consumer health" [14].

To ensure safe and wholesome meat to all consumer at all the different stages of meat processing, good hygiene practice, and standard operating procedures must be instituted up to the point of packaging methods, distribution, and the marketing of the meat and its products. Meat must be free from bacterial contamination and extrinsic contaminants such as toxin, chemical residues that would be injurious to humans eating it [15].

Raw beef sold at various butcher shops do undergo considerable amount of handling and contacts with microbes of different sources. The multiplication of pathogenic bacteria can be allowed by keeping meat at ambient temperature for a long time and this can be commonly done in various butcher shops in the market [4].

The testing against microbes provides a way to measure how well the meat operator has controlled the slaughtering, dressing, and production processes in order to minimize and control contamination [16]as well, the bacterial counts in the meat are used as an acceptable indicator for its quality test[3] .

The hygienic and quality control methods used in meat and it's products, especially in food catering, butcher shops, abattoirs, etc., have been recommended in many countries around the world[17]. "Without proper hygienic control, the environment in abattoir and butcher' s area can act as important sources of bacterial contamination and this would have a great impact on public health [18].

"Meat quality control is a system that regulates the measure of extrinsic materials such as chemical residues, toxins, pathogenic microorganisms and putrefied tissues, which could be present in meat and are deleterious to human health" [15]. Millions of people in the world suffer from different kinds of illness due to meat contamination annually [19]. "Market sanitation and meat inspection in the tropics are of relative more importance than development of preservation and processing techniques as most meat is consumed fresh" [20].

Therefore, the objectives of this study are aimed to determine the effect of time of meat purchase on the level of bacterial contamination of beef from specific abattoirs and butcher shops in Yenagoa, Bayelsa state.

II. MATERIALS AND METHOD

2.1 Study Location

This study was carried out at the Department of Animal Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. Bayelsa State is located within latitude 4.6640300 and longitude 6.0369870 East [21]. Bayelsa share boundary with Delta State North, Rivers State East and Atlantic Ocean in the southernmost parts. The climate is humid with a mean annual rainfall of 2909 mm and the average annual temperature of 26^{o}C and mean relative humility of 85%, respectively [22].

2.2 Source of Meat Samples

Meat samples were acquired from two abattoirs (Etegwe market and Swali market) and butcher shops from two different markets (Swali market and Etegwe market) in Yenagoa, Bayelsa State, Nigeria.

2.3 Sample Collection

A total of twenty-four beef samples were used for the experiment. Six beef samples were purchased from each abattoir, three in the morning and three in the evening making a total of twelve beef samples from the abattoirs. Furthermore, six beef samples (three in the morning and three in the evening) were purchased from selected butcher shops in both of the market sources, making a total of twelve beef samples from butcher shops. Four grams of each sample were cut into well labelled sterile containers and put in an ice-box carrier and transported to the laboratory where a quantitative test was carried out.

2.4 Experimental Design

The experimental design used for the experiment was split plot design. A total of twenty-four beef samples were used for the experiment and the experiment lasted for a week.

Duration of purchase	Abattoir		Butcher shops	
	Etegwe	Swali	Etegwe	Swali
Morning	R1 $A_{EM}1$	R1 $A_{SM}1$	R1 $B_{EM}1$	R1 $B_{SM}1$
	R2 $A_{EM}2$	R2 $A_{SM}2$	R2 $B_{EM}2$	R2 $B_{SM}2$
	R3 $A_{EM}3$	R3 $A_{SM}3$	R3 $B_{EM}3$	R3 $B_{SM}3$
Evening	R1 $A_{EE}1$	R1 $A_{SE}1$	R1 $B_{EE}1$	R1 $B_{SE}1$
	R2 $A_{EE}2$	R1 $A_{SE}2$	R2 $B_{EE}2$	R2 $B_{SE}2$
	R3 $A_{EE}3$	R1 $A_{SE}3$	R3 $B_{EE}3$	R3 $B_{SE}3$

EM – Etegwe Morning; EE – Etegwe Evening; SM – Swali Morning; SE – Swali Evening; R – Replicate

Table 1: Showing Experimental Layout

2.5 Data Collection

The bacterial contamination was assayed at the Microbiology laboratory, Federal Medical Centre, Yenagoa, Bayelsa state. All the samples were examined to determine the level of contamination with respect to the time of purchase. The biochemical tests carried out include Indole test, coagulase test and catalase test.

2.5.1 Indole Test

"Indole test was usedin the identification ofenterobacteria that breakdown the amino acid tryptophan with the release of indole. Tryptophan is idolized by tryptophanase to produce three possible end products; indole, pyruvate and ammonia. Indole production is detected by Kovac' s or Ehrlich' s reagent which contains hydrochloric acid and P-dimethylaminobenzaldehyde in amyl alcohol. Sterile tubes containing 4ml of tryptophan broth was inoculated aseptically by taking the growth from 18-24hrs culture. The tube was incubated at 37^{o}C for 24-28hrs. 0.5ml of Kovac' s reagent was added to the broth culture and the presence or absence of ring was observed" [23]

2.5.2 Coagulase Test

"This test was used to the identify *Staphylococcus aureus* which produce the enzyme coagulase. The Coagulase causes the plasma to clot by converting fibrinogen to fibrin with visible agglutination. Tube coagulase test was performed in sterile tubes by adding 0.5 ml of broth culture of the selected isolates to 0.5 ml of citrated plasma. After mixing the contents, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 ml of uninoculated sterile broth and 0.5 ml of citrated plasma. The tubes were monitored for clot formation at 30-minutes intervals for the first four (4) hours and then after 24-hour incubation. The reaction was considered positive if a clot was visible within the tube and negative if no degree of clotting was visible"[24]

2.5.3 Catalase Test

"This test was used to separate those bacteria that produce the enzyme catalase, such as staphylococci from non-catalase producing bacteria such as streptococci. The tube catalase test was performed by adding 4-5 drops of 3% H_2O_2 into a test tube. Using a wooden applicator, a small amount of organism from a well-isolated colony (18-24 hours prior to the test) was collected and placed in the test tube. The tube was placed against a dark background and observed for immediate bubble formation at the end of the wooden applicator stick"[25]

2.6 Statistical Analysis

Data collected for bacterial contamination were analyzed using Descriptive Statistics and subjected to Two-Way ANOVA using SPSS Version 25. When the analysis of variance indicates the existence of significant effect, Duncan Multiple Range Text was used to locate the means that are significantly different from each other at $P<0.01$.

III. RESULT

3.1 Effect of Time of Meat Collection on Bacteria Colonies at Etegwe Abattoir

The Effect of Time of Meat Collection on Bacteria Colonies at Etegwe Abattoir is shown in Figure 1 below. The results revealed that *Staphylococcus epidermis* (363 CFU/g) was the only Bacterium present in meat samples collected in the morning from Etegwe Abattoir. However, in the evening *Staphylococcus epidermis* (478 CFU/g), *Staphylococcus aureus* (77 CFU/g), *Pseudomonas fragi* (163 CFU/g) and *Escherichia coli* (140 CFU/g)were present. According to the results in the chart, *Staphylococcus epidermis*(478 CFU/g) was found to be the highest while *Staphylococcus aureus* (77 CFU/g) had the lowest value from the meat collected in the evening at Swali Butcher.

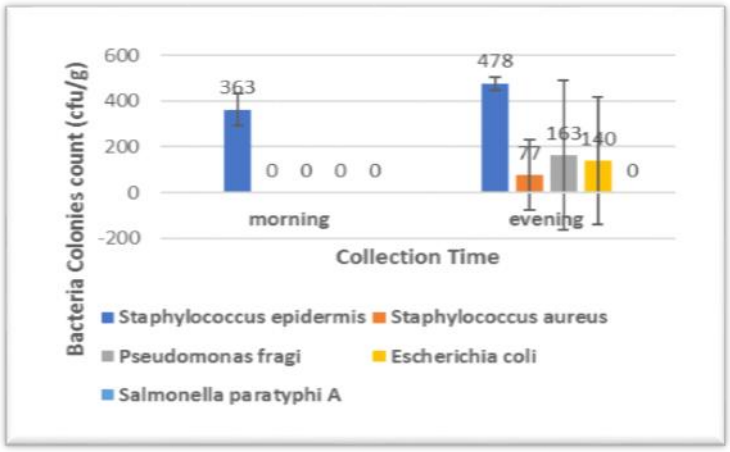

Figure 1: Effect of Time of Meat Collection on Bacteria Colonies at Etegwe Abattoir

3.2 Effect of Time of Meat Collection on Bacteria Colonies at Swali Abattoir

The Effect of Time of Meat Collection on Bacteria Colonies at Swali Abattoir is shown in Figure 2 below. According to the results *Staphylococcus epidermis* (400 CFU/g) was the only bacterium present in meat samples collected in the morning from Swali Abattoir. However, *Staphylococcus epidermis* (427 CFU/g) and *Escherichia coli* (97 CFU/g) were the bacteria present in the evening samples. Among the bacteria isolates, *Staphylococcus epidermis* had the highest value (427 CFU/g) while *Escherichia coli* (97 CFU/g) had the least.

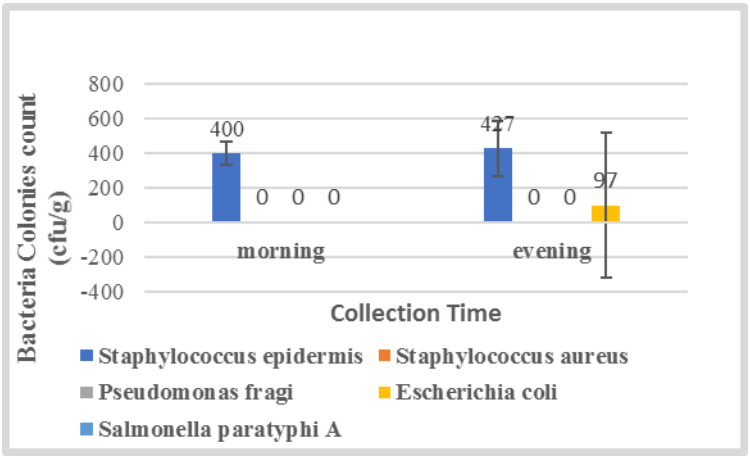

Figure 2: Effect of Time of Meat Collection on Bacteria Colonies at Swali Abattoir

3.3 Effect of Time of Meat Collection on Bacteria Colonies at Etegwe Butcher

The Effect of Time of Meat Collection on Bacteria Colonies at Etegwe Butcher is shown in Figure 3 below. The results revealed that *Staphylococcus epidermis* and *Staphylococcus aureus* were the only bacteria present in meat samples collected in the morning from Etegwe Butcher, with *Staphylococcus epidermis* (381 CFU/g) having the highest value and *Staphylococcus aureus* (31 CFU/g) having the least. However, in the evening samples *Staphylococcus epidermis* (410 CFU/g), *Staphylococcus aureus* (220 CFU/g), and *Escherichia coli* (70 CFU/g) were present. According to the results in chart, *Staphylococcus epidermis*(410 CFU/g) had highest value while *Escherichia coli* (31 CFU/g) had the lowest value in meat collected in the evening at Etegwe Butcher.

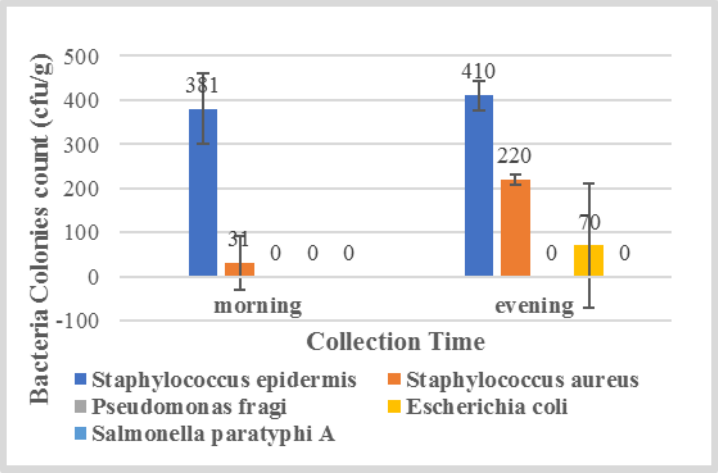

Figure 3: Effect of Time of Meat Collection on Bacteria Colonies at Etegwe Butcher

3.4 Effect of Time of Meat Collection on Bacteria Colonies at Swali Butcher

The Effect of Time of Meat Collection on Bacteria Colonies at Swali Butcher is shown in Figure 4 below. The results revealed that *Staphylococcus epidermis* (400 CFU/g) bacterium was present in the meat samples collected in the morning from Swali Butcher. However, *Staphylococcus epidermis* (427 CFU/g), *Salmonella paratyphi* A (190 CFU/g), and *Escherichia coli* (97 CFU/g) were present in the evening samples. From the results in the chart, *Staphylococcus epidermis*(400 CFU/g) had the highest value while *Escherichia coli*(97 CFU/g)had the least value from the meat collected in the evening at Swali Butcher.

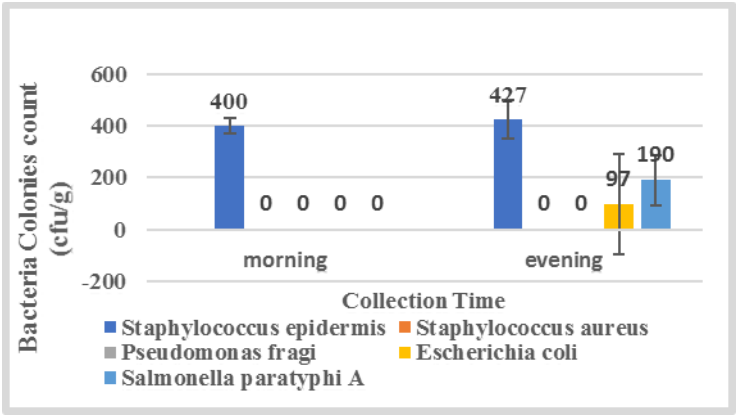

Figure 4: Effect of Time of Meat Collection on Bacteria Colonies at Swali Butcher

3.5 Interactive Effect of Time of Meat Collection on Bacteria Colonies at Etegwe and SwaliAbattoir

The Interactive Effect of Time of Meat Collection on Bacteria Colonies at Etegwe and Swali Abattoir is shown in Figure 5 below. Results revealed that *Staphylococcus epidermis*, with a mean value of 300 CFU/g was the only bacterium present in meat samples collected in the morning from both Abattoirs. However, *Staphylococcus epidermis* (300 CFU/g), *Staphylococcus aureus* (67 CFU/g), *Pseudomonas fragi*(83 CFU/g) *and Escherichia coli* (117 CFU/g) were the bacteria present in meat samples collected in the evening from both Abattoirs. From the results in the chart, *Staphylococcus epidermis* recorded the highest colony forming units of 300 CFU/g while *Staphylococcus aureus* recorded the least colony forming units (67 CFU/g).

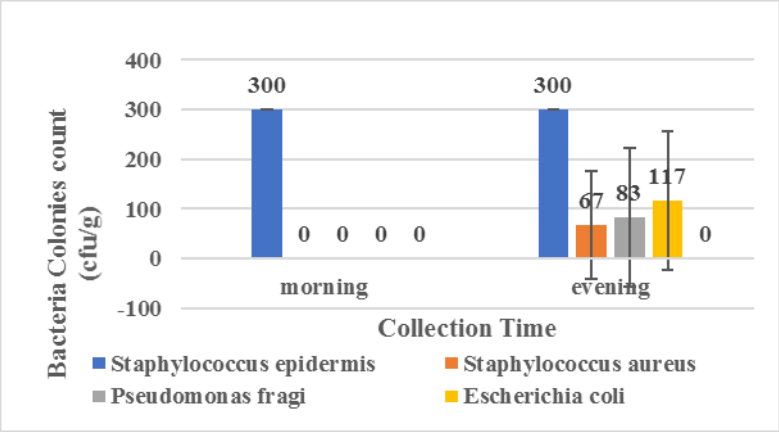

Figure 5: Interactive Effect of Time of Meat Collection on Bacteria Colonies at Etegwe and Swali Abattoir

3.6 Interactive Effect of Time of Meat Collection on Bacteria Colonies at Etegwe and Swali Butcher

The Interactive Effect of Time of Meat Collection on Bacteria Colonies at Etegwe and Swali Butcher is shown in Figure 6 below. From the results below, it was revealed that *Staphylococcus epidermis* (391 CFU/g) and *Staphylococcus aureus* (15 CFU/g) were the only bacteria present in meat samples collected in the morning from Butcher shops in both markets. However, *Staphylococcus epidermis* (419 CFU/g), *Staphylococcus aureus* (110 CFU/g), *Escherichia coli* (84 CFU/g) and *Salmonella paratyphi* A. (95 CFU/g) were all present in the evening samples. From the results, *Staphylococcus epidermis*(419 CFU/g) recorded the highest value while *Escherichia coli* (84 CFU/g) recorded the least value.

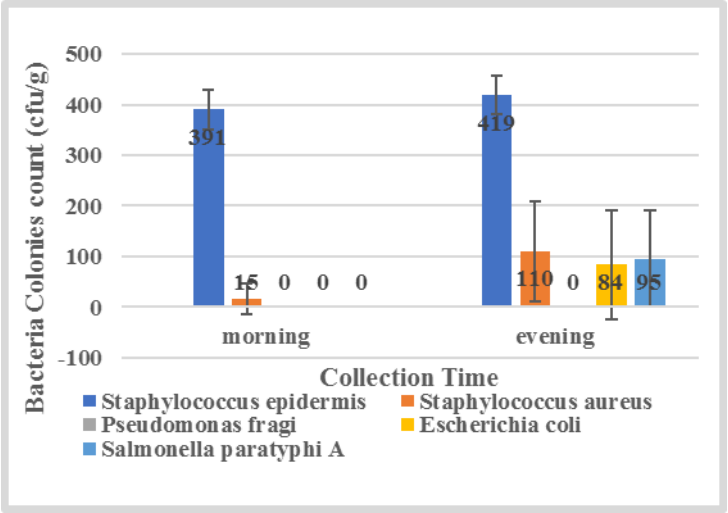

Figure 6: Interactive Effect of Time of Meat Collection on Bacteria Colonies at Etegwe and Swali Butcher

IV. DISCUSSION

This study examined the effect of time of purchase on the bacteria contamination of meat from Abattoir and Butcher shops in Bayelsa State. The market sources used for the experiment were Swali Market and Etegwe market.

Results from the effect of time of meat collection on bacteria colonies at Etegwe abattoir is shown in Figure 1. The results revealed that only one bacterium (*Staphylococcus epidermis*) was isolated from samples collected in the morning while for samples collected in the evening, about four bacteria isolates (*Staphylococcus epidermis*, *Staphylococcus aureus*, *Pseudomonas fragi* and *Escherichia coli*)were recorded. According to the results in the chart, *Staphylococcus epidermis* was found to have the highest count (478 CFU/g) while *Staphylococcus aureus* had the lowest count (77 CFU/g) from the meat samples collected in the evening at Etegwe Abattoir. Among the bacteria isolates, only the bacterium *Staphylococcus aureus* (77 CFU/g) was within the acceptable range of the Hazard Analysis and Critical Control Point system (100 CFU/g or less) for food safety. However, all bacteria count recorded except that of *Staphylococcus aureus* (77 CFU/g) were within the intermediate permissible ranges (100 CFU/g to 1000 CFU/g) of the Hazard Analysis and Critical Control

Point system. Furthermore, all bacteria count recorded were out of the guidelines of good manufacturing practice maximum permissible range (between 105 and 107 CFU/g) of total microbial contamination of raw meat. Meat can be contaminated during the process of slaughtering by microbes in the intestines of the animals, hands of the handlers, the knives used and also by the microbes in the surface in which the animals are slaughtered. However, the increase in the number of bacteria isolates as shown in Figure 1 indicates microbes usually multiply depending on how the meats are handled and stored between the morning and evening hours.

The effect of time of meat collection on bacteria colonies at Swali Abattoir (Figure 2) showed *Staphylococcus epidermis* to be the only bacterium present in meat samples collected in the morning. In the evening, *Staphylococcus epidermis* and *Escherichia coli* were the bacteria present with *Staphylococcus epidermis* (427 CFU/g)also having the highest value (similar to that of Etegwe Abattoir) and *Escherichia coli* (97 CFU/g) having the least value. The colony counts of *Staphylococcus epidermis* in both morning and evening samples(400 CFU/g and 427 CFU/g respectively) were within the intermediate permissible ranges (100 CFU/g to 1000 CFU/g) of the Hazard Analysis and Critical Control Point system (FAO). However, the count of *Escherichia coli* was lower than the maximum permissible range (102 and 104 CFU/g) of *Escherichia coli* and Enterobacteriaceae contamination in raw meat [26]; and [27]. According to [28], meat processed in abattoirs prior to being conveyed to butcher shops has considerable number of microbes which multiplies during transportation or at the butcher shops depending on the mode of transportation, vehicle used and how it was handled and kept at retail points.

The effect of time of meat collection on bacteria colonies at Etegwe butcher shops is shown in Figure 3. The results revealed that *Staphylococcus epidermis* and *Staphylococcus aureus* (381 CFU/g and 31 CFU/g respectively) were the only bacteria present in meat samples collected in the morning from Etegwe Butcher shops with *Staphylococcus epidermis* having the highest count between the two bacteria isolates. However, samples collected in the evening recorded the presence of *Staphylococcus epidermis*, *Staphylococcus aureus* and *Escherichia coli* having bacteria counts of 410 CFU/g, 220 CFU/g and 70 CFU/g respectively. Furthermore, the results in Figure 3 also revealed that *Staphylococcus epidermis* had the highest count in both morning and evening samples (381 CFU/g and 410 CFU/g respectively) while *Escherichia coli* had the lowest count in meat samples collected in the evening but was absent in meat samples collected in the morning.The colony counts of *Staphylococcus epidermis* in both morning and evening samples(381 CFU/g and 410 CFU/g respectively) were within the intermediate permissible ranges (100 CFU/g to 1000 CFU/g) of the Hazard Analysis and Critical Control Point system (FAO). However, the *Staphylococcus aureus*count of meat samples collected in the evening was lower than the range of HACCPS but within permissible range in evening samples. On the other hand, *Escherichia coli* was absent in the morning samples but was lower (70 CFU/g) than the maximum permissible range (102 and 104 CFU/g) of *Escherichia coli* and Enterobacteriaceae contamination in raw meat [26]; and [27].

The effect of time of meat collection on bacteria colonies at Swali Butcher as shown in Figure 4 also reveals that *Staphylococcus epidermis* was present in meat samples collected both in the morning and evening from Swali Butcher shops. However, *Salmonella paratyphi* A and *Escherichia coli* were present only in the evening samples. From the results in Fig 4, it was observed that *Staphylococcus epidermis* had the highest count while *Escherichia coli* had the least count from the meat collected in the evening at Swali Butcher. The colony counts of *Staphylococcus epidermis* present in the meat samples collected in the morning and evening (400 CFU/g and 427 CFU/g respectively) and *Salmonella paratyphi* A (190 CFU/g) present in the evening samples were within the ranges of HACCPS. However, the colony counts of *Escherichia coli* (97 CFU/g) present in the evening samples was lower than the ranges of HACCPS. Furthermore, from the results of this study, it can be observed that both samples collected from Etegwe butcher shops and Swali butcher shops recorded the highest bacteria count from *Staphylococcus epidermis* and the least from *Escherichia coli*.

Moving further, the interactive effect of time of meat collection on bacteria colonies at both Etegwe Abattoir and Swali Abattoir (Figure 5) revealed *Staphylococcus epidermis* (300 CFU/g) to be the only isolate in meat samples collected in the morning from both Abattoirs. However, the bacteria analysis on the evening samples revealed *Staphylococcus epidermis* (300 CFU/g), *Staphylococcus aureus* (67 CFU/g), *Pseudomonas fragi*(83 CFU/g) *and Escherichia coli* (117 CFU/g)to be present in the meat samples collected in the evening from both abattoirs. From the results in the chart, *Staphylococcus epidermis* recorded the highest colony forming units of 300 CFU/g while *Staphylococcus aureus* recorded the least colony forming units (67 CFU/g).

For the Interactive effect of time of meat collection on bacteria colonies at Etegwe and Swali Butcher shops (Figure 6), only two isolates were recorded to be present in meat samples collected in the morning. Among the two isolates, *Staphylococcus epidermis* (391 CFU/g) had the highest colony forming units while *Staphylococcus aureus* (15 CFU/g) had the least. However, four bacteria isolates, *Staphylococcus epidermis* (419 CFU/g), *Staphylococcus aureus* (110 CFU/g), *Escherichia coli* (84 CFU/g) and *Salmonella paratyphi* A. (95 CFU/g) were found to be present in the evening samples. From the results, *Staphylococcus epidermis*(419 CFU/g) recorded the highest colony forming units while *Escherichia coli* (84 CFU/g) recorded the least. The

high value of *Staphylococcus epidermis* recorded from both markets could be attributed to the way and manner in which meats were handled in the Abattoir as *S. epidermidis* in particular is the most frequently isolated species from human epithelia [28].

The result of this study reveals that there was a great difference between the bacteria counts of morning and evening samples which implies that there was a great increase in bacteria counts with the advancement of time. This increase implies that it is better to purchase meat in the morning or immediately the animal is slaughtered than in the evening or after the meat had been exposed for a long time [29]. The high level of microbial counts in the evening samples could be attributed to the mode of transportation, handling methods and contact with contaminated equipment and this is in agreement with [30]. Furthermore, "meats at ambient temperature for long durations tends to favour the multiplication of bacteria already present in meats" [30], and this is a common practice among meat sellers in both market sources.

V. CONCLUSION AND RECOMMENDATION

From the results of this study, it can be concluded that the bacteria contamination of meat increased with advancement of time. The bacteria count of the evening samples were higher compare to that of the morning samples. Also, it can also be seen that more bacteria isolates were recorded in the evening samples.

Furthermore, among the different bacteria isolated from the samples collected from both the abattoir and butcher shops in both market sources, *Staphylococcus epidermis* had the highest occurrence and also recorded the highest count.

Hence, it is recommended that it is better to purchase meat in the morning or immediately the animal has been slaughtered than in the evening or after the meat had been exposed for a long time because of the observed increase in microbial loads in the evening samples which increases the chances of meat-borne infection to humans.

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