Morphological and Biochemical Characterization of Bacteria Isolated from Bush Meat Sold at Different Locations in Ado-Ekiti, Nigeria

OGUNLADE AYODELE OLUWAYEMISI, AFOLABI OMOLARA OLAJUMOKE,
JEJE OLUMUYIWA ABIDEMI

Department of Food Technology
The Federal Polytechnic Ado Ekiti, P.M B 5351, Ekiti state
NIGERIA

Abstract: The term "bush meat" stands for meat that has been sourced from wild animals and is meant for human consumption. Bush meat also include a large variety of wild species that are eaten as food. The microorganisms that ultimately bring about the spoilage in meat are either present at the time of slaughter, introduced by workmen, cutting tools, water and air during dressing. This study was aimed to carry out the morphological and biochemical characterization of bacteria isolated from bush meat sold at various locations in Ado-Ekiti. A total of 5 samples of bush meat were purchased from 5 different bush meat vendors in Ado-Ekiti (Aba Erinfun, Adebayo, Oja Oba, Odo-Ado and Poly junction), and thereafter transported to the laboratory in a sterile foil paper. The result showed that total aerobic count ranged from 1.0×10^5 to 9.20×10^3 Cfu/g. The bacterial species identified in the bush meat samples were *Salmonella typhi, Klebsiella pneumonia, Enterobacter aerogenes, Yersinia enterocolitica, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Proteus sp, Shigella sp, Micrococcus luteus* and *Pseudomonas aeruginosa*. The highest isolates were found in samples purchased from Odo Ado, while the lowest were found in samples purchased from poly junction in Ado Ekiti. The presence of these bacteria on the bush meat indicated that the bush meats are contaminated and can pose a serious threat to the health of consumers. Hence, efficient awareness should be carried out to educate meat handlers and consumers on the adverse effects of lack of proper personal and environmental hygiene and sanitation.

1 Introduction

Meat is animal's flesh that is used as food. Meat is considered as the most important and readily available source of animal protein consumed by humans. However, meat is the most perishable of all staple foods since it contains sufficient nutrient needed to support the growth of microorganisms (Huda *et al*., 2010). Bush meat includes a large variety of wild species that are eaten as food (Bennett and Robinson, 2000; Wilkie *et al*., 2005; Nasi *et al*., 2011). Vertebrates, however, contribute to almost all the bush meat consumed. As many as 129 wild vertebrate species are known to be traded and consumed in west and central Africa (Petrozzi *et al*., 2010). By class, mammals are the most common, followed by reptiles, birds and amphibians; mammals also dominate the bush meat trade in terms of individual animals and biomass sold. Bush meat is also a significant source of revenue for many forest families (Milner-Gulland *et al*., 2003). Consumers often consider bush meat as wholesome, safe alternative to commercially produced meat on sale at grocery stores. In some regions, it is preferred to farm-raised meats for its taste or based on the perception that industrial meats contain chemicals and additives. Moreover, bush meat also plays a special role in the cultural and spiritual identity of indigenous peoples (Siren, 2012). The microorganisms that ultimately bring about the spoilage in meat are either present at the time of slaughter, introduced by workmen, cutting tools, water and air during dressing. This study was therefore aimed at carrying out the

morphological and biochemical characterization of bacteria isolated from bush meat sold at various locations in Ado-Ekiti.

2. Materials and Methods

2.1 Collection of Samples

A total of five (5) bush meat samples were purchased from five different locations in Ado Ekiti viz: Aba Erinfum, Adebayo, Oja Oba, Odo-Ado and Poly junction. The Samples were labelled appropriately as sample A, B, C, D and E. The names of the market, date and time of collection were also indicated. Samples were transported in sterile polythene bags to the laboratory for analysis.

2.2 Sterilization of Materials and Disinfection of Working Area

All glass wares were washed with detergent and water and then rinsed thoroughly with distilled water and sterilized by dry heat in a hot air oven at a temperature of 160˚C for 2 hours. Pipettes and test tubes were plugged with absorbent cotton wool and wrapped with aluminium foil paper. Inoculating loops were flamed to red hot with the aid of Bunsen burner flame, and used immediately on cooling. Working surfaces were disinfected by swabbing using cotton wool soaked with 75% ethanol before and after any experiment.

2.3 Preparation and Sterilization of Culture Media

The media used for the isolation of the test organisms were nutrient agar. The media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121℃ for 15 min. It was allowed to cool to 45℃ before pouring into sterile petri dishes

2.4 Microbiological Analysis

Isolation of microorganism from the sample, determination of total viable counts (microbial load), direct and microscopic observation according to the method of Olutiola *et al*. (2000).

2.4.1 Identification of Bacteria Isolates

The bacterial isolates were identified by using morphological and biochemical tests such as coagulase test, catalase test, oxidase test, indole test, urea production, nitrate reductions, motility test, spore test, hydrogen sulphide production test, oxidase production, citrate utilization test, nitrate reduction test, Voges Proskauer and methyl red test. (Fawole and Oso, 2004) and Olutiola *et al*. (2000)

3 Results and Discussion

Table1: Conventional Biochemical Characterization of Bacteria Isolates of Bush meat purchased from Aba Erinfun in Ado – Ekiti.

S	G	Cat	Ind	Co	Oxid	M/R	Probable organisms
A₁	-	+	-	-	+	⊣	*Salmonella trphi*
A₂	-	+	-	-	+	⊣	*Salmonella typhi*
A₃	-	+	-	-	+	⊣	*Salmonella typhi*
A₄	-	+	-	-	-	⊣	*Shigella dysentery*
A₅	-	+	-	-	+	-	*Proteus mirabilis*
A₆	-	+	-	+	-	-	*Pseudomonas aeruginosa*
A₇	-	+	-	+	-	-	*Pseudomonas aeruginosa*
A₈	-	+	-	+	-	-	*Pseudomonas aeruginosa*
A₉	-	+	+	+	+	-	*Pseudomonas aeruginosa*

Key: + means positive, - means negative

Sample A- Bush meat from Erinfun Market

S-sample, G- Gram reaction, Cat- catalase, Ind-Indole, Co-Coagulase, Oxid-Oxidase, M/R-Methyl red

Table 2: Conventional Biochemical Characterization of Bacteria Isolates of bush meat purchased from Adebayo in Ado – Ekiti.

S	G	Cat	Ind	Co	Oxid	M/R	Probable organisms
B_1	-	+	+	-	+	+	*Escherichia coli*
B_2	-	+	-	-	+	+	*Escherichia coli*
B_3	-	+	-	-	+	+	*Salmonell typhi*
B_4	-	+	-	-	+	-	*Proteus mirabilis*
B_5	-	+	-	-	+	-	*Proteus mirabilis*
B_6	-	+	+	-	+	-	*Proteus mirabilis*

Key: + means positive, - means negative

Sample A- Bush meat from Adebayo

S-sample, G- Gram reaction, Cat- catalase, Ind-Indole

Co-Coagulase, Oxid-Oxidase, M/R- Methyl red

Table 3: Conventional Biochemical Characterization of Bacteria Isolates of bush meat purchased from Oja Oba in Ado – Ekiti.

S	G	Cat	Ind	Co	Oxid	M/R	Probale orgasms
C_1	-	+	+	-	+	+	*Escherichia coli*
C_2	-	+	+	-	+	+	*Escherichia coli*
C_3	-	+	-	+	-	-	*Pseudomonas aeruginosa*

Key: + means positive, - means negative

Sample A- Bush meat from Oja Oba

S-sample, G- Gram reaction, Cat- catalase, Ind-Indole

Co-Coagulase, Oxid-Oxidase, M/R- Methyl red

Table 4: show the Conventional Biochemical Characterization of Bacteria Isolates of bush meat purchased from Odo Ado in Ado – Ekiti.

S	G	Cat	Ind	Co	Oxid	M/R	Probable organisms
D_1	-	+	-	-	+	·	*Enterobacter aerogenes*
D_2	-	+	-	-	+	·	*Enterobacter aerogenes*
D_3	-	+	-	-	+	·	*Enterobacter aerogenes*
D_4	-	+	+	-	+	·	*Escherichia coli*

S	G	Cat	Ind	Co	Oxid	M/R	Probable organisms
D5	-	+	+	-	+	·	Escherichia coli
D6	-	+	-	-	+	·	Salmonella typhi
D7	-	+	-	-	+	·	Proteus mirabilis
D8	-	+	-	-	+		Proteus mirabilis
D9	-	+	-	-	+	·	Proteus mirabilis
D10	-	+	-	-	+	·	Proteus mirabilis
D11	-	+	-	+	-	·	Pseudomonas aeruginosa
D12	+	-	-	-		·	Enterococcus faecalis
D13	+	+	-	-		·	Staphylococcus aureus
D14	+	+	-	-		·	Staphylococcus aureus
D15	+	+	-			·	Staphylococcus aureus

Key: + means positive, - means negative

Sample A- Bush meat from Erinfun Market

S-sample, G- Gram reaction, Cat- catalase, Ind- Indole

Co-Coagulase, Oxid-Oxidase, M/R- Methyl red

Table 5: Conventional Biochemical Characterization of Bacteria Isolates of bush meat purchased from Poly junction in Ado – Ekiti.

S	G	Cat	Ind	Co	Oxid	M/R	Probable organisms
E1	-	+	+	-	+	+	Escherichia coli
E2	-	+	-	-	-	+	Shigella dysentery
E3	-	+	+	-	-	+	Shigella dysentery
E4	-	+	+	-	-	-	Yersinia enterocolitica
E5	-	+	-	-	+	-	Proteus mirabilis
E6	-	+	+	+	+	-	Aeromonas hydrophilia
E7	+	-	-	-	-	-	Enterococcus faecalis

Key: + means positive, - means negative

Sample A- Bush meat from Poly Junction

S-sample, G- Gram reaction, Cat- catalase, Ind-Indole

Co-Coagulase, Oxid-Oxidase, M/R- Methyl red

4. Discussion

The result of this study revealed that *Salmonella typhi, Klebsiella pneumonia, Enterobacter aerogenes, Yersinia enterocolitica, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Proteus spp, Shigella spp, Micrococcus luteus* and *Pseudomonas aeruginosa* were the common pathogenic bacteria found associated with bush meat sold in different locations in Ado-Ekiti. The presence of *Escherichia coli, Shigella spp* and *Salmonella typhi* were attributed to the contamination of the meat sample by man through handling and processing. Its main habitat is human and animals and found mostly in the nose, throat and skin of healthy individuals. The microorganisms

isolated in this study has been reported earlier by Adedeji *et al.* (2011) who isolated similar organisms from fresh shrimps sold at selected meat markets in southwest Nigeria. Okareh and Erhahon, (2015) reported that *Staphylococcus spp* has pathogenic strains which could cause food poisoning due to the heat stable *Staphylococcus* enterotoxin which is resistant to gastrointestinal enzymes. *S. aureus*, a normal flora of human skin and mucous membrane is one of the most common causes of boils, impetigo and folliculitis and in some cases, bacteraemia and infections of the bones and wounds (Herman *et al.,* 2011).

In a similar study carried out by Meenakshi (2010), *Bacillus cereus, staphylococcus aureus, Shigella spp* and *Streptococcus sp*. were all found to be associated with meat. During handling of meat, the natural flora of meat environment will be contaminated with organisms associated with man, such as *Staphylococcus aureus* which was isolated in this investigation can grow well at $30^0c – 37^0C$. In a related development Tomiće *et al.,* (2017), isolation and identification of *Staphylococcus aureus* and *Bacillus cereus* from the fresh meat also supports the outcome of this study.

5 Conclusion

In conclusion, there are serious safety concerns related to the consumption of meat and meat product because of the presence of pathogenic bacteria. However, several techniques exist in the prevention of the growth of pathogenic microorganisms during distribution and storage of processed meat. Huss *et al.,* (2000) observed that the hazards related to contamination, recontamination or survival of biological hazards during processing could be controlled by applying good manufacturing practice and good hygiene practice. The presence of the bacteria isolated on the bush meat indicated that the bush meats are contaminated and can pose a serious threat to the health of consumers. Hence, efficient awareness should be carried out to educate meat handlers and consumers on the adverse effects of lack of proper personal and environmental hygiene and sanitation.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Ogunlade, Ayodele collected the sample and carried out the research, Afolabi O.O and Jeje O.A carried out the experiment together with Ogunlade, Ayodele. They all harmonized the write up