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Assessment of Microbiological Quality Associated with Ready- to- Eat Bush Meat Sold at Rumuokoro Market in Rivers State

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Authors' contributions

This work was performed in collaboration among all authors. Author IMI designed the study, performed the statistical analysis and managed the analyses of the study. Author BCA wrote the protocol and first draft of the manuscript. Author UAO managed the literature searches. All authors read and approved the final manuscript.

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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. Totally 24 samples were collected and analyzed 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 ten (10) fold serial dilution was used. *Staphylococcus* spp, *Pseudomonas* spp, *Bacillus* spp, and *Escherichia coli* were isolated. 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 grass-cutter so there was a significant difference (P <0.005). The occurrence of *Staphylococcus aureus* isolated from antelope (26.9%) was higher when compared to grass- cutter (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 is significantly difference (P<0.005) from grass cutter. But the occurrence of *Aspergillus*

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spp and *Penicillium* spp were higher in grass cutter 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 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 warm salt solution, proper washing and well cooked before consumption.

Keywords: Microbiological; bush-meat; food hygiene; grass-cutter; consumers.

1. INTRODUCTION

The forest and woodland are often referrred to as bush, so the wild animals derived from the bush are being hunted and consumed as bush-meat [1]. Bush- meat constitutes a vast array of species ranging from Donkeys, Leopard, Monkeys, Grass cutters (*Thryonomys, Swindenanns*), African elephant, Antelope (*Alcalaphinae*) [1].

Most Ready-to-eat bush meats 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 from microbial attack such as bacteria and fungi or toxic substances produced by these bacteria. It is posing serious health threat or danger to the individual or group of individuals who rely on this type of meat as a source of food, particularly when not adequately cooked before consumption [1].

Similarly, ready-to-eat bush meat 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. Bush-meat 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 [2]. Moreover, ready- to-eat bush-meat with high-fat content should not be kept for a long duration but utilized as soon as possible after cooking to avoid intensive rancidity [3].

Furthermore, these bush meat must be continuously examined for spoilage-related off-odor, which is the result of incorrect preparation and or drying of the meat. Bush-meat with signs of deterioration must be rigorously sorted out and not be cooked [2].

In the same vein, some crucial noticeable factors 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 [4-6]. 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 bacteria and fungi when not properly handled which subsequently could lead to food-borne illness/ diseases among consumers because these microbes are referred to as ubiquitous thereby causing deterioration to bush-meat reducing its acceptability and economic benefits to the humans [6]. When these microorganisms invade the bush- meat, it has the capacity of disfiguring the pleasant appearance of the bush-meat, changing the odor to that of offensive, and possibly changing the taste content to a soured taste which in turn would not be palatable to the consumer [4]. 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 bush-meat samples were used for this study. Twelve (12) each from two species of bush-meat 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 [7]. The samples were grated with a grater on the work bench aseptically. A gram of each sample was weighed into a sterile beaker containing ten mils (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 9mls 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 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 37^0C for 24hrs while, the fungal plate was incubated at room temperature for five days [8].

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 methlylene blue agar, while Fungal count was determined using Saboraud Dextrose Agar. The samples were incubated for 24hrs at 37^0C.

2.5 Isolation of Microorganism

After the incubation, single discreet colonies from the growth media plate were 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 4^0C.

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 color, elevation form, margin, and surface growth of the organisms. Microscopic observation involved gram staining procedure which is used to differentiate between Gram-positive from negative [9].

2.7 Gram Staining Technique

Gram staining was done as described by Anele et al. [10] “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

Indole test, Sugar fermentation test, Oxidase test, Citrate test, Catalase test, Methyl Red Voges Proskauer test (MRVP), Motility test, and Triple Sugar Iron test were some of the biochemical tests carried out as described by [9-10].

2.9 Statistical Analysis

The results obtained from this study were edited, coded and subjected to different statistical analysis. Mean occurrence was determined for various samples. Analysis of variance (ANOVA) was used to determine the significance at 95% confidence interval [10].

3. RESULTS

3.1 Mean Count of Microorganisms Associated with Ready-to- Eat Bush-Meat

Antelope bush meat as shows in Table 1 had higher TAPC (8.09± 0.15log10CFU/g), compared to Grass-cutter (7.62± 0.9log10CFU/g). Coliform Count (CC), and Fungal Count (FC) were however higher on Antelope meat (6.74± 0.17 and 4.03± 0.54log10cfu/g) compared to CC and FC on Grass-cutter (6.46± 0.51 and 3.85 ± 0.47log10cfu/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

The bacterial and fungal on the ready-to- eat bush meat as indicated in Table 2 presented *Staphylococcus aureus* (26.9%), *Pseudomonas* spp (23.1%) and *Bacillus* spp (30.8%) isolate on the investigated Antelope had higher occurrence than Grass-cutter samples, while *Escherichia coli* (25.0%) was higher in grass-cutter than Antelope sample occurrence of *Aspergillus* spp and *Penicillium* spp were higher on Grass cutter samples (57.1%) and (42.9%) respectively compared to the Antelope meat (55.6%) and (44.4%). There was a significant difference (P<0.005).

4. DISCUSSION

From the result obtained, it was observed that microbial loads on the sample obtained from Antelope were higher than the one obtained from the grass-cutter. This shows that the microbial quality of grass-cutter is relatively better when compared to Antelope. The high microbial count in ready – to- eat bush meat sold at Rumuokoro market in Rivers state is an indication of improper cooking and handling of ready-to-eat bush meat by producers and retailers. Most bacteria and fungi isolated are soil pathogens and opportunistic pathogens. Similar values were obtained from [11]. The presence of *Staphylococcus* spp indicates that consumers of ready- to – eat bush meat 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 Grass-cutter could be as a result of improper handling by retailers. Similar findings were recorded by [12] who also investigated some bacterial count like *E coli.Staphylococcus aureus*, *Bacillus* spp with yeast, and mold on 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

Table 1. Mean count of microorganisms associated with ready-to- eat bush- meat sold at Rumuokoro market, Port Harcourt, Rivers State

Samples	TAPC	CC	EC	FC
Antelope	8.09± 0.15	6.74±0.17	5.08±1.61	4.03± 0.54
Grass-cutter	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	Grass cutter	Total no of isolates	Frequency of occurrence (%)
Bacteria				
Staphylococcus spp	7(26.9%)	4(25.0%)	11	28.9
Pseudomonas spp	6(23.1%)	2(12.5%)	8	21.1
Bacillus spp	8(30.8%)	3(18.8%)	11	28.9
Escherichia coli	5(19.2%)	3(25.0%)	8	21.1
Total	**26**	**12**	**38**	**100**
Fungi				
Aspergillus spp	4(57.1%)	5(55.6%)	9	52.6
Penicillium spp	3(42.9%)	4(44.4%)	7	47.4
Total	**7**	**9**	**16**	**100**

may occur at the selling site during cutting and chopping using the same material and products [13]. In this regard, it was observed that the raw meats as well as an ingredient were cut, chopped using the same material and products. Numerous factors may be responsible for the spoilage of fresh ready- to-eat bush meat, such as conditions of evisceration, and exposure to ambient temperature and relative humidity. Macroscopic alterations like the presence of moisture, mold, maggots, and a nauseating smell are often observed on the meat at the selling point to consumers. This situation can be explained by illegal practices by salesmen, which involve the injection of water into smoked bush meat or soaking of smoked bush-meat to add volume to the dried muscle tissue. The amount of illegal, bush meat 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 [14]. 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 *Leuconostoe* 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 [4]. *Bacillus* spp and *Staphylococcus* spp are usually found in the environment and on people's hands which could be detrimental to the bush-meat and consumption when not managed adequately [2].

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 bush-meat 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 hand 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. James O, Sam AO. A fresh look at bush meat, Christ church, New Zealland. 2007;1- 11.
2. Oliver SP, Boor KJ, Murphy SC, Murinda SE. Food safety hazards associated with consumption of raw milk. Foodborne Pathogens and Disease. 2009;6(7):793-806.
3. Fa JE, Peres CA, Meeuwing J. Bush meat exploitation in tropical forests: an Intercontinential comparison. Conservation Biology. 2002;16:232-241.
4. David NS, Cole SS, Rodertson JK. Food Microbiology, 6th Edition. Wright and Son LTD, New York. 1999;10-25.
5. Murray PR. Principle of preservation of shelf stable dried meat product. American Society for Microbiology. 2005;7:1-52.
6. Willey JM, Sherwood LM, Woolverton CJ. Microbiology 7th Edition: New York; Mcgrraw Hill USA. 2007;476.
7. Harrigan UF, Maccan MF. Laboratory Method in Food and Diary Microbiology. Nostrand Coy Publishers New York, USA. 1976;17-21.
8. Davies G. Bush meat and International Development Conservation. Biology. 2002; 16(3):587-589.
9. Cheesbrough M. District Laboratory Practice in Tropical Countries, low price Edition, Part 2, United Kingdom, Cambridge University Press. 2007; 64-71.
10. Anele BC, Ikeh IM, Stanley HO. Isolation of Microbes Associated with Automated Teller Machine (ATM) keypads studied at

Rumuokoro Port Harcourt, Rivers state. South Asian Journal of Research in Microbiology. 2021;11(1):10-17.
11. Jones MJ, Tanya VN, Fonken DN, Mbagung CM. Microbiological and Nutritional qualities of dried meat product. The Journal of Food Technology in Africa. 2011;4:1-4.
12. Bryan FI, Jermini M, Schmitt R, Chilufya M, Mwanzana M, Matoba A, Mfume E, Chibiya H. Hazard associated with holding and reheating food at vending sites in a small town in Zambia. Journal of Food Protection. 1997;60:391-398.
13. Mosupye FM, Von Holy A. Microbiological hazard identification and exposure assessment of street food vending in Johannesbrug, South Africa. International Journal of Food Microbiology. 2000;61(2): 137-145.
14. Malaisse F, Parent G. Rodents of the Miombo woodland area: A nutritional and Ecological approach. Ecology of Food and Nutrition. 1982;11:211-216.

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