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Isolation and Identification of Bacteria Associated with Suya (Roasted Meat Product) Sold in Dutsinma Local Government Area, Kastina State

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Authors' contributions

This work was carried out in collaboration between all authors. Author JBO designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author IM managed the analyses of the study. Author CGO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A study was carried out to isolate and identify Bacteria associated with suya (roasted meat product) sold in Dutsinma metropolis. Bacteriological analysis was carried out on the thirty (30) unspiced and thirty (30) spiced the samples collected from five (5) different retail outlets for identification and isolation using microscopy and biochemical test. The Prevalence of occurrence of the bacteria isolates was highest for *Escherichia coli* with 25.9%, *Staphylococcus epidermidis* with 24.5%, *Bacillus cereus* with 21.0%, *Klebsiella pneumoniae* with 11.8%, *Staphylococcus aureus* with 8.4% *Streptococcus faecalis* with 6.3%, and *Salmonella sp* with 2.1%. The mean aerobic plate count were in order of 10^6 (cfu/g) with the highest value for unspiced suya samples at 2.65 and that of spiced suya samples was 2.95. Occurrence of such organisms in ready-to –eat food constitutes a food safety issue which calls for urgent response in the education of suya producers on the hazards, Critical Control Points and the importance of personal hygiene and clean environment. Critical limits for the critical control points identified in this study are proposed.

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1. INTRODUCTION

Meat is an animal product. Meat is the flesh of animals which serves as food; there are different types of meat from different types of animals, *e.g.* pork meat (pig), mutton (sheep) and beef (cow). Generally, meat is excellent in supplying high quality protein, vitamins and mineral salts [1], thus they are essential for the growth, repair and maintenance of body cells which is necessary for our everyday activities. Consumption of meat could be traced back in history to the period when primitive man ate raw flesh of animals. But as man developed, he domesticated wild animals. Beef have been the major supply of meat in Nigeria as a result of extensive and semi-intensive cattle production system in Nigeria by Fulani and Hausa people of the Northern Nigeria [2].

Due to the chemical composition and characteristic, meat are highly perishable foods which provide an excellent medium for growth of many hazardous microorganisms that can cause infection in human and also lead to meat spoilage and economic loss. The most important bacterial meat spoilage is caused by lactic acid bacteria which is physiologically related group of fastidious and ubiquitous gram-positive organisms. These include many species such as *Lactobacillus, Leuconostoc, Pediococcus and Streptococcus species.*

In the world today, traditionally processed meat products are consumed in different countries, amongst which is the meat delicacy called 'Suya'. "Suya" is a traditional barbecue, smoked or roasted obtained from thinly sliced boneless meat and marinated with various spices such as clove, ginger, pepper, salt, peanut cake, vegetable oil as well as food additives and flavorings [3,4] enjoyed as a delicacy in West Africa [5]. It is a street-vended food which provides a source of inexpensive, convenient and often nutritious menu for cities, urban and rural areas; a major source of income for vast number of persons and creates opportunity for self-employment. It originated from the Hausa people of Northern Nigeria, and other countries surrounding Northern Nigeria like Chad, Sudan and Niger [6], as a result of extensive and semi-intensive cattle production system by the Fulani and Hausa people of Northern Nigeria [2]. This leads to the production of ready – to – eat beef products such as suya, kilishi, balangu and kundi. Suya is however the most popular as its consumption has extended to other part of the country [6].

However, the preparation process carried out under largely unhygienic conditions and the risk of contamination is very high. Since meat has a high nutritive value, microorganisms could easily grow on it. The possible sources of contamination are through slaughtering of sick animals, washing the meat with dirty water, improper handling by butchers, contamination by flies, processing close to sewage or refuse dumps sites, spices, transportation and use of contaminated equipment such as knife and other utensils [7]. The slaughtering process affords extensive contamination of sterile tissue with gram-negative enteric bacteria from animal intestine including *Salmonella species* and *Escherichia coli* as well as contaminant such as gram-positive *Lactic cocci* associated with humans, animals and the environment. *Enterococci* and *Clostridia* have been isolated from lymph node of red meat animals [8].

The high ultimate pH of meat generally makes it very susceptible for microbial growth even under the best handling or manufacturing conditions and practice [9]. Even meat gotten from freshly slaughtered, healthy animals which is supposed to have no, or very low microbial populations, could be contaminated to an unsafe level at the point of consumption. Microorganisms grow on meat causing visual, textural and organoleptic changes when they release metabolite. The smoke produced as a number of effects including preservative effect resulting from the deposition of organic compounds all presents in the smoked product (Suya meat). [10]. A preservative effect is also induced by the surface drying that occurs to the extent of 30% total weight loss in hot smoked product. Antioxidant effect is produced by the phenol deposited unto the product.

The microbial load in meat and meat product increases as long as growth conditions are favorable. The factor influencing microbial growth includes acidity, pH, temperature, water activity. Gaseous requirement, nutrient and competition of microbes for the nutrient. Controlling these factors implies maintaining long shelf life of meat and meat product but proper preservation of meat could be achieved by the combination of two or more preservation method which includes drying, salting and high temperature [11].

1.1 Aim and Objectives

It is imperative to note that the tremendous growth in the production and consumption of "Suya" in Nigeria has made it a great concern to study and to know its microbial quality. This study is therefore aimed at isolating and identifying bacteria associated with "Suya" sold in Dutsinma metropolis of Katsina state, Nigeria with the following objectives:

1. To isolate, microbial species associated with suya meat.
2. To identify the microbial species associated with the suya meat.

1.2 Definition of Terms

Suya: is originated from Northern part of Nigeria is grilled meat coated with ground chili pepper, peanut powder, and other local spices.

Kilishi: is made from meat that has been cut into very thin slices which is then spread out to dry. A special preparation of chili pepper, spices and local herbs is prepared into paste which is slightly brushed on both sides.

Balangu: refers to meat that has been grilled over wood/coal fire, specifically no seasoning is applied to bring out the natural flavor of the particular type of meat which may be goat, sheep or cow meat.

Kundi: is a relish intermediate moisture meat (dried meat) product, produced in the Northern part of Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Dutsin-Ma community of Katsina State. Dutsin-Ma is a local government area (LGA) in Katsina state, Nigeria. Its headquarters are in the town of Dutsin Ma. The local government has an area of 527 km^2 and a population of 69,671 at the 2006 census [12]. Dutsin-Ma became a local government in 1976. The inhabitants of the local government are predominantly Hausa and Fulani by tribe. Historically, their main occupation is farming and animal rearing. Currently other occupations found in the community include traders, vulcanizers, students, civil servants and self employed individuals.

2.2 Samples Collection

Sixty (60) skewers of suya meat were obtained randomly from suya vendors at popular suya spots from Wednesday market, Isa Kaita junction and Yarar Dole, Abuja road and Hospital road in Dutsinma LGA, with display of suya (spiced and unspiced samples) meats. The suya samples were wrapped in sterile aluminium foil papers to avoid further contamination and then transported to the Microbiology Laboratory of Federal University Dutsinma for microbial analysis.

2.3 Samples Preparation

A suya piece from each sample was removed from the skewers, aseptically cut into thin smaller pieces using sterile knife and mashed in a sterile laboratory type mortar and pestle. 1g of the mashed suya meat was weighed and several dilutions were achieved up to 5 fold (10-5) for each prepared samples using 1ml from stock homogenate and 9ml of sterile distilled water for the serial dilution experiment. This was carried out in order to obtain discrete colony.

2.4 Determination of Total Viable Count and Coliform Count

A five -fold serial dilution was made for the suya meat samples in appropriate dilution tubes. 0.1 ml of the homogenized sample was taken from 1-5 folds dilution and dispensed in sterile Petri dishes by pour plate method thus was allowed to settled and gel afterward inoculated plates were incubated at 370 c for 24 hours (If after 24 hrs, there is no growth, allow the incubation to 48 hrs) as described by [13]. The media of choice are MacConkey agar and nutrient agar. The MacConkey agar is a differential medium used in the differentiation of lactose fermenters though it grows on non lactose fermenters. Developed colonies were counted to obtain total viable count and coliform counts respectively. Discrete colonies were purified by sub-culturing into nutrient agar plates and were subsequently identified using standard methods [14].

2.5 Microscopy and Colonial Identification

Characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopically) for colonial appearance, size, elevation, form, edge, consistency, colour, odour,

opacity, haemolysis and pigmentation hence result was recorded. The isolates were characterized and identified based on their cultural characteristics, gram reaction and biochemical reaction as follows:

2.6 Gram Reaction

This was carried out to differentiate gram positive from gram-negative organisms. *Staphylococcus aureus* and *Escherichia coli* were used as control organisms.

2.7 Methods

A wire loop was sterilized in Bunsen burner and allowed to cool then a loopful of growth was collected from the agar plate and applied on a clean grease-free slide then a drop of normal saline was added, emulsfied and heat fixed by passing over a flame three times. The smear was flooded with crystal violet for 30-60 seconds and then covered with iodine for 30-60 seconds and then washed off; it was decolorized with acetone until no colour runs off the slide and rinsed immediately. The slide was covered with safranin for 1minute and then washed off with clean water. The slide was kept in a rack to air dry after wiping the back with cotton wool.

The stained smear was then examined microscopically under oil immersion at 100x objective lens. Gram positive bacteria appeared dark purple while gram-negative bacteria appeared red.

2.8 Data Presentation and Statistical Analysis

Colony forming unit (cfu/g) was used to estimate the number of viable bacteria cells in a sample and simple descriptive statistics was used to determine the prevalence rate of bacteria isolated.

3. RESULTS

3.1 Aerobic Plate Count from Samples in Wednesday Market

A total number of sixty (60) "suya" samples were collected from five (5) different retail outlets each within Wednesday Market, Isa Kaita/Yarar Dole and Hospital Road/ Abuja road areas of Dutsin-ma for bacteriological analysis. Thirty (30) out of the total number taken were collected with spice (spiced) while thirty (30) others were not spiced (unspiced). From this study, seven (7) different species of bacteria were isolated and identified in both the spiced and the unspiced "suya" samples with.

Table 1: shows the total aerobic plate count (tapc) in (cfu/g) for twenty (20) Wednesday Market, samples collected from the five (5) retail outlets within two weeks (aerobic plate count for both spiced and unspiced "suya" samples) and also relates the mean aerobic plate count (cfu/g) for both spiced and unspiced "suya" samples collected from five (5) different suya vendor from Wednesday market. The mean for aerobic plate count (cfu/g) was 2.55×10^6 for sample A, 2.45×10^6 for sample B, 2.65×10^6 for sample C, 2.55×10^6 for sample D, 2.65×10^6 for sample E. Sample C and E have the highest colony forming unit (cfu/g) followed by sample A, D and B while that of spiced was 2.95×10^6 for sample A, 2.89×10^6 for sample B, 2.85×10^6 for sample C, 2.86×10^6 for sample D, 2.88×10^6 for sample E. Sample A have the highest colony forming unit (cfu/g) followed by sample B, E and C.

3.2 Aerobic Plate Count from Sample at Isa Kaita Junction/Yarar Dole Junction

Table 2: shows the total aerobic plate count (tapc) in (cfu/g) for twenty (20) Isa Kaita Junction/Yarar Dole junction, sample collected from the five (5) retail outlets within two weeks (aerobic plate count for both spiced and unspiced "suya" samples) and also relates the mean aerobic plate count (cfu/g) for both spiced and unspiced "suya" samples collected from five (5) different suya vendor from Isa Kaita Junction/Yarar Dole junction aerobic plate count (cfu/g) was 2.1×10^6 for sample A, 2.2×10^6 for sample B, 1.75×10^6 for sample C, 2.3×10^6 for sample D, 2.35×10^6 for sample E. Sample E have the highest colony forming unit (cfu/g) followed by sample, D, B, A and C while that of spiced was 2.75×10^6 for sample A, 2.75×10^6 for sample B, 2.5×10^6 for sample C, 2.75×10^6 for sample D, 2.6×10^6 for sample E. sample A,C and B have the highest colony forming unit (cfu/g) followed by sample E, and D.

3.3 Aerobic Plate Count from Sample at Abuja Road/Hospital Road

Table 3: shows the total aerobic plate count (tapc) in (cfu/g) for twenty (20) Isa Kaita Junction/Yarar Dole junction, sample collected from the five (5) retail outlets within two weeks

(aerobic plate count for both spiced and unspiced "suya" samples) and also relates the mean aerobic plate count (cfu/g) for both spiced and unspiced "suya" samples collected from five (5) different suya vendor from Abuja road/Hospital road aerobic plate count (cfu/g) was 1.89×10^6 for sample A, 2.73×10^6 for sample B, 2.1×10^6 for sample C, 2.37×10^6 for sample D, 2.3×10^6 for sample E. Sample B have the highest colony forming unit (cfu/g) followed by sample, D, E, C and A while that of spiced was 2.73×10^6 for sample A, 2.41×10^6 for sample B, 1.88×10^6 for sample C, 2.8×10^6 for sample D, 2.68×10^6 for sample E. Sample A have the highest colony forming unit (cfu/g) followed by sample E,B, D and C.

Table 1. Total aerobic plate count (tapc) in (cfu/g) for twenty (20) "Wednesday market" samples collected from the five (5) retail outlets. For first week and second week

Retail outlet	Unspiced "Wednesday market" samples. (cfu/g)		Mean aerobic plate count (cfu/g)	Spiced "Wednesday market" samples. (cfu/g)		Mean aerobic plate count (cfu/g)
	Sample 1 First week	Sample 2 Second week	$\sum=\underline{x}$ n	Sample 1 First week	Sample 2 Second week	$\sum=\underline{x}$ n
Sample A	2.50×10^6	2.60×10^6	2.55×10^6	2.90×10^6	2.99×10^6	2.95×10^6
Sample B	2.20×10^6	2.69×10^6	2.45×10^6	2.84×10^6	2.94×10^6	2.89×10^6
Sample C	2.60×10^6	2.70×10^6	2.65×10^6	2.79×10^6	2.90×10^6	2.85×10^6
Sample D	2.30×10^6	2.80×10^6	2.55×10^6	2.90×10^6	2.82×10^6	2.86×10^6
Sample E	2.70×10^6	2.60×10^6	2.65×10^6	2.86×10^6	2.89×10^6	2.88×10^6

Table 2. Total aerobic plate count (tapc) in (cfu/g) for twenty (20) "Isa Kaita/Yarar Dole junction" samples collected from the five (5) retail outlets. For third week and fourth week

Retail outlet	Unspiced "Isa Kaita/Yarar Dole" samples		Mean aerobic plate count (cfu/g)	Spiced "Isa Kaita/Yarar Dole samples		Mean aerobic plate count (cfu/g)
	Sample Third week (cfu/g)	Sample 4 fourth week (cfu/g)	$\sum=\underline{x}$ n	Sample 3 Third week (cfu/g)	Sample 4 fourth week (cfu/g)	$\sum=\underline{x}$ n
Sample A	1.9×10^6	2.3×10^6	2.1×10^6	2.9×10^6	2.6×10^6	2.75×10^6
Sample B	2.5×10^6	1.9×10^6	2.2×10^6	2.7×10^6	2.8×10^6	2.75×10^6
Sample C	2.0×10^6	1.5×10^6	1.75×10^6	2.5×10^6	2.5×10^6	2.5×10^6
Sample D	2.2×10^6	2.4×10^6	2.3×10^6	2.9×10^6	2.6×10^6	2.75×10^6
Sample E	2.5×10^6	2.2×10^6	2.35×10^6	2.5×10^6	2.7×10^6	2.6×10^6

Table 3. Total aerobic plate count (tapc) in (cfu/g) for twenty (20) "Abuja road /Hospital road" samples collected from fifth week to sixth week

Retail outlet	Unspiced "Abuja road/Hospital road" samples		Mean aerobic plate count (cfu/g)	Spiced "Abuja road /Hospital road" samples		Mean aerobic plate count (cfu/g)
	Sample 5 fifth week (cfu/g)	Sample 6 Sixth week (cfu/g)	$\sum=\underline{x}$ n	Sample 5 fifth week (cfu/g)	Sample 6 Sixth week (cfu/g)	$\sum=\underline{x}$ n
Sample A	2.00×10^6	1.78×10^6	1.89×10^6	2.70×10^6	2.75×10^6	2.73×10^6
Sample B	2.13×10^6	1.97×10^6	2.73×10^6	2.14×10^6	2.68×10^6	2.41×10^6
Sample C	1.69×10^6	1.82×10^6	2.1×10^6	2.00×10^6	1.75×10^6	1.88×10^6
Sample D	2.10×10^6	2.63×10^6	2.37×10^6	2.90×10^6	2.70×10^6	2.8×10^6
Sample E	2.20×10^6	2.40×10^6	2.3×10^6	2.69×10^6	2.66×10^6	2.68×10^6

Table 4. Frequency of occurrence of bacterial isolates from various retail outlet in Dutsinma

Microorganisms	Number of occurrence				
	Wednesday market	Isa Kaita/Yarar Dole	Hospital Road/ Abuja road	Total	Percentage %
Staphylococcus aureus	5	3	4	12	**8.4**
Staphylococcus epidermidis	18	7	10	35	**24.5**
E.coli.	23	7	10	37	**25.9**
Bacillus sp.	18	4	8	30	**21.0**
Kleibsella pneumoniae	7	5	5	17	**11.8**
Streptococcus sp.	4	2	3	9	**6.3**
Salmonella sp.	2	Nil	1	3	**2.1**
Total				143	**100**

3.4 Frequency of Occurrence of Bacterial Isolates from Various Retail Outlets in Dutsinma

Table 4: relates the frequency of occurrence of each bacterium isolated in "suya" sample collected from each retail outlet and the overall percentage of the occurrence of each bacterium isolated and identified. Percentage of occurrence of bacterial isolated in relation to all the retail outlets is highest for *Escherichia coli* with 25.9% which probably may arise from the use of non portable water during washing of raw meat. This is also in agreement with the report of [2]. The presence of *Staphylococcus epidermidis* (24.5%) may be due to contamination from aerial spores carried in the air. This agrees with the report of cross contamination from meat handlers during processing, since it is normal flora of the skin [15]. *Staphylococcus aureus* was also found in the "suya" meat product due to poor personal hygiene. *Staphylococcus aureus* are found in human nose, throats, skin and 50-60% of normal people are carriers thus shows *8.4%* occurrence while *Salmonella sp, Klebsiella pneumoniae, Streptococcus sp* and *Bacillus Sp* shows 2.1%, 6.3%, 11.8% and 21.0% respectively. *Salmonella sp* can survive improper heating of suya meat product during processing. [16] stated that the presence of *Salmonella sp* as contaminant could be attributed to inadequate heating of meat product during its preparation.

4. DISCUSSION

In the present study, the microorganisms isolated were *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella sp, Klebsiella pneumoniae and Staphylococcus epidermidis*. The result is in accordance with the report of [17] which stated that microbiological analysis of meat samples in Awka, urban of Anambra State, indicating contamination of meat samples with various bacterial species including *Staphylococcus aureus,* and some enteric bacteria.

Prevalence of occurrence of bacteria isolated in relation to all the retail outlets is highest for *Escherichia coli* with 25.9% which probably may arise from the use of non portable water during washing of raw meat. This is also in agreement with the report of [2,18]. The presence of *Staphylococcus epidermidis* (24.5%) may be due to contamination from aerial spores carried in the air. This agrees with the report of cross contamination from meat handlers during processing, since it is normal flora of the skin, [15]. *Staphylococcus aureus* was also found in the "suya" meat product due to poor personal hygiene. *Staphylococcus aureus* are found in human nose, throats, skin and 50-60% of normal people are carriers thus shows *8.4%* occurrence while *Salmonella sp, Klebsiella pneumoniae, Streptococcus sp* and *Bacillus Sp* shows 2.1%, 6.3%, 11.8% and 21.0% respectively. *Salmonella sp* can survive improper heating of suya meat product during processing. [16] stated that the presence of *Salmonella sp.* as contaminant could be attributed to inadequate heating of meat product during its preparation.

From Table 3, the significant rise in bacterial load in both spiced and unspiced "suya" samples could be as a result of poor processing method, poor hygiene practices, improper and unhygienic handling of the meat product, bad sanitation operations and use of unclean utensils. Which agrees with [19] that one of the major sources of contamination arises from the handlers during preparation and display of meet product for sale. However an important factor which significantly contributes to the great increase in the count is the location of the retail outlets which are situated in motor parks where "suya" can be

easily contaminated by aerial spores or bacterial spores carried in the air and several other insects, such as flies.

The mean aerobic plate count for spiced "suya" samples is higher when compared to that of the unspiced "suya" samples for all the retail outlets which may be due to the additional contamination arising from the spices used, or poor handling of the spices during preparation, this is in consonance with [2] who stated that contamination of meat product could arise from spice and use of contaminated utensils, and that spice may also be heavily loaded with microorganism which serve as good source of contamination of meat products .

5. CONCLUSION

Some bacteria isolated and identified in the "suya" meat product are of public health importance thus their presence in such meat product continues to be considered as major causes of gastro-intestinal disorders, food poisoning and food borne diseases.

Considering the fact that suya meat constitutes a great source of protein which is needed for body building and repair of worn out tissue in human, adequate steps must be taken to prevent contamination and spoilage by microorganisms. Hence, improvement in the microbial quality of suya meat is very important.

The microbes isolated from the suya meat indicate that the standards of preparation and preservation have not improved much over the years and facilities used for preparation are not sterile.

6. RECOMMENDATIONS

Quality control unit should be established in meat processing industries in Nigeria and Hazard Analysis Critical Control Point (HACCP) concept should be applicable to the processing and renderings of meat and suya meat beef products .This will go a long way in reducing contamination and spoilage of meat products.

Proper animal husbandry, hygienic slaughter, adequate meat inspection, proper meat transportation sanitation of utensils and equipment, portable drinking water and proper storage of meat should all be employed to reduce microbial contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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