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Research Article

Microbial Investigation Of Bacteria Isolated From Raw Beef Samples Sold In Swali Market, Yenagoa Metropolis, Bayelsa State, Nigeria

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ABSTRACT

This paper's goal is to assess, via standard microbiological procedures, the microbial investigation of bacteria isolates found in raw beef sold in Yenagoa metropolis' Swali market, Bayelsa State, Nigeria. Six (6) months was dedicated to conducting this research. On nutrient agar for the Swali market, the total viable counts of the raw beef samples collected from the markets vary from 10.9 x 102 Cfu/ml to 11.9 x 102 Cfu/ml for vendors A, B, and C, and from 9.1 x 102 Cfu/ml to 10.3 x 102 Cfu/ml on cetrimide agar for vendors A, B, and C respectively. On nutrient agar, the raw beef samples from Vendor B had the highest bacterial count 11.9 x 102 Cfu/ml, however on cetrimide agar, the sample from Vendor C had the highest bacterial count 10.3 x 102 Cfu/ml for the Swali market. Salmonella typhimurium, Pseudomonas aeruginosa, Bacillus cereus, Micrococcus luteus, and Staphylococcus aureus were among the bacteria found in the raw beef samples. The percentage of bacteria isolates found in raw beef samples from Swali Market shows that Bacillus cereus has the lowest percentage of isolates occurrence (10%), while Salmonella typhimurium, Micrococcus luteus, Staphylococcus aureus, and Pseudomonas aeruginosa have the highest percentages of isolates occurrence (22.5% occurrence each, accounting for 90%).

INTRODUCTION

Unlike industrialized nations where humans are rarely troubled by parasites in meat due to strong standards of meat hygiene and improved parasite control in animal agriculture, developing countries place a high priority on meat hygiene (Mahangaiko et al., 2015). According to Hocquette et al. (2018), meat, especially beef, is a significant source of critical nutrients for humans, including vitamins, trace elements, proteins, and essential amino acids. According to Godfray et al. (2018), meat consumption is lowest in low-income countries and highest in high-income nations like Finland. Demand for meat is rising in Africa,

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where eating meat may be seen as a status symbol, particularly in low-income nations (Zerabruk et al., 2019). Yet, poor sanitation and handling procedures along the meat supply chain could result in a significant level of bacterial contamination of the meat and its contact surfaces (Rani et al., 2017). The meat deteriorates easily due to bacterial development facilitated by its high protein content and adequate water activity. According to Azuamah et al. (2018), spoiled meat is unappealing and unfit for consumption, which results in significant financial loss. There may be hazards to food safety connected to the production and consumption of meat (Li et al., 2019). Foodborne illnesses are widespread throughout the world with distinct geographical variations (Hoffmann et al., 2017). The significant danger of bacterial contamination of meat by several types of pathogens makes foodborne diseases associated with meat a global public health concern (Tesson et al., 2020). Cattle, a major reservoir for these infections and a major source of raw beef contamination, are frequently found to carry non-typhoidal Salmonella enterica, Campylobacter, and Shigatoxin-producing Escherichia coli (STEC) (Li et al., 2019). The most frequent bacterial pathogens in Europe that cause foodborne illnesses are Campylobacter, Salmonella, STEC, and enteropathogenic Yersinia. Of these, Salmonella and STEC pose the greatest risks to public health when it comes to contaminated raw beef (EFSA and ECDC, 2019). Meat is among the many items in the EU that contain Listeria monocytogenes. Despite being a relatively uncommon illness, listeriosis is one of the worst foodborne illnesses, with a high fatality rate, particularly in elderly and immunocompromised patients (Chlebicz & Slizewska 2018). According to Zhang et al. (2020), there is a possibility that antimicrobial-resistant bacteria can infect humans through beef. Particularly gram-negative bacteria are becoming a bigger issue since they are resistant to vital antimicrobials used in human health, such as quinolones and third-generation cephalosporines (World Health Organization, 2017). In Nigeria, there is a growing consumer demand for beef (Famubo et al., 2020). Our goal was to investigate bacterial contamination on the surface of raw beef at the retail level in Nigeria since, despite improvements in hygiene measures, standards of hygiene are still low across the meat production chain. Additionally, we aimed to compare the levels of bacterial contamination and the presence of foodborne pathogens on raw beef surfaces between raw beef from small-scale businesses in Yenagoa City, Bayelsa State, Nigeria.

MATERIALS AND METHODS

Collection of samples

The research was conducted in the city of Yenagoa, Bayelsa State, using sterile bags to gather samples from retail meat shops at random from three retail vendors in the Opolo market (Vendor A, Vendor B, and Vendor C). The samples were then sent to the Niger Delta University Biological Science Laboratory for analysis.

LABORATORY MEDIA PREPARATIONS

The study used three different media: Cetrimide Agar (CA), Nutrient Agar (NA), and both. The media were set up in compliance with the manufacturer's guidelines.

SAMPLE PREPARATION AND INNOCULATION

MEAT SAMPLES

Using a sterile knife, 1 gram (1g) of beef sample three retail beef vendors (A, B and c) was diced into fine particles and added to 9 milliliters of regular saline solution to create 10 milliliters of stock solution. The procedure for serial dilution was followed by Pelczar et al. (2002). After thoroughly swirling one milliliter (1 ml) of stock solution into dilution 10-1, one milliliter (1 ml) of dilution 10-1 was transferred into dilution 10-2.

The process was carried out up to 10-6 dilution. Using the pour plate method, the samples were plated in triplicate. One milliliter of the diluted sample (10-3) was taken and put into a sterile petri dish. Next, 20 milliliters of the melted agar media were added to the plates. To achieve a uniform growth, the inoculum in the media was thoroughly mixed by carefully swirling the plates. The molten inoculation media in the petri dishes were let to solidify. To determine the total viable count, all nutrient agar-inoculated samples were inverted and incubated at 370C for a full day (Bhandare et al., 2009). Additionally, samples inoculated on cetrimide agar were inverted and incubated for a full day at 370C. Random selections were made of colonies based on Colonies were streaked on new nutrient agar plates to sub-culture them after being chosen at random based on their diverse cultural traits. To get pure isolates, the streaked plates were incubated for 24 hours at 370C. To identify the bacterial isolates, biochemical testing, gram staining methods, and bacterial motility tests were used.

INTERPRETATION OF MICROBIAL GROWTH

Petri dishes with between 30 and 300 colonies on cetrimide and nutrient agar plates were chosen, and the colonies were counted using a colony counter. The viable count was then calculated by multiplying the count by the reciprocal of the suitable dilution factor and expressed in CFU/g/cm2.

RESULTS AND DISCUSSION

EVALUATION OF MICROBIAL LOAD FROM BEEF SAMPLES FOR BACTERIA

The table below displays the microbial load of the different beef samples from three market outlets for both bacteria.

Table 1: Total Bacterial Counts for Beef Samples in Market (Swali Market)

SAMPLES	MEDIA	NO. OF COLONIES	MEAN	DILUTION FACTOR	TCFU CFU/ML	SAMPLES	MEDIA
VENDOR A		I	ii	iii			
NA	Nutrient Agar	92	108	128	109	10^{-3}	10.9 X 10^2
CA	Cetrimide Agar	84	88	100	91	10^{-3}	9.1 X 10^2
VENDOR B		I	ii	iii			
NA	Nutrient Agar	100	132	124	119	10^{-3}	11.9 X 10^2
CA	Cetrimide Agar	84	108	100	97	10^{-3}	9.7 X 10^2
VENDOR C		I	ii	iii			
NA	Nutrient Agar	108	120	124	117	10^{-3}	11.7 X 10^2
CA	Cetrimide Agar	96	100	112	103	10^{-3}	10.3 X 10^2

According to table 1, which represents the Swali Market outlet, the total bacterial count for the beef samples from Vendor A was 10.9 X 102 cfu/ml for nutrition agar and 9.1 X 102 cfu/ml for cetrimide agar. For nutritional agar and cetrimide agar, Vendor B's samples had a total bacterial count of 11.9 X 102 cfu/ml and 9.7 X 102 cfu/ml, respectively, while Vendor C's samples had a total

bacterial count of 11.7 X 102 cfu/ml and 10.3 X 102 cfu/ml, respectively. Three duplicates of the material were plated, and the plates with luxuriant growths were chosen. The total number of colonies was divided by three to get the mean values. To get the total colony forming units (TCFU), this was then multiplied by the dilution factor.

BACTERIA ISOLATES FROM MEAT SAMPLES FROM THE THREE RETAIL VENDORS

The three retail beef suppliers' beef samples were found to have the following isolates of bacteria. The three retail beef vendors' beef samples contained the following bacteria samples that were isolated and identified: Bacillus cereus (Isolate 5) had the lowest frequency of all the bacteria isolates in the beef samples, while Staphylococcus aureus (Isolate 1), Micrococcus luteus (Isolate 2), Pseudomonas aeruginosa (Isolate 3), and Salmonella typhimurium. (Isolate 4) occurred at equally high rates.

CELLULAR MORPHOLOGY AND BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATED FROM MEAT SAMPLE FROM THE THREE RETAIL MARKETS

Table 2: Cellular Morphology and Biochemical Characteristics of Bacteria isolated from Meat Vendor (A) in Swali Market

Isolate Number	1	2
Colonial Characteristics	Oval, mucoid, wavy, umbonate, diffusible green colony	Colorless, transparent with dark centers
Gram Stain	-	-
Cellular Morphology	Carved rods	Capsulated rod
MOT	+	+
CAT	+	+
OXID	+	-
INDOLE	+	-
LAC	-	-
H2S	-	+
GAS	-	-
GLU	+	+
CIT	+	+
Identified Isolates	Pseudomonas aeruginosa	Salmonella typhimurium

Table 3: Cellular Morphology and Biochemical Characteristics of Bacteria isolated from Meat Vendor (B) in Swali Market

Isolate Number	1	2	3	4
Colonial Characteristics	Oval, mucoid, wavy, umbonate, diffusible green colony	Circular, pinhead, smooth, entire, convex, bright yellow colony	Large R-type, irregular opaque colonies, smooth and moist colonies	Circular, pinhead, smooth, entire, convex, yellow colony
Gram Stain	-	+	+	-
Cellular Morphology	Carved rods	Cocci in clusters	Rods	Cocci in clusters
MOT	+	-	+	-
CAT	+	+	+	+
OXID	+	+	-	_
INDOLE	+	+	-	+
LAC	-	-	-	+
H2S	-	-	-	-
GAS	-	-	-	-

GLU	+	+	+	+
CIT	+	+	+	+
Identified Isolates	Pseudomonas aeruginosa	Micrococcus luteus.	Micrococcus luteus.	Bacillus cereus

Table 4: Cellular Morphology and Biochemical Characteristics of Bacteria isolated from Meat Vendor (C) in Swali Market

Isolate Number	1	2	3
Colonial Characteristics	Circular, pinhead, smooth, entire, convex, bright yellow colony	Circular, pinhead, smooth, entire, convex, yellow colony	Colorless, transparent with dark centers
Gram Stain	+	+	-
Cellular Morphology	Cocci in clusters	Cocci in clusters	Capsulated rod
MOT	-	-	+
CAT	+	+	+
OXID	+	-	-
INDOLE	+	+	-
LAC	-	+	-
H2S	-	-	+
GAS	-	-	-
GLU	+	+	+
CIT	+	+	+
Identified Isolates	Micrococcus luteus	Staphylococcus aureus	Salmonella typhimurium

Keys: (LAC) Lactose; (H2S) Hydrogen Sulfuride; (GLU) Glucose; (CIT) Citrate; (+) Positive; (-) Negative; MOT) Motility; (CATA) Catalase; (OXID) Oxidase; and so on. Tables 2, 3, and 4 report the colonial characteristics of the various bacteria isolates, as well as their Gram Stain reaction, cellular morphology, and the various biochemical tests that were conducted.

Table 5: Percentage of occurrence of Bacteria Isolated from Raw Beef Samples in Swali Market

Sample Vendor	Staphylococcus aureus	Micrococcus luteus	Pseudomonas aeruginosa	Salmonella typhimurium	Bacillus cereus
Vendor A	-	-	+	+	-
Vendor B	+	+	+	-	+
Vendor C	+	+	-	+	-
Total	22.5%	22.5%	22.5%	22.5%	10%

The percentage of occurrence of bacteria isolates present in beef samples (Table 5), from Swali Market showed Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa and Salmonella typhimurium all having the same highest occurring bacteria isolate of 22.5% occurrence each and Bacillus cereus has lowest occurred bacteria isolate with 10% occurrence.

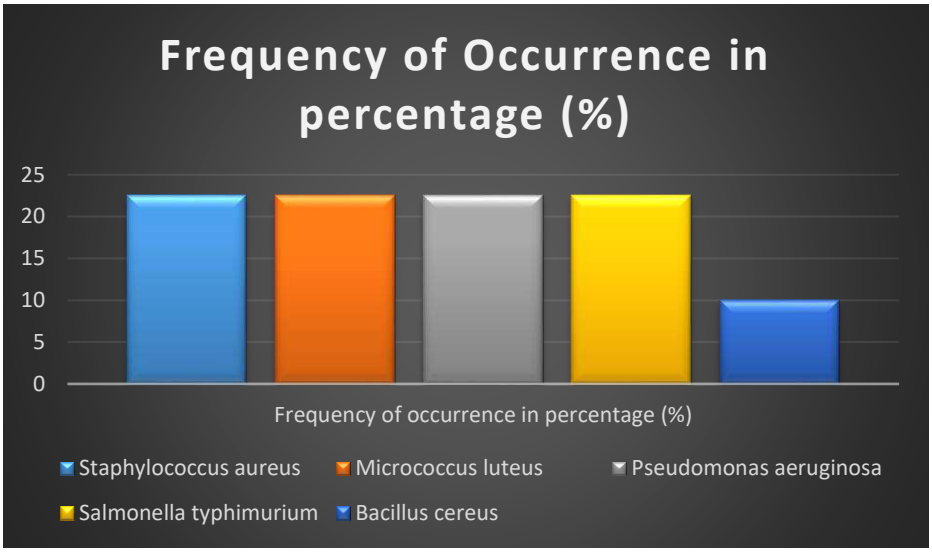

Fig 1. Frequency of occurrence in percentage (%) of bacteria isolates from Raw Beef Samples in Swali Market

Bacteria isolates

A graph (Fig. 1) that displays the frequency of occurrence as a percentage (%) plotted against the isolates of bacteria, with Bacillus cereus having the lowest percentage frequency of occurrence at 10%, while Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa and Salmonella typhimurium all having the same highest frequency occurring bacteria isolate of 22.5% occurrence each.

DISCUSSION

Samples of raw beef were obtained for this investigation from three vendors in the Swali Market in Yenagoa Metropolis, Bayelsa State. According to the analysis's findings, microorganisms that were recovered from the raw beef samples included Bacillus cereus, Pseudomonas aeruginosa, Micrococcus luteus, Salmonella typhimurium, and Staphylococcus aureus, in that order. This conclusion agrees with some of Teshome's et al., 2020 previous research. The highest percentage occurrence was recorded on Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa and Salmonella typhimurium with all having the same percentage of occurrence (22.5%) each and the lowest percentage occurrence recorded for Bacillus cereus (10%), were found in the bacteria isolates obtained from beef samples purchased from three vendors in Swali Market Yenagoa Bayelsa State.

It is significant for public health that harmful bacteria isolates occur in raw beef. It is a sign of contamination when these bacteria are present. Given that Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Micrococcus luteus and Bacillus cereus are present in soils and/or water, their presence may indicate contamination from meat handlers or water. These contaminations may be as a result of poor handling from slaughter by the butchers, the water used in washing the meat since Swali market is located at bank of yenagoa river in Bayelsa State capital.

CONCLUSION

Bacterial contamination levels of raw beef samples were significantly higher in Swali Market Yenagoa Bayelsa State, indicating poor working hygiene and sanitation along the meat production chain in the state. Gram positive and gram-negative bacteria were found to be prevalent in fresh beef, according to the microbiological analysis of the meat. The investigation found that the meat sold in the Swali market hads high bacterial level. This could be caused by poorly sanitized slaughterhouses, inadequate hygiene

standards, and carcasses that were dressed on the floor. High total viable counts could potentially be the cause of these issues, which could endanger public health.

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