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BACTERIOLOGICAL EXAMINATION OF SUYA MEAT SOLD IN ENUGU METROPOLIS

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ABSTRACT
This study focused on the bacteriological analysis of roasted suya meat sold in Enugu metropolis. Twelve (12) samples of roasted fresh suya meat were purchased from roadside marketers from different locations in Enugu and were transported in sterile sample containers to the Microbiology laboratory of Godfrey Okoye University Enugu. The samples were cultured on Mac Conkey and Nutrient agar respectively after swabbing the surfaces of the fresh suya meat. Pure cultures of the isolates were carried out and their colony morphologies taken and then stored in nutrient agar slaunt. The isolates were then identified using Gram stain and other biochemical test. The isolates were identified as *Staphylococcus aureus* (35%), *Escherichia coli* (15%), *Streptococcus* species (15%), *Pseudomonas spp* (35%). The most frequently isolated organisms were *Staphylococcus and Pseudomonas*. The total viable bacterial counts ranged from $1.9x10^3 - 3.8x10^3$ cfu/g whereas, total coliform count ranged from $1.1x10^3$-$3.0x10^3$ cfu/g on Nutrient agar and Mac Conkey agar respectively. The result revealed that the hygienic condition of the meat (suya) was below acceptable standard for human consumption.

KEYWORDS: *Meat, E-coli*, *Staphylococcus aureus,* Mac Conkey agar, Enugu State.

INTODUCTION
Meat is a major source of protein and an important source of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of

body cells which is necessary for our everyday activities. Meat could be traced back to human history, then when primitive men use raw flesh of dead 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. (Umoh, 2004).

Suya meat is a boneless lean meat of mutton, beef, goat or chicken meat staked on sticks, coated with sauces, oiled and then roasted over wood using a fire from charcoal. It is a traditionally processed meat product and is usually not done with strict hygiene condition because they are still done locally. That is served hot and sold along streets, at clubs, picnics centers, and restaurants and within institutions. Suya meat is one of the intermediate moisture products that are easy to prepare and highly relished. Meat is a major source of protein and an important source of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of body cells which is necessary for our everyday activities. Meat could be traced back to human history, then when primitive men use raw flesh of dead 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. (Umoh, 2004). Suya meat, is a spicy skewered meat which is a popular food item in various parts of Nigeria (Eke et al, 2014). It is traditionally prepared by the Hausa people of northern Cameroon, Nigeria, Niger, and some parts of Sudan (where it is called agashe). Suya is generally made with skewered beef, ram, or chicken (AFP, 2012). The thinly sliced meat is marinated in various spices which include peanut cake, salt, vegetable oil and other flavorings, and then barbecued (egbebi and seidu, 2014). Suya meat is a boneless lean meat of mutton, beef, goat or chicken meat staked on sticks, coated with sauces, oiled and then roasted over wood using a fire from charcoal. It is a traditionally processed meat product and is usually not done with strict hygiene condition because they are still done locally. That is served hot and sold along streets, at clubs, picnics centers, and restaurants and within institutions. Suya meat is one of the intermediate moisture products that are easy to prepare and highly relished. 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, handling by butchers, contamination by flies, processing close to sewage or refuse dumps environment, spices, transportation and use of contaminated equipment such as knife and other utensils. (Igyor and *Uma*, 2005*)*. 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 (Lawries, 2000, Alexander *et al*. 1998). It has become very popular as a street delicacy in several countries, particularly those in West Africa (Inyang et al., 2005). Literature has it that microbial organisms isolated from "Suya" are of public health significance, as study conducted on "Suya" (dried smoked meat) sold in Ado and Akure, South West Nigeria revealed bacteria, molds, yeast and fungi (Egbebi and Seidu, 2011). Osho (2004) also evaluated the bacteria contamination of "Suya" processed in Abeokuta, South western Nigeria and found up to 103 cfu/g entero-bacteriaceae in 40% of the 622 samples collected; more than 104 cfu/g aerobic mesophiles including *Staphylococcus aureus* in all collected samples. Inyang *et al* (2005) also evaluated the bacterial quality of *Suya* sold in Markurdi, Northern Nigeria and concluded that feacal coliforms were the main bacterial contaminants although they occurred within acceptable limit. Meanwhile Edema *et al.,* (2008), who evaluated the microbial hazards associated with, processing of suya meat, reported that processing water, meat processing slabs, utensils, spices and raw meat revealed contamination with potential pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, Salmonellae species and aflatoxigenic molds with aerobic mesophilic counts in the order of 105 cfu with the highest value (7.17) observed in the packaging material and the lowest value (1.47) observed in the processing water. Microorganisms grow on meat causing visual, textural and organoleptic changes when they release metabolite (Jackson *et al*. 2001). A lot of factors affect the growth of microorganisms on meat. These factors include temperature, pH, water availability, presence of nutrients, moisture, acidity (intrinsic factors), gaseous requirement, atmosphere of storage (extrinsic factors) (Nester *et al. 2001*). This work is aimed at determining the microbial quality of suya meat sold in Enugu.To isolate, characterize and identify microbial species associated with suya meat and to offer useful information to the consuming public.

MATERIAL AND METHODS

All 12 samples of suya meat were collected at random at popular suya spots in Enugu. The suya meat samples were swabbed with sterile cotton swabs and re susppended in 9mls of distilled water for two folds serial dilution. The suya piece from each sample was removed from the skewers, 1g of the suya meat was weighed and then aseptically introduced into 9ml

of sterile distilled water, properly shaken and sieved before a twofold dilution was carried out. A two -fold serial dilution was made for the suya meat samples in appropriate dilution tubes. The media of choice are Mac Conkey agar and nutrient agar. The Mac Conkey agar is a differential medium used in the differentiation of lactose fermenters though it grows on non lactose fermenters. Nutrient agar is a supportive medium for the growth of most non-fastidious microorganisms and also used to enrich media with blood serum. 1ml of each dilution was pipetted and plated on nutrient agar and Mac Conkey agar using the spread method. Incubation was 37^0C for 24hours. Developed colonies were counted to obtain total viable count and coliform counts respectively. Discrete colonies were purified by subculturing into nutrient agar plates and were subsequently identified using standard methods. (Bichanan and Gibbo, 1974). The samples were inoculated aseptically with a wire loop on the prepared. Mac Conkey and Nutrient agar plates and incubated at 37°C between 18hours and 24hours. Then, the plates were read for growth of organisms.

The isolates were characterized and identified based on their cultural characteristics and biochemical reaction according to Buchanan, *et al* (1994). To differentiate gram position from gram-negative organisms a gram reaction test was carried out. *Staphylococcus aureus* and *Escherichia coli* were used as control organisms, 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, emulsified and heat fixed by passing over a flame three times. The smear was flooded with crystal violet for 30-60seconds and then covered with iodine for 30-60seconds 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. to differentiate those bacteria that produce enzyme catalase such as *Staphylococcus aureus* and *Escherichia coli* were used as positive and negative controls respectively. To differentiate those bacteria that produce enzyme catalase such as *Staphylococcus aureus* and *Escherichia coli* were used as positive and negative controls respectively a catalase test was carried out as follows; Three milliliters (3ml) of hydrogen peroxide solution was poured into a sterile test tube. Then a sterile glass rod was used to collect several colonies of the test organisms and inoculate into the hydrogen peroxide solution. It was observed for immediate active bubbling for positive

test. Coagulase test was used to identify *Staphylococcus aureus* which produces the coagulase enzyme which cause plasma to clot by converting fibrinogen to fibrin. The slide method was used. The method used was a drop of sterile distilled water was placed on each end of a sterile slide. Then a colony 3of the test organism was emulsified on each spot to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or clotting of the organisms within 10seconds. Plasma was not added to the second suspension which serves as control. Oxidase test was carried out to identify bacterial species that will produce the cytochromeoxidase enzyme. *Pseudomonas aeruginosa* and *Escherichia coli* were employed as positive and negative controls respectively. This followed a piece of filler paper was placed in a clean Petri dish and 2-3 drops of fresh or nascent oxidase reagent was added. A colony of test organism was collected using a glass rod and smeared on the filter paper and observed. Blue-purple color within few a seconds showed a positive test. For Urease test, the test was aimed at identifying *Enterobacteria* that produce urease enzyme, which hydrolyze urea to give ammonia and carbon dioxide. *Proteus* and *Salmonella* were used as control positively and negatively controls respectively. The procedure was that the test organism was heavily inoculated into Christensen's urea broth in a bijou bottle using a sterile wire loop and incubated at 35^{0}C- 37^{0}C for 18-24hours and examined, thereafter a pink color in the medium showed positive test. Citrate test was based on the ability of an organism to use citrate as its source of carbon. It was used to identify the *Enterobacteria*. The method involved Simon's citrate agar medium was prepared in a slant bijou bottle, and then using a sterile wire loop was used to inoculate the test organism onto the slant medium and incubated at 35^{0}C for 48hours after which it was examined for color formation. A bright blue color in the medium gave a positive citrate test. *Klebsiella pneumonia* and *Escherichia coli* were employed as positive and negative controls respectively. Vogues-poskauer test was used to identify members of the *Enterobactiaceae* that produce acetymythylcarbinol (acetone) a natural product formed from pyruvic acid in the course of glucose fermentation. Buffered glucose broth was inoculated with the test organism and incubated at 37^{0}C for 3days. Three milliliters (3ml) of nephtol was then added followed by 3ml of sodium hydroxide solution, mixed well and allowed to stand for 1hour at room temperature. The formation of a pink color in the medium within 1hour indicates a positive result. *Klebsiella pneumonia* and *Escherichia coli* were used as positive and negative controls respectively. The indole test was carried out for indole production by test organism which is important in identifying

enterobacteria. A sterile wire loop was used to inoculate a colony of test organism into 2ml of peptone water containing tryptophan. The tube was stoppered and incubated at 37^0C for 24hours. Kovac's reagent was added to the medium. Observation of red coloration on the surface layer within 10minutes showed a positive result. The carbohydrate fermentation test was used to determine the ability of bacteria to utilize different sugars. Examples are mannitol, glucose, lactose and sucrose. The method include, the four sugar solutions were prepared and poured into test tubes well stopped with Durham tube for gas collection. The sugar was autoclaved after which a loopful of test organisms was introduced into the sugar solution (Buchana and Caibbons. 1994). A change in color from pink to yellow shows fermentation and collection of gas bubbles in the Durham tube shows gas production which is a positive test. A control was set up without the organism inoculated. Methyl red test was carried out to identify *Enterobacteria* based on the ability to produce and maintain stable acid end product from glucose fermentation. *Escherichia coli* was used as positive control. Glucose phosphate peptone water was used for inoculation of test organisms and incubated for 48 hours at 37^0c after which few drops of methyl red solution was added to the culture and read immediately. Formation of red color ismmediately showed a positive test (Buchanan, *et al,* 1994).

RESULTS

Suya samples collected randomly were carefully analyzed for their microbial profile. The total via3ble count ranged from 1.9x10^3 – 3.8x10^3. Whereas total coliform count ranged from 1.1x10^3 -3.0x10^3 as shown in Table 1. The characterization and identification result is presented on Table 2. The isolates were identified as *Staphylococcus* (35%), *Pseudomonas* (35%), *Streptococcus* (15%) and *Escherichia coli* (15%). The most frequently isolated organism was *Staphylococcus and Pseudomonas* species.

Table 1: Total Viable And Coliform Counts (10^3 CFU/G).

S/N	MAcCONKEY AGAR (coliform x10^3)	NUTRIENTAGAR (total viable countx10^3)
1	1.1	1.9
2	1.2	2.0
3	1.3	2.1
4	1.4	2.2
5	1.5	2.3
6	2.0	2.4
7	2.3	2.5
8	2.6	3.2
9	2.9	3.4

10	3.0	3.8
11	2.8	3.2
12	1.7	2.4

Table 2: Characterisation/Identification Of Isolates.

Isolate	Gram reaction.	Shape	Indole	Citrate	Voges prauskaur	Methyl Red	Frutose	Oxidase	Urease	MannitolL	Catalase	Lactose	Glucose	Sucrose	PROBABLE ORGANISM
R_1	+	Cocci Cocci	-	+	+	-	-	-	-	+	+	+	+	A/G	*Staphylococcus spp*
R_2	+	Cocci	+	+	+	+	-	-	+	+	-	+	+	+	*Streptococcus spp*
R_3	-	rod	+	-	-	-	+	-	-	+	+	+	A/G	+	*E. coli*
R_4	-	rod	-	+	-	-	-	+	-	-	+	-	D	-	*Pseudomonas spp*
R_5	-	rod	+	-	-	-	+	-	-	+	+	+	A/G	+	*E. coli*
R_6	+	Cocci	-	+	+	-	-	-	-	+	+	+	+	A/G	*Staphylococcus spp*
R_7	-	Rods cocci	-	-	-	-	-	+	+	-	+	-	-	-	*Pseudomonas spp*
R_8	+	Cocci in cluster	-	+	+	-	-	-	-	+	-	+	+	A/G	*Staphylococcus spp*
R_9	-	Rods	+	+	-	-	-	+	+	-	+	-	+	-	*Pseudomonas spp*
R_{10}	+	Cocci in Chains	-	+	+	+	-	-	+	+	-	-	A/G	+	*Streptococcus spp*
R_{11}	-	Rod	+	-	-	-	+	-	-	+	+	+	A/G	+	*E. coli*
R_{12}	+	Cocci in cluster	-	-	+	-	-	-	-	+	+	+	+	A/G	*Staphylococcus spp*

KEYS: + Positive, **-** negative, A = acid production, G =gas production.

Table 3: Frequency of Occurrence of Isolates.

ISOLATE	FREQUENCY	PERCENTAGE (%)
Staphylococcus spp	6	35
Escherichia coli spp	4	16
Pseudomonas spp	6	35
Streptococcus spp	4	15
Total	**20**	**100**

DISCUSSION

Meat basically contains all the nutrients necessary for microbial growth and metabolism, making it susceptible to microbial contamination. In view of the microbial quality of meat and meat products, proper hygiene must be ascertained to ensure safety from infection after consumption of such products and to promote quality assurance. In the present study, the microorganisms isolated were *Staphylococcus* species, *Escherichia coli and Pseudomonas* species. The result was in consonance with the report of (Chukwura et al 2002) which stated that microbiological analysis of meat samples in Enugu State, indicated contamination of meat samples with various bacterial species including *Staphylococcus aureus* and some enteric bacteria. (Gilbert, 2001) also affirm that meat preserved with a certain amount of salt by so permit the growth of *Staphylococcus aureus* whereas, the presence of some members of the family of *Enterobacteriacea* family is due to contamination from intestine of slaughtered animals. Four organisms were isolated from the suya sample in view of the of the unhygienic condition of meat handling in Nigeria, the organisms isolated in this sturdy are the organisms usually implicated in meat spoilage could always be suspected in connection with meat contamination and spoilage.

The presence of *Staphylococcus* species agrees with the report of cross contamination from meat handlers during processing, since it is normal flora of the skin., (Gilbert et 2001). Raw meat is usually carried on the body by butcher in Nigeria due to lack of education Dada et *al* 1993, confirmed that coliform are introduced from the water used for washing the meat which of course is always contaminated. This is also in agreement with the report of Umoh (2004) that the also presence of *Escherichia coli* probably may arise from the use of non – portable water during washing of raw meat. The meat also showed presence of *Pseudomonas aeruginosa*, which usually occurs in soil, vegetation and surfaces of plants, humans and animals (Field, 2002). On the whole, the major sources of microbial contamination of suya meat appear to be handling by butchers and the use of contaminated water and equipment. So control of suya meat contamination can be achieved if aseptic techniques are employed during preparation of suya.

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