

ASSESSMENT OF MICROBIOLOGICAL QUALITY ASSOCIATED WITH READY-TO-EAT **BUSHMEAT** SOLD AT RUMUOKORO MARKET IN RIVERS STATE

ABSTRACT

The study was carried out to investigate the Microbiological quality of microorganisms associated with **ready-to-eat** bush meat sold at Rumuokoro market in Rivers state. Total numbers of 24 samples were collected and analysed using different media such as Nutrient agar for Total aerobic plate count (TAPC), MacConkey agar for **the** coliform count, Eosin methylene blue for *Escherichia coli* (EC) and Potato Dextrose Agar for Fungal count (FC) and folds serial dilution was used. Four different microorganisms were isolated such as *Staphylococcus spp*, *Pseudomonas spp*, *Bacillus spp*, and *Escherichia coli*. The total aerobic plate count (TAPC). *E. coli* count (EC). Coliform count (CC) and Fungal count (FC) isolated from antelope were higher when compared to **grasscutter** so there was a significant difference ($P < 0.005$). The occurrence of *Staphylococcus aureus* isolated from antelope (26.9%) was higher when compared to **grasscutter** (25.0%). However the occurrence of *Pseudomonas spp* and *Bacillus spp* isolated from Antelope (23.1% and 30.8%) were higher when compared to grass cutter (12.5% and 18.5%) while the occurrence of the above organisms isolated on both Antelope **was** is significantly **different** ($P < 0.005$) from **grasscutter**. But the occurrence of *Absidia spp* and *Penicillium spp* were higher in **grasscutter** sample (57.1%) and (42.9%) compared to antelope (55.6%) and (44.4%), respectively, although the mean difference was statistically significant ($P < 0.005$) so there was a significant difference. It is hereby recommended that most handlers should always wash hands before and after handling the meat as improper hand washing is the number one cause of **food-borne** illness. Consumers of such meat should learn food hygiene practices such as, soaking the meat in a warm salt solution, proper washing, and **well-cooked** before consumption.

Keywords: Microbiological, **Bushmeat**, Food Hygiene, **Grasscutter**, Consumers.

1. Introduction

The forest and woodland **are** often **referred** to as bush, so the wild animals derived from the bush are being **hunted** and consumed as **bushmeat** (James and Sam, 2007). **Bushmeat** constitutes a vast array of species ranging from Donkeys, Leopard, Monkeys, **Grasscutter** (*Thryonomys*, Swindenanns), African **elephants**, Antelope (Alcalaphinae) (James and Sam, 2007).

Most Ready-to-eat **bushmeats** sold in **the** market especially in West Africa are usually those produced locally by drying with smoke and cooking. Hence, the method does not make the

~~meat-free totally free~~ from microbial ~~attacks~~ such as bacteria and fungi or toxic substances produced by ~~these~~ bacteria, thus posing serious health ~~threats~~ or danger to the individual or group of individuals who ~~basically~~ rely on this type of meat as a source of food, particularly when not adequately cooked before consumption (James and Sam, 2007).

Similarly, Ready-to-eat ~~bushmeat~~ usually serves as a source of income as it can be exported or sold within to generate capital or money, cheaper protein source thereby resulting in massive consumption than an alternative source of protein. ~~Bushmeat~~ can be ready-to-eat when they are properly dried which is a complex process with many crucial steps starting from the slaughtering of the animal, carcass trimming selection of the raw material, proper cutting, and ~~pre-treatment~~ of the pieces to be dried (Oliver *et al.*, 2009). More also, Ready-to-eat ~~bushmeat~~ with a ~~high-fat~~ content should not be kept for a long duration but utilized as soon as possible after cooking ~~in order~~ to avoid intensive rancidity (Fa *et al.*, 2002).

Furthermore, these bush meat must be continuously examined for ~~spoilage-related~~ off-odour, which is the result of incorrect preparation and or drying of the meat. ~~Bushmeat~~ with signs of deterioration must be rigorously sorted out and not ~~to~~ be cooked (Oliver *et al.*, 2009).

In the same vein, ~~there are~~ some crucial noticeable factors ~~that~~ ~~enhance~~ the microbial contamination of Ready-to-eat bush meat such as water activity or availability, pH- value, redox potentials, moisture, temperature, relative humidity, and nutrient content (David *et al.*, 1999; Murray, 2005; Willy *et al.*, 2007). Bush meats are commonly consumed by different people especially in Nigeria irrespective of their age and race, because of the nutritive value or nature of the meat. But they are also subjected to microbial attack and proliferation ~~basically~~ bacteria and fungi when not properly handled which subsequently could lead to ~~foodborne~~ illness/ diseases among consumers ~~due to the fact that~~ ~~since~~ these microbes are refers to as ubiquitous ~~in nature~~, thereby causing deterioration to bush meat reducing its

acceptability and economic benefits to the humans (Willy *et al.*, 2007) When these microorganisms invade in the **bushmeat**, it has the capacity of disfiguring the pleasant appearance of the **bushmeat**, change the odour to that of offensive and possibly change the taste content to a soured taste which in turn would not be palatable to the consumer (Davies, 2002). Therefore, it is imperative to underscore the microbiological qualities of ready-to-eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state, and its suitability for human consumption.

2. Materials and Methods

2.1 Study Area

The study was primarily based on Rumuokoro market Port Harcourt, Rivers state.

2.2 Sample Size/Collection

A total of 24 **ready-to-eat bushmeat** samples were used for this study. 12 each from two species of the **bushmeat** were collected from the same market at Rumuokoro, Port Harcourt, Rivers state. The collected samples were transferred into a sterile **aluminum** foil to prevent contamination and then transported to Microbiology Laboratory Complex Madonna University, Elele Rivers state for bacterial analysis without further delay.

2.3 Preparation and Dilution of Sample for Analysis

The preparation and dilution of food homogenate **were** done using standard methods adopted by (Harrigan and MacCane, 1976). The sample was grated with a grater on **the workbench** aseptically. A gram of each sample was weighed into a sterile beaker containing ten **millilitres** (10ml) of peptone water to form the stock. Six more different sterile test **tubes** were arranged on a test tube rack and labelled in ascending order from 10^{-2} to 10^{-6} containing 9ml of peptone water for serial dilution. The stock was mixed thoroughly and 1ml was transferred into another test tube 10^{-2} containing 9ml of ~~distilled or~~ **(you said that you have used paptone water for serial dilution, is not it?)** peptone water using a pipette. 1ml was also transferred from the test tube 10^{-2} to the next and so on, till the last test tube 10^{-6} for serial dilution. Petri dishes were also arranged in duplicates representing the media, dilution factor used, and the date of work. 1ml of each sample was dispensed into each **duplicate**, the dilution factor. The media were poured **with** respect to the label on each plate, it was gently swirled and allowed to solidify. The bacterial plates were carefully packed and incubated at 37°C for 24hrs while, the fungal plate was incubated at room temperature for five days (Davies, 2002).

2.4 Enumeration of Bacterial and Fungal Count

The sample was cultured on various media using the pour plate technique. The aerobic count was determined using Nutrient agar (NA), the coliform count was determined using MacConkey agar, *Escherichia coli* was determined using Eosin methylene blue agar, while Fungal count was determined using Sabouraud Dextrose Agar. The samples were incubated at 37°C for 24hrs.

2.5 Isolation of Microorganism

After the incubation, single discrete colonies from the growth media plate was sub-cultured by streaking into a fresh growth media plate until pure isolates were obtained using Nutrient agar to serve as stock culture and stored in the refrigerator at 4°C.

2.6 Morphological and Cultural Examination

After the incubation, the macroscopic and microscopic characteristics of the microbial growth on various media were observed and recorded. The macroscopic observation involved examination of the colour, elevation form, margin, and surface growth of the organisms. Microscopic observation involved Gram staining procedure which was used to differentiate between Gram-positive from negative. (Cheesbrough, 2007).

2.7 Gram Staining Technique

Gram staining was done as described by Anele *et al.*(2021) “A loopful of water was placed in a grease-free sterile slide and then a portion of the organism was spread to make a smear. The smear was air-dried and heat-fixed. The smear was covered with crystal violet and allowed to stand for one minute, the stain was washed off and excess water was drained. The smear was covered with Gram’s iodine and allowed to stand for one minute. The excess iodine was drained off and rinsed gently. 75% alcohol was also used as a decolourizer and spread on the smear until the drops coming off the slide were a pale violet colour, for 20seconds. The slide was washed gently with water. The smear was counterstained with safranin for 120 seconds. It was washed with water and the smear was allowed to blot dry. A drop of the immersion oil was placed on the smear and the slide was viewed under the microscope at the oil immersion objective. Gram-positive cells appeared purple under the microscope and Gram-negative cells appeared pink or red under the microscope.

2.8 Biochemical test

The biochemical tests that were carried out were as follows: Indole test, Sugar fermentation test, Oxidase test, Citrate test, Catalase test, Methyl Red and Voges Proskauer test (MR/VP), Motility test, and Triple Sugar Iron test as described by (Cheebrough, 2007; Anele *et al.* 2021).

2.9 Statistical Analysis

The results obtained from this study were edited, coded, and subjected to different statistical investigations. Mean occurrence was determined for various samples. Analysis of variance (ANOVA) was used to determine the significance at 95% internal ANOVA (Anele *et al.*, 2021).

3. RESULTS

3.1 Mean count of microorganisms associated with Ready-to-eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state

Antelope bushmeat as shows in Table 1 had higher TAPC ($8.09 \pm 0.15 \log_{10}$ CFU/g), compared to Grasscutter ($7.62 \pm 0.9 \log_{10}$ CFU/g). CC and FC were however higher for Antelope meat (6.74 ± 0.17 and $4.03 \pm 0.54 \log_{10}$ CFU/g) compared to ~~CC and FC on~~ Grasscutter (6.46 ± 0.51 and $3.85 \pm 0.47 \log_{10}$ CFU/g), although the mean difference was statistically significant ($p < 0.05$).

3.2 Percentage distribution of microorganisms isolated from dried Ready-to-eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state

The bacterial and fungal count of the Ready-to-eat bushmeat as indicated in Table 2 presented *Staphylococcus aureus* (26.9%), *Pseudomonas spp* (23.1%), and *Bacillus spp* (30.8%) isolates for the investigated Antelope which had higher occurrence than Grasscutter samples, while *Escherichia coli* (25.0%) was higher in grasscutter than Antelope sample. The occurrence of *Aspergillus spp* and *Penicillium spp* were higher for Grasscutter samples

(57.1%) and (42.9%) respectively compared to the Antelope meat (55.6%) and (44.4%) respectively. There was a significant difference ($P < 0.005$).

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Table 1: Mean count of microorganisms associated with Ready-to-eat bushmeat sold at Rumuokoro market, Port Harcourt, Rivers state

Samples	TAPC (log₁₀ CFU/g)	CC (log₁₀ CFU/g)	EC (log₁₀ CFU/g)	FC (log₁₀ CFU/g)
Antelope	8.09±0.15	6.74±0.17	5.08±1.61	4.03±0.54
Grasscutter	7.62±0.19	6.46±0.51	4.83±1.53	3.85±0.47

Keys:

TAPC= Total Aerobic Plate Count

CC= Coliform Count

EC= *Escherichia coli*

FC= Fungal Count

Table 2: Percentage distribution of microorganisms isolated from dried Ready-to-eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state

Isolates	Antelope	Grasscutter	Total n° of isolates	Frequency of occurrence (%)
Bacteria				
<i>Staphylococcus</i> spp	7(26.9%)	4(25.0%)	11	28.9
<i>Pseudomonas</i> spp	6(23.1%)	2(12.5%)	8	21.1
<i>Bacillus</i> spp	8(30.8%)	3(18.8%)	11	28.9
<i>Escherichia coli</i>	5(19.2%)	3(25.0%)	8	21.1
Total	26	12	38	100
Fungi				
<i>Aspergillus</i> spp	4(57.1%)	5(55.6%)	9	52.6
<i>Penicillium</i> spp	3(42.9%)	4(44.4%)	7	47.4
Total	7	9	16	100

4. Discussion

From the result obtained, it was observed that microbial loads of samples obtained from Antelope were higher than the ones obtained from grasscutter. This shows that the microbial quality of grasscutter meat was relatively better when compared to Antelope meat. The high microbial count in Ready-to-eat bushmeat sold at Rumuokoro market in Rivers state is an indication of improper cooking and handling of Ready-to-eat bushmeat by producers and the retailers. Most bacteria and fungi isolated are soil pathogens and opportunistic pathogens. Similar values were obtained from (Jones *et al.*, 2011). The presence of *Staphylococcus* spp indicates that consumers of Ready-to-eat bushmeat sold at Rumuokoro market are at risk of contracting food-borne intoxication. The significant difference observed in Total aerobic plate count (TAPC) in Antelope when compared to Grasscutter could be as a result of improper handling by retailers. Similar findings were recorded by (Bryan *et al.*, 1997) who also investigated some bacterial counts like *E coli*, *Staphylococcus aureus*, *Bacillus* spp with yeast, and mold for this meat product, and it was as a result of inadequate hygienic practice by the producers and the retailers. Furthermore, additional bacterial contamination of raw material may occur at the selling site during cutting and chopping using the same material and products (Mosupye and Von Holy, 2000). In this regard, it was observed that the raw meats, as well as ingredient, were cutted, chopped using the same material and products (for the two lines underlined, this idea is already mentioned, it is a repetition). Numerous factors may be responsible for the spoilage of fresh ready-to-eat bushmeat, such as conditions of evisceration, and exposure to ambient temperature, and relative humidity. Macroscopic alterations like the presence of moisture, mould, maggots and a nauseating smell are often observed on the meat at the selling point to consumers (the authors did not mention the cold chain and the storage conditions). This situation can be explained by illegal practices by salesmen, which involve the injection of water into smoked bushmeat or soaking of smoked

bush meat in order to add volume to the dried muscle tissue. The amount of illegal, bushmeat entering has increased in recent years, with the increased demand for farmed bush meats. Consumption of bush meat can constitute a threat to public health because some of them are not properly preserved (Malaisse, 1982). In the developed world, studies of some bacteria that can be isolated from Ready-to-eat bush meat include the genus, *Lactobacillus*, *Acinetobacter*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Pseudomonas*, and *Leuconostoc*, etc. Some of the fungi that can also be isolated are *Aspergillus spp* and *Penicillium spp*. Transmission of enteric pathogens to humans through the consumption of contaminated food such as meat has been reported (David *et al.*, 1999). *Bacillus spp* and *Staphylococcus spp* are usually found in the environment and on people's hands which could be detrimental to the bushmeat and consumers when not managed adequately (Oliver *et al.*, 2009).

5. Conclusion

Due to the high cost of fresh meat, some people go for Ready- to- eat bush meat as an alternative source of meat and protein. Therefore, producers and wholesalers of dried bush meat and cooked bush meat should be careful in handling and storage to avoid further contamination as consumers of these meats could be at risk of serious health challenges. Thus the users of these meats should be careful not to consume these meats uncooked.

6. Recommendations

Since the preservation of ready-to-eat bush meat is by drying in smoking, cooking, or refrigerating method, hence the following recommendations should be maintained:

1. The bushmeat should be preserved in such a way that it is not in direct contact with soil to avoid contamination by soil microorganisms.
2. It should not be kept in a wet environment, as this could lead to microbial growth.

3. Handlers should always wash **hands** before and after handling as improper hand washing is the major cause of **food-borne** illness.
4. Consumers of such meat should learn food hygiene practices such as, soaking the meat in **a** warm salt solution, proper washing, and **well-cooked** before consumption

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