Original Article

Isolation and Characterization of Bacteria Contaminants of Commercial Suya Meat along Sakponba Road, Benin City

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ABSTRACT

Background: Suya meat is a traditionally prepared spicy beef product which is commonly made by the Northerners of Nigeria known as the Hausas, it is a perishable food due to its chemical composition and characteristics, giving it a perfect setting for the growth of various dangerous bacteria that can cause human infection, as well as meat deterioration and economic loss. **Methods:** Forty (40) samples of suya meat were collected from vendors around Sakpoba Road in Benin City, which were Igun, Ogbelaka, Erie, First, Second, Third, Saint Saviour, Ewaka, Nomayo, and Erediawa respectively and was immediately taken to the laboratory for analysis. Bacterial were isolated using standard microbiological, biochemical and molecular techniques. Further identification of the isolates was carried out using 16S ribosomal ribonucleic acid (16S rRNA) gene primer. **Results:** The most prevalent Characterization of the bacterial isolates using the 16S rRNA gene sequence analysis shows the isolates to include the following; *Enterobacter cloacae* strain D4, *Escherichia coli* strain SMM1138, *Escherichia coli* strain ATDS48, *Escherichia coli* strain Huaian, *Klebsiella pneumoniae* strain KP5296, *Myroides odoratimimus* strain 7BD125, *Proteus mirabilis* strain LCX7, *Providencia rettgeri* strain Jilu0102. Most of this microorganisms isolated are pathogenic and are toxic when ingested in contaminated suya meat. The Prevalence of occurrence of the bacteria isolates was highest for *Escherichia coli* with 25% and *Staphylococcus aureus* with 25% respectively, *Bacillus cereus* with 15%, *Klebsiella pneumoniae* with 12.5%, *Pseudomonas* spp with 10%, *Citrobacter* spp with 5%, and *Salmonella* spp with 7.5%. The mean aerobic plate count were in order of 10^3 (cfu/g) with the highest value for 20.8%. **Conclusion:** Occurrence of such organisms in ready-to-eat suya meat constitutes a food safety issue which calls for urgent response in the education of suya meat producers on the hazards, Critical Control Points and the importance of personal hygiene and clean environment should be handled adequately.

Keywords: Clean Environment, Personal Hygiene, Standard Microbiological Techniques and Suya Meat.

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1. INTRODUCTION

Due to the difficulty of establishing causative linkages between food contamination and consequent sickness or death, the burden of foodborne diseases on public health and welfare, as well as on economies, has been greatly underestimated [1]. Suya meat is boneless, lean sheep, cattle, goat, or chicken flesh that has been marinated in sauces, oiled, and then grilled over a charcoal fire. It is a traditionally processed meat product that is usually not done under stringent hygienic settings because it is still done locally. Suya meat is a delicious and easy-to-prepare food with medium moisture content. Meat is a perishable food due to its chemical composition and characteristics, giving it a perfect setting for the growth of various dangerous bacteria that can cause human infection, as well as meat deterioration and economic loss. The most common cause of bacterial meat degradation is lactic acid bacteria, a medically related group of fastidious and widespread gram-positive organisms.

Few examples are *Lactobacillus, Leuconostoc, Pediococcus,* and *Streptococcus*[2]. Microorganisms could easily grow on meat since it has a high nutritional value. Possible sources of contaminations are washing the meat with dirty water, handling by butchers, contamination by flies, processing near sewage or garbage dumps, spices, transportation, and use of contaminated equipment such as knives and other utensils [3]. Even under the best handling or manufacturing circumstances and methods, meat's high final pH makes it particularly susceptible to microbial development. Following the developments, some other studies found occasional incidences of gastroenteritis and infection symptoms after consuming "Suya," indicating that the product does pose a food safety risk [3, 4]. Microorganisms were recovered from "Suya" sold in Ondo and Akure, South West Nigeria, are of public health concern, as a study conducted revealed that marketed suya within the cities contain bacterial, molds, yeast, and fungi [5, 6, 7]. Investigation of bacterial quality of suya sold in Markurdi, Northern Nigeria, revealed that feacal coliforms were the most common bacterial contaminants, but they were found in tolerable quantities[3]. Meanwhile, Edema *et al.*[7], who investigated the microbial hazards associated with suya meat processing, found contamination with potential pathogens such as *Bacillus cereus, Staphylococcus aureus, Salmonella* spp, and aflatoxigenic molds with aerobic mesophilic counts in the order of 10^5 cfu/g in processing water, meat processing slabs, utensils, spices, and raw material, with the highest value (7.17) observed in the packaging materials. It is critical to highlight that the enormous increase in the production and consumption of "Suya" in South–South zone of Nigeria had made it a major concern to researchers based on its microbial quality[10].

Food poisoning can cause acute gastro enteritis. It's a disease caused by ingesting food contaminated with harmful germs or chemicals. *Salmonella typhi, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens* and *Bacillus cereus* are some of the microorganisms found in Suya that cause food poisoning when consumed. Some of these microorganisms in meat cause off-flavors, rendering the meat unfit for consumption and lowering its taste value [9,10,11].

Pathogenic and non-pathogenic organisms that may infect man's food chain throughout time may act as a reservoir of antimicrobial resistance genes, according to research. Pathogenic organisms may transmit resistance genes to non-pathogenic strains, making them antibiotic resistant [7]. The existence of resistant bacteria in meals of animal origin, according to Courvalin and Weber [5], poses a possible health risk. Abakpa *et al.* [1] reported the presence of antibiotic resistant bacteria in meat products and poultry.

This study therefore focused on the isolation and characterization of bacteria contaminants of hawked suya-meat in order to investigate the level of hygiene with its production.**2.**

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MATERIALS AND METHODS

2.1 Sample Collection

Spiced roasted lean cow meat (suya) samples were purchased from major junctions around Sakponba road in Benin metropolis; these include Igun, Ogbelaka, Erie, First, Second, Third, Saint Saviour, Ewaka, Nomayo, and Erediawa. A total of fourty (40) samples were collected; four (4) Suya meat samples were collected from each junction on different days for a period of three weeks between July and August, 2021. All the samples were collected in sterile wide-mouthed jars and kept in their original package, labeled and transported immediately to the Benson Idahosa University Microbiology Laboratory for analysis.

2.2 Sterilization of Materials

The materials used in this study were subjected to various forms of sterilization before and after use. The glass wares, which included test tubes, conical flasks, and pipettes, were thoroughly washed with detergents, rinsed properly with water, and drained. Subsequently, these materials were covered with foil paper and sterilized in a hot air oven at 170^0C before being used. The media used in this study were prepared according to the instructions of the manufacturers[12, 13]. Prepared media and distilled water were autoclaved at 121^0C for 15 minutes. Hardware like the inoculating loop was heated to redness in an open flame before and after use. The work bench was swabbed using 70% alcohol for disinfection before analysis was made.

2.3 Isolation and Identification of Bacterial Isolates

Samples were serially diluted by adding 1 gram of homogenized meat sample into 9ml of sterile peptone water and 1 ml of the Spiced roasted lean cow meat (suya) into 9ml of sterile water (BPW; Becton Dickinson, Sparks, MD, USA). About 0.1 ml of the serially diluted samples was inoculated onto Mannitol Salt agar (Oxoid, UK), MacConkey agar (Oxoid, UK) [13, 14, 15], Eosin Methylene Blue agar (Oxoid, uk) and Nutrient agar and incubated for 24 hrs. Colonies which appeared on MacConkey agar was used for coliform count while nutrient agar was used for total aerobic count, Mannitol salt agar was used for Staphylococcus count, and Eosin Methylene Blue was used for *Esherichia coli* count. Pure cultures of all colonies were subsequently made on all the agar types after which colonies of the pure cultures were picked and maintained on agar slants for further analysis [6]. The bacterial isolates were presumptively identified using their cultural, morphological, and biochemical characteristics according to the methods of MacFaddin [17]. The following tests were carried out; Gram staining, Motility, Catalase, Oxidase, Coagulation, Citrate, Indole and Sugar fermentation.

2.4 Biochemical Identification

2.4.1 Indole Test

The test was used to differentiate member of Enterobacteriaceae family as part of the IMVIC test. Indole production was used to test whether the organism can oxidize tryptophan resulting in the formation of indole, pyruvic acid and ammonia. The indole production during the reaction was detected by adding Kovac's reagent (dimethyl aminobenzaldehyde), a cherry–red layer on the top of the test tube indicates positive, the absence of this cherry –red layer indicates negative result.

2.4.2 Oxidase Test

Oxidase test was used to check the presence of the electron transport chain in the final phase of aerobic respiration of the isolate. In the oxidase test, an artificial final electron acceptor (N, N, N, N'–tetramethyl phenylenediamine dihydrochloride) was used in the place of oxygen. This acceptor changes colour to a dark blue or purple when it takes the electron from the last element cytochrome oxidase in the electron transport chain. A sterile swab was used to obtain a small amount of the isolate, a drop of oxidase reagent will be placed onto the culture, positive reactions turn the bacteria violet to purple

immediately or within 10 to 30 seconds, absence of a violent to purple colour will be regarded as a negative result.

2.4.3 Catalase Test.

Catalase test was used to differentiate *Staphylococci* (catalase-positive) from *Streptococci* (catalase-negative). The enzyme, catalase was produce by bacteria that respired using oxygen, and protect them from the toxic by product of oxygen metabolism. This was done by transfer of a small amount of a colony directly to a clean glass slide using toothpick or sterile loop or needle. It is then followed by a drop of hydrogen peroxide. Presence of bubbles indicates a positive reaction and absence of bubbles indicate a negative reaction.

2.4.4 Motility Test

This test was carried out in test tubes, of a semi – solid medium (1.3g of nutrient agar). Fifteen (15) ml of the agar was dispensed into the tubes, autoclaved and allowed to gel. Using an alcohol sterilized and flamed straight needle. Each isolate was inoculated by stabbing to half of its depth and incubated at 37^0c for 24 hours. Growth away from the inoculation line was recorded as evidence of motility while organisms that grew only on the area where they are inoculated was regarded as negative.

2.4.5 Coagulate Test

The test done by putting a drop of serum to a clean glass slide, it was followed by the addition of a colony. The presence of clump or particles indicates a positive result and absence of clump or particles indicates a negative result.

2.4.6 Citrate Test

A citrate test was performed as a part of the IMVIC test to differentiate member of the Enterobacteriaceae[18]. Simon citrate agar was prepared and dispenses 5ml into each test tube and autoclaved. After autoclaving, the test tube was slanted and allowed to solidify, then the organism was streak inside the test tube and incubate. After 24hrs of incubation the colour changed from green to blue indicate positive reaction and no colour change indicate a negative reaction.

2.4.7 Sugar Fermentative Test

Four (4) sugar was utilized; glucose, fructose, lactose and sucrose. The isolates were inoculated into the respective cooled sterilized tubes containing 5 ml of the basal medium (Peptone water), inverted Durham tube to detect gas production, 1ml of the 1% sugar solution (sterilized by steam sterilization for 30 min) and methyl red indicator. The inculcated tubes were incubated an aerobically at 37^0C for 48 hours. After incubation, the tubes were observed for acid and gas production by the respective isolate. Acid production was indicated by the formation of a yellow colour while gas evolution was determined by the formation of bubbles and spaces within the inverted Durham tube.

2.5 Antibiotics Susceptibility Testing

Antibiotics susceptibility profiles was determined using the Kirby – Bauer disc diffusion test. Briefly, respective colonies was picked and streaked on Mueller – Hinton blood agar (Oxoid) using a sterile swab. The antibiotics discs (Oxoid) was laid onto the surface of the agar plates using a multi – disc and sterile forceps. The plates were incubated at 30^0C over-night. The diameters of inhibition zones were measured using ruler and the various zones of inhibition were determined and recorded.

2.6 Molecular Identification of Bacterial Isolates Using 16S rRNA Sequence Analysis

2.6.1 DNA Extraction Protocol

Broth culture 1000uL was centrifuged at 10,000rpm for 5 min to get pellets, the supernatant was decanted and 1 ml of DNA Extraction Buffer (DEB) containing proteinase K (0.05mg/ml) was added and vortexed. Then 50l of 20% Sodium Dodecyl Sulphate (SDS) was added and incubated in a water bath at 65^0C for 30 minutes. Then the tubes were allowed to cool to room temperature,

about 100 l of 7.5M Potassium Acetate was added and mixed briefly and thereafter centrifuged at 13000rpm for 10 minutes. The supernatant was transferred into new autoclaved tubes and 2/3 volumes of cold Isopropanol/Isopropyl alcohol added and the tubes gently inverted 3 – 5 times and the tubes was thereafter incubated at -20^{0}C for 1 hour. It was then be centrifuged at 13000rpm for 10 minutes and the supernatant discarded. Five hundred microlitre (500l) of 70% ethanol was added and then centrifuged for 5 minutes at 13000 rpm the supernatant carefully discarded with the DNA pellet intact. All traces of ethanol were removed and the DNA pellets dried at 37^{0}C for 10 – 15 minutes. The DNA pellets were re-suspended in 50l of Tris – EDTA (TE) buffer and an aliquot of the DNA was stored at – 20^{0}C for further analysis.

2.6.2. Gel Integrity and Purification of Amplified Product

The integrity of the DNA and PCR amplification was checked on 1% and 1.5% agarose gel respectively. The buffer (1XTBE buffer) was prepared and subsequently used to prepare agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60^{0}C and stained with 3l of 5.0g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4l of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel. After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents.

2.7 DNA Extraction Using ZR Fungal/Bacterial DNA Miniprep (Manufactured by Zymo Research)

The following steps were used for DNA extraction, 2mLs of bacterial cells broth was placed into a ZR Bashing TM Lysis Tube. This was followed by the addition of 750µl Lysis solution to the tube and secured in a bead fitted with 2ml tube holder assembly and processed at maximum speed > 5 minutes. The ZR bashing bead TM lysis tube was centrifuged in a micro centrifuge at > 10,000g for 1 minute. Then, 400µl supernatant of the solution was transferred to a Zymo-Spin TM IV Spin filter (orange top) in a collection tube and centrifuge at 7,000g for 1 minute. Then, 1,200µl of fungal /bacterial DNA binding buffer was added to the filtrate in the collection tube and was transferred to 800µl of the mixture in a Zymo-Spin TM IIC column in a collection tube and centrifuge at 10,000g for 1 minute. The flow was discarded through the collection tube and repeated for three times. Then, 200µl DNA pre-wash buffer was added to the Zymo-Spin TM IIC column in new collection tube and centrifuge at 10,000g for 1 minute. Also, 500µl fungal/bacterial DNA wash buffer was added to the Zymo-Spin TM IIC column and centrifuge at 10,000 x g for 1 minute. The Zymo-Spin TM IIC column was transfer to a clean 1.5 ml microcentrifuge tube and add 100µl (35µl minimum) DNA elution buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA [11].

2.8. Electrophoresis for DNA and PCR

In the electrophoresis of DNA and PCR, 1g of agarose for DNA and 2g of agarose for PCR was used**.** Agarose powder was mixed with 100mL 1xTAE in a microwavable flask and microwave for 1-3 min until the agarose is completely dissolved (but do not over boil the solution) as some of the buffer will evaporate and thus alter the final

percentage of agarose in the gel [11]. Then, allow agarose solution cool down to about 50°C (about when you can comfortably keep your hand on the flask), about 5mins. Then add 10µL EZ vision DNA stain. Then Pour the agarose into a gel tray with the well comb in place and place newly poured gel at 4°C for 10-15 mins or let sit at room temperature for 20-30 mins, until it has completely solidified [19,20].

2.9 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA X were used for all genetic analysis.

2.9.1 Cycling Conditions

Initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 56˚C for 30secs and elongation at 72˚C for 45sec. Followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10 ˚C forever.

2.10 Statistical Analysis

All experiments were performed in triplicates and the results expressed as the means± Standard Deviation (SD) using Microsoft Excel. One – way analysis of variance (ANOVA) analysis was conducted using SPSS software version 23.

3. RESULTS AND DISCUSSION

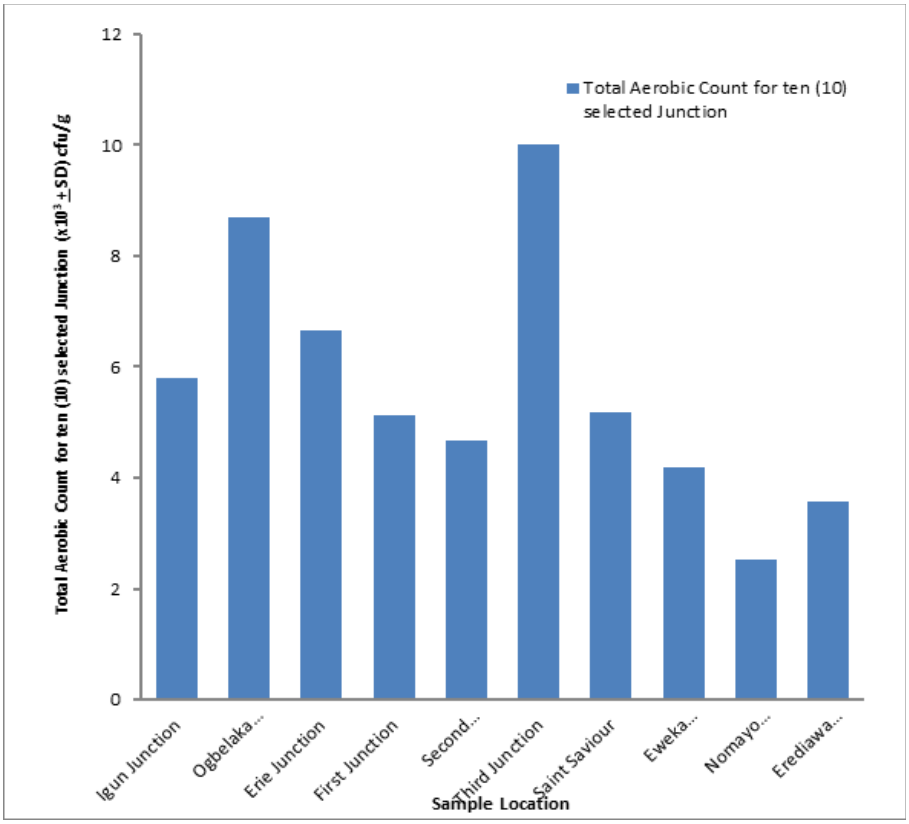

Figure 1: Total aerobic count for Ten (10) selected junction in Benin metropolis with third junction having the highest aerobic count while Nomayo junction having the lowest aerobic count.

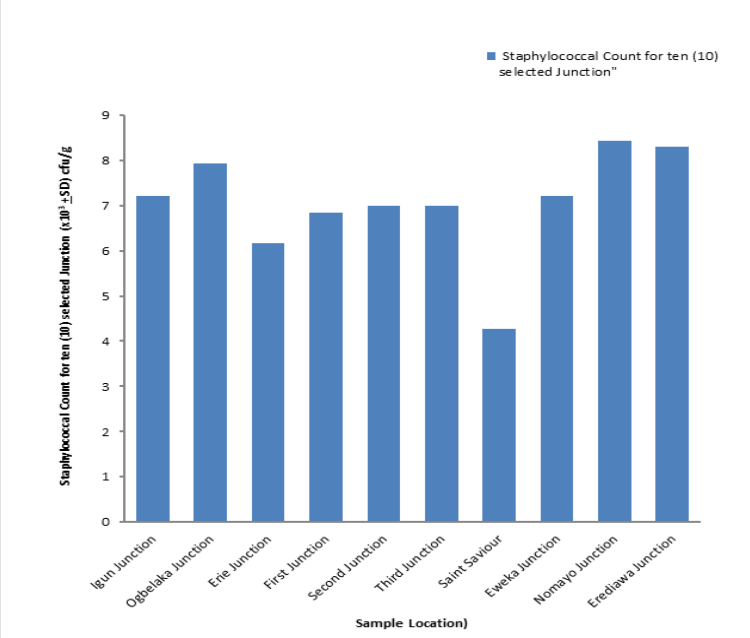

Figure 2: Total Staphylococcal count for ten (10) selected junction in Benin metropolis with Nomayo junction having the highest Staphylococcal count while Saint Savior junction having the lowest Staphylococcal count**.**

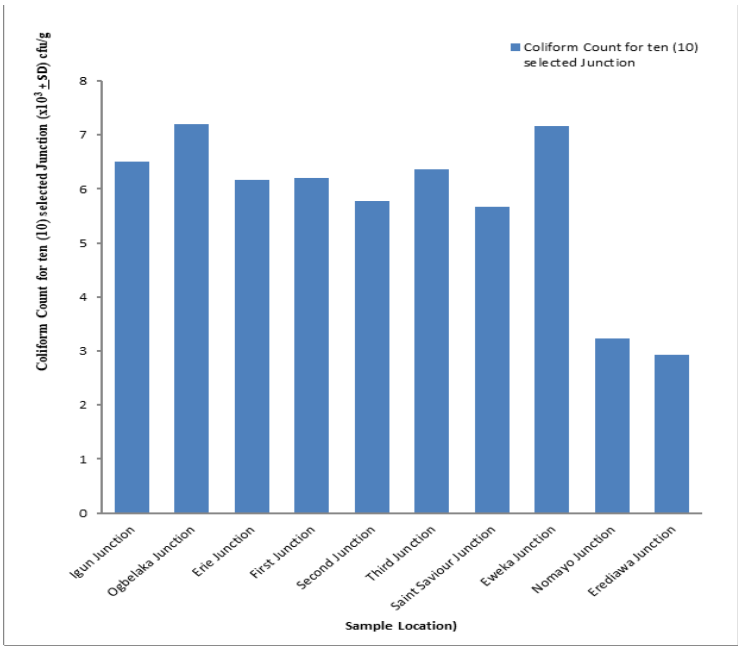

Figure 3: Total Coliform count for ten (10) selected junction in Benin metropolis with Ogbelaka and Eweka lunction having the highest Coliform count while Erediawa junction having the lowest Coliform count.

Figure 1 shows the total aerobic count recorded for spiced lean cow meat (suya) obtained from different junctions in Benin Metropolis. The total aerobic count for spiced lean cow meat (suya) ranged from 2.53 × 10^3 cfu/g recorded for suya meat purchased from Nomayo junction to 10.00 × 10^3 cfu/g purchased in third junction in Sakponba road in Benin Metropolis.

Figure 2 shows the total Staphylococcal count recorded for spiced lean cow meat (suya) obtained from different junctions in Benin Metropolis. The total Staphyloccal count for spiced lean cow meat (suya) ranged from 4.27 × 10^3 cfu/g recorded for suya meat purchased from saint saviour junction to 8.43 × 10^3 cfu/g purchased in Erediawa junction in Sakponba road in Benin Metropolis.

Figure 3 show the total Coliform count recorded for spiced lean cow meat (suya) obtained from different junctions in Benin Metropolis. The total Coliform count for spiced lean cow meat (suya) ranged from 2.93 × 10^3 cfu/g recorded for suya meat purchased from Erediawa junction to 7.20 × 10^3 cfu/g purchased in Ogbelaka junction in Sakponba road in Benin Metropolis.

Figure 4 show the total *Escherichia coli* count recorded for spiced lean cow meat (suya) obtained from different junctions in Benin Metropolis. The total *Escherichia coli* count for spiced lean cow meat (suya) ranged from 1.40 × 10^3 cfu/g recorded for suya meat purchased from Igun junction to 6.80 × 10^3 cfu/g purchased in Erie in Sakponba road in Benin Metropolis.

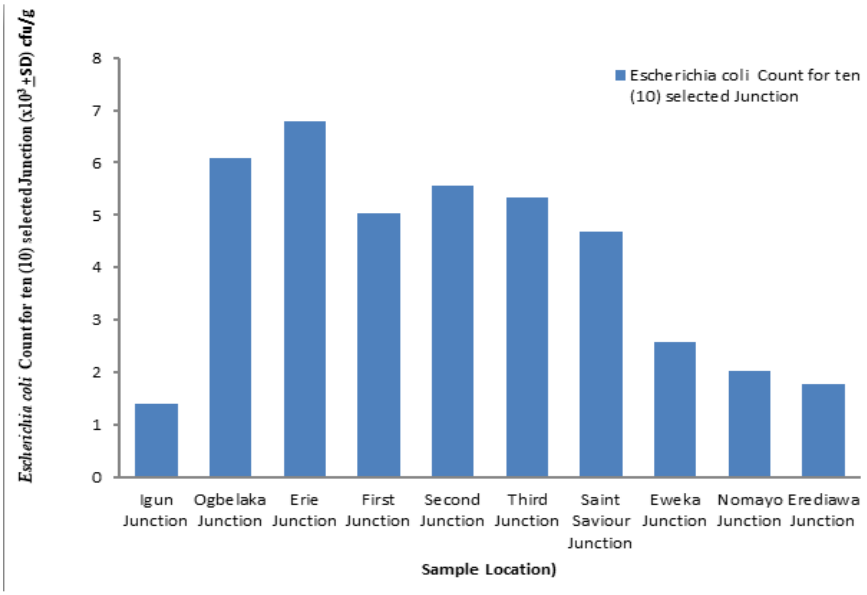

Figure 4: Total *Escherichia coli* count for ten (10) selected junction in Benin metropolis with Erie junction having the highest *Escherichia coli* count while Igun Junction having the lowest *Escherichia coli* count.

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Table 1: Morphological and Biochemical Characteristics of Bacteria Isolated from Suya Meat

Cultural Characteristics	Morphology	Motility	Gram Stain	Glucose	Fructose	sucrose	Lactose	Catalase	Oxidase	Coagulate	Citrate	Indole	Probable organism
Golden Yellow	Cocci in bunch	*+ve*	*+ve*	AG	AG	AG	AG	*+ve*	*-ve*	*-ve*	*+ve*	*-ve*	*Staphylococcus aureus*
Irregular	Single rods	*+ve*	*+ve*	A	A	A	A	*+ve*	*-ve*	*-ve*	*+ve*	*-ve*	*Bacillus spp*
Creamy	Single rods	*-ve*	*-ve*	A	A	A	A	*+ve*	*-ve*	*+ve*	*+ve*	*-ve*	*Klebsiella spp*
Green Metallic	Single rods	*-ve*	*-ve*	A	A	A	A	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*Escherichia coli*
Creamy, Reddish	Single rods	*+ve*	*-ve*	A	A	A	AG	*+ve*	*-ve*	*+ve*	*-ve*	*-ve*	*Pseudomonas spp*
Smooth, Reddish	Paired rods	*+ve*	*-ve*	AG	AG	AG	AG	*+ve*	*-ve*	*-ve*	*-ve*	*+ve*	*Salmonella spp*
Entire whitish	Single rods	*-ve*	*-ve*	AG	AG	AG	AG	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*Citrobacter spp*

A = Colour change, AG = Colour change and bubbles, +ve = Positive, -ve = Negative

Table 1 show the morphological and Biochemical characteristics of bacterial isolated from suya sample based on Biochemical test. Seven probable organisms were identified and their cultural characteristics were stated. Four (4) sugars were utilized: glucose, fructose, lactose and sucrose for sugar fermentation test to observe for acid and gas production by the isolates. Other test such as catalase, oxidase, coagulate, citrate and indole test was done to characterized the bacterial isolated.

Table 2: Organisms Isolated from Suya Samples from the Various Locations along Sakponba Road, Benin City, Based on Biochemical Test

Location	Number of Sample	Number with Organism Isolated	Isolates
Igun junction	4	3(75%)	*Staphylococcus aureus, Bacillus species, Escherichia coli, Salmonella specie.*
Ogbelaka junction	4	4(100%)	*Staphylococcus aureus, Bacillus species, Klebsiella species, Escherichia coli, Pseudomonas species, Salmonella species.*
Erie junction	4	4(100%)	*Staphylococcus aureus, Klebsiella species, Escherichia coli, Pseudomonas species, Citrobacter species.*
First junction	4	4(100%)	*Staphylococcus aureus, Bacillus species, Klebsiella species, Escherichia coli, Pseudomonas species.*
Second junction	4	4(100%)	*Staphylococcus aureus, Bacillus species, Klebsiella species, Escherichia coli, Pseudomonas species, Salmonella species.*
Third junction	4	4(100%)	*Staphylococcus aureus, Bacillus species, Klebsiella species, Escherichia coli, Pseudomonas species, Salmonella species, Citrobacter species.*
Saint Saviour junction	4	4(100%)	*Staphylococcus aureus, Bacillus species, Klebsiella species, Escherichia coli, Pseudomonas species, Salmonella species, Citrobacter species.*
Eweka junction	4	3(75%)	*Staphylococcus aureus, Bacillus species, Escherichia coli, Pseudomonas species, Citrobacter species.*
Nomayo junction	4	4(100%)	*Staphylococcus aureus, Bacillus species, Klebsiella species, Escherichia coli, Pseudomonas species, Salmonella species, Citrobacter species.*
Erediawa junction	4	3(75%)	*Staphylococcus aureus, Bacillus species, Klebsiella species, Escherichia coli.*
Total	40	37(92.5)	

Table 2 shows the organisms isolated from suya samples from the various locations along Saponba road, based on biochemical test. A total of eighty (80) isolates were recorded, four (4) samples was collected from ten (10) different junctions. The microorganisms isolated from each junction were recorded according to the location of the samples and the percentage of samples with growth of organisms was also recorded.

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Table 3: Percentage Distribution of Bacteria Species Isolated from Suya Sample from Sakponba Road, Benin City, Based on Biochemical Test

Location	ISOLATES							
	Staphylococcus aureus	*Bacillus species*	*Klebsiella species*	*Escherichia coli*	*Pseudomonas species*	*Salmonella species*	*Citrobacter species*	Total Organism
Igun junction	2	2	-	3	-	1	-	8
Ogbelaka junction	2	1	1	2	1	1	-	8
Erie junction	1	-	1	2	1	-	1	6
First junction	2	1	2	2	1	-	-	8
Second junction	2	1	1	2	1	1	-	8
Third junction	3	2	1	2	1	1	1	11
Saint Saviour junction	2	1	2	1	1	1	1	9
Eweka junction	1	1	-	2	1	-	-	5
Nomayo junction	2	1	1	2	1	1	1	9
Erediawa junction	3	2	1	2	-	-		8
	20(25%)	12(15%)	10(12.5%)	20(25%)	8(10%)	6(7.5%)	4(5%)	80(100%)

Table 3 shows the percentage distribution of bacterial species isolated based on biochemical test. A total of seven (7) organisms were isolated. The total number of organisms based on different junction was also recorded. The total percentage of each organism was recorded based on biochemical test of the percentage distribution of the bacterial isolates.

Table 4: Antibiotics Resistance Profile of Bacteria Isolated from Spiced Roasted Lean Cow Meat (Suya): Resistant Isolates, N (%)

Antibiotics	*Bacillus species (n=12)*	*Citrobacter species (n=4)*	*Klebsiella species (n=10)*	*Escherichia coli (n=20)*	*Pseudomonas species (n=8)*	*Salmonella species (n=6)*	*Staphylococcus aureus (n=20)*
Pefloxacin (µg)	12(100)	4(100)	10(100)	11(55)	7(87.5)	5(83.3)	12(60)
Gentamycin (µg)	10(83.3)	4(100)	10(100)	14(70)	8(100)	5(83.3)	20(100)
Ampiclox (µg)	5(41.6)	2(50)	5(50)	7(35)	8(100)	5(83.3)	10(50)
Cefuroxime (µg)	5(41.6)	0(00)	5(50)	7(35)	6(75)	5(83.3)	10(50)
Amoxicillin (µg)	11(91.7)	2(50)	10(100)	18(90)	7(87.5)	5(83.3)	20(100)
Ceftriaxone (µg)	0(00)	0(00)	1(10)	0(00)	2(25)	3(50)	2(10)
Ciprofloxacin (µg)	5(50)	1(25)	5(50)	4(40)	2(25)	3(50)	8(40)
Streptomycin (µg)	6(50)	4(100)	6(60)	12(60)	5(62.5)	5(83.3)	12(60)
Cotrimoxazole (µg)	11(91.7)	4(100)	9(90)	20(100)	8(100)	5(83.3)	16(80)

Table 4 shows the antibiotics resistance profile of the isolate. Isolates showed 92.1% resistance towards **Cotrimoxazole** and 91% resistance towards Gentamycin while **Amoxicillin** showed 86% resistance and Pefloxacin shows 83.7% resistance towards isolates. Also, isolates showed 68% resistance towards Streptomycin, 58.6% resistance towards Ampiclox, 47.8% resistance towards Cefuroxime, 40% resistance towards **Ciprofloxacin** and 13.6% resistance towards **Ceftriaxone**. Isolates were more sensitive to **Ciprofloxacin** and **Ceftriaxone**. *Staphylococcus aureus* recorded 57.5%, *Bacillus* species recorded 64.2%, *Klebsiella* species recorded 65%, *Escherichia coli* recorded 54.5%, Pseudomonas species recorded 71.3%, *Salmonella* species recorded 76.6% and *Citrobacter species* recorded 52.5%.

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Table 5: Molecular Identification of Isolates Using 16SrRNA

Isolate Similarity in Gene Bank	Similarity (%)	p-value
Enterobacter cloacae	86.15%	7e-165
Escherichia coli	91.23%	2e-34
Escherichia coli	86.21%	2e-38
Escherichia coli	74.75%	0.002
Klebsiella pneumoniae	96.07%	0
Myroides odoratimimus	75.34%	1e-34
Proteus mirabilis	91.70%	0
Providencia rettgeri	81.49%	0

Table 5 Show the molecular identification of bacteria species isolated from spiced roasted lean cow meat (suya) sample purchased in Benin Metropolis,' isolate SMI showed 91.23% similarity to *Klebsiella pneumoniae* strain KP5296, isolate SM4 show 86.15% similarity to *Escherichia coli* strain ATDS48. Isolate 1 has 91.23% Pairwise Identity to *Escherichia coli* strain SMM1138 with NCBI accession number MT115976.1. The e value is 2e-34. Isolate 2 has 75.34% Pairwise Identity to *Myroides odoratimimus* strain 7BDI25 with NCBI accession number MH725660.1. The e value is 1e-34. Isolate 3 has 81.49% Pairwise Identity to *Providencia rettgeri* strain Jilu LO102 with NCBI accession number KX904719.1. The e value is 0. Isolate 4 has 86.15% Pairwise Identity to *Enterobacter cloacae* strain D4 with NCBI accession number MG714873.1. The e value is 7e-165. Isolate 5 has 96.07% Pairwise Identity to *Klebsiella pneumoniae* strain KP5296 with NCBI accession number MK386777.1. The e value is 0. Isolate 6 has 86.21% Pairwise Identity to *Escherichia coli* strain ATDS48 with NCBI accession number MZ413289.1. The e value is 2e-38. Isolate 7 has 74.75% Pairwise Identity to *Escherichia coli* strain Huaian1232 with NCBI accession number MN314198.1. The e value is 0.002. Isolate 8 has 91.70% Pairwise Identity to *Proteus mirabilis* strain LCX7 with NCBI accession number KY646084.1. The e value is 0.

3.2 DISCUSSION OF RESULTS

The isolates were further identified using molecular methods and sequencing. The bacteria isolated in this study include *Staphylococcus aureus* 7 (25%), *E. coil 6* (25%), *Bacillus* species 5 (15%). *Pseudomonas* species 3 (10%), *Klebsiella* species 4 (12.5%), *Citrobacter* species 2 (5%), and *Salmonella* species 2 (5%). These bacteria have been reported to be transferred into food through direct means (such as from knives, slicers, or conveyor belts) or indirectly (as from floors or control panels) [13]. Wafaa *et al.* [14] opined that the presence of enteric and fecal bacteria like *Salmonella sp.,* and *Escherchia coli* in foods is an indication of fecal pollution. *Staphylococcus aureus* has been isolated from equipment, especially in dairies [15]. These bacteria can also be transferred to food by cross- contamination. *Staphylococcus aureus* has the ability to produce toxins in foods which may result to food borne intoxications. *Pseudomonas aeruginosa 3(10%)* was isolated in this study, It has been classified as a dominant bacterial genus in food processing environments. *Pseudomonas* is also the most important genus for spoilage of food stored aerobically at low temperatures [21, 22]. This group of bacteria produces extracellular enzymes in large amounts and degrades foods, resulting in off–favours and off –tasting foods [21, 23]. Ralyea *et al.* [20] opined that *Pseudomonas* sp. could recontaminate spiced lean cow meat (suya).

According Ralyea *et al.* [20], the characterization of the bacteria after sanitation processing equipment showed that *Pseudomonas* sp. were dominant in number and were also the most abundant protease producers among the residential bacteria. *Bacillus spp.* was isolated in this study 5(15%), this bacteria is recognized as spoilers of spiced lean cow meat (suya) and may also spoil other types of food, such as bread, they do this by producing extracellular enzymes [20]. According to Granum [9] some *Bacillus* spp. may produce different types of toxins that cause disease due to preformed toxins in food (emetic type) or internal infection (diarrheal type) and it is frequently found in food and in food processing environments, especially meat. Granum and Baird [9] suggested that *Bacillus cereus* gastroenteritis is as a result of the enterotoxin produced by this organism in the intestinal tract. Although *Bacillus cereus* can contaminate food from the environment, but the most likely source of *Bacillus cereus* in food are raw materials. *Escherichia coil* was also present in other food samples 6 (25%), this organism belongs to the family enterobacteriacae which is widely distributed in the environment. *Escherichia coli* spread in the environment through contaminated food and water and have been reported to cause diarrheal illness, urinary tract infections, meningitis, sepsis, wound infections and nosocomial pneumonia. Reports has also shown that the Enterohamorrhagic *Escherichia coli* (EHEC) sub group can cause food borne illness as the *Escherichia coli* 0157:H7 strain which cause severe and potentially fatal illness as Hemorrhagic colitis which is characterized by bloody diarrhea and severe abdominal pain [15, 21]. The frequency of *Salmonella* spp 2 (7.5%) isolated in this study was low compared to the other bacteria; *Salmonella* cause salmonellosis due to the production of enterotoxin and cytotoxin bacteria [26, 27]. This bacterium is also responsible for typhoid fever, salmonellosis and gastrocenteritis with symptoms of fever, vomiting and diarrhea. Septicemia and ulcers have been associated with severe cases of typhoid fever. Studies and laboratory investigation records have shown that typhoid fever is endemic in Nigeria [17].

The occurrence of antibiotic-resistant bacteria in this study is a potential health threat [8], as several reports has showed that resistance can be treated [22]. The presence of food borne pathogens that may not respond to antibiotic creates complications in the treatment of these foods borne illness. According to Iwamoto *et al.* [13] the clinical management of some food borne illness has been complicated due to the issue of antimicrobial resistant bacteria [8]. Studies revealed that resistant organisms used are mostly found in the hospitals where antimicrobial substances are commonly found, but there are indications that resistance is currently found almost everywhere in the community. Using 16S rRNA gene sequence and PCR amplification of the 16S rRNA gene, molecular phylogeny analysis confirmed the presence of *Enterobacter cloacea* strain D4, *Proteus mirabilis* strain LCX7, and *Escherichia coli SMM1138.*

4. CONCLUSION

The occurrence of organisms in ready-to-eat suya meat constitutes a food safety issue which calls for urgent response in the education of suya meat producers on the hazards, Critical Control Points and the importance of personal hygiene and clean environment should be handled adequately. The public health laboratory service should take it as a pain to educate suya sellers on food preparation. Suya sellers should try and make the environment for the preparation clean and good to avoid bacterial. The utensils used for the preparation should be kept clean always since microorganisms may develop in them if poorly handled. Most of these organisms investigated in this study are pathogenic and toxic when ingested in contaminated suya meat. Consumers should minimize their level of consumption so as to avoid the buildup of bacterial in the body system.

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Conflict of interests

The authors declared that there is no conflict of interest in this study.

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