

## Microbial Population and Antibiotic Resistance in Suya and Kilishi Processed Meat in Lafia Nasarawa State, Nigeria

Agade, Y. I., Faith, E.A\*, Zanwa, I. A

Department of Animal Science, College of Agriculture Lafia, Nasarawa State, P.M.B. 33, Nasarawa State, Nigeria

### \*Corresponding author:

Faith, E.A

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**Abstract:** This study was conducted to isolate and identify microbial population and antibiotic resistance profile of suya and kilishi in Lafia Local Government Area of Nasarawa State, Nigeria. A total of sixteen beef samples (Suya =8) and (kilishi =8) were collected with swab stick and inoculated in 2mls of peptone water; then brought to the laboratory for culture. The 2mls of the various sample were then added to 10mls of peptone water and 1/10 dilution were made and sub-cultured on MacConkey agar and Eosin methylene blue agar (EMB agar) and incubated at 37°C for 24 hr. The plate were then read, for colony count and bacteria identification using Gram staining and biochemical reagent. The mean total coliform count between suya and kilishi beef sample examined were  $250 \times 10^4$  (cfu/ml) and  $470 \times 10^4$  (cfu/ml). Four strain of bacteria were isolated in the beef sample. The isolates were identified as *Klebsiella spp*, *Escherichia coli*, *Proteus spp* and *Pseudomonas spp*, by comparing their morphological and biochemical characteristics with standard reference organisms. Of the four bacteria isolates from both suya and kilishi sample analyzed, *Klebsiella spp* was the highest with 25% followed by both *Escherichia coli*, *Proteus spp* with 6.25% where as 0.00% was recorded for *Pseudomonas spp* respectively. The results of all the isolates were sensitive and resistance to different antibiotic used. All the isolates were sensitive to gentamicin, pefloxacin and ciprofloxacin and resistance to augmentin and amoxicillin. Aseptic techniques should be adequately employed in the meat industries so as to reduce microbial load of meat and its products for safe consumption by consumers and thus prevent food-borne diseases or infections.

**Keywords:** Microbial, culture, isolation, antibiotics, suya, kilishi.

### INTRODUCTION

Meat is an edible animal flesh which comprises principally the muscular tissue, and also includes internal organ called viscera such as heart, liver, kidney, intestine and bladder [1].

Food security is a complex issue, where animal proteins such as meats, meat products, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants [2]. Food borne infections and illnesses is a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide [3].

According to Yunusa [4] the chemical composition of meat varies considerably with age, species, degree of fatness of animal, and the part of carcass involved etc. Because of the enormous value of

meat in the diet, there exist large markets for meet and meat products worldwide at varying money value hence their demands increase day by day across the globe. Meat products are obtained when raw meat or preserved meat (cured meat) are altered in form by grinding, pressing, drying and other processes then augmented in flavour by smoking, spicing or blending with other food. These meat products are subjected to combination of several basic processing steps before reaching their final form. Therefore meat products are also termed as processed meat. Micro-organisms that occur in meat and meat products most times are responsible for food borne illness.

These micro-organisms are *Bacillus anthraxis*, *Clostridium butilism*, *Escherichia coli*, *Salmonellatyphii*, *Shigella sp*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Proteus vulgaris*, *Pseudomonas*, *Leuconostoc spp*, *Lactobacillus sp*,

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*Micrococcus* sp, *Mycobacterium tuberculosis*, *Vibrio* sp [5].

There exist different types of meat product ranging from the industrially processed corned beef, ham, bacon sausage to the indigenous Nigeria traditionally processed ready-to-eat meat product such as “*balangu*” (roasted meat), “*kilishi*, *suya* and many more”. Extrinsic factors such as temperature, relative humidity, O<sub>2</sub> availability and other factors including chemical and physical properties of food (meat) affect microbial growth. Meanwhile the processing of meat products includes those prepared product for consumption, increases the quality, texture, flavour, colour as well as reduce bacterial contaminant of meat used. On heating, frying or smoking, meat losses its moisture which results in increasing the concentration of other nutrients thus protein and fats are present in larger amount per unit weight in dried meat samples than in their fresh forms.

Suya is a spicy, traditional stick meat product that is commonly produced by the Hausas in Northern Nigeria from beef. Where rearing of cattle are an important pre-occupation and major source of livelihood for the people [6]. 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 [7]. Suya is a popular, traditionally processed, ready to eat Nigerian meat product, which may be served or sold along the streets, in club houses, at picnics, parties, restaurants and within institutions [8].

It is a mass consumer fast food. Its preparation and sales along the streets are usually not done under strict hygienic condition because they are still done locally with crude tools. On the other hand Kilishi (Nigerian beef cracker) is tropical intermediate moisture content meat product prepared from beef slices, infused in slurry of groundnut paste and spices and sundried. Kilishi is comparable to other beef crackers produced and eaten across the globe: (Somalia); qwanta-(Ethiopia); kaddia-(Middle East); jerky-North (America) and charque-(South America) [9]. The relative shelf stability of kilishi at room temperature makes it a popular rich nourishing snack [10]. The production of kilishi leaves the product dry, crispy and unfavorable for microbial proliferation and the end product would have a considerable shelf life if it is well packaged and kept sufficiently dry [11].

The Enterobacteriaceae group of bacteria is the most challenging bacterial contaminant to raw and processed meat products worldwide. *E. coli*, *Klebsiella* species are the most predominant species in all food poisoning cases associated with some meat products, Due to the rising incidence of food borne infections, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat products, it depends greatly on investigating the causative agents in food (meat products), eliminating them to ensure food safety and to protect public health from microbial contamination of food [12].

The sources of these contaminations have been linked to poor hygienic conditions of the handler and environment, raw meat, spices and package materials as well as cross that has led society to antibiotic resistance-a serious health problem world-wide which is now trying to be solved by many approaches [13].

Once the bacteria has produced toxin, the food can be extensively and properly cooked killing the bacterial without destroying their toxin. Many of the toxin are gene based that is carried on plasmid. The intensity of the sign and symptoms may vary with amount of contaminated food ingested and susceptibility of individual to toxins. This study was conducted to isolate and identify microbial population and antibiotic resistance profile of processed meat (suya and kilishi).

## MATERIALS AND METHODS

### Study Area

The study was conducted at the Suya and Kilishi selling spots in Lafia Local Government Area of Nasarawa State. The location lies within latitude 08° 33 North and longitude 08° 33 East at an attitude of 181.53m (570ft) above sea level with annual rainfall of 1311.75mm.

### Sample Collection

Different categories of meat samples: Suya (8) and kilishi (8) were collected with swab stick and inoculated in 2mls of peptone water; then brought to the laboratory for culture. The 2mls of the various sample were then added to 10mls of peptone water and 1/10 dilution were made and sub-cultured on MacConkey agar and Eosin methylene blue agar (EMB agar) and incubated at 37°C for 24 hr.

The plate were then read, for colony count and bacteria identification using Gram staining and biochemical reagent, such as indole, urease, oxidase etc.



**Plate-1: Roasted ready-to-eat (suya and kilishi) meat samples displayed on tray and newspaper with spice expose to the environment**

#### **Determination of total viable count and coliform count**

A two -fold serial dilution was made for the suya and kilishi meat samples in appropriate dilution tubes. The media of choice are MacConkey agar and nutrient agar. The MacConkey agar is a differential medium used in the differentiation of lactose fermenters although 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 MacConkey agar using the spread method. Incubation was at 37°C 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 [14].

#### **Procedure for Culturing Samples**

The samples were inoculated aseptically with a wire loop on the prepared. MacConkey and Nutrient agar plates and incubated at 37°C between 18hours and 24hours. Then, the plates were read for growth of organisms.

#### **Procedure for identification of the organisms**

The isolates were characterized and identified based on their cultural characteristics and biochemical reaction as follows:

#### **Gram reaction**

This was carried out to differentiate gram positive from gram-negative organisms. *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.

#### **Motility Test**

Motility test was aimed at identifying motile bacteria. A drop of normal saline was placed on a sterile slide and colony of test organism was suspended and emulsified and then covered with a cover slip. The slide was examined microscopically using 10x and 40x objective lens. Movement in different directions gave a positive test.

#### **Coagulase Test**

This 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.

A drop of sterile distilled water was placed on each end of a sterile slide. Then a colony of 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

This was carried out to identify bacterial species that will produce the cytochrome oxidase enzyme. *Pseudomonas aeruginosa* and *Escherichia coli* were employed as positive and negative controls respectively. A piece of filter 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 seconds showed a positive test.

### Urease Test

This 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 positive and negative controls respectively. The test organism was heavily inoculated onto Christensens urea broth in a bijou bottle using a sterile wire loop and incubated at 35 °C- 37°C for 18-24hours and examined, thereafter a pink color in the medium showed positive test.

### Indole Test

This 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°C for 24hours. Kovac's reagent was added to the medium. Observation of red coloration on

the surface layer within 10minutes showed a positive result.

### Methyl Red Test

This 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°C after which few drops of methyl red solution was added to the culture and read immediately. Formation of red color immediately showed a positive test.

### Statistical analysis

Data were analyzed using frequency and percentages of the statistical package for social sciences (SPSS version 22).

### RESULTS

Sixteen samples of beef; 8 samples from suya and 8 samples from kilishi in Lafia selling point in Nasarawa state Nigeria were analyzed microbiologically for the incidence of gram negative bacteria. Table-1 shows the total viable coliform counts in suya and kilishi on Nutrient agar and Mac Conkey agar. The mean total coliform count between suya and kilishi beef sample examined were  $250 \times 10^4$  (cfu/ml) and  $470 \times 10^4$  (cfu/ml) respectively.

**Table-1: Total viable coliform count in the beef samples collected**

Meat type	
Suya	$250 \times 10^4$ (cfu/ml)
Kilishi	$470 \times 10^4$ (cfu/ml)

Four strain of bacteria were isolated in the beef sample. The isolates were identified as *Klebsiella spp*, *Escherichia coli*, *Proteus spp* and *Pseudomonas spp*, by comparing their morphological and biochemical characteristics with standard reference organisms as presented in (Table-2). The suya sample recorded the growth of *Klebsiella spp* while the growth of *Escherichia coli*, *Proteus spp* and *Pseudomonas spp* were not observed in the suya samples studied. The growth of *Klebsiella sp*, *Escherichia coli*, *Proteus spp*

were identified in the kilishi whereas there was not any growth of *Pseudomonas spp* (Table-2).

The result of Table-2 also showed that of the four bacteria isolates from both suya and kilishi sample analyzed, *Klebsiella spp* was the highest with the percentage of 25% followed by both *Escherichia coli*, *Proteus spp* with the percentage of 6.25% where as 0.00% was recorded for *Pseudomonas spp* respectively.

**Table-2: Frequency and percentage distribution of the isolated bacteria from the examined samples**

Bacterial isolate	Suya	Kilishi	% of occurrence
<i>Klebsiella spp</i>	3	1	25.0
<i>Escherichia coli</i>	No growth	1	6.25
<i>Proteus spp</i>	No growth	1	6.25
<i>Pseudomonas</i>	No growth	No growth	0.00

Result of table-3 showed antibiotic resistance and sensitivity to the bacterial isolates of the beef sample. The results indicates that all the isolates were sensitive and resistance to different antibiotic used. All

the isolates were sensitive to gentamicin, Pefloxacin and ciprofloxacin while resistance to augmentin and amoxicillin.



**Table-3: Antibiotic sensitivity and resistance in the isolated bacterial species from the beef samples (suya and kilishi)**

Bacterial	Sensitivity	Intermediate	Resistance
<i>Klebsiella Spp</i>	PFX <sup>3+</sup>	=	AUG
	GEN <sup>3+</sup>	=	NIT
	CPLX <sup>3+</sup>	=	AMX
	TET <sup>2+</sup>	=	COT
<i>Escherichia coli</i>	COT <sup>3+</sup>	=	AMX
	CPFX <sup>3+</sup>	=	AUG
	TET <sup>3+</sup>	=	CRO
	PFX <sup>3+</sup>	=	
<i>Proteus spp</i>	GEN <sup>3+</sup>	=	
	OFLX <sup>3+</sup>	=	AMX
	CPFX <sup>3+</sup>	=	AUG
	TET <sup>3+</sup>	=	CRO
	PFX <sup>3+</sup>	=	NIT
<i>Pseudomonas spp</i>	GEN <sup>3+</sup>	=	
	GEN+ <sup>3+</sup>	=	COT
	OFLX <sup>3+</sup>	=	AMX
	CPFX <sup>3+</sup>	=	TET
	PFX <sup>3+</sup>	=	AUG
		=	CRO
		=	NIT

PFX =Pefloxacin, CPLX= Ciprofloxacin, GEN= Gentamicin, OFLX=Ofloxacin, AMX = Amoxicillin, CEF = Cefurantoin; COT = Cotrimoxazole; TET=Tetracycline; GEN = Gentamicin; NIT = Nitrofurantoin; AUG= Augmentin, CRO=Ceftriaxone,

## DISCUSSION

The results showed that the study of bacterial isolation and identification of meat were contaminated with four strains of pathogenic bacteria. The positive samples of meat isolate may have been as a result of human contamination, a uniform pattern of human-associated strains was not observed. This result is in agreement with other reports that *Pseudomonas spp* frequently are present in low numbers on raw meat surface which occurs infrequently [15].

The growth of *Klebsiella spp* observed in this present study was as a result of poor sanitation, handling of meat with unwashed hands, vegetable specie etc.

*Klebsiella spp* is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar.

*Klebsiella* is a common opportunistic pathogen for humans and other animals, as well as being resident or transient flora (particularly in the gastrointestinal tract). Other habitats include sewage, drinking water, soils, surface waters, industrial effluents, and vegetation. Until recently, almost all these *Klebsiella* have been identified as one species.

The problem may be attributed to a number of possible sources, including the natural resistance of

species to certain possible transfer of antibiotic resistance among species. The present study demonstrated that road side meat sample from suya vendors in Lafia Local Government of Nasarawa state were heavily contaminated with *klebsiella spp* than the kilishi. This represent a high level of contamination which indicates a potential breakdown of hygiene at various stages of the meat processing. *Klebsiella spp* is most likely transmitted by hand of the meat workers. Other also reported the *staphylococcus aureus* as a source of meat contamination [16] and this report is not in agreement with the present findings of this work as *staphylococcus aureus* was not isolated in this study. *Escherichia coli* and *Proteus spp* were the second most frequent meat borne pathogens isolated in this study from suya and kilishi. *E. coli*, which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. *E. coli* strain has previously been isolated from meat samples [17]. *E. coli* was identified in the kilishi beef samples examined although the bacterium was not identified in the suya beef sample analyzed. However, diarrhea caused by enterotoxigenic *E. coli* is highly prevalent in young children in developing countries as well as in travelers. It spreads through contaminated water and food [18]. The potentially high mortality associated with *E. coli* strain infection, therefore make its presence in any food material worrisome and of serious public health concern as most of the outbreaks recorded has been traced to consumption of beef contaminated with the *E. coli* strain [17].

The present study demonstrated that meat samples from butchers shop in Lafia Nasarawa state were contaminated with *E.coli* and *Proteus spp*, high contamination level in examined meat products may indicates unsanitary conditions of raw meat production from which it is produced. They are indicators of fecal pollution at slaughterhouse which begins from skinning and direct contact with knives and workers hands. Also, during evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses. Undercooked meat products have caused many meats poisoning incident associated with *E.coli* which is present in the faeces, intestine and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process. This result was remarkably different from those previously reported (78%) and (31%) [19, 20].

Four organisms were isolated from the suya and kilishi sample in view of the of the unhygienic condition of meat handling in Nigeria, the organisms isolated in this study are the organisms usually implicated in meat spoilage and could always be suspected in connection with meat contamination and spoilage. The meat (suya and kilishi) do not showed the presence of *Pseudomonas spp*, which usually occurs in soil, vegetation and surfaces of plants, humans and animals [17].

The results of all the isolates were sensitive and resistance to different antibiotic used. All the isolates were sensitive to gentamicin, pefloxacin and ciprofloxacin while resistance to augmentin and amoxicillin and this findings is in line with the report of Chen *et al.*, [21] who reported resistance to these panel of antibiotics tetracycline, ampicillin, nitrofurantoin and chloramphenicol in poultry meat in China and Nigeria respectively. All isolates of also exhibited antimicrobial resistance and sensitivity to a minimum of one antibiotics used in this study.

## CONCLUSION

Meat constitutes a great source of protein which is needed for body building and repair of worn out tissue in human. Improvement in the meat quality of meat is very important and adequate steps must be taken to prevent contamination and spoilage by microorganisms. The organisms isolated from the meat indicate that the standards of preparation and preservation have not improved much over the years and facilities used for preparation are not sterile. Aseptic techniques should be adequately employed in the meat industries so as to reduce microbial load of meat and its products for safe consumption by consumers and thus prevent food-borne diseases or infections.

## RECOMMENDATION

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 (suya and kilishi inclusive) as this will go a long way in reducing contamination and spoilage of meat products. Proper animal husbanding, 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. More research work should be done by professionals (microbiologist inclusive) and scientist in area of preservation of meat for a long time with shelf life of not less than one year, a breakthrough will encourage a long period of storage, thus preventing contamination as well as the caution use of antibiotics is recommended during treatments of life cattle as well as ensuring the withdrawal period before slaughter.

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