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Contamination Level of Microbes Isolated From Raw Beef Meat Presearved in a Cash Crunched Nigerian Economy

Amadi-Ikpa C.N.[1*], Aguma I.[2], Inyang G.E.[3]
Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt
Corresponding Author: Amadi-Ikpa C.N. chidi.amadiikpa@iaue.edu.ng

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ABSTRACT

The contamination level of microbes isolated from raw beef meat preserved in a cash crunched Nigerian economy was investigated due to spoilage by microbes as observed in the market space. Beef meat samples were purchased from abattoirs, separated and preserved in three parts namely: the salt treated part, the cold/refrigerated treated part and the non-treated part. The meat samples were analyzed using standard microbiological procedure and result showed a significant difference in the microbial load, which were significant in terms of meat spoilage. Thirty-nine microbial isolates were obtained belonging to four species namely: Staphylococcus aureus, Streptococcus faecalis, Escherichia coli and candida sp. Following this, the use of salt as a preservative agent raised the contamination level of the meat. Thus, other forms of preservation should be considered, aimed at cushioning the likelihood of poor Government policies in future, considering the fact that energy supply via electricity is poor.

INTRODUCTION

Nigeria cash swap policy failed in its implementation and thus, resulted to hardship amongst citizens and to the economy (Igbinadolor, 2023). According to Ariemu (2023) Nigerian citizens groaned with the challenge of low cash flow, which in-turn affected the purchase of fuel. This challenge was accompanied with poor electricity supply. Alternatively, the purchase of fuel to run power plant for preservation of raw meat via refrigeration was an issue. Improper food storage and preservation due to inadequate power supply compromised the sale of meat during the period. As leftover meat at the close of market were taken back home for preservation. According to Magar (2021) and Rahman et al. (2018) preservation of beef meat by refrigeration is one of the most widely used method, amongst other methods such as sun drying, use of chemicals etc. Preservation by refrigeration enhances the meat safety, shelf life and appearance (Magar, 2021). The Nigerian economic policy affected business operations as meat vendors had difficulties preserving their beef meat. Olu-Taiwo et al. (2021) and Norrung et al. (2009) accordingly agreed on the need for fuel (hydrocarbon) to run power plant in the absence of electricity. This became an issue of concern as the challenge jeopardized meat preservation which resulted to spoilt meat displayed in the market. The meat which could be contaminated by microbes (Rani et al., 2023) is justified effectively preserved. However, in the absence of effective preservative measure, the normal microbial flora in / on the meat may evade tissues, proliferate and initiate spoilage (Rani et al., 2023). Certain bacteria such as Campylobacter sp., Shigella sp., Escherichia coli and Salmonella sp. have been isolated from beef meat resulted from spoilage (Olu-Taiwo et al., 2021). Studies have shown that consumption of under-cooked beef meat are linked to the transmission of pathogens (Norrung et al., 2009). These pathogens evade raw meat through cross contamination during the handling, processing, distribution and marketing (Norrung et al., 2009). Thus, the study acknowledged the level of microbial interference associated with raw meat displayed for human purchase in a cash crunched economy with respect to the meat vendors' adoptive preservative approach. Thus, the study comparatively, analyzed the level of contamination of beef meat sold in Nigerian market for microbial infestation considering the fact that the meat is either preserved with salt or refrigerated or not treated by any means.

LITERATURE REVIEW

Nigeria cash swap policy failed in its implementation and thus, resulted to hardship amongst citizens and to the economy (Igbinadolor, 2023). According to Ariemu (2023) Nigerian citizens groaned with the challenge of low cash flow, which in-turn affected the purchase of fuel. This challenge was accompanied with poor electricity supply. Alternatively, the purchase of fuel to run power plant for preservation of raw meat via refrigeration was an issue. Improper food storage and preservation due to inadequate power supply compromised the sale of meat during the period. As leftover meat at the close of market were taken back home for preservation. According to Magar (2021) and Rahman et al. (2018) preservation of beef meat by refrigeration is one of the most widely used method,

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METHODOLOGY

Description of the Market Operations

The market operations of the study area, Rumuwoji Market, otherwise called Mile One market is on a daily basis. Meat of various sizes and types are amongst food item sold in the market. Meat such as pork meat, poultry meat, cow leg, beef meat etc. are all sold in the market. The sale of beef meat has received more attention due to the high demand and availability of abattoir in the area. On event the meat are not sold off, the left-over meat is preserved for the following day market.

Collection of Beef Meat Samples and Study Design

Beef meat were purchased from the Rumuwoji market from ten different sales point in a sterile bag and put in an ice-block cooler. The beef samples were purchased in parts, bagged properly, and taken to the Biology Laboratory of Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, were some parts were preserved by (i) salting, (ii) refrigeration (iii) and some other part kept without preservation.

Preparation of Meat Samples for Microbial Analysis

The preparation of the sliced beef sample was carried out as adopted by Kang (2018) in a sterile condition. A portion of the beef meat (1g) was chopped off and the piece introduced into a sterile freshly prepared 9 ml peptone water. The diluted peptone water was then mixed by vortexing and the component served as stock for further dilutions of 10-3 which was used for the analysis (Amadi-Ikpa et al., 2020).

Preparation of Media

The following agar: Mannitol salt agar, Eosin Methylene blue agar, Sabauraud Dextrose agar and Nutrient agar were procured and prepared for the analysis. Media preparation were carried out as directed by the manufacturer and then poured on a sterile petri dish.

Enumeration of Bacterial Load on the Sliced Beef Sample

Bacteriological load on the beef sample were determined by inoculating 0.1 volumes of 10-3 dilutions obtained from the stock into: Mannitol salt agar medium, Eosin Methylene blue agar medium, Sabauraud Dextrose medium agar and Nutrient agar medium for the enumeration and isolation of Staphylococcus aureus, Escherichia coli heterotrophic fungi and heterotrophic bacteria respectively.

Characterization of the Isolates

All isolates were characterized microscopically, macroscopically and biochemically.

Microscopic Examination of the Isolates

The Gram staining technique was carried out to differentiate the bacteria isolates into Gram positive and Gram-negative bacteria based on their cell wall composition. In carrying out this analysis, a loopful of a 24 hours culture was picked and used to make a smear on a clean, grease-free slide. The smear was heat fixed by passing over a flame and then the slide flooded with crystal violet for 1 minute and rinsed with water. The smeared slide was again flooded with Gram's iodine for 1 minute and rinsed with water. Thereafter, the smeared slide was decolorized using 95% alcohol for 30 minutes. Furthermore, the slide was counter stained with safranin reagent for 30 seconds, rinsed with water, allowed to air-dry and viewed under microscope. Cells that appeared purple were recorded as Gram-positive cells while cells that appeared pink, Gram-negative cells.

For the fungi microscopic identification, a drop of Lactophenol reagent was put into a clean -glass slide, followed by the introduction of the fungal isolate using a sterile Nichrome. The preparation was then observed under a light microscope and the observation noted (Franco-Duarte et al., 2019).

Macroscopic Examination of the Isolates

All bacteria and fungi isolates were examined macroscopically as observed by Franco-Duarte et al. (2019). The color, size, elevation. edge, opacity and shape of the bacterial and fungal isolates were observed critically.

Biochemical Characterization of Bacterial Isolates

The following biochemical test, Voges-Proskauer, Citrate, Indole, Catalase, Capsule, Motility, Coagulase, Methly Red assay were carried out (Franco-Duarte et al., 2019).

Voges Proskauer Test

A loopful of the bacterium was inoculated into 10ml sterile Voges Proskauer broth medium prepared according to manufacturer's instructions. The tube was then incubated at 35 - 37 degrees centigrade for 48 hours after which 0.6ml of 5 percent – naphthol and 0.2ml of 40 percent Potassium Hydroxide reagent was added to the broth culture. An inference of red coloration indicated Voges Proskauer positive test while an absence of red coloration did not indicate Voges Proskauer test.

Citrate Test

The Citrate test was adopted to evaluate the ability of the isolate to utilize Sodium Citrate as its sole source of carbon and inorganic ammonium salt as its only source of nitrogen. Isolates that can grow on this medium turn the bromothymol blue colored indicator from green to blue. Simon citrate agar was prepared. A sterile wire loop was used to pick a loopful of the isolate and streaked on slant surface. The tube was then incubated at 370C for 24hrs. Change in color from green to blue was indicative of positive result while no change in color was indicative of a negative result.

Indole Test

This test was used to determine the ability the isolate to split the amino acid tryptophan to form pyruvic acid, ammonia and indole using the enzyme tryptophanase. A loopful of the bacterium was inoculated into sterile peptone water and incubated at 37 degrees centigrade for 48hrs. Thereafter, 0.3 - 0.5 ml of Kovac's reagent was added using a Pasteur's pipette. An inference of a red ring on the medium indicated an indole utilization while a yellow ring indicated the absence of indole.

Catalase Test

The catalase test was done to determine the ability of the isolates to breakdown Hydrogen Peroxide into Oxygen and Water. The text involved the introduction of the text bacteria into 3% Hydrogen Peroxide solution. Rapid production of effervescence indicated the enzyme catalase while the absence of catalase was indicative of weak effervescence.

Motility Test

Semi solid nutrient agar was used for this test. The media were prepared, introduced into a test tube and the test organisms picked with a sterile straight wire into the media by stabbing. Thereafter, the medium was incubated at 37 degrees centigrade for 24 - 48 hours. An inference of growth in a diffused form, from the line of stab through the medium indicated a positive result, whereas growth only along the line of stab indicated a negative result.

Capsule

Capsule staining test was done to determine the presence of capsule in the bacterium. The procedure was carried by adding a few drops of crystal violet onto the test bacteria on a clean microscopic slide, then stirred and viewed under a light microscope. An inference of a light blue appearance on the microscope signified encapsulated cell, while the reverse signified the cell was not capsulated.

Methyl Red Test

The Methyl Red test was done and used to determine the ability of the isolates to produce and maintain stable acid end products from Glucose fermentation. The test involved taken a loopful of the test bacteria and the bacteria inoculated into 10ml sterile Methyl Red broth medium prepared according to manufacturer's instructions. The tube was then incubated at 35 - 370C for 48 hrs. after incubation, 5-6 drops of methyl red reagent was added. Development of red color indicated, Methyl Red while Voges Proskauer was indicated with a red color on the surface of the medium.

Coagulase Test

The slide method was employed and the test involved placing the test organism on a clean sterile microscopic slide with an addition of two to three drops of saline and a human plasma, the component was then mixed thoroughly and allowed to clot. Clotting after few minutes indicated coagulase positive while an absence of clot indicated a coagulase negative.

Sugar (Lactose and Glucose) Fermentation Test

This sugar test was done to evaluate the ability of the isolate to utilize sugar (lactose and glucose) to produce acid. Peptone broth and the sugar at 1% each was compounded with a methyl red indicator for the purpose of the analysis. The medium was sterilized and after sterilization, a loopful of the test organism was introduced into the test tubes and then incubated at 35 -370C for 24 - 48 hrs. Change in color from red to yellow and gas production indicated a positive sugar fermentation test, while in the absence of no change in color depicted negative sugar utilization.

RESULTS

Bacteria Load on/in the Beef Meat

Table 1 shows the mean Staphylococcal counts of 1.1 x 104cfu/g, 8 x 103cfu/g, and 4 x 103cfu/g derived from salt treated beef meat, Non-treated Beef Meat and refrigerated beef meat samples respectively. This showed a significant difference at a probability level of greater than 0.05. Heterotrophic bacteria load on the salt treated beef meat was 1.5 x 105cfu/g, while the non-treated meat and refrigerated meat were 1 x 105cfu/g and 5 x 104cfu/g respectively. The differences in these counts were statistically significant (P>0.05). Heterotrophic fungi had counts of 9 x 103cfu/g, 3 x 104cfu/g, and 1.9 x104 cfu/g for salt treated, non-treated and refrigerated beef meat samples respectively, showing a significant difference at P<0.05. Escherichia showed counts of 1 x 103cfu/g for salt treated meat, 2.5 x104cfu/g counts for non-treated meat samples and for refrigerated meat, 5 x 103cfu/g counts were derived, at a significant difference (P>0.05).

Table 1. Mean Bacteria Load in the Beef Meat

Microbial Parameters/	**Salt treated Beef Meat (CFU/g) (n= 10)**	**Non- treated Beef Meat (Left Over (CFU/g) (n =10)**	**Refrigerated Beef Meat (CFU/g) (n=10)**	**T-test**
Staphylococcal	1.1×10^4	8×10^3	4×10^3	P < 0.05
Total Heterotrophic Bacteria	1.5×10^5	1×10^7	5×10^4	P < 0.05
Total Heterotrophic Fungi	9×10^3	3×10^4	1.9×10^4	P < 0.05
Escherichia	1×10^3	2.5×10^4	5×10^3	P < 0.05

Key=(CFU/g) Coliform Forming Unit Per gram. N=Number of Samples

Morphological and Colonial Characterization of the Bacteria Isolates

Table 2 showed the morphological and colonial characterization of the bacteria isolates. The color of the colonies on the various culture plates revealed white and grey colors with small sized colonies that had low elevation. The edges of the colonies were curved and they appeared opaque. Gram reaction indicated a grape-like clustered appearance under a purple background for Gram positive bacteria while for the Gram-Negative bacteria indicated a pink darkly stained background.

Table 2. Colonial and Morphological Characterization of the Isolates

Isolates	Gram.Reac	Color	Shape	Edge	Opacity	Size	Elevat.	Edge
Escherichia coli	-	Green	Round	Curve	Opaque	Small	Low	Curve
Strep. Faecalis	+	Grey	Rod	Curve	Opaque	Small	Low	Curve
Staph. aureus	+	Yellow	Round	Curve	Opaque	Large	Low	Curve

Key: -= Negative, +=Positive

Biochemical Reaction of the Bacteria Isolates

Table 3 showed the biochemical reaction of the bacteria isolates obtained, the Gram-negative bacteria were distinguished with a positive lactose and motility test, which indicated Escherichia coli. The Gram-positive isolate reported a negative lactose and citrate expression property, thus indicated Staphylococcus aureus. Staphylococcus aureus were distinguished positive for motility and citrate expressions. The Gram-positive isolate reacted negatively, to Methyl red, citrate, indole, catalase and lactose reagents. However, Voges Proskaurer reagent reacted positive to the isolate, Streptococcus faecalis.

Table 3. Biochemical Characterization of the Isolates

Bacteria	Coa	MR	VP	Lac	Cat	Glu	Indol	Cit	Cap	Motility
E.coli	-	+	-	+	+	+	+	-	+	+
S. aureus	+	+	+	+	+	+	-	+	+	_
S. faecalis	-	-	+	-	-	-	-	-	-	-

Note: Cat = Catalase, Glu = Glucose, Indol=Indole, Cit= Citrate MR= Methyl Red, VP= Voges Proskaurer, Lac = Lactose, Cap. = Capsule, Coa.= Coagulase, Gram React. =Gram Reaction.

Table 4, showed the fungi phenotypic features for Candida sp. with Creamy white and smooth phenotypic features.

Table 4. Phenotypic Features of Heterotrophic Fungal Isolates

Macroscopic Feature	Probable Fungi
Creamy white and smooth	*Candida* sp.

Frequency Distribution of Bacterial and Fungal Isolates

Table 5 showed the frequency distribution of microbes derived from salt treated, non- treated and refrigerated beef meat samples. Escherichia coli, Staphylococcus aureus, Streptococcus feacalis and Candida sp. accounted for 7.7%, 33.3%, 41.0% and 60% respectively.

Table 5. Percentage Frequency Distribution of the Microbes

Microbial Parameters/	Salt treated Beef (n= 10)	Non-treated Beef Meat (Left Over (n =10)	Refrigerated Beef Meat (n=10)	Frequency	Percentage (%)
Escherichia coli	2	0	1	3	7.7
Staph. Aureus	7	5	1	13	33.3
Strep. Faecalis	9	5	2	16	41.0
Candida sp.	1	4	2	7	18

Key: (n)= Number of samples

DISCUSSION

Heterotrophic bacteria load in left over / non-treated meat were higher and significantly different from counts obtained in salt and refrigerated meat due to inappropriate processing as noted by Barcenilla et al. (2022). Barcenilla et al. (2022) stated that heterotrophic counts have been used as criteria to predict the shelf-life of meat. Loads observed with non-treated meat fell between 107 and 108 cfu/g and this satisfied in general the beginning of changes in organoleptic property of meat which includes: changes in appearance, taste and smell (Barcenilla et al., 2022 ; Manas, 2016). The loads of Escherichia coli in both persevered and non-preserved beef samples were significantly different. Andritsos et al. (2012) stated that the loads of Escherichia coli may be due to the meat encounter with faces during butcher, so their increased load specifically in left over/ non-treated meat may have resulted from insufficient hygiene. However, the presence of Escherichia coli in the other preservative methods is also noted as a frequent finding in studies (Alvarez-fernandez et al., 2013). Basically, Alvarez-fernandez et al. (2013) identified anti-bacterial resistance in Escherichia coli isolated from conventionally and organically reared poultry. Staphylococcal counts in the meat samples showed the salt treated meat samples had higher loads than the refrigerated meat. Basically, the less loads as reported in refrigerated meat may be due to cold temperature as reported by Gill (1996). Gill (1996) noted that cold temperature could stall the growth of microorganisms, in which case temperature had effect on the meat. However, salt treated meat favored the growth of Staphylococcus aureus, which are salt-loving. The use of salt to preserve meat does not guarantee protection against microbes (fungal) proliferation or infestation as the meat according to Stuart (2009) are predisposed to microbial growth. Although, the presence of Staphylococcus aureus can be challenging due some strains which have the capacity to cause food borne infection.

Heterotrophic fungi load in the meat samples were significantly, different probably because the fungal; Candida spp. which were common in the study are frequently predisposed to invade humid environmental conditions which the meat promotes (Bhatnagar & Ehrlich, 2002). According to Wang et al. (2021), yeast and molds were primarily present on the surface of meat however, Li et al. (2003) did not identify yeast as the most dominant microorganism in meat but mold. The occurrence of Candida sp. in this study could be associated with airborne fungal spore, which may have entered the meat before preservation (Bhatnagar & Ehrlich, 2002).

CONCLUSIONS AND RECOMMENDATIONS

The contamination level of microbes isolated from raw beef meat preserved in a cash crunched Nigerian' economy was significantly high. Although, refrigeration of beef meat still emerged as the best method, were it inhibited the proliferation of microbes. Other form of preservation should be considered to cushion the effect of poor Government policies in future, considering the fact that energy supply via electricity has a huge role to play in the reduction of meat wastage and losses.

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