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Microbial Examination of Bacteria Isolated from Raw Beef Samples Sold in Opolo Market, Yenagoa Metropolis, Bayelsa State, Nigeria

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ABSTRACT: The objective of this paper is to evaluate the microbial examination of bacteria isolates in beef sold in Opolo market Yenagoa metropolis, Bayelsa State Nigeria using standard microbial techniques. The research was carried out for a period of four (4) months. Total viable counts of beef samples obtained from the market ranges from 7.3 X 10^2cfu/ml to 9.9 X 10^2cfu/ml for vendors a, b and c on nutrient agar and 6.4 X 10^2cfu/ml to 8.0 X 10^2cfu/ml on cetrimide agar for vendors A, B and C for the three vendors. Beef samples from Vendor B had the highest bacterial count 9.9 X 10^2cfu/ml on nutrient agar while the sample from Vendor C had the highest bacterial count 8.0 X 10^2cfu/ml on cetrimide agar. The Bacteria encountered in the beef samples were *Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella typhimurium* and *Bacillus cereus*. The percentage of occurrence of bacteria isolates present in beef samples from Opolo Market showed *Micrococcus luteus*as the highest occurring bacteria isolate with 55% occurrence and *Salmonella typhimurium*as lowest occurring bacteria isolate with 5% occurrence.

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Beef is the name for meat from mature cow. It's a good source of protein, fat, phosphorus, enzyme, water, and other nutrients. A variety of Microorganisms especially bacteria can grow on meat because it's one of the most perishable foods (Mayr *et al*., 2003). Generally, fresh raw meat has been linked to a number of meat borne conditions and intoxication in numerous nations (Mukhopadhyay *et al*., 2009). This is due to the fact that bacteria, both pathogenic and non-pathogenic, can migrate from the gastrointestinal system of cattle to the meat when erroneously and deficiently reused. Up to the stage of consumption, different processing styles of fresh meat fresh meat goes through infect it with bacteria. One of the leading causes of food- borne illness is tainted raw meat (Bhandare *et al*., 2007). *Salmonella*, *Staphylococcus* and other pathogens interlace in food poisoning and intoxication, as well as bacteria from deterioration slime, bruises, and poor odour on shells of meat, has been reported by experimenters. Along the food chain, beef could be defiled during processing, distribution, retail marketing, and handling or drug (Zhao *et al*., 2001).Implicit sources of impurity of meat include sources that are directly linked to the beast itself similar as its skin or fecal material (Kumar *et al*., 2014). External sources of meat impurity include the bloodbath house terrain, the retail outlet terrain; the vehicle used for the transport of the meat from the bloodbath house and of course the meat instructors (Kumar *et al*., 2014). The crude meat processing tools due to lack of needed tools could also serve as a source of meat impurity (Salihu 2010). Bacteria associated with fresh meat have been reported to include *Staphylococcus aureus, Salmonella spp., Escherichia coli, Listeria monocytogenes, Lactobacillus spp., Acinetobacter spp., Clostridium perfringens, Pseudomonasspp, Brochothrixthermosphacta, Clostridium botulinum*and sometimes*Yersinia enterocolitica*. Most of these organisms can beget not only the deterioration of meat but foodborne infections (Carrizosa 2017). The rubrics *Pseudomonas*, *Acinetobacter*, *Moraxella* and *Flavobacterium*are the

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most generally reported cause of deterioration of meat stored under aerobic condition whereas the predominant spoilage associated with meat stored under anaerobic condition include Gram-positive bacteria, particularly lactic acid bacteria (LAB) and *Brochothrix thermosphacta* (Carrizosa 2017; Doulgeraki 2012). Meat is regarded as putrefied when it's unfit for human consumption. Meat may be subordinated to corruption by its own enzyme, microbial action and due to fat oxidation causing textural or organoleptic change when microorganisms release metabolites (Ukut 2010). These changes affect in unwelcome odour and/or unusual taste. The atmosphere in the slaughterhouse and the approach espoused by different bumblers could compromise safety and wholesomeness of the meat. Aseptic practices in massacre houses distance post-processing of beef is associated with implicit health trouble to consumers due to presence of pathogens in meat and defiled outfit. Effluent from massacre houses are known to contribute to impurity of both shells and groundwater since during processing in massacre house, blood, fat, odour urine and meat towels are discharged into wastewater courses (Adeyemo *et al*., 2002). For aseptic reasons butcher houses use large amount of water in processing operations which in turn produce large amount of wastewater. After food creatures are killed in bloodbath houses, meat is transported by meat-van to different retail meat outlets and vended to consumers. Impurity of the meat could be in retail meat shops due to contact with particular particulars (tables, logs, hooks, balances and cutters), insects and canvases (Adzitey *et al*., 2011a). Meat is regarded as putrefied when it's unfit for mortal consumption. Meat may be subordinated to corruption by its own enzyme, microbial action and due to fat oxidation causing textural or organoleptic change when microorganisms release metabolites (Ukut 2010). These changes affect in unwelcome odour and/or unusual taste. Digestive enzymes, microbiological pollutants and lipid oxidation each contribute to the corruption of meat and meat products' quality (Adzitey *et al*., 2011b. The process of meat corruption is attributed to lipid oxidation, protein breakdown and the loss of other important factors. When the face where food is handled is largely defiled by pathogenic microorganisms, food safety is compromised which is a threat to public health (Soyiri *et al*., 2008). Meat is a favorable medium for growth of a number of microorganisms because it's rich in nutrients and humidity. Retail meat is considered both a significant source for food- borne infections and an implicit carrier for the dispersion of resistant bacteria in the community (Bouzid 2015). Lack of ultramodern bloodbath house installation, the actuality of small retail outlets andnon-compliance with the aseptic product protocols have been reported as the major challenges hampering aseptic meat product (Kumar *et al*., 2014; Bouzid 2015). Also, lack of quality control examinations and enforcement can be regarded as a reason for the incurious station of meat instructors to aseptic handing of meat. In order to alleviate the prevalence of food-borne conditions, experts endorse for proper and frequent threat assessment (Nekouei 2018). Food of beef origin has a tendency to spoil fleetly and become a veritable source of gastrointestinal conditions in tropical surroundings, affecting the health of consumers (Akinro *et al*., 2009). The reason for illness which could lead to death in extreme conditions is due to biochemical and microbiological changes in the meat that affect in the conformation of noxious compound (Soyiri *et al*., 2008). Despite the high rate of consumption of meat, studies are lacking on the microbial elaboration of beef quality in the study area hence the need for the present study. Hence, the ideal of this paper is to estimate the microbial examination of bacteria isolates in beef vended in Opolo request Yenagoa megalopolis, Bayelsa State Nigeria.

MATERIALS AND METHODS

Collection of samples: The study was carried out in Yenagoa metropolis of which samples from retail meat shops were collected randomly from three retail vendors from in Opolo market in Yenagoa city of Bayelsa State coded (Vendor A, Vendor B and Vendor C). The samples were collected with sterile bags and taken to the Niger Delta University Biological Science Laboratory for analysis.

Laboratory Media Preparations: Three media used in the study are Nutrient Agar (NA), and Cetrimide Agar (CA). The media were prepared according to the manufacturer's instructions.

Sample Preparation and Inoculation

Meat Samples: 1 gram (1g) of meat sample was chopped to fine particles using sterile knife and transferred into 9ml of normal saline solution to make 10ml of stock solution. Serial dilution was performed as described by Pelczar *et al*. (2002).One millilitre (1 ml) of stock solution was transferred into dilution 10^{-1}, this was swirled properly and then 1ml of dilution 10^{-1} was transferred into dilution 10^{-2}. The procedure was done up to dilution 10^{-6}.The samples were plated in triplicates using pour plate method. From the diluted sample (10^{-3}), 1ml was taken and poured into sterile petri-dish after which 20ml of the molten agar media was poured in the plates. The plates were swirled properly to mix the inoculum in the media in order to obtain an even growth. The petri-dishes with molten inoculated media were allowed to solidify. All

samples inoculated in the nutrient agar were inverted and incubated at 37^0C for 24 hours in order to get the total viable count (Bhandare *et al*., 2009). Samples inoculated on cetrimide agar were also inverted and incubated at 37^0C for 24 hours. Colonies were picked at random according to their various cultural characteristics and were sub-cultured by streaking on fresh nutrient agar plates. The streaked plates were incubated at 37^0C for 24 hours in order to obtain pure isolates. Biochemical tests, gram staining techniques and bacterial motility test were carried out to identify the bacterial isolates.

Interpretation Of Microbial Growth: Petri dishes containing 30 - 300 colonies on the nutrient agar plates and cetrimide agar plates were selected and counted using the colony counter, enumerated and multiplied by the reciprocal of the appropriate dilution factor to obtain the viable count and expressed in CFU/g/cm^2.

RESULTS AND DISCUSSION

Evaluation of Microbial Load from Beef Samples for Bacteria: The microbial load of the various beef samples from three market outlets for both bacterial are shown in table 1.

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

Samples	Media	No. Of colonies			Mean	Dilution factor	TCFU CFU/ML
Vendor A		**i**	**ii**	**iii**			
NA	Nutrient Agar	72	84	64	73	10^{-3}	7.3 x 10^2
CA	Cetrimide Agar	60	80	52	64	10^{-3}	6.4 x 10^2
Vendor B		**i**	**ii**	**iii**			
NA	Nutrient Agar	80	68	148	99	10^{-3}	9.9 x 10^2
CA	Cetrimide Agar	48	68	96	71	10^{-3}	7.1 x 10^2
Vendor C		**i**	**ii**	**iii**			
NA	Nutrient Agar	60	64	156	93	10^{-3}	9.3 x 10^2
CA	Cetrimide Agar	68	72	100	80	10^{-3}	8.0 x 10^2

From table 1 which is Opolo Market outlet, the total bacterial count for beef samples from Vendor A was 6.4 x 10^2cfu/ml and 7.3 x 10^2cfu/ml for forcetrimide and nutrient agar, respectively.. Vendor B total bacterial count was 7.1 x 10^2cfu/ml and 9.9 x 10^2cfu/ml for cetrimide and nutrient agar, respectively. Vendor C total bacterial count was 8.0 x 10^2cfu/ml and 9.3 x 10^2cfu/ml for cetrimide and nutrient agar, respectively. The samples were plated in triplicates and the plates with luxuriant growths were chosen. Mean values were obtained by dividing the total number of colonies by 3. This was further multiplied by the dilution factor to obtain the total colony forming units (TCFU).

Bacteria Isolates from Meat Samples from Three Retail Vendors: The following bacteria isolates where identified to be present in the beef samples from the three retail beef vendors. The bacteria samples isolated and identified are *Staphylococcus aureus* (isolate 1) which occurred in 21 of the beef samples from the three retail beef vendors. *Micrococcus luteus* (isolate 2) was isolated and identified and it occurred on 10 of the beef samples from the three retail beef vendors. Isolate 3 identified organisms is *Pseudomonas aeruginosa* and it occurred in 15 of the beef samples from the three retail beef vendors. Isolate 4 identified organisms is *Salmonella* spp occurred 6 of the beef samples from the three retail beef vendors.

Table 2: Cellular Morphology and Biochemical Characteristics of Bacteria isolated from Beef Sample from Opolo Market (Vendor A)

A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Circular, pinhead, smooth, entire, convex, yellow colony	+	Cocci in clusters	-	+	-	+	+	-	-	+	-	*Staphylococcus aureus*
2	Circular, pinhead, smooth, entire, convex, bright yellow colony	+	Cocci in clusters	-	+	+	+	-	-	-	+	+	*Micrococcus luteus*

Keys: (+) Positive; (-) Negative; (MOT) Motility; (CATA) Catalase; (OXID) Oxidase; (LAC) Lactose; (H_2S) Hydrogen Sulphide; (GLU) Glucose; (CIT) Citrate. Key: A = Isolate Number; B = Colonial Characteristics; C = Gram Stain; D = Cellular Morphology; E = MOT; F = CATA; G = OXID; H = Indole; I = LAC; J = H2S; K = GAS; L = GLU; M = CIT; NN = Identified Isolates

Table 3: Cellular Morphology and Biochemical Characteristics of Bacteria isolated from Beef Sample from Opolo Market (Vendor B)

A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Oval, mucoid, wavy, umbonate, diffusible green colony	-	Carved rods	+	+	+	+	-	-	-	+	+	*Pseudomonas aeruginosa*
2	Circular, pinhead, smooth, entire, convex, bright yellow colony	+	Cocci in clusters	-	+	+	+	-	-	-	+	+	*Micrococcus luteus*
3	Circular, pinhead, smooth, entire, convex, yellow colony	+	Cocci in clusters	-	+	+	+	+	-	-	+	+	*Staphylococcus aureus*

Keys: (+) Positive; (-) Negative; (MOT) Motility; (CATA) Catalase; (OXID) Oxidase; (LAC) Lactose; (H_2S) Hydrogen Sulphide; (GLU) Glucose; (CIT) Citrate. Key: A = Isolate Number; B = Colonial Characteristics; C = Gram Stain; D = Cellular Morphology; E = MOT; F = CATA; G = OXID; H = Indole; I = LAC; J = H2S; K = GAS; L = GLU; M = CIT; NN = Identified Isolates

Cellular Morphology and Biochemical Characteristics of Bacteria Isolated from Meat Sample from the Three Retail Markets: The colonial characteristics of the different bacteria isolates, their Gram Stain reaction, cellular morphology and the different biochemical test carried out are reported on tables 2, 3 and 4. The table shows the percentage of occurrence of bacteria isolated from beef samples and it is observed that *Micrococcus luteus* have the highest occurrence at 55%.

Table 4: Cellular Morphology and Biochemical Characteristics of Bacteria isolated from Beef Sample from Opolo Market (Vendor C)

A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Oval, mucoid, wavy, umbonate, diffusible green colony	-	Carved rods	+	+	+	+	-	-	-	+	+	*Pseudomonas aeruginosa*
2	Circular, pinhead, smooth, entire, convex, bright yellow colony	+	Cocci in clusters	-	+	+	+	-	-	-	+	+	*Micrococcus luteus*
3	Colourless, transparent with dark centers	-	Capsulated rod	+	+	-	-	-	+	-	+	+	*Salmonella sp.*

Keys: (+) Positive; (-) Negative; (MOT) Motility; (CATA) Catalase; (OXID) Oxidase; (LAC) Lactose; (H_2S) Hydrogen Sulphide; (GLU) Glucose; (CIT) Citrate.

Key: A = Isolate Number; B = Colonial Characteristics; C = Gram Stain; D = Cellular Morphology; E = MOT; F = CATA; G = OXID; H = Indole; I = LAC; J = H2S; K = GAS; L = GLU; M = CIT; NN = Identified Isolates

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

Sample Vendors	*Staphylococcus aureus*	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
Vendor A	+	+	-	-
Vendor B	+	+	+	-
Vendor C	-	+	+	+
TOTAL	20%	55%	20%	5%

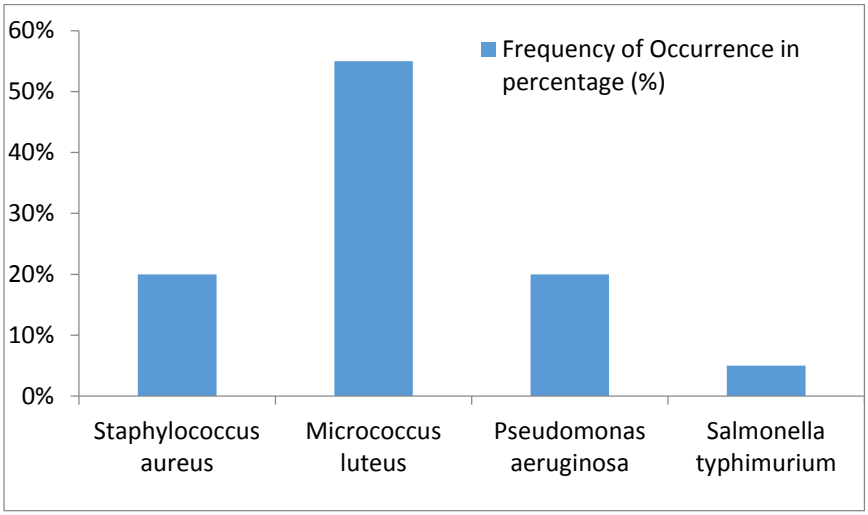

Fig 1. Frequency of occurrence in percentage (%) of bacteria isolates from Beef Samples in Opolo Market.

Bacteria isolates: A graph showing the frequency of occurrence in percentage (%) plotted against bacteria isolates with *Micrococcus luteu* shaving the highest percentage occurrence of 55%, *Salmonella typhimurium* had the least percentage occurrence of 5% and *Bacillus cereus* having no occurrence.

In this study, beef samples were collected from three vendors from Opolo Market in Yenagoa Metropolis in Bayelsa State. The results obtained from the analysis showed that bacteria isolated from the Beef samples include *Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella typhimurium* and *Bacillus cereus* respectively. This result is in line with some earlier works carried out by Iroha *et al.* (2011)Bacteria isolates gotten from beef samples from the various vendors from Opolo Market reported *Micrococcus luteus* to having the highest percentage occurrence of 55%, *Salmonella typhimurium* had the least percentage occurrence of 5% and *Bacillus cereus* having no occurrence. The occurrence of pathogenic bacteria isolates in beef is of public health importance. The presence of these bacteria is an indication of contamination. The presence of *Micrococcusluteus, Pseudomonas aeruginosa* and *Salmonella typhimurium* could be as a result of contamination from meat handlers and/or water since the organisms are found in soils and/or water. The presence of *Staphylococcus aureus* in the beef samples could possibly occur through the processing methods of

hands and arms coming into contact with the beef or by coughing or sneezing (Sobukola *et al*., 2009; Okonko *et al*., 2008 [a,b,c,d] and 2009 [a,b]).

Conclusion: Beef which is the main source of protein for humans is highly vulnerable to spoilage. The microbial examination in beef revealed that both gram positive and gram negative bacteria were common in fresh beef. The results obtained from the study had a high bacteria count for beef sold in Opolo market. This may be due to carcasses which were dressed on the floor and low level of hygiene and poor slaughter house sanitation which could also be responsible for high total viable counts which pose a risk to public health.

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