Original Research Article

Molecular Characterization of Bacteria Associated with Vended Suya Meat in Port Harcourt

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ABSTRACT

The contamination of vended food with microorganisms especially pathogenic microbes is a public health hazard which could result to gastroenteritis. The aim of this study was to identify by molecular techniques bacteria associated with vended suya meat in part of Port Harcourt. Forty (40) ready to eat suya meat were randomly bought from 10 vendors across four locations: Rumuokoro, Rukpokwu, Nkpolu and Choba. Total heterotrophic bacteria and total coliform bacteria in samples were analyzed

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using standard microbiological techniques. Ranges of the total heterotrophic bacterial and total coliform bacterial counts of suya meat in the various locations were: Rumuokoro (1.00×10^5 to 2.78×10^6 and 0.00×10^4 to 1.35×10^5), Choba (8.1×10^5 to 2.73×10^6 and 9.0×10^4 to 1.75×10^6), Nkpolu (2.0×10^5 to 1.95×10^6 and 0.00×10^5 to 9.5×10^5 CFU/g) and Rukpokwu (1.30×10^5 to 7.95×10^5 and 0.00×10^5 to 7.55×10^5 CFU/g). There were significant differences ($p \leq 0.05$) in the THB and TCB counts across the vendors in the respective locations. Twenty-eight bacterial isolates: *Staphylococcus delphini, Staphylococcus lugdunensis, Bacillus subtilis, Staphylococcus pasteuri, Paenibacillus pectinilyticus, Lysinibacillus fusiforms, Bacillus aerius, Serratia nematodephila, Providencia alcalifaciens, Klebsiella singaporensis, Pseudomona aeruginosa, E. coli, Pseudomonas fluorescens* and *Proteus myxofaciens* were identified from the vended suya meat. The molecular characterization of 16S rRNA of the isolates showed 99-100% similarity to other species in the NCBI data base. The evolutionary distances computed were in agreement with the phylogenetic placement of the 16S

rRNA of the isolates *Providencia* and the *Bacillus* sp respectively and revealed a closely relatedness to *Providencia stuartii* and *Bacillus flexus respectively*. The 16S rRNA of *Bacillus, Pseudomonas* and *Lysinibacillus* sp revealed a closely relatedness to *Bacillus flexus, Pseudomonas aeruginosa* and *Lysinibacillus fusiformis.* The frequency of occurrence of bacterial isolates across the locations were: *Pseudomonas aeruginosa* (7.14), *Bacillus flexus* (7.14), *Bacillus* sp (14.29), *Staphylococcus* sp (14.29), *Staphylococcus lugdunensis* (10.71), *Proteus* sp (10.71), *Lynsibacillus macroides* (3.57), *E. coli* (10.71), *Serratia* sp (10.71), *Klebsiella* sp (7.14) and *Providencia alcalifaciens* (3.57). These bacterial genera could pose serious health challenge especially if they are consumed in quantities required to cause infections as many have been linked to cause gastroenteritis and other forms of infections. Proper hygiene compliance during preparation and packaging is recommended to eliminate or reduce microbial population and types.

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Key words: Suya, molecular characterization, Bacteria

Introduction

Suya is a spicy traditional stick meat product that is commonly produced by the Hausa's in Northern Nigeria, where rearing of cattle are an important pre-occupation and major source of livelihood for the people (Edema *et al.,* 2008). Igene and Mohammed (2008) opined that it is a popular, traditionally processed, ready to eat Nigerian meat product that could be served or sold along the streets, in club houses, at picnics, parties, restaurants and within institutions. Potential health risks are associated with contamination of street vended food by pathogens during handling and preparation stages. Vendors are often poorly educated, unlicensed, untrained in food hygiene and they work under crude unsafe conditions with little

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or no knowledge about the causes and dangers of food borne diseases (Barro *et al.,* 2007). This statement is supported by Vilar *et al.* (2000) who also opined that the preparation and sales of suya meat in the streets is done with little or no hygiene since they are mostly prepared with crude tools. The fact that there are sporadic cases of gastroenteritis and symptoms of food infection after consumption of suya by some individuals, indicates that the product constitutes food hazard risk (Odusote and Akinyanju, 2003; Inyang *et al*., 2005). Some of these microorganisms could arise from the normal flora or transient flora of the vendor since they rarely wash their hands, and materials such as plates and knifes are kept on tables that are not well cleaned. Sometimes, these microbes could arise from the ingredients, spices such as onions, tomatoes, peppers, etc which are packaged together with the suya meat before delivery to the consumer. According to Amala and Onwuli (2017), spices which have no known antimicrobial properties in the quantity or concentration used in packaging suya meat could be a direct source or contributor to the contamination of the suya meat. Also,in a previous study conducted by Igyor and Uma (2005) possible sources of contamination could be through slaughtering of sick animals, washing the meat with contaminated 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. Thus, consumption of the suya meat and these ingredients are considered one of the major causes of gastroenteritis (Amala and Onwuli, 2017). Local methods to monitor the safety and quality of meat have depended on regulatory inspection and sampling regimes, but these ways cannot guarantee total consumer protection unless 100% inspection and sampling are employed as this level of inspection is impractical for

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various economic and logistic reasons (Falegan *et al.,* 2017). Effective intervention to reduce contamination of beef begins with determining potential sources of contamination. Tissues under the hide of healthy cattle are usually sterile (Anderson, 2012), consequently, tissues become contaminated during the slaughtering process. Sources of meat contamination during slaughter maybe classified as those associated with the animal, processing practices, Abattoir facilities and employees. The extent to which Potential contamination sources become hazardous to public health depends on management and unpredictable events or factors. Even in the best managed slaughter facilities, contamination may still occur. Fortunately, most bacterial Colonies which have been isolated from beef have been non-pathogenic, although human pathogens such as *Salmonella, Campylobacter* and *Listeria* have been isolated (Dickson and Anderson, 2012). Due to the increased consumption of suya, there is a need to carryout regular microbiological quality assessment so as to determine the bacterial contamination and to avoid infection from its consumption. There is paucity of information concerning the bacterial load and molecular characterization of vended suya meat sold in Port Harcourt. Thus, this study was aimed at investigating the microbial quality of vended suya meat and characterization of the bacterial isolates using biochemical and molecular methods.

Materials and Method

Study Area

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The study was carried out in Obio-Akpor Local Government Area of Rivers State. The study area is heavily populated with numerous suya spots scattered across the four locations. The locations were Rumuokoro, Rukpokwu, Nkpolu and Choba with the following coordinates; 40° 52'01''N and 60^o59'51''E, 40° 53'48" N and 70° 00'05" E, 40° 52' 09" N and 60^o58'35" E, 40^o53' 55" and 60° 54' 21" E, respectively. The suya samples were collected randomly from 10 vendors in these four locations and the study was for a period of 3 months.

Sample Collection

A total of 40 suya meat samples were used for this study. Ten (10) samples were randomly bought from ten vendors in each location. The samples were collected in sterile sample containers to avoid contamination, labeled accordingly and transported to Microbiology Laboratory, Rivers State University, for analysis. Weekly sampling was carried out for a period of one month.

Enumeration and Isolation of Bacteria

Sterile forceps was used to transfer 10g of each sample into conical flask containing 90ml of sterile normal saline. The prepared stock solution (10^{-1} dilution) was agitated to dislodge the microbes attached to the meat. Ten-fold dilution was carried out serially until 10^{-6} dilution was achieved. Aliquot (0.1ml) of the 10^{-3} dilution was inoculated in duplicates onto the surface of prepared nutrient and MacConkey agar plates and plates were spread evenly using a sterile bent glass rod. The plates were incubated for 24 hours and after incubation, colonies were observed, counted and recorded. Discrete colonies were isolated based on their colonial differences. A sterile wire loop was used to pick discrete colonies and subcultured on freshly prepared nutrient agar plates. Subculturing of isolates were done repeatedly until pure isolates were obtained.

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Characterization and Identification of Isolates

The isolates were identified based on Morphological characteristics (Gram staining), biochemical tests and molecular method.

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Molecular Method

The method described by Robinson and Wemedo (2019) was used in identifying the bacterial isolates. In this method, 24 hours old cultures of the isolates were transferred separately into Luria Bertani (LB) medium and incubated for 24 hours. After incubation, five milliliters of the turbid overnight broth culture of the isolate in LB was spun at 14000rpm for 3 min. The cells were re-suspended in 500µl of normal saline and heated at 95 ^{o}C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro centrifuge tube and stored at -20 ^{o}C. The Nanodrop1000 spectrophotometer was used to quantify the extracted DNA. Amplification of the 16S rRNA was carried out according to the methods of Saitou and Nei (1987). The 27F and 1492R primers on ABI 9700 Applied Biosystems thermal cycler in a total volume of 25µl for 35 cycles were used to amplify the 16S rRNA of the rRNA genes of isolates. The PCR mix was composed of the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl). The forward and reverse primers at a concentration of 0.4M and the extracted DNA representing the template. The conditions of the PCR were adjusted: initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator. The BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa was used in sequencing. Phylogenic analysis was carried out by editing resulting sequences with the aid of the bioinformatics algorithm Trace edit tool

having downloaded similar sequences from the National Center for Biotechnology Information (NCBI) data base using BLASTN. Downloaded sequences were aligned using ClustalX and the evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

Statistical Analysis

The mean and standard deviation of the enumerated colonies were computed using SPSS (version 22). Two-way ANOVA was used in checking for significant difference while the Duncan was used in separating the means.

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Results

The total heterotrophic bacterial and total coliform count per gram of vended suya meat in Rumuokoro is presented in Table 1. Results showed that the total heterotrophic bacterial and total coliform count ranged from 1.00×10^5 to 2.78×10^6 and 0.00×10^4 to 1.35×10^5 CFU/g, respectively. The result also showed that the highest total heterotrophic bacterial load was recorded from suya meats in Vendors 10 while Vendors 7 had the least heterotrophic bacterial load. Vended suya meats in Vendors 1, 2, 5, 6 and 7 had no coliform load while coliforms were detected in vended suya meats in Vendors 3, 4, 8 and 9 of the Rumuokoro location.

The total heterotrophic bacterial and total coliform count per gram of vended suya meat in Choba location is presented in (Table 2). Results of the total heterotrophic bacterial load and total coliform load in this location ranged from 8.1×10^5 to 2.73×10^6 and 9.0×10^4 to 1.75×10^6 CFU/g, respectively. The highest total heterotrophic bacterial load was recorded in Vendors 6 while the highest coliform

load was recorded in Vendor 4. Vendor 10 had the least total heterotrophic bacterial load while Vendors 9 had the least coliform counts.

The result for the total heterotrophic bacterial and total coliform count per gram of vended suya meat in Nkpolu location is presented in Table 3. The results showed that the total heterotrophic bacterial load and total coliform ranged from 2.0×10^5 to 1.95×10^6 and 0.00×10^5 to 9.5×10^5 CFU/g. The results also showed that the highest total heterotrophic bacterial and coliform load was recorded in Vendors 5 and 9, respectively while the least total heterotrophic bacterial load was recorded in Vendor 3. Vendors 3, 4, 6 and 9 had no coliform counts.

The result for the total heterotrophic bacterial and total coliform count per gram of vended suya meat in Rukpokwu location is presented in Table 4. The results showed that the total heterotrophic bacterial load and total coliform counts ranged from 1.30×10^5 to 7.95×10^5 and 0.00×10^5 to 7.55×10^5 CFU/g. Vendor 5 had the highest bacterial load while Vendors 4 had the least bacterial load. The Vendors with no coliform counts were Vendors 2, 5, 6 and 7, while Vendors 3 had the highest coliform counts.

Table 1: Microbial Load (CFU/g) of Vended Suya Meat in Rumuokoro

Vendors	THB ($\times 10^5$)	TCC ($\times 10^4$)
1	2.15 ± 1.06^{ab}	$0.00 \pm .00^a$
2	3.75 ± 9.19^b	$0.00 \pm .00^a$
3	27.80 ± 1.06^e	13.5 ± 1.63^b
4	2.05 ± 3.54^{ab}	7.00 ± 4.24^b

5	1.50 ± 4.24^{a}	$0.00\pm.00^{a}$
6	1.60 ± 4.24^{a}	$0.00\pm.00^{a}$
7	1.00 ± 1.41^{a}	$0.00\pm.00^{a}$
8	19.60 ± 9.89^{d}	5.00 ± 2.82^{b}
9	16.50 ± 1.55^{c}	5.00 ± 4.24^{b}
10	27.80 ± 9.89^{e}	6.00 ± 4.24^{b}

*Means with same superscript down the column show no significant difference $(P \leq .05)$

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Table 2: Microbial Load (CFU/g) of Vended Suya Meat in Choba

Vendors	THB ($\times10^{6}$)	TCC ($\times10^{5}$)
1	2.05 ± 2.12^{b}	6.60 ± 1.41^{d}

2	2.05±3.54 [b]	5.85±1.91 [cd]
3	2.06±4.24 [b]	2.75±2.05 [ab]
4	2.20±5.73 [bc]	17.5±6.36 [e]
5	1.88±2.33 [b]	2.00±1.34 [e]
6	2.73±1.62 [d]	3.30±1.84 [abc]
7	2.66±1.48 [cd]	1.50±1.41 [ab]
8	1.87±8.48 [b]	1.20±1.41 [a]

9	2.08±9.19 [b]	0.90±1.41 [a]
10	0.81±3.54[a]	4.25±7.07 [bcd]

*Means with same superscript down the column show no significant difference ($P \leq .05$)

Table 3: Microbial Load (CFU/g) of Vended Suya Meat in Nkpolu

Vendors	THB ($\times 10^6$)	TCC ($\times 10^5$)
1	1.55±0.07[bcd]	5.50±0.21[d]
2	1.35±0.92[abcd]	4.50±0.21[cd]
3	0.20±0.14[a]	0.00±0.00[a]

4	0.30 ± 0.14^{ab}	0.00 ± 0.00^{a}
5	1.95 ± 0.76^{d}	2.50 ± 0.07^{bc}
6	1.75 ± 0.92^{cd}	0.00 ± 0.00^{a}
7	0.35 ± 0.35^{ab}	0.00 ± 0.00^{a}
8	0.53 ± 0.42^{abc}	9.50 ± 0.21^{e}
9	0.50 ± 0.28^{abc}	0.00 ± 0.00^{a}
10	1.65 ± 0.35^{cd}	2.50 ± 0.07^{bc}

*Means with same superscript down the column show no significant difference ($P \leq .05$)

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Table 4: Microbial Load (CFU/g) of Vended Suya Meat in Rukpokwu

Vendors	THB ($\times 10^5$)	TCC ($\times 10^5$)
1	1.40 ± 0.42^{a}	2.90 ± 2.97^{b}
2	2.95 ± 0.21^{a}	0.00 ± 0.00^{a}
3	7.95 ± 0.21^{b}	1.22 ± 1.38^{b}
4	1.30 ± 0.14^{a}	7.55 ± 9.12^{b}
5	5.30 ± 5.94^{ab}	0.00 ± 0.00^{a}
6	2.05 ± 0.07^{a}	0.00 ± 0.00^{a}
7	5.00 ± 0.85^{ab}	0.00 ± 0.00^{a}

8	2.55 ± 0.35^{a}	1.90 ± 1.56^{b}
9	1.60 ± 0.28^{a}	4.60 ± 4.81^{b}
10	3.90 ± 0.57^{ab}	2.90 ± 2.97^{b}

*Means with same superscript show no significant difference ($P \leq .05$)

Microbial Isolates

Results of the isolates obtained from vended suya meat showed that twenty-eight bacterial isolates belonging to *Staphylococcus delphini, Staphylococcus lugdunensis, Bacillus subtilis, Staphylococcus pasteuri, Paenibacillus pectinilyticus, Lysinibacillus fusiforms, Bacillus aerius, Serratia nematodephila, Providencia alcalifaciens, Klebsiella singaporensis, Pseudomona aeruginosa, E. coli, Pseudomonas fluorescens* and *Proteus myxofaciens* were identified. These bacterial isolates showed very high similarity/ relatedness to those in the data base of the automated bacterial identification system (ABIS).

Table 5. Distribution of Bacterial Isolates in the Different Locations

Isolates	Rumuokoro	Rukpokwu	Nkpolu	Choba	Frequency
Pseudomonas aeruginosa	+	-	-	+	2 (7.14)
Bacillus flexus	+	-	+	-	2 (7.14)
Bacillus sp	+	+	+	+	4 (14.29)
Staphylococcus sp	+	+	+	+	4 (14.29)

Staphylococcus lugdunensis	-	+	+	+	3 (10.71)
Proteus sp	+	-	+	+	3 (10.71)
Lynsibacillus macroides	-	+	-	-	1 (3.57)
E. coli	-	+	+	+	3 (10.71)
Serratia sp	+	+	-	+	3 (10.71)
Klebsiella sp	-	+	+	-	2 (7.14)
Providencia alcalifaciens	-	+	+	-	1 (3.57)

Key: + = Bacteria isolated; - = bacteria not isolated

Molecular Characterization

The obtained 16S rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates showed a percentage similarity to other species at 99-100%%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates II(C) and B(A)8 within the *Providencia* and the *Bacillus* sp respectively and revealed a closely relatedness to *Providencia stuartii* and *Bacillus flexus respectively*. The 16S rRNA of the isolates B1, B2 and B3 were placed within the Bacillus*, Pseudomonas* and *Lysinibacillus* sp and revealed a closely relatedness to *Bacillus flexus, Pseudomonas aeruginosa* and *Lysinibacillus fusiformis* respectively (Fig 1).
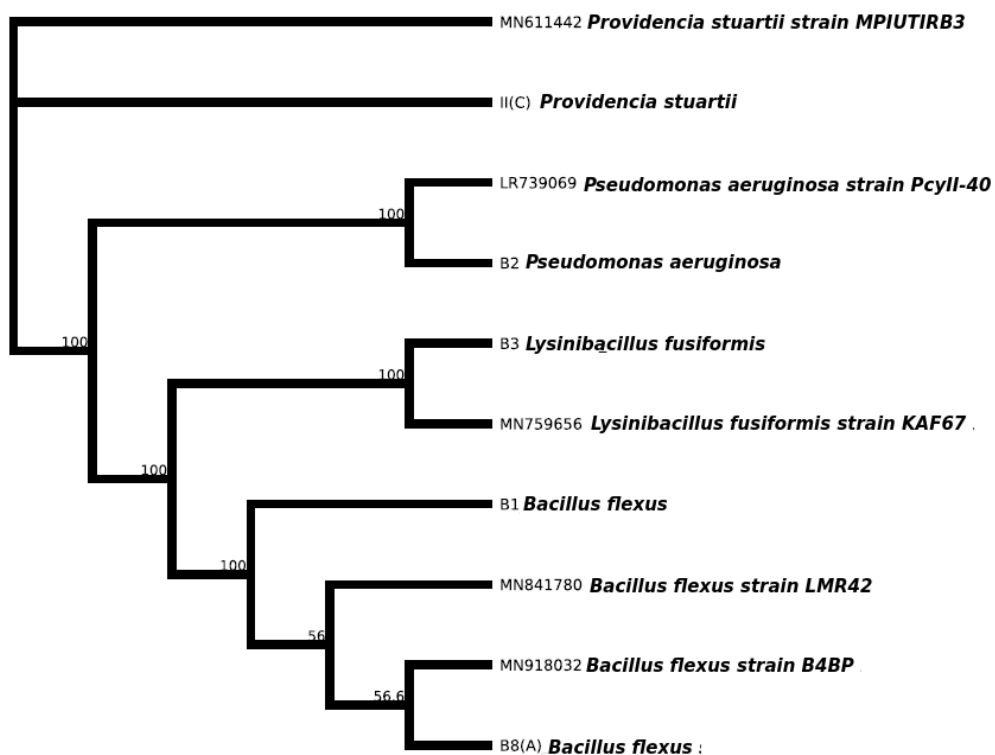
MN611442 *Providencia stuartii strain MPIUTIRB3*

II(C) *Providencia stuartii*

LR739069 *Pseudomonas aeruginosa strain PcyII-40*

B2 *Pseudomonas aeruginosa*

B3 *Lysinibacillus fusiformis*

MN759656 *Lysinibacillus fusiformis strain KAF67*

B1 *Bacillus flexus*

MN841780 *Bacillus flexus strain LMR42*

MN918032 *Bacillus flexus strain B4BP*

B8(A) *Bacillus flexus*

Fig 1: Phylogenetic tree showing the evolutionary distance between the bacterial isolates

Discussion

Suya meat (beef suya) is a special delicacy that is prepared and spiced in different form by different vendors. This delicacy is well accepted and consumed in different parts of Nigeria and is mostly sold in the evening or at night especially in Rivers State. Contamination of the ready to eat suya meat by microorganisms could pose serious health risks. The microbial load of the suya meat in this current study showed varied microbial load across the different sellers and the locations. More so, the total heterotrophic bacterial load of the suya meat in this study were higher than the 4.33-4.87 $\log_{10}$cfu/g bacterial load of suya in Port Harcourt reported by Amala *et al.* (2017) and the 3.36- 6.23 $\log_{10}$ cfu/g bacterial load of suya meat in Maiduguri, Nigeria (Ogbonna *et al.,* 2012). The total heterotrophic bacterial load of suya meats in this current study did not agree with the result of 2.8-5.47log10 cfu/g of suya meats in Lagos, Nigeria (Hassan *et al.,* 2014., Manyi *et al.,* 2014). The total heterotrophic bacterial load in this current study were higher than the 2.85x10^{5}CFU/ML reported by Falegan *et al.* (2017) of suya meat samples in Ado-Ekiti Metropolis, Ekiti State, Nigeria. The coliform count in this current study were detected only in suya meat from few vendors. The coliform load in this current study does not agree with Falegan *et al.* (2017) who reported no coliform load in suya meats from Ado-Ekiti State, Nigeria. The total coliform (3.3 x 10^7/g) reported by Ologhobo *et al.* (2009) of suya meats are higher than the total coliform counts in this current study. The total heterotrophic bacterial and coliform load of suya meats in the locations showed varied counts which were also significant across the vendors.

The total heterotrophic bacterial (THB) load and total coliform counts of the vended suya meats in Rumuokoro showed statistical significance (P ≤ 0.05) across the vendors. The THB counts in vendors 3 and 10 were significantly higher than THB counts recorded in suya meats from vendors 1 to 9. Also, the THB of vendors 8 and 9 were significantly higher than those recorded in vendors 1, 2, 4, 5, 6 and 7, respectively. The coliform counts recorded in vendor 3 of the Rumuokoro location was not

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significantly different (P ≥0.05) from those recorded in vendors 4, 8, 9 and 10, respectively but were significantly different from vendors 1, 2, 5, 6 and 7 which recorded no coliform. The total heterotrophic bacterial load and total coliform counts of the vended suya meats in Choba locations showed statistical significance (P ≤ 0.05) across the vendors. The THB of vendors 6 which had no significant difference with those recorded in vendor 7 was significantly higher than the THB recorded for vendors 1, 2, 3, 4, 5, 8, 9 and 10, respectively. Similarly, the coliform counts recorded in vendors 4 and 5 of the Choba location were significantly higher (P ≤ 0.05) than the coliform counts recorded in the other vendors. The THB load of vended suya meat in Nkpolu showed great significant differences across the vendors. The THB recorded in vendor 5 was significantly higher (P ≤ 0.05) than THB counts recorded in vendors 3, 4, 8 and 9, respectively but showed no significant difference (P≥0.05) with THB counts recorded in vendors 1, 2, 6 and 9, respectively. The coliform counts recorded in this location across the vendors showed that suya meat from vendor 8 which had the highest coliform counts was significantly higher (P ≤ 0.05) than coliform counts recorded from the other 9 vendors. There were also significant differences (P ≤ 0.05) recorded in vended suya meats obtained from vendors in the Rukpokwu locations.

The microbial contamination of the meat samples from the different vendors in their respective locations could be attributed to the poor handling, environmental factors as well as unhygienic methods involved in processing the meat. This agreed with Odusote and Akinyanju (2003) who opined that microbial contamination of suya meat was as a result of processing suya meat in unhygienic conditions. The process of roasting suya meat (meat barbeque) is known to be the major critical control point which ensures eradication of microbial contaminants thereby leading to a safe suya meat. According to Ogunbanwo *et al.* (2004), roasting of meat ensures that the meat is void of microbial contaminants. Although, previous study has suggested that contamination of the ready to eat suya meat could arise from the addition of spices, post roasting handling, storage and the addition

of other additives including slices of fresh onions and tomatoes (Amala *et al.,* 2017). The onset of gastroenteritis and other food borne related symptoms have been reported by previous study after the consumption of suya meats (Inyang *et al.,* 2005).

Most of the bacterial isolates recovered in this current study have been isolated from suya meat by previous study. Orpine *et al.* (2018) isolated *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella sp, Klebsiella pneumoniae* and *Staphylococcus epidermidis.* Also, Amala *et al.* (2017) identified five bacteria: coagulase positive *S. aureus, E. coli, Klebsiella* spp, *Pseudomonas aeruginosa* and coagulase negative *S. aureus.* Falegan *et al.* (2017) amongst the microorganisms isolated from vended suya meat in Ado-Ekiti, Nigeria detected the presence of *S. aureus, E. coli* and *Bacillus* sp which are also among the bacterial isolates obtained in this study. The frequency of occurrence of bacterial isolates in this study were; *P. aeruginosa* (7.14%)*, Bacillus flexus* (7.14%), *Bacillus* sp (14.29), *Staphylococcus* sp (14.29), *S. lugdunensis* (10.71), *Proteus* sp (10.71), *Lynsibacillus macrolides* (3.57), *E. coli* (10.71), *Serratia* sp (10.71), *Klebsiella* sp (7.14%)*,* and *Providencia* sp (3.57)*. Bacillus* sp and *Staphylococcus* sp were the predominant bacterial isolates followed by *Proteus* sp, *E. coli* and *Serratia* sp while *Lynsibacillus macrolides* and *Providencia* sp were the least occurring bacterial isolates. Amongst the bacterial isolates obtained from suya meat in Port Harcourt by Amala *et al.* (2017), *Staphylococcus aureus* was the predominant isolates and this result agreed with the findings in this study. Findings in this study do not agree with Orpine *et al.* (2018) who reported that *E. coli* was the predominant bacterial isolates from suya meats in Dutsinma Local Government Area, Kastina State, Nigeria. The presence of *E. coli* in suya meats in this study could be attributed to indirect or direct contamination arising from faecal origin. *E. coli* is known to be the most predominant bacteria in the human and animal intestines (Prescott *et al.,* 2008). *Staphylococcus aureus* which was isolated from the suya meat could be due to poor hygiene of handlers since the bacterium is commonly found in the nose, skin and throats of humans (Orpine *et*

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al., 2018; Prescott *et al.,* 2008). *Salmonella* sp have been reported to survive in suya meats that are not properly heated during the preparation of stage, thus, the presence of *Salmonella* sp in this study could be attributed to improper heating of suya meat (Adams and Moss, 1999). Also, the presence of *P. aeruginosa, Providentia* sp, *Bacillus* sp, *Micrococcus* sp, *Proteus* sp, *Serratia* sp and *Klebsiella* sp could be attributed to poor hygienic measures or the use of contaminated water or materials contaminated with these microbes. This is in agreement with Gilbert and Harrison (2001) who suggested that cross contamination arising from environmental sources as well as the handlers during processing of the suya meat could lead to microbial contamination.

Conclusion

This study has shown that the bacterial load of vended suya meats were at very high levels and that the bacterial isolates encountered could contain pathogens which could predispose consumers of serious gastroenteritis. Strict hygiene during preparation and packaging should be a top priority by vendors. Also, siting of suya stands should be done in clean environments.

References

REFERENCES

Vilar, I., García, F. M. C. and Prieto, B. (2000). A survey on the microbiological changes during the manufacture of dry-cured lacon, a Spanish traditional meat product. J Appl Microbiology; 89:1018–1026.

Igyor, M.A. and Uma, E.N. (2005). Bacterial quality of a smoked meat product (Suya). Nigeria Food Journal; 23:233-242.

Falegan, C.R., Akoja, S.O. and Oyarekua, M.A. (2017). Microbiological assessment of suya (sliced roasted beef) in Ado-Ekiti Metropolis, Ekiti State, Nigeria. MOJ Biol Med.; 2(3):266–269

Anderson, D.C. (2012). Use of cereal residues in beef cattle production systems. Journal of Animal Science.; 46(3):849–861.

Dickson, J.S. and Anderson, M.E. (2012). Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. Journal Food Protection; 55(2):133–140.

Edema, M. O., Osho, A. T., Dials, C. L. (2008). Evaluation of microbial hazard associated with processing of suya (a grilled meat product). Science Research Essays. 3(12), 621 – 626.

Inyang, C.U., Igyor, M.A. and Uma, E.N. (2005). Bacterial quality of a smoked meat product (Suya). Nigerian Food Journal; 23(1):239–242.

Amala, S. Enoch and Onwuli, D. O. (2017). Bacterial burden of suya and suya spiced ingredients sold in some parts of Port Harcourt, Nigeria. International Journal of Current Research; 9 (8): 55665-55668.

Robinson, V.K. and Wemedo, S. A. (2019). Molecular Characterization of Indoor Air Microorganisms of a Model Primary Health Care in Port Harcourt, Rivers State, Nigeria. Asian Journal of Biotechnology and Genetic Engineering; 2(1): 1- 9

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4:406-425.

Jukes, T.H. and Cantor, C.R. (1969). Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism, (pp. 21-132) Academic Press, New York.

Cheesebrough, M. (2005). District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, London, UK; 143 – 156.

Ogbonna, I. O., Danladi, M. S., Akinmusirs, O. and Odu, C. E. (2012). Microbiological safety and proximate composition of suya stored in ambient temperature for six hours from Maiduguri, Northern Nigetria. International Journal of Food Safety, 14: 11-16

Manyi, M.M., Ola, F.I. and Innocent, O. (2014). Microbial and parasitic quality of suya: a roasted beef sold in Makurdi, Benue State, Nigeria. African Journal of Microbiology Research, 8(35), 3235-3242.

Hassan, I. A., Emun H. O. and Adekunle E. O. 2014). Microbial quality of ready to eat barbecue meat (suya) sold on the street of Lagos State. International Journal of Advances in pharmacy, Biology and Chemistry, 3(4), 973-982.

Odusote, K.A. and Akinyanju O.O. 2003. Hazard associated with suya preparation, Consumer bulletin, 2(2), 20-24.

Ologhobo, A. D., Omojola, A. B., Ofongo, S. T., Moiforay, S. and Jibir, M. (2010). Safety of street vended meat products - chicken and beef suya. African Journal of Biotechnology; 9 (26): 4091-4095.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap.

Evolution, 39:783-791