

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

ABSTRACT

The contamination of vended food with microorganisms especially pathogenic microbes is a public health hazard that 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. Sampling was carried out for a period of 3 months (September to November, 2019). The total heterotrophic bacterial counts of the vended suya meat for Rumuokoro, Rukpokwu, Nkpolu, and Choba were 1.04×10^6 , 3.4×10^6 , 1.49×10^6 and 2.04×10^6 CFU/g, respectively. While the total coliform counts of the vended suya meat for Rumuokoro, Rukpokwu, Nkpolu and Choba were 0.36×10^5 , 3.21×10^5 , 2.45×10^5 and 6.39×10^5 CFU/g, respectively. The total heterotrophic bacterial counts of vended suya meat bought from vendors in Choba were significantly higher ($P \leq .05$) than those bought from the Rumuokoro and Rukpokwu vendors. Similarly, the coliform counts of the suya meat bought from Choba vendors were significantly higher ($P \leq .05$) higher than the coliform counts of vended suya meat bought from vendors in Rumuokoro and Nkpolu. Twenty-eight bacterial isolates: *Staphylococcus delphini*, *Staphylococcus lugdunensis*, *Bacillus subtilis*, *Staphylococcus pasteurii*, *Paenibacillus pectinolytic*, *Lysinibacillus fusiformis*, *Bacillus aerius*, *Serratia nematodephila*, *Providencia alcalifaciens*, *Klebsiella singaporensis*, *Pseudomonas 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* spp respectively and revealed a close relatedness to *Providencia stuartii* and *Bacillus flexus* respectively. The 16S rRNA of *Bacillus*, *Pseudomonas*, and *Lysinibacillus* spp revealed a close relatedness to *Bacillus flexus*, *Pseudomonas aeruginosa*, and *Lysinibacillus fusiformis*. The frequency of occurrence of bacterial isolates across the locations was: *Pseudomonas aeruginosa* (7.14), *Bacillus flexus* (7.14), *Bacillus* spp (14.29), *Staphylococcus* sp (14.29), *Staphylococcus lugdunensis* (10.71), *Proteus* sp (10.71), *Lysinibacillus macroides* (3.57), *E. coli* (10.71), *Serratia* spp (10.71), *Klebsiella* spp (7.14) and *Providencia alcalifaciens* (3.57). These bacterial genera could pose serious health challenges especially if they are consumed in quantities required to cause infections as many have been linked to causing gastroenteritis and other forms of infections. Proper hygiene compliance during preparation and packaging is recommended to eliminate or reduce microbial populations and types.

Keywords: Suya, molecular characterization, total coliform count, total heterotrophic count

Introduction

Suya is a spicy traditional stick meat product that is commonly produced by the Hausas in Northern Nigeria, where the rearing of cattle is 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 clubhouses picnics, parties, restaurants, and within institutions. Potential health risks are associated with contamination of street vended food by pathogens during the handling and preparation stages. Vendors are often poorly educated, unlicensed, untrained in food hygiene and they work under crude unsafe

conditions with little or no knowledge about the causes and dangers of foodborne 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 (Oduote 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 knives 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 that 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 the 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 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 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 may be 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 a 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

The study was carried out in Obio-Akpor and Port Harcourt City 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°59'51"E, 40° 53'48" N and 70° 00'05" E, 40° 52' 09" N and 60°58'35" E, 40°53' 55" and 60° 54' 21" E, respectively. The suya samples were collected randomly from 10 vendors in these four locations and the study was carried out for a period of 3 months.

Sample Collection

A total of 40 roasted meat (suya) 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 3 months.

Enumeration and Isolation of Bacteria

The method of Amadi *et al.*, (2014) was adopted with slight modification. In this method, sterile forceps were used to transfer 10g of each sample into a 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. An aliquot (0.1ml) of the 10^{-3} dilution was inoculated in duplicates onto the surface of the prepared nutrient and MacConkey agar plates and the 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 them on freshly prepared nutrient agar plates. Subculturing of isolates was done repeatedly until pure isolates were obtained.

Characterization and Identification of Isolates

The isolates were identified based on Morphological characteristics (Gram staining), biochemical tests, and Molecular method. The biochemical tests such as citrate test, coagulase test, Voges Proskauer test, Methyl red test, fermentation of sucrose, glucose, mannitol and lactose, growth on MacConkey agar, oxidase test, indole test, motility, and urease test were carried out according to Cheesbrough (2005).

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 °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 microcentrifuge tube and stored at -20 °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, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C 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. Phylogenetic 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 descriptive statistics on Minitab 19 while One-way ANOVA was carried out to check for a significant difference. The Turkey-Pairwise comparison was used in mean separation on Minitab 19.

Results

The bacteriological analysis of the vended suya meat samples from the different locations is presented in Table 1. Results showed that the total heterotrophic bacterial counts of the vended suya meat for Rumuokoro, Rukpokwu, Nkpolu and Choba were 1.04×10^6 , 3.4×10^6 , 1.49×10^6 and 2.04×10^6 cfu/g, respectively. The results showed that the vended suya meat from Choba had the highest heterotrophic bacterial load followed by vended suya meats from Nkpolu while the least heterotrophic bacterial load in vended suya meat was recorded in the Rukpokwu location. Results also showed that the total coliform counts of the vended suya meat for Rumuokoro, Rukpokwu, Nkpolu and Choba were 0.36×10^5 , 3.21×10^5 , 2.45×10^5 and 6.39×10^5 cfu/g, respectively. The highest coliform counts in vended meat were recorded in those bought from the vendors in Choba followed by those bought in Rukpokwu location while the least coliform counts were recorded in vended suya meat bought from Rumuokoro. Statistically, there were significant differences in the total heterotrophic bacterial counts and coliform counts of vended suya meat bought from the various locations. The total heterotrophic bacterial counts of vended suya meat bought from vendors in Choba were significantly higher ($P \leq .05$) than those bought from the Rumuokoro and Rukpokwu vendors. Similarly, the coliform counts of the suya meat bought from Choba vendors were significantly higher ($P \leq .05$) higher than the coliform counts of vended suya meat bought from vendors in Rumuokoro and Nkpolu.

Table 1: Total Heterotrophic Bacterial and Coliform counts (CFU/g) of Vended Meat (Suya)

Location	THB ($\times 10^6$)	TCC ($\times 10^5$)
Rumuokoro	1.04 ± 1.11^{bc}	0.36 ± 6.05^b
Rukpokwu	0.34 ± 2.51^c	3.21 ± 5.65^{ab}
Nkpolu	1.49 ± 1.51^{ab}	2.45 ± 3.25^b
Choba	2.04 ± 5.32^a	6.39 ± 6.71^a

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

Keys: THB = Total heterotrophic bacteria, TCC = Total coliform count

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 pasteurii*, *Paenibacillus pectinilyticus*, *Lysinibacillus fusiformis*, *Bacillus aerius*, *Serratia nematodephila*, *Providencia alcalifaciens*, *Klebsiella singaporensis*, *Pseudomonas 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
<i>Pseudomonas aeruginosa</i>	+	-	-	+	2 (7.14)
<i>Bacillus flexus</i>	+	-	+	-	2 (7.14)
<i>Bacillus</i> sp	+	+	+	+	4 (14.29)
<i>Staphylococcus</i> sp	+	+	+	+	4 (14.29)
<i>Staphylococcus lugdunensis</i>	-	+	+	+	3 (10.71)
<i>Proteus</i> sp	+	-	+	+	3 (10.71)
<i>Lysinibacillus macroides</i>	-	+	-	-	1 (3.57)
<i>E. coli</i>	-	+	+	+	3 (10.71)
<i>Serratia</i> sp	+	+	-	+	3 (10.71)
<i>Klebsiella</i> sp	-	+	+	-	2 (7.14)
<i>Providencia alcalifaciens</i>	-	+	+	-	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 close 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 close relatedness to *Bacillus flexus*, *Pseudomonas aeruginosa* and *Lysinibacillus fusiformis* respectively (Fig 1).

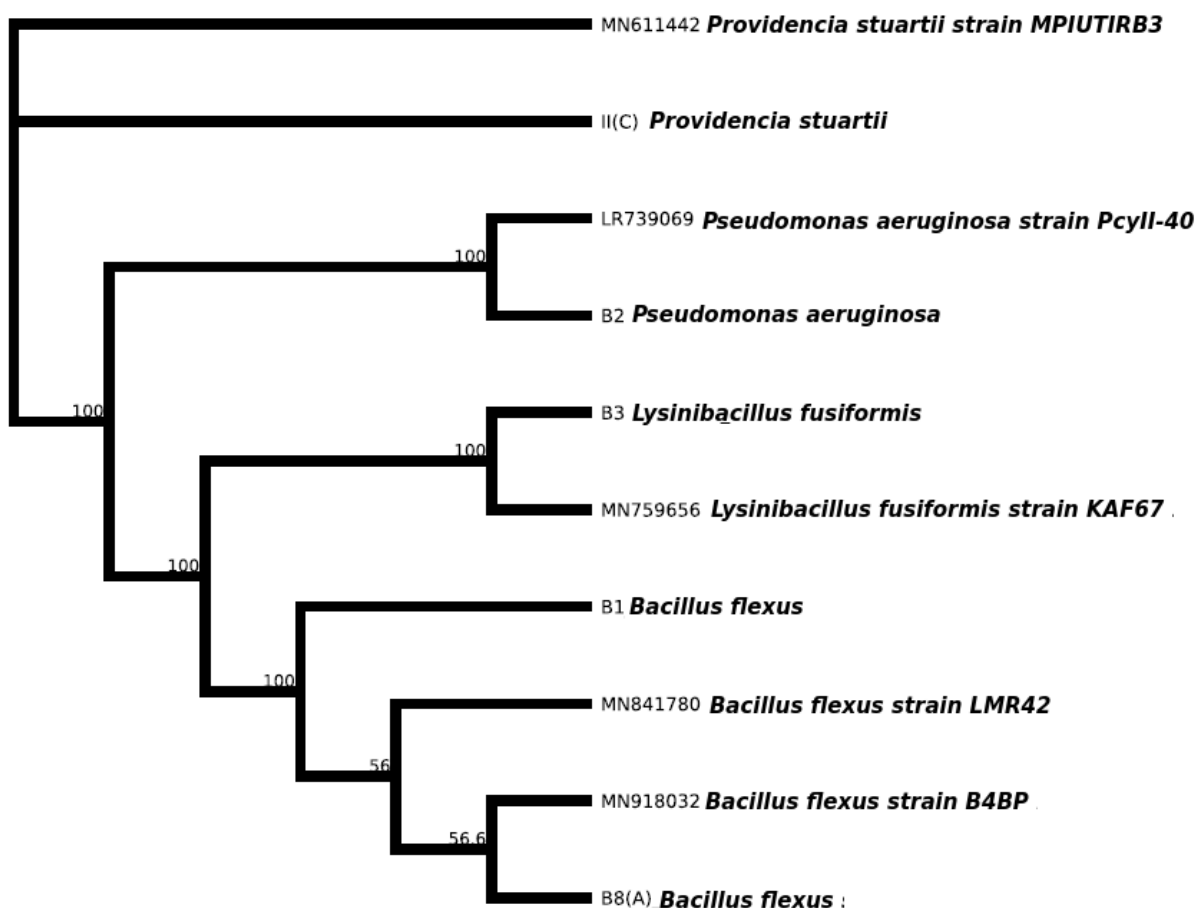


Fig 1: Phylogenetic tree showing the evolutionary distance between the bacterial isolates

Discussion

Suya meat (beef suya) is a special delicacy that is prepared and spiced in a 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 locations. More so, the total heterotrophic bacterial load of the suya meat in this study were higher than the 4.33-4.87 log₁₀cfu/g bacterial load of suya in Port Harcourt reported by [Amala and Onwuli \(2017\)](#) and the 3.36- 6.23 log₁₀ 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 was higher than the 2.85x10⁵CFU/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 10⁷/g) reported by [Ologhobo et al. \(2010\)](#) of suya meats is 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 and coliform counts are higher than the limit of <10⁶ and <100 CFU/g recommended limit of the Centre for Food Safety, Food and Environmental Hygiene Department (2014).

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 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 that ensures eradication of microbial contaminants thereby leading to safe suya meat. According to Ogunbanwo *et al.* (2004), roasting 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 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 the 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 was; *P. aeruginosa* (7.14%), *Bacillus flexus* (7.14%), *Bacillus spp* (14.29), *Staphylococcus spp* (14.29), *S. lugdunensis* (10.71), *Proteus spp* (10.71), *Lynsibacillus macrolides* (3.57), *E. coli* (10.71), *Serratia spp* (10.71), *Klebsiella spp* (7.14%), and *Providencia spp* (3.57). *Bacillus spp* and *Staphylococcus spp* were the predominant bacterial isolates followed by *Proteus spp*, *E. coli*, and *Serratia spp* while *Lynsibacillus macrolides* and *Providencia spp* 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 isolate 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 isolate 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 al.*, 2018; Prescott *et al.*, 2011). *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*, *Providencia 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 was at very high levels and that the bacterial isolates encountered could contain pathogens that could predispose consumers to 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.

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