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Bacterial Assessment of Retailed Smoked Edible Frog (*Hoplobatrachus occipitalis*)

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Abstract
This research work evaluated the bacteriological safety of smoked edible frog (Hoplobatrachus occipitalis) retailed in three selected markets in Ota metropolis, Ogun State, Nigeria. Three different markets namely Iju-Ota, Sango and Ijoko markets were visited and smoked edible frogs were procured. The study was conducted to determine if there is any potential risk of foodborne infection from consuming these smoked edible frogs by the populace. Microbiological analyses were carried out using nutrient Agar, mannitol salt agar, mac conkey agar, Salmonella shigella agar and eosin methylene blue agar (EMBA) and conventional methods were used to identify the bacteria isolated. The results revealed that samples from Sango market had the highest heterotrophic bacterial counts and ranged from 2.57 x 10^7 cfu/g to 3.47 x 10^7 cfu/g among the three markets. Total coliform counts ranged from 1.23 x 10^5 cfu/g to 2.04 x 10^5 cfu/g, total enterobacteriaceae counts ranged from 8.91 x 10^2 cfu/g to 1.29 x 10^3 cfu/g, total staphylococcal counts ranged from 1.82 x 10^5 cfu/g to 2.63 x 10^5 cfu/g and that of Salmonella shigella counts ranged from 8.71 x 10^2 cfu/g to 1.15 x 10^3 cfu/g. The bacterial isolates in this study belong to six genera. Presence of these bacterial isolates is highly suggestive that these products were prepared and sold under condition that would permit the survival and multiplication of various microorganisms. Proper processing and cooking of the smoked edible frog meat will get rid of these pathogens, thereby preventing foodborne infections.

Keywords: Analyses, Bacteria, Foodborne infection, Populace, Smoked Frog.

INTRODUCTION
The importance of meat to human cannot be overemphasized, as they are obtained from several sources and it serves as the major source of nutrient and vitamins to the body. Meats contain essential classes of food such as carbohydrates, proteins, fats, vitamins and minerals, they provide the nutritional requirements of man in the appropriate quantities (Agbidye *et al*., 2009). The provision of these nutritional quantities becomes a major problem in most developing countries such as Nigeria leading to under or malnutrition. However, the relative high cost of beef, the commonest meat and red meat health related problems, have now attracted the focus of research to other sources of meat or alternatives which would be less costly and easy to consume with little or no health risk (Paoletti, 2005).

In a view to reduce such menace in Nigeria, some lesser known animals which can serve as protein sources are studied for their nutritive and non-nutritive value for human consumption. One class of such known animals that could be considered for this purpose is the amphibian (Onadeko *et al*., 2011).

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Amphibians are one of the most threatened groups of vertebrates, with at least one-third of over 6,000 known species being threatened with extinction (Stuart, 2004). Many reasons are attributed to the decline of amphibians' species such as global warming, habitat destruction and modification, pollution, disease, etc. Over-exploitation, being one of the major causes is rarely mentioned while other causes have gained much research interest (Halliday, 2008).
Many species of Anuran (class Amphibia) are eaten in many parts of the world. Recently, there has been an increasing trend in the exportation of frogs from developing to the developed countries.

Frogs are now reared on large scale for both local consumption and for export in countries like Malaysia, Brazil, Indonesia, Mexico, France and USA. High consumption rates were reported in Italian and French restaurants and in holiday villages in Turkey (Baygar and Ozyur, 2010).

The reason for humans preferring frog over others meat is not really known but it may have something to do with their abundance. Normally, frog's meat is not only appreciated for its exquisite flavour and texture but as a source of protein of high biological value. Although in Brazil, frogs are marketed as whole carcass, frog meat is usually commercialised as fresh or frozen legs in the international market, with unfrozen legs obtaining higher prizes. Frogs have been used in the production of infant food (Rodrogues *et al.,* 2014).

A survey of edible amphibian species in South Western Nigeria showed farmers as being the highest consumers of frog followed by secondary school teachers and then housewives. They also eat not just *Hoplobatrachus occipitalis* but other species (Onadeko *et al*., 2011). Furthermore, in India, due to a rise in agricultural pests and mosquitoes, the processing and export of frogs was banned as they are used for biological control of these pests. Subsequently, the populations of frogs have largely increased and import of pesticides and insecticides has dropped by 40% (Mohneke *et al*., 2010).

Harmful microbes were isolated from *Hoplobatrachus occipitalis* in Igwuruta, Rivers state that could pose health implications to the consumers. They include *Escherichia coli, Salmonella typhi, Vibrio cholera and Shigella sp* (Ibietela and Awuzie, 2017). The most frequently isolated bacteria from frog sample were identified as *Micrococcus species, Staphylococcus aureus* and *Staphylococcus epidermis* while the most frequently isolated fungi were identified as *Aspergillus niger* and *Penicillium oxalicum.* (Ogoanah *et al,* 2017).

Therefore, processing and storage techniques often result in bacterial overgrowth in smoked frog. The presence of these bacteria in smoked frog cause a major hazard to human health as it is a commonly consumed product among average people. Therefore, this study is a further contribution to the knowledge of bacterial flora of smoked Frog and to create the awareness of the pathogen to would-be consumers in markets in Ota metropolis, Ogun State, Nigeria.

MATERIALS AND METHOD

Study Area

The study area is Ota in Ado-Odo, Ota Local Government Area of Ogun State, a town geographically located between latitude $6^{0}38^{1}$N to $6^{0}41^{1}$N and longitude $3^{0}8^{1}$E and $3^{0}12^{1}$E. The climate of the area is tropical and is characterized by rain forests vegetation.

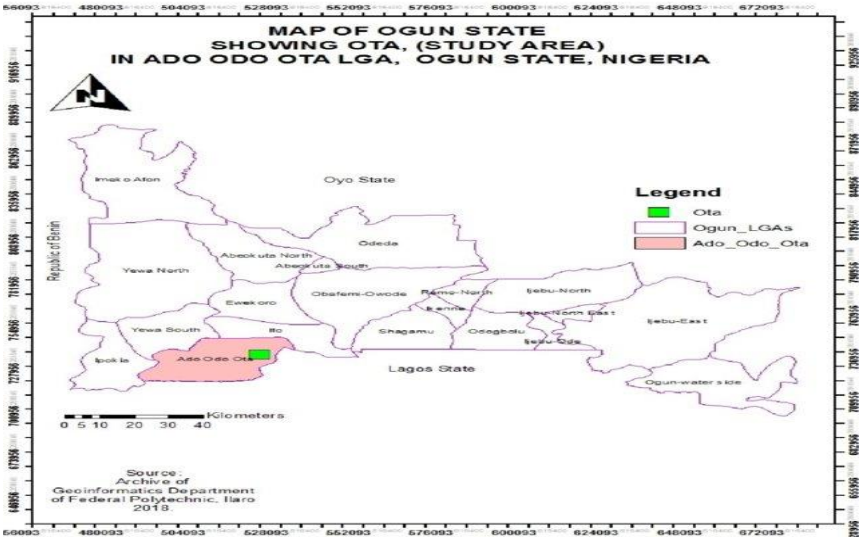

Figure 1: Map of Ota, Ogun State, Nigeria (Ajala, 2019)

Sample Collection

Ten smoked edible frog samples were collected at random in each of the three selected markets in Ota (Iju-Ota, Sango and Ijoko), making a total of thirty (30) samples which were transported to Microbiology laboratory in Bells University of Technology, Ota for analyses

Plate 1: Picture of samples of smoked edible frog

Microbiological Analytical Procedures

The method of microbiological analysis described by Da Silva *et al.,* (2018) was adopted for the analysis of the frog samples. Such microbiological parameters determined are Total Heterotrophic Bacterial Count (THBC),Total Staphylococcal Count (TSC), Total Enterobacteriaceae Count (TEBC), Total Coliform Count (TCC) and test for presence or absence of *Salmonella spp* respectively using Nutrient Agar (NA), Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMBA), MacConkey Agar (MCA) and Salmonella Shigella Agar (SSA).

Smoked Frog Sample Preparation for Analysis

The smoked frog samples were portioned into three: head (H), trunk (TR) and tail (TL) parts, each of which was aseptically pounded into pieces. Five (5) grams each of the three samples was weighed into 45ml of normal saline after which it was serially diluted to 10^{-5} (Douglas and Amuzie, 2017) by adding 1ml of the initial dilution to 9.0ml of normal saline. Using pour plate method, an aliquot of 1ml of 10^{-3} to 10^{-5} was added to each of the sample of the smoked frog; homogenate was then aseptically inoculated into separate petri dishes and freshly prepared molten cooled nutrient agar was added to each plate and were allowed to solidify. The inoculated plates were then inverted and incubated aerobically at 37^{0}C for 24 hours after

which the plates were examined for growth. The discrete colonies which developed were counted and the mean count were recorded as Total heterotrophic bacterial counts and expressed as colony forming unit per gram (cfu/g).

Data Presentation

Data collected on the microbial load of the smoked frog from the different markets were analysed for their central tendencies using descriptive statistics i.e. bar charts in figures. The mean of the values of the total microbial counts (cfu/g) are presented in tables.

RESULTS

The results of this investigation revealed that the value of the microbial counts in *Hoplobatrachus occipitalis* obtained from the three selected markets i.e. Iju-Ota, Sango and Ijoko markets in Ota metropolis showed differences among the markets, as presented in the bar charts in the figures.

Mean microbial loads observed in the head (H), trunk (TR) and leg (L) parts of the smoked edible frog samples purchased from the three selected markets in Ota are presented in this section.

Total Heterotrophic Bacterial counts (THBC) of smoked edible frog samples

The mean THBC of the Heads, Trunks and Legs of smoked edible frog samples of the three selected markets are shown in Table 1. The results ranged from the least value of 2.95×10^5 cfu/g (Trunk, Iju-Ota market) to the highest value of 3.47×10^7 cfu/g (Trunk and Leg, Sango market).

Table 1: Mean total heterotrophic bacterial counts (THBC) of smoked edible frog parts retailed in three selected markets

Part	Iju-Ota Market Counts (cfu/g)	Sango Market Counts (cfu/g)	Ijoko Market Counts (cfu/g)
Trunk	2.95×10^5	3.47×10^7	2.04×10^7
Head	2.63×10^6	2.57×10^7	2.29×10^7
Leg	6.03×10^5	3.47×10^7	1.23×10^7

Total Staphylococcal Counts (TSC) of smoked edible frog samples

The mean TSC values of the three parts of the smoked edible frog samples among the selected markets are presented in Table 2. The values ranged from 1.78×10^3 cfu/g (Trunk, Iju-Ota market) to 2.63×10^5 cfu/g (Trunk, Sango market).

Table 2: Mean total staphylococcal counts (TSC) of smoked edible frog parts retailed in three selected markets

Part	Iju-Ota Market Counts (cfu/g)	Sango Market Counts (cfu/g)	Ijoko Market Counts (cfu/g)
Trunk	1.78×10^3	2.63×10^5	1.95×10^5
Head	4.57×10^3	1.82×10^5	1.82×10^5
Leg	2.88×10^3	2.24×10^5	1.70×10^5

Total Coliform Counts (TCC) of smoked edible frog samples

The result showed differences in the mean TCC values of the smoked edible frog samples among the selected markets (Table 3). The trunk samples of Sango market showed the highest mean value of 2.04 x 10^5 cfu/g while the least value of 7.24 x 10^2 cfu/g was recorded in Iju-Ota market.

Table 3. Mean total coliform counts (TCC) of smoked edible frog parts retailed in three selected markets

Part	Iju-Ota Market Counts (cfu/g)	Sango Market Counts (cfu/g)	Ijoko Market Counts (cfu/g)
Trunk	7.24 x 10^2	2.04 x 10^5	1.91 x 10^5
Head	1.95 x 10^4	1.23 x 10^5	1.91 x 10^5
Leg	3.39 x 10^3	1.58 x 10^5	1.62 x 10^5

Total Enterobacteriaceae Counts (TEBC) of smoked edible frog samples

The mean TEBC values of the smoked edible frog samples of the three selected markets is presented in Figure 1. The trunk part sample from Iju-Ota market recorded the least value of 0.33 ± 0.12 log cfu/g while same trunk part from Sango market had the highest value of 3.11±0.01 log cfu/g.

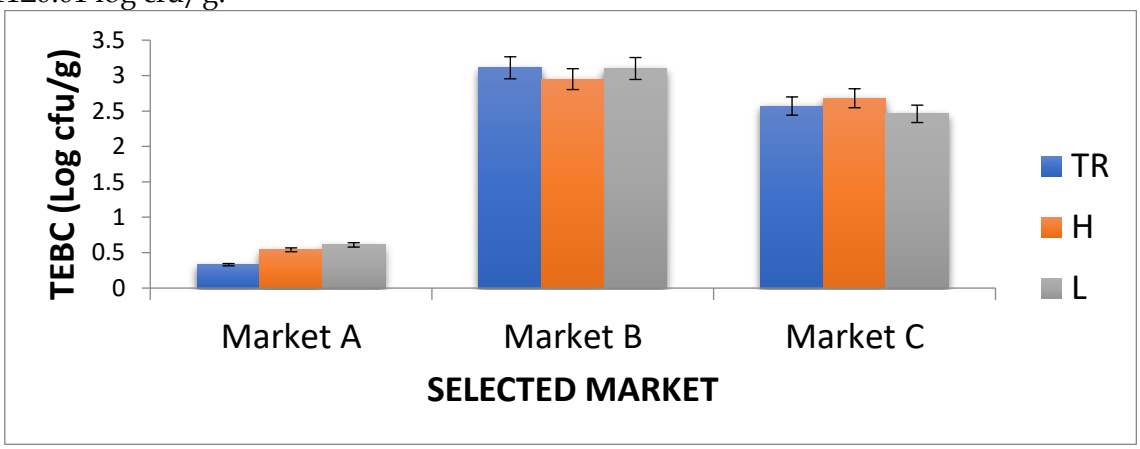

Figure 1: Mean Total Enterobacteriaceae Counts value of smoked edible frog parts retailed in three selected markets

Key: Market A=Iju-Ota; Market B=Sango; Market C=Ijoko; TR=Trunk part; H=Head part; L=Leg Part

Enumeration of Total *Salmonella shigella* Counts of smoked edible frog

There were differences in the smoked edible frog samples in terms of the the occurrence of *Salmonella*. Absence of *Salmonella* was only recorded in frog samples from Iju-Ota market. The other two markets (Sango and Ijoko) recorded significant growth of *Salmonella* in their samples, ranging from 2.36 ± 0.17 to 3.06 ± 0.12 log cfu/g (Figure 2).

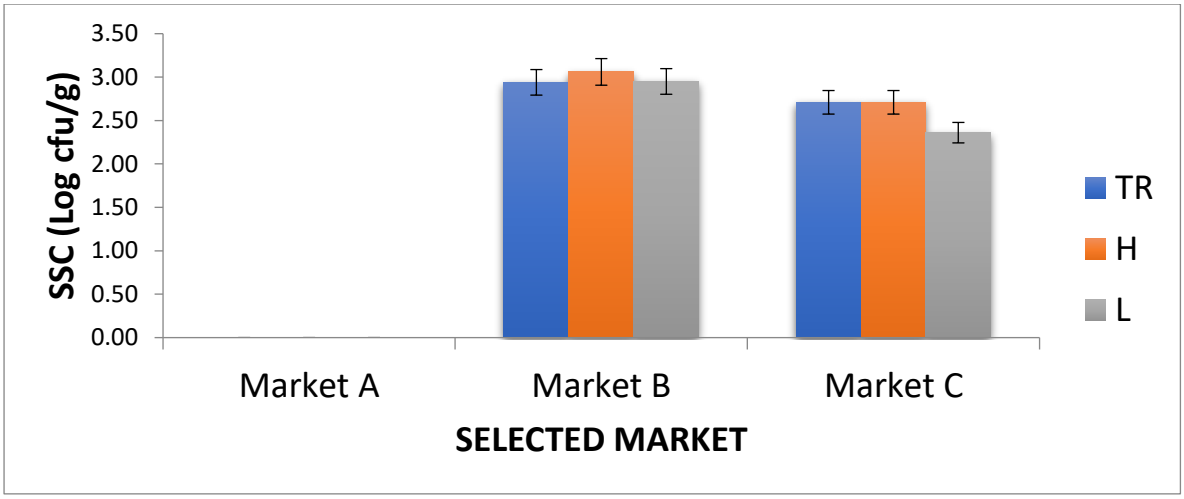

Figure 2: Mean Total *Salmonella shigella* Counts of smoked edible frog parts retailed in three selected markets
Key: Market A=Iju-Ota; Market B=Sango; Market C=Ijoko; TR=Trunk part; H=Head part; L=Leg Part

DISCUSSION

Microorganisms are ubiquitous and our foods including smoked–dried frogs are not excluded (Adewole and Olajubu, 2020). According to International Commission on Microbiological Specification for Food (ICMSF, 1986), good quality smoked edible frog should have counts of total bacteria of less than 10^7 per gram, the total staphylococcal counts should not exceed 10^4 per gram, *Salmonella spp* must not be detected in 25 grams of the product. Total enterobacteriaceae and total coliform counts should not exceed 10 grams and 100 grams respectively per sample. The high quantity of total bacterial, total enterobacteriaceae, total coliform as well as staphylococcal and salmonella colonies made the smoked edible frog samples especially from both Sango and Ijoko markets unacceptable, as the microbial counts was slightly above the ICMSF recommended limits and human may suffer from various diseases after consuming those smoked edible frogs.

Highest microbial growth was observed from nutrient agar for all samples while the least was observed on *Salmonella shigella* agar. This is because nutrient agar is a general purpose agar which allows growth of the various physiological groups present (Douglas and Amuzie, 2017). The results obtained in the study are highly suggestive that these products were prepared and sold under condition that would permit the survival and multiplication of various organisms.

In this study, the bacteria isolated from the smoked edible frog samples which cut across all samples from the three different markets include *Salmonella sp, Klebsiella sp, Staphylococcus aureus, Enterobacter sp, Bacillus sp,* and *Escherichia coli.* Meanwhile, Codjo *et al* (2022) reported the presence of formidable bacteria such as *Bacillus anthracis*, *Escherichia coli*, *Salmonella* sp., *Enterobacter* sp., *A. hydrophila* and *Staphylococcus* sp. in frog to justify the health risk to which these animals are exposed in the natural environment both for their breeding and for human consumption.

Meanwhile, the bacterial isolates identified from the smoked frog were members of both the gram-positive and gram-negative groups, which were also common flora/inhabitants of the soil and water environments. However, more gram-negative bacteria were isolated than gram-positive bacteria. This was in agreement with the work of Douglas and Amadi (2019) who isolated more gram-negative than gram-positive bacteria in edible frog. Most of the

organisms isolated are likely to be pathogenic since the origin is from man either as skin microorganisms or as coliforms, which have specific pathogenic tendencies (ability to cause diseases especially when consumed at high rates). Association of these organisms with smoked edible frogs is of great significant health importance as their presence suggested high level of contamination indicating the possible risks of foodborne infection and poisoning to human consumers (Douglas and Amuzie, 2017). In this study, *Escherichia coli* was one of the six genera of bacteria isolated and Ogbalu and Douglas (2016) reported a similar pathogenic serotype *Escherichia coli 0157* gotten from infected frog meat which resulted in colitis with bloody diarrhea and may produce hemolytic uremic syndrome. This has raised red flag concerning the available frog products in our markets.

Feacal coliforms in the frog samples is a natural occurrence and difficult to remove before preparation (Sukran *et al.,* 2009). This coupled with inadequate hygiene; mishandling, improper storage as well as poor transportation could lead to an increase in the microbial load of the smoked edible frogs. The aged, children as well as immune-compromised patients may be adversely affected with consumption of improperly processed smoked edible frog (*Hoplobatrachus occipitalis)* with great economic losses and loss of man-hours (Ibitela and Awuzie, 2017).

CONCLUSION

The study has revealed the presence of some bacteria associated with smoked edible frogs *(Hoplobatrachus occipitalis)*. The pathogens from this source of meat can be transmitted to man both actively and passively, since the smoked edible frog is veritable protein source.

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