Journal of Advances in Biological and Basic Research 01[05] 2015
www.asdpub.com/index.php/jabbr

Original Article

Microbiological quality assessment of suya sold in Yenagoa Metropolis, Nigeria

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Keywords:
Food,
Microorganisms,
Suya,
Yenagoa metropolis

Abstract

This study assessed the microbial quality of suya sold in Yenagoa metropolis, Nigeria. Triplicate sample of suya were purchased from six communities in Yenagoa metropolis. Standard microbiological procedures were used for determining the microbial diversity and density. Results for the total heterotrophic bacteria, total coliform and total fungi ranged from 3.93 – 4.98, 2.94– 3.33 and 2.84 – 3.48 Log cfu/g, respectively. Analysis of variance showed that there were no significance differences (P>0.05) among the suya sample vended in some location in Yenagoa metropolis, Bayelsa state, Nigeria. Six and four bacterial and mould isolates were identified and among them the highest and least occurrence frequencies were S*taphylococcus aureus*(28.1%) and *Proteus* species (9.3%) for bacteria and *Aspergillus niger*(39.7%) and *Mucor* species (11.8%) for mould respectively. Other bacterial isolates include *Escherichia coli, Bacillus, Micrococcus* and *Pseudomonas* species and fungi isolates were *Aspergillus flavus* and *Penicillium* species. The implication of microbial diversity and density on potential consumers of suya in Yenagoa metropolis were discussed.

1. Introduction

Food is an essential resource needed for survival of the human body to enhance normal functioning of the system as well as growth [1]. As such food play essential role in human lives. Thus, a high level of food safety is needed to ensure safety from diseases or dangers that may come from foods [2]. Food substances provide protein, carbohydrate, vitamins, and minerals. Till date, most foods that humans consume are mainly from plants and animals (including aquatic and terrestrial life forms). Food are contaminated by microorganisms in public places such as schools, sidewalks, festival areas and others; since it is widely exposed to the air [2].

Specifically, meat which is derived from the flesh of animals are consumed for food purposes can be highly contaminated [3]. Meats consumed in developing countries like Nigeria are the from wild, which is often referred to as "bush meat" (viz. bush pig, guinea fowl, deer, antelope, rabbit, squirrel, rat) or domestic animals (such as sheep, cattle, goat, camel, chickens, turkey, ducks, pig, geese) [3]. Meat constitutes a significant amount of animal protein that is eaten. Similarly, human also obtain their protein sources from fisheries [4] and vegetation i.e. plants [3].

According to Olayinka and Sani [4], meat is the most perishable of all important foods and this is as a result of their chemical composition, which contain enough nutrients required for microbial growth and sufficient quantity of these constituents exist in fresh meat. Meat is a major source of high quality protein, fat, carbohydrate, vitamins and minerals and is delicious, palatable and easily digestible [6]. Hassan *et al*. [7], Nwakanma *et al.* [8] also described meat as major source of protein and important source of vitamins for most people in many parts of the world. Meat is essential for the growth, repair and maintenance of body cells which are necessary for everyday activities. Typically, the consumption of meat could be traced back in history to the period when primitive man ate raw flesh of animals and later developed the art of domestication of wild animals [7, 8]. Meat begins to deteriorate after slaughtering leading to chemical and physical changes [9]. Odey *et al*. [9] also noted that the initial microbial density plays a role in the determination of food product shelf-life.

In Nigeria, meat is processed into several forms including 'Tsire' or 'Suya', Kilishi, and Balangwu. Ready to eat food (including suya) processing is a major source of livelihood for people probably due to unemployment and failed family and community values [10]. This is because they do not require any further processing prior to consumption and are vented in public places especially in the evening between 6 – 10pm. Some of the popular locations where Suya is found include along streets, in club houses, restaurants, picnics and homes [11].

Suya originated from the Hausa people of northern Nigeria, where rearing of cattle is an important occupation. Suya is a boneless meat steak, coated with sauces, oiled and then roasted over wood using a fire from charcoal [12]. Suya from animals such as mutton, beef or goat or chicken are usually staked on sticks, coated with sauces, oiled and then roasted over wood hot charcoal [8, 11]. The meat used for suya production is typically trimmed from associated connective tissues, nerves and vessels [11]. According to Adenaike *et al*. [11], the meat is artificially sliced into very thin continuous sheets which are then cut into pieces and then staked on sticks, spiced with groundnut powder/flour, salt, vegetable oil and flavourings such as monosodium glutamate or others, before arranging them round hot charcoal for toasting.

The production of suya is mostly carried out in environments lacking quality control. The processing of suya in environment with poor sanitary condition could predispose its potential consumers to pathogens of public health importance. Suya could be contaminated by microbes in processing utensils, water and handling processes. Some of the microbes that could be introduced into the suya during handling could make it prone to microbial spoilage. The activities of microbes could lead to contamination of food and changes in physical and nutritional attributes of the suya.

Several studies have been conducted on suya vended in some states in Nigeria, including Lagos State [7, 13], Oyo state [14], Anambra state [12], Rivers state [9], Enugu state [8, 15], Ekiti and Ondo state [16], Benue state [17], Borno state [18], south western Nigeria [19]. Hence this study is focused on the microbial quality of suya vended in Yenagoa metropolis, Bayelsa state of Nigeria.

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2. Materials and methods

2.1. Field Sampling

Triplicate suya samples sold in five locations in Yenagoa metropolis (viz. Akenfa, Agudama-Epie, Edepie, Opolo, Kpansia, and Amarata) Bayelsa state, between 6:30 – 9:30pm were purchased. The suya samples were packaged in sterile Ziploc bag and analysis was carried out approximately 12 – 16 hours later.

2.2 Sample preparation

Twenty grams of each Suya sample was blended using blender (BLG-450, Binatone) in 180 ml of deionized and sterile water. The blender was washed and rinsed thrice using sterile and de-ionized water prior to re-use.

2.3 Enumeration microbial counts

Microbial counts were enumerated using three media i.e. Nutrient Agar (for total heterotrophic bacteria count), MacConkey Agar (for the enumeration of *Enterobacteriaceae* family), Potato dextrose agar (for mould and Yeast). The media were prepared according the manufacturers' instruction. Pour plate techniques described by Pepper and Gerba [20] and Benson [21] were used for total colony forming unit (TCFU). About 0.1 ml of each serial sample was plated in the various media. Agar plate for total heterotrophic bacteria count was incubated at 37°C for 24 – 48 hours; mould and yeast were incubated at 30°C for 3-4 days; bacteria of the *Enterobacteriaceae* family were incubated at 37ºC for 24hours. The colonies that grew on the various medium were counted and expressed as colony forming units (cfu)/g of the suya.

2.4 Microbial identification

2.4.1 Bacteria Identification

The biochemical tests were carried out using the guide of Cheesbrough [22] and Benson [21]. All the bacteria isolates were cultured on Nutrient Agar prior to use for biochemical tests (viz. gram reaction, motility, indole, catalase, coagulase, oxidase, urease, citrate and Litmus test). Thereafter, the resultant characteristics were compared with those of known taxa using scheme of Cheesbrough [22] and Bergey's Manual of Determinative Bacteriology by Holt *et al*. [23]. Based on gram reaction, the gram positive cocci organisms were streaked onto Mannitol Salt Agar (MSA) plate and incubated at 37°C for 24 hours. The presence of yellowish pigments in MSA indicated *Staphylococcus aureus*. Also, the pure cultures from MacConkey agar were streaked in Levine's Eosin Methylene Blue (EMB) Agar and incubated at 37º C for 24 hours. The presence of small nucleated colonies with greenish metallic sheen indicates *E. coli*[20, 21]. The presence of swarming growth on blood agar medium after incubation indicated *Proteus* species.

2.4.2 Microscopic identification of fungi procedures

Both microscopic and macroscopic techniques were employed for the identification of the mould. The microscopic morphology was determined using Lactophenol cotton blue stain as described by Pepper and Gerba[20] and Benson [21]. 0.lml of 95% ethanol was pipetted with the glass pipette and a fragment of well grown culture was carefully collected from the agar slant with wire loop into the ethanol placed on the glass slide were it was spread with the ethanol. It was allowed to dry by air for few minutes. 0.1ml of lactophenol blue stain was pipetted into the glass slide; cover slide was used to cover the glass slide before it was viewed microscopically. Macroscopic examination of the mould isolates were compared with pictures from Pepper and Gerba[20], Ellis *et al*. [24] and Benson [21].

2.5 Statistical Analysis

Statistical Package for Social Sciences (SPSS) software version 20 was used for the statistical analysis of the log transformed microbial counts. Descriptive statistics i.e. mean and standard error values were expressed. A one-way analysis of variance was carried out at $\alpha = 0.05$ and Tukey test statistics was used for mean separation.

3. Results

The microbial density of Suya sold in Yenagoa metropolis, Bayelsa state, Nigeria is presented in Table 1. The total heterotrophic bacteria, total coliform and total fungi ranged from 3.93 – 4.98 Log cfu/g, 2.94– 3.33 Log cfu/g and 2.84 – 3.48 Log cfu/g, respectively. There was no significance difference ($P>0.05$) among the suya sample vended in some locations in Yenagoa metropolis, Bayelsa state, Nigeria.

Table 1: Microbiological population of Suya sold in Yenagoa metropolis, Nigeria

Location	Total Heterotrophic Bacteria, Log cfu/g	Total coliform, Log cfu/g	Total Fungi, Log cfu/g
Akenfa	4.45±0.67a	2.94±0.30a	2.84±0.34a
Edepie	4.04±0.37a	2.96±0.50a	3.24±0.33a
Agudama-Epie	4.16±0.21a	3.26±0.34a	3.46±0.48a
Opolo	4.98±0.19a	3.16±0.40a	3.48±0.30a
Kpansia	3.93±0.10a	3.15±0.44a	3.07±0.25a
Amarata	4.73±0.61a	3.33±0.46a	3.29±0.49a

Each value is expressed as mean ± standard error (n = 3); The same alphabet along the column indicates that it is not significantly different at $P>0.05$ according to the Tukey HSD Statistics

The microbial isolates found in Suya sold in some location in Yenagoa metropolis, Nigeria is presented in Table 2. The isolates include *Staphylococcus aureus*, *Escherichia coli, Bacillus, Micrococcus, Pseudomonas* and *Proteus* species (bacteria) and *Aspergillus niger, Aspergillus flavus, Penicillium* and *Mucor* species (fungi). Among the bacterial diversity, *Staphylococcus aureus*(28.1%) and *Proteus* species (9.3%) has the highest and least occurrence frequency respectively (Figure 1). While for mould, *Aspergillus niger* (39.7%) and *Mucor* species (11.8%) had the highest and least occurrence frequency (Figure 2).

Table 2: Microbial isolates found in Suya sold in some location in Yenagoa metropolis, Nigeria

Microbes	Akenfa	Edepie	Agudama- Epie	Opolo	Kpansia	Amarata
Bacteria						
Escherichia coli	+	-	+	+	+	+
Bacillus species	+	+	-	+	+	-
Staphylococcus aureus	+	+	+	+	+	+
Psudomonas species	-	+	+	-	+	-
Micrococcus species	-	+	-	+	-	-
Proteus species	+	-	-	+	-	-
Fungi						
Aspergillus niger	+	+	+	-	+	+
Aspergillus flavus	-	+	-	+	-	+
Penicillium species	+	-	-	+	+	-
Mucor species	-	-	+	-	-	-

+ = Present; - = Absent

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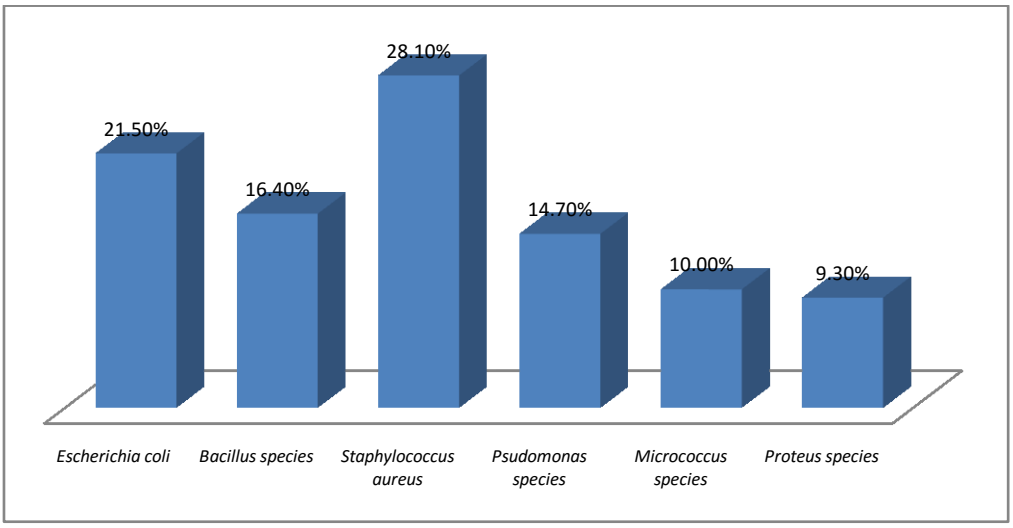

Figure 1: Cumulative frequency of occurrence of the bacteria isolates found in Suya sold in Yenagoa metropolis, Nigeria

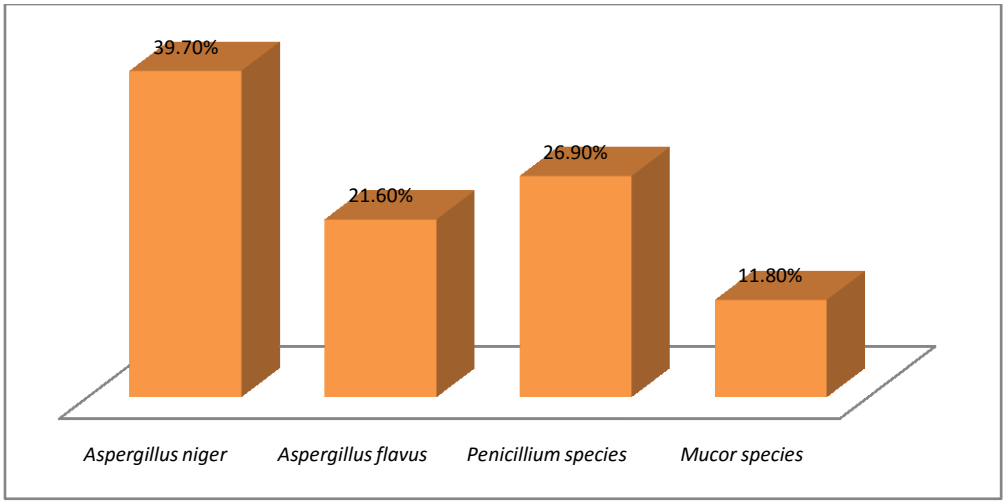

Figure 2: Cumulative frequency of occurrence of the mould isolates found in Suya sold in Yenagoa metropolis, Nigeria

4. Discussion

Based on Table 1, lack of variations in microbial population among the suya sample from different location in Yenagoa metropolis, suggests that the processors and handling prior to when the products get to the consumers indicated similar hygienic level and processing methods [25]. The microbial population is within the acceptable land tolerable limits for total aerobic bacteria and fungi counts. However, ICMSF [26] cited in Olopade *et al.* [27], Izah *et al.* [25] reported the limits for total aerobic bacteria and fungi counts in the order $\leq 10^3$ to be acceptable, 10^4 to 10^5 to be tolerable for ready to eat food. Also the authors reported that coliform should not be found in ready to eat food hence allowable limit of 0.00.

The microbial population reported in this study falls within the range previously reported in some Nigerian cities. For instance, Hassan *et al.* [7] reported microbial density of barbecue meat (Suya) from different location of Lagos State (i.e. Oshodi, Surulere Mushin, Ebute meta, Island, Ikeja, Ojota, Ketu, Ikorodu and Shomolu) in the range of 0.06 - 9.4 x 10^6cfu/g (Total Viable count), 0.05 – 6.35 x 10^5 Cfu/g (total coliform counts), 0.125 -2.6 x 10^6cfu/g (*Staphylococcus* counts) and 1.5 x 10^3 – 1.5x10^5 cfu/g (yeast/mould). Afolabi and Odubanjo[14] reported microbial counts from chicken and beef suya in the range of 0.70 – 1.50 x 10^6cfu/g (total viable count), 1.00 - 5.00 x 10^5 cfu/g (total coliform counts) and 1.00 - 7.00 x 10^5cfu/g (mould/yeast) from some suya spots at different locations in Oyo town, Oyo state. Onuorah *et al.* [12]) reported microbial counts from Tsire-Suya (a spicy, popular, ready-to-eat, boneless, beef product that is stacked on slender wooden sticks and cooked by roasting using a glowing fire from charcoal) sold in Awka, Anambra state in the range of 0.9 - 1.5 x 10^4cfu/g (total viable counts), 0.5 - 1.0 x 10^4cfu/g (total coliform counts) and 1.0 – 5.0 x 10^3cfu/g (faecal coliform counts). Odey *et al.* [9] reported total viable count for bacterial counts (1.4 - 2.3 x 10^5cfu/g), total coliform counts (1.0- 3.5 x10^5cfu/g) in meats (i.e. *Kilishi*, goat, beef and suya) sold in Calabar, Cross Rivers. Amadi *et al.* [28] reported aerobic plate count and total coliforms in Suya vented in Bori Metropolis, in Rivers state ranged from 0.24 – 1.39 x 10^5cfu/g and 2.7 – 6.2 x 10^4cfu/g respectively. Nwakanma*et al.* [8] reported bacterial counts and coliforms counts from Suya meat sold in Enugu metropolis in the range of 1.9- 3.8x 10^3cfu/g (total viable bacteria) and 1.1 -3.0x 10^5cfu/g (coliforms). Egbebi, and Seidu[16] reported bacteria and fungi counts as 0.30 –0.4 × 10^5cfu/g and 0.10 – 0.2 × 10^5cfu /g respectively in Ado Ekiti, Ekiti state and 0.3 – 0.85 × 10^5cfu/g and 0.10 – 0.22 × 10^5 cfu/g in Akure, Ondo state. Manyi *et al.* [17] reported aerobic colony counts, coliforms counts and mould and yeast counts in the range of 4.91 – 7.27 Log cfu/g, 2.33 – 4.32 and 2.05 – 4.35 Log cfu/g respectively in suya meat vented in Makurdi, Nigeria. Uzeh *et al.* [13] reported total viable count of tsire-suya as 7x 10^2 – 171 x 10^2cfu/g, staphylococcal counts as 1x10^2 - 12x10^2cfu/g and total coliforms counts as 1x$10^2$to 42x10^2cfu/g vended in some locations in Lagos. Ogbonna *et al.* [18] studied microbial density of suya consumed within Maiduguri metropolis and reported total viable counts (0.0 - 8.08 Log cfu/g); *Escherichia coli* counts (0.0- 5.48 Log cfu/g); Staphylococcal counts (0.0 -6.0 Log cfu/g); and yeast and mould counts (0.0-4.30 Log cfu/g). The level of microbial density found in suya could be due to nutrient level. Amadi *et al.* [28] reported that Suya is a rich source of essential

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nutrient for humans and for microbial growth and survival, thus its nutritional composition and characteristics makes it an excellent medium for microbial proliferation leading to deterioration and loss in its organoleptic properties. Again, the microbes may have entered the suya through spices used in its preparation. For instance, Shamsuddeen[29] reported mean aerobic plate counts, Staphylococcal counts, fungal counts and coliform as $2.96x10^8$, $1.73x10^7$, $1.04x10^5$cfu/g and MPN of >2400/g respectively in spices spice used in the production of *Kilishi*(a traditionally dried and grilled meat product).

Based on Table 2, Figure 1 and 2, the group of microbes identified from this study is comparable to the findings of other authors in different part of Nigeria. Hassan *et al*. [7] reported *Escherichia coli*, *Staphylococcus species*, *Pseudomonas sp, Clostridium septicum*, *Micrococcus* species and *Bacillus alvei* (bacteria), *Mucorracmosios, Geomyces panorus, Penicillum* species and *Aspergillus* species (fungi) as microbial isolates found in suya in some location of Lagos state. Afolabi and Odubanjo[14] reported *Bacillus*, *Escherichia*, *Pseudomonas* and *Staphylococcus* species (bacteria), *Aspergillus* and *Penicillium* species (fungi) as microbial isolates found in Suya sold at different suya spot in Oyo town, Oyo state. Onuorah *et al*. [12] reported *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Klebsiella aerogenes, Pseudomonas aeruginosa* and *Streptococcus pyogenes* as bacterial found in Tsire-Suya sold in Awka, Anambra state. Manyi *et al*. [17] reported *E.coli, Staphylococcus aureus, Salmonella, Shigella, Klebsiella, Streptococcus, Bacillus, Enterococcus* and *Pseudomonas* species (bacterial), *Aspergillus, Rhizopus, Penicillium* species and yeasts as microbes found in Suya vented in Makurdi metropolis, Benue state, Nigeria. Uzeh *et al* [13] reported the presence of *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. Egbebi and Seidu [16] reported *Aspergillus, Rizopus, Penicillium, Mucor* species (mould), *Rhodotorula, Candida, Saccharomyces* species (yeast), *Proteus*, *Salmonella, Staphylococcus, Streptococcus, Pseudomonas, Klebsiella, Bacillus* species and *E. coli* (bacteria) as microbes found in suya meat vented in Akure in Ondo state and Ado-Ekiti in Ekiti state. Edema *et al*. [19] reported the occurrence of *Bacillus cereus*, *Staphylococcus aureus*, Salmonellae and aflatoxigenic molds (*Aspergillus flavus* and *Aspergillus parasiticus*) from utensils and hands of suya processors during slicing, staking onto sticks, spicing and holding at ambient temperature in six *Suya*s pots in South-western Nigeria. Also, Shamsuddeen[29] reported the presence of *E. coli, Salmonella* species, *Staphylococci* and *Clostridium perfringens* in spice used in the production of Kilishi.

The frequency of occurrence of microbes in this study showed slight similarity with previous authors work. Afolabi and Odubanjo[14] reported the occurrence frequency of bacteria found in suya sold in Oyo town as 45% (*Bacillus* species) 15% (*Pseudomonas* species), 20% each (for *Staphylococcus* species and *Escherichia coli*) (bacteria), 66.7% (*Aspergillus* species) and 33.3% (*Penicillium* species) (fungi). Onuorah *et al* [12] also reported the frequency of bacterial occurrence in Tsire-Suya as 34.3% (*Escherichia coli*), *Staphylococcus aureus*(14.3%), *Bacillus cereus* (17.1%), *Klebsiella aerogenes* (11.4%), *Pseudomonas aeruginosa* (14.3%) and 8.6% (*Streptococcus pyogenes*). Enem and Onyekwodiri [15] reported the occurrence frequency of bacteria isolated from 'suya' sold from seven centers in Nsukka, Enugu state, as 37.2% (*Staphylococus* species), 26.6% (*Streptococus* species), and 14.8% (*Eschrichia coli*) 12.5% (*Bacillus* species) and 8.9% (*Salmonella* species). Odey *et al*. [9] reported bacterial isolated occurrence frequency found in Suya sold in Calabar, Cross Rivers state include *Staphylococcus aureus* (21.43%), *Escherichia coli* (14.20%), *Streptococcus* species (14.29%), *Salmonella* species (14.20%), *Bacillus* species (21.43%), *Pseudomonas* species (7.14%) and *Proteus* species (7.14%). Amadi *et al*. [28] reported *Escherichia coli* (16%), *Staphylococcus aureus*(20%), *Listeria Monocytogenes*(4%), *Bacillus subtilis*(4%), *Klebsiella pneumonia* (4%), *Staphylococcus epidermidis*(8%), *Streptococcus agalactiae*(8%), *Micrococcus luteus* (16%), *Shigella* species (8%), *Yersinia pestis*(12%) as microbes found in suya vented in Bori metropolis, Rivers state. Nwakanma *et al*. [8] reported *Staphylococcus aureus*(35%), *Escherichia coli* (15%), *Streptococcus* species (15%), *Pseudomonas spp*(35%) for bacterial isolates found in suya meat sold in Enugu metropolis.

Typically, the main sources of microbial contamination of suya meat appear to come from butchers and the use of contaminated water condiments and equipment [7, 8], post-processing contaminant or as a result of poor processing [13], use of dirty hands, table tops and dirty processing and other unhygienic practices [18]. Also, unwashed hand could be used in handling the suya, sneezing or coughing also occurs which could enter the product into the product to causing contamination [18].

The microorganisms isolated in this study were the organisms usually implicated in meat spoilage and unhygienic condition of meat handling [7]. Onuorah *et al*. [12] reported that the occurrence of these bacteria isolates in suya is risky to its consumer thus of public health especially *Escherichia coli, Bacillus*, *Staphylococcus aureus* and *Streptococcus pyogenes*, which are known to produce potent enterotoxins which when ingested via food can cause a sudden onset of illness within three to four hours, characterized by nausea, vomiting and diarrhea. Uzeh *et al*. [13] reported that the incidence of *Escherichia coli* in suya may be as a result of poor hygiene, while *S. aureus*and *B. cereus* have been implicated in food borne illnesses.

The presence of *Escherichia coli* suggests that the contaminants may be from faecal origins [30]. Ogbonna *et al*. [18] also reported that *E. coli* is used to assess the sanitary quality of food product, hence the presence in suya in high proportion is a challenge. *Staphylococcus aureus* produces toxins that could cause food poisoning and toxic shock [30]. Some of the diseases that could result from these microbes include bacteremia/septicemia, endocarditis (*Bacillus* species), and urinary tract infection (*Proteus* species) [1, 30, 31].

Mould detected in suya samples from Yenagoa metropolis is known to cause diseases because they can produce mycotoxins. For instance, *Aspergillus* species produces aflatoxins[1, 30]. These moulds reduce the biological value of the meat via enzymatic degradation of meat components) [5]. These typically occur through metabolic interactions with bacteria pathogens [5].

5. Conclusion

Meat is one of the most perishable sources of animal protein that is consumed by human irrespective of race, socio-economic and gender. In attempt to prevent deterioration and quest for convenient source of animal protein, animal flesh is processed into suya. Suya is a major meat processed from beef, cattle, goat, chicken etc. Suya is sold in several public places including, streets, markets, restaurants and public places during functions such as birthday and marriage ceremonies. This study evaluated the microbial quality of suya vended in Yenagoa metropolis, Bayelsa state, Nigeria. The microbial density showed that the suya sold in Yenagoa within the acceptable and tolerable limits for ready to eat food. While the presence of coliforms suggested that is unfit for consumption. The occurrence of microbes in the suya may have stemmed from handling, preservation strategies, and poor hygienic level from the slaughtering point to point of suya processing. With improved hygiene, the potentials risk of food borne disease associated with suya could be reduced.

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