



BACTERIOLOGICAL QUALITY OF BEEF SOLD AT DIFFERENT RETAIL POINTS IN OWO METROPOLIS

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ABSTRACT: *The present study aimed to investigate the bacteriological characteristics of fresh beef sold in various retail centers within the Owo communities, located in Ondo State, Nigeria. Samples were collected from six different locations using a systematic random sampling method, with two samples collected from each location—one in the morning (M) and the other in the evening (E). The standard procedure for isolating microorganisms was followed. Morphological and biochemical characterization of isolates are presented in Table 1, while Table 2 displays bacterial counts on the fresh beef samples over a period of ten days. On the first day of analysis, the sample from Ikare Junction exhibited the highest bacterial counts (152 x 10⁵cfu/g for the morning sample and 276 x 10⁵cfu/g for the evening sample), which was statistically significant ($p \pm > 0.05$). Conversely, the sample from Ehin-ogbe had the lowest bacterial count (36 x 10⁵cfu/g) in the morning, while the sample from Ijebu had 116 x 10⁵cfu/g in the evening. There was a noteworthy ($p \pm > 0.05$) disparity between morning and evening samples across all locations, with the evening samples consistently showing higher bacterial counts. This discrepancy could be attributed to prolonged exposure to contamination over time. Table 1 also revealed the probable organisms according to the biochemical and morphological characterization of the isolates. The isolates were *E. coli*, *Enterobacter spp.*, *Serratia spp.*, *Citrobacter spp.*, *Staphylococcus aureus*, *Staphylococcus epidemidis*, *Bacillus subtilis*, *K. pneumoniae*, *Lactobacillus spp.*, *Leuconostoc spp.*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Preteus mirabilis*, *Salmonella spp.* and *Shigella spp.* The presence of these organisms in fresh beef depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering and processing of fresh beef. Their presence indicates a public health hazard and gives a warning signal for the possible occurrence of food-borne intoxication. Proper hygiene practices should be observed during handling, marketing, and calls for concerted efforts on the part of relevant authorities to check the trend since it is a public health challenge.*

KEYWORDS: Beef, Bacteriological, Owo, Pathogenic, Retail points.



INTRODUCTION

The bacteriological quality of beef is of paramount importance for ensuring consumer safety and public health. Contaminated beef can harbor various pathogens, including bacteria such as *Escherichia coli* (*E. coli*), *Salmonella* spp., and *Campylobacter* spp., posing significant health risks to consumers if ingested. Therefore, understanding the bacteriological quality of beef sold at different retail points is crucial in assessing the potential risks associated with consumption and implementing effective control measures to safeguard public health. This study aims to investigate and analyze the bacteriological quality of beef available at various retail points, especially, open-air markets. By examining samples collected from different sources, we seek to identify potential differences in microbial contamination levels and assess the efficacy of current food safety practices in place across different retail settings in Owo. The findings of this research hold significant implications for stakeholders in the food industry, regulatory agencies, and consumers alike. Identifying potential sources of contamination and understanding the factors influencing microbial presence in beef can inform the development of targeted interventions to mitigate risks and improve overall food safety standards. Through comprehensive analysis and interpretation of data, this study endeavours to contribute valuable insights into enhancing the bacteriological quality of beef throughout the retail supply chain, thereby promoting consumer confidence and ensuring the delivery of safe and wholesome food products to the public.

LITERATURE REVIEW

Food security is a complex issue, where animal proteins such as meats, meat products, fish, and fishery products are generally regarded as high-risk commodities concerning pathogen contents, natural toxins, and other possible contaminants and adulterants (Yousuf *et al.*, 2008). Foodborne infections and illnesses are a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide. Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety (Adak *et al.*, 2005). In the developing world, foodborne infection leads to the death of many children and the resulting diarrheal disease can have long-term effects on children's growth as well as on their physical and cognitive development. In the industrialized world, foodborne infection causes considerable illness, heavily affecting healthcare systems (Adak *et al.*, 2005). According to Clarence *et al.* (2009), food-borne diseases are diseases resulting from ingestion of bacteria, toxins, and cells produced by microorganisms present in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and the susceptibility of the individuals to the toxin. Meat is the most perishable of all important foods since it contains sufficient nutrients needed to support the growth and development of microorganisms (Magnus, 2001). The chief constituents of meat are water, protein and fat, phosphorus, iron, and vitamins are also contained in meat. The major primary unit of meat is called carcass. It represents the ideal meat after the head, legs, hide, intestine, viscera and blood have been removed. The edible parts of a carcass include lean flesh, fat flesh, and edible glands or organs such as the heart, liver, kidney tongue, and brain. The age and sex of the animal have a major influence on the quality of meat that is produced from animals. Most meat has a high water content corresponding to a water activity of approximately 0.99 which is suitable for microbial growth (Rao *et al.*, 2009). Meat is considered to be spoiled when it is unfit for human consumption. Meat is subjected to



changes by its own enzyme, by microbial action and its fat may be oxidised chemically. Microorganisms grow on meat causing visual, textural, and organoleptic changes when they release metabolites (Jackson *et al.*, 2001). Tissue from healthy animals is sterile; however, it has been pointed out that during slaughter, dressing, and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract but that more added from knives, cloths, air, carts, and equipment in general. External contamination of meat is a constant possibility from the moment of bleeding to consumption (Lawrie, 2004). Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors) (Rombout and Wout, 2004), however, the factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest *et al.*, 2005).

Foodborne microbiological hazards may be responsible for as many cases of illness as possible each year and are thus an important food safety challenge. To lower the incidence of foodborne disease, many experts and stakeholders urge the development of a science- and risk-based food safety system, in which decision-makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks (Batz *et al.*, 2005). Such a system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum (Batz *et al.*, 2005). So, increasing meat quality assurance per microbial load assessment is deemed necessary (Yousuf *et al.*, 2008).

It has been reported that gram-negative bacteria account for approximately 69% of the cases of bacterial food-borne disease (Clarence *et al.*, 2009). Turtura (1991) reported that the most frequent coliforms identified on meat were *E. coli*, and *E. agglomerate*, and less frequent strains are of the genera *Klebsiella*, *Shigella sonnie*, and *Proteus*. *E. coli* and *S. aureus* are normal flora in humans and animals, their presence in foods are indication of excessive human handling (Clarence *et al.*, 2009). Members of the gram-negative bacteria e.g. *E. coli* are widely distributed in the environment. Contaminated food and water are the major sources by which the bacteria are spread. *Escherichia coli* is commonly used as a surrogate indicator, its presence in food generally indicates direct and indirect fecal contamination (Clarence *et al.*, 2009). Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag *et al.*, 2008).

The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contamination are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel, and the physical facilities themselves are all implicated (Rombouts and Nouts, 2004).



Aims and Objectives:

This study aims to assess the bacteriological safety of beef sold in Owo, with the following objectives:

- 1) Identification of prevalent bacterial pathogens associated with beef contamination.
- 2) Evaluation of the efficacy of existing control measures in reducing bacterial contamination and
- 3) Proposing recommendations for improving bacteriological quality assurance in the beef industry

MATERIALS AND METHODS

Sample Collection

Samples of meats were obtained from meat vendors at four popular meat spots in Owo communities, Ondo State. The samples were immediately wrapped in sterile aluminium foil to prevent contamination and then transported to the Microbiology Laboratory of Science Laboratory Technology for microbiological analysis.

Sample Preparation

The meat samples were thoroughly ground using a previously sterilized pestle and mortar, 11g were transferred into 99ml of sterile peptone water, and then shaken thoroughly to make a homogenous mixture (these served as stock solutions for each sample). Serial dilutions were made using 1 ml from the stock homogenate and 9 ml of the sterile distilled water. Several dilutions were made, up to 5 folds (i.e.,10⁵) for each sample, to obtain discrete colonies as described by Banwart (2012).

Media Preparation

Nutrient Agar (NA) for total aerobic plate count, Mannitol salt agar (MSA) for Staphylococcus, and desoxycholate citrate agar (DCA) for Salmonella/Shigella were prepared according to the manufacturer's instructions, and used in this study for the enumeration of bacteria, as well as for pure culture selection of the microorganisms. All glassware used in the analysis was sterilized in a hot oven at $170 \pm 5^\circ\text{C}$ for at least two hours, while the media and distilled water were sterilized by autoclaving at 121°C for 15 min at 15 psi (Abdullahi *et al.*, 2019; Igwegbe *et al.*, 2019a). Plating was carried out in triplicate and the pour plate method was used to make the viable counts (Quinn *et al.*, 2002; Jay *et al.*, 2005; Vipul *et al.*, 2012; Igwegbe *et al.*, 2019b). In this method, one (1) ml of the inoculums was mixed thoroughly in molten plate count agar held in a hot water bath at $47 \pm 2^\circ\text{C}$. The agar was allowed to set; the plates were inverted and then incubated at 32°C for 24 – 48 hours for bacterial counts (including mesophilic and thermophilic spore formers) and at 25°C (Cheesbrough 2006). For each dilution, the viable colonies, which appeared colorless, in the three plates were counted and the means were calculated.



Isolation of the Microorganisms

The isolation of *Escherichia coli* was achieved following the methods described by Jay *et al.* (2005) and Abdullahi *et al.* (2019); that of *Staphylococcus aureus* and *Staphylococcus epidermidis* were isolated by the methods described by Stanley *et al.* (2015) while *Pseudomonas aeruginosa* and *Klebsiella spp.* were isolated by the methods described by MacFaddin (2000). The isolated organisms were further subjected to Gram-staining techniques as described by Cheesbrough (2006).

Microscopic Examination and Identification of Colonies

The characterization and identification of the colony isolates were achieved by morphological examination of the colonies in the plates for their appearance, size, elevation, form, edge, color, and odor and the observations were Abdullahi *et al.* (2009) properly noted. The biochemical tests including oxidase, urease, catalase and coagulase reactions, citrate utilization, Voges-Proskauer, motility, sugar fermentation, methyl red, and indole production tests were also carried out as described by Cheesbrough (2006).

Statistical Analysis

This study was designed as a complete randomized design (CRD) and the results obtained were subjected to a one-way analysis of variance (ANOVA). The test for significance among means was carried out using Tukeys's Honest Significant Difference (HSD) Test at a 5% level of significance (Dean *et al.*, 2017).

Table 1: Morphological and Biochemical Characterization of Isolates

GRAM	SHAPE	MOT	CAT	COAG	CIT	GLU	SUC	LAC	ORGANISM
-	Rod	+	+	N.D	-	+	+	+	<i>E. coli</i>
-	Rod	+	+	N.D	+	+	-	+	<i>Enterobacter spp</i>
-	Rod	+	+	N.D	+	+	-	+	<i>Serratia spp</i>
-	Rod	+	+	N.D	+	-	+	+	<i>Citrobacter spp</i>
+	Cocci	-	+	+	-	+	+	+	<i>Staphylococcus aureus</i>
+	Cocci	-	+	-	-	+	-	+	<i>Staphylococcus epidermidis</i>
+	Rod	+	+	N.D	-	+	+	+	<i>Bacillus subtilis</i>
-	Rod	-	+	N.D	+	+	+	+	<i>K. preumomiae</i>
+	Rod	-	-	N.D	-	+	+	+	<i>Lactobacillus spp</i>
+	Rod	-	-	N.D	-	-	+	+	<i>Leuconostoc spp</i>
-	Rod	-	+	N.D	-	+	-	-	<i>Pseudomonas aeruginosa</i>
+	Cocci	-	-	N.D	+	+	+	-	<i>Enterococcus faecalis</i>
-	Rod	-	+	N.D	-	+	+	-	<i>Preteus mirabilis</i>
-	Rod	-	+	N.D	-	+	+	+	<i>Salmonella spp</i>
-	Rod	-	+	N.D	-	+	+	+	<i>Shigellaspp</i>

Key: + = positive, - = negative, N.D = not done



Table 2: Bacterial count on the fresh beef samples from retail outlets in Owo ($\times 10^5$ cfu/g)

Location	Day 1 M	Day 1 E	Day 2 M	Day 2 E	Day 3 M	Day 3 E	Day 4 M	Day 4 E	Day 5 M	Day 5 E	Day 6 M	Day 6 E	Day 7 M	Day 7 E	Day 8 M	Day 8 E	Day 9 M	Day 9 E	Day 10 M	Day 10 E
Emure	144 ^b	218 ^a	65 ^b	108 ^a	118 ^a	77 ^b	86 ^b	121 ^a	68 ^b	103 ^a	53 ^b	87 ^a	105 ^b	157 ^a	62 ^b	101 ^a	76 ^b	132 ^a	71 ^b	101 ^a
OkeOgun	131 ^b	207 ^a	97 ^b	177 ^a	105 ^a	118 ^a	56 ^a	89 ^a	76 ^b	97 ^a	49 ^b	103 ^a	106 ^b	176 ^a	75 ^b	128 ^a	102 ^b	132 ^a	107 ^b	139 ^a
EhinOgbe	36 ^b	201 ^a	42 ^b	98 ^a	122 ^b	174 ^a	98 ^b	127 ^a	86 ^b	117 ^a	95 ^b	132 ^a	102 ^b	167 ^a	92 ^b	126 ^a	104 ^b	152 ^a	109 ^b	121 ^a
Ikare Junction	152 ^b	276 ^a	133 ^a	107 ^a	120 ^a	94 ^b	129 ^b	157 ^a	66 ^b	92 ^a	73 ^b	148 ^a	81 ^b	144 ^a	67 ^b	117 ^a	87 ^b	129 ^a	101 ^b	170 ^a
Ijebu	90 ^b	116 ^a	109 ^a	122 ^a	109 ^b	132 ^a	125 ^b	167 ^a	109 ^b	118 ^a	88 ^b	107 ^a	72 ^b	97 ^a	89 ^b	164 ^a	107 ^b	155 ^a	92 ^b	148 ^a
Poly Gate	124 ^b	196 ^a	153 ^a	101 ^b	105 ^a	118 ^a	107 ^a	119 ^a	132 ^b	151 ^a	78 ^b	93 ^a	102 ^b	118 ^a	101 ^a	176 ^a	58 ^b	97 ^a	69 ^b	111 ^a

Means \pm with different superscripts on the same row are significantly ($p \geq 0.05$)

Key: M = Morning; E = Evening

Table 3: Summary of Bacterial Count on the Fresh Beef samples from retail Outlets in Owo Metropolis ($\times 10^3$ cfu/g)

Location	Morning	Evening
Emure	848.00 \pm 2.00 ^b	1214.00 \pm 0.18 ^a
Okeogun	904.00 \pm 2.79 ^b	1366.00 \pm 0.02 ^a
Ehin Ogbe	886.00 \pm 1.00 ^b	1415.00 \pm 0.12 ^a
Ikare Junction	1009.00 \pm 0.00 ^b	1434.00 \pm 0.11 ^a
Ijebu Owo	990.00 \pm 2.75 ^b	1325.00 \pm 0.21 ^a
Poly Gate	1029.00 \pm 1.00 ^{ab}	1280.00 \pm 0.01 ^a
Total	5666.00\pm2.12^b	8038.00\pm0.15^a

Means \pm with different superscripts on the same row are significantly ($p \geq 0.05$)



DISCUSSION

The bacteriological analysis of fresh meat samples sourced from various retail outlets in Owo was conducted. Table 1 provides insight into the potential organisms based on the biochemical and morphological characteristics of the isolates, while Table 2 details the bacterial counts observed in the fresh beef samples collected over a ten-day period, both in the morning and evening. On the first day of assessment, the sample from Ikare Junction exhibited the highest bacterial counts for both morning and evening samples (152×10^5 cfu/g and 276×10^5 cfu/g respectively), while Ehin-ogbe had the lowest count (36×10^5 cfu/g) in the morning, and Ijebu recorded 116×10^5 cfu/g in the evening. Notably, there was a significant ($p \pm > 0.05$) elevation in bacterial counts in evening samples across most locations, possibly due to prolonged exposure to bacterial contamination at the retail points. Table 3 summarizes the bacterial counts from fresh beef samples sourced from different retail outlets, indicating a consistent statistical ($p \pm > 0.05$) disparity between morning and evening collection sessions. Moreover, bacterial counts from Ijebu and Poly gate were consistently higher over the ten-day period compared to other samples. This signifies the high level of contamination of the samples from those locations. The high bacterial loads found on the samples may be due to the water used in washing the meat before selling. Furthermore, the presence of moisture in food is directly related to water activity and the higher the water content, the more susceptible the food will be to microbial spoilage and unfavourable chemical reactions (Zukal and Incze, 2010). Table 2 also revealed the probable organisms according to the biochemical and morphological characterization of the isolates. The isolates were *E. coli*, *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *K. pneumoniae*, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Preteus mirabilis*, *Salmonella* spp. and *Shigella* spp. The presence of these organisms in fresh beef depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering and processing of fresh beef. From the results obtained, fresh beef sample were contaminated with high level of *K. pneumoniae*, *Enterobacter* spp., *P. aeruginosa*, *E. coli*, *Salmonella* spp. and *Pseudomonas* spp. This agrees to previous reports by Clarence *et al.* (2009) who reported *S. aureus*, *E. coli*, *Klebsiella*spp and *Pseudomonas*spp in meat pie and Okonko *et al.*(2009) who reported *Enterobacter* sp., *S. aureus*, *E. coli*, *Proteus* sp, *Salmonella* sp., *Citrobacter*sp, *Klebsiella* sp, *Pseudomonas* sp., and *Serratia* sp. in a study on seafood products. Okonko *et al.* (2009) also reported the presence of *S. aureus*, *Enterobacter* sp., *P. aeruginosa*, and *E. coli* in palms of all the frozen food processors/handlers and water used by them. Actually, they consider the detectable presence of pathogens like *Salmonella* sp. as an indicator of adulteration (Yousuf *et al.*, 2008). Moreover, fecal coliforms as *Escherichia coli* are generally considered indisputable indicators of fecal contamination from warm-blooded animals (Yousuf *et al.*, 2008). The traditional method of examining the microbiological safety, storage stability, and sanitary quality of foods is to test representative samples of the final product for the presence of pathogens or spoilage organisms (Igwegbe *et al.*, 2019). Different microbial groups (e.g., aerobic plate counts and yeast and moulds); and indicator bacteria such as Coliforms are used as an indicator of sanitation per gram or milliliter of a product (Olaoye *et al.*, 2010). The occurrence of such bacteria as *Staphylococcus aureus* and *E. coli* in the meat samples investigated in this study should be of public health concern; due to the facts that they have been implicated in various diseases of man (Gilbert and Harrison, 2001; Ogbonna *et al.*, 2012; Falegan *et al.*, 2017). Gilbert and Harrison (2001) made a similar observation and attributed it to the salt content of the preserved meat which permits the growth of



Staphylococcus aureus. In addition, it could be due to the fact that humans are also the primary reservoirs of *Staphylococcus aureus*. It has been reported that about 40% of healthy individuals harbor *Staphylococcus aureus* in their throats, nasal cavities, infected cuts and sores (Lawley *et al.* (2012). Thus, careless manual handling of fresh beef by retailers might have been the main cause of the higher cellular counts of *Staphylococcus aureus* in. Furthermore, temperature abuse during processing can result to high cellular counts of *Staphylococcus aureus* in the muscles and offal. Staphylococcal enterotoxins are produced by *Staphylococcus aureus*, and most food poisoning strains produce enterotoxin A (Lawley *et al.*, 2012). Also, the presence of *E. coli* may be as consequence of careless slaughtering operations and the use of non-potable water during washing of the fresh beef. This is also in agreement with the findings of Umoh (2004); Tijjani and Jumare (2014). The fresh beef also showed presence of *Pseudomonas aeruginosa*, which occurs in soil, vegetation, and the surface of plants, animals and humans (Field, 2002). Four organisms were isolated from the beef samples in view of the unhygienic condition of meat handling in Nigeria. The organisms isolated in this study are the organisms usually implicated in beef spoilage and could always be suspected in connection with contamination and spoilage. The presence of *Staphylococcus* species agrees with the report of cross-contamination from fresh beef handlers during processing since it is a normal flora of the skin (Gilbert and Harrison, 2001). Most butchers in Nigeria, lacking knowledge of hygiene, usually carry raw meat on the body and use contaminated water to wash the raw meat. It is generally recognized that illnesses due to the consumption of contaminated foods are perhaps the most widespread health problems in the contemporary world today and an important cause of reduced economic productivity. Recent studies examining the morbidity and mortality of foodborne diseases have confirmed the significant public health burden posed by these diseases. In developed countries, it is estimated that one-quarter to one-third of the population is made ill each year because of foodborne diseases. In developing countries, including Nigeria, the burden is much more severe; therefore there is a need to regularly survey the food preparation, handling and storage techniques to ensure the safety of the food on one hand, and the health of the consumers on the other hand.

CONCLUSION

The presence of pathogenic *K. pneumoniae*, *Salmonella* spp. and *E. coli* among others, encountered in fresh meat from retailers in Owo is alarming. Their presence indicates a public health hazard and gives a warning signal for the possible occurrence of food-borne intoxication.

RECOMMENDATIONS

- 1.) The presence of these organisms in fresh beef should receive particular attention.
- 2.) Beef handlers and sellers should be educated on the adverse effects of lack of proper personal and environmental hygiene and sanitation;
- 3.) Veterinary personnel should inspect the animals to be slaughtered before the beef is sold to the general public;



4.) Good processing and handling practices should be adhered to strictly by butchers and those selling the beef, the water used in processing the beef should be sterile. Also, the equipment must be washed properly before and after use;

5.) Further regulatory and educational efforts are needed to improve the safety of the product.

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