PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (5) 1477-1483 May 2024

Bacteriological Analysis of Ready to Eat Suya Meat Sold In Adolor and Oluku Area of Benin City, Nigeria

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ABSTRACT: Traditionally processed meat product is usually done without observing strict hygiene conditions, which could cause foodborne illnesses. As a consequence of microorganisms exceeding acceptable limits, hence, the objective of this paper was to determine the bacteriological analysis of ready-to-eat suya meat sold in Adolor and Oluku area of Benin City, Nigeria. Freshly roasted suya meat samples were obtained from two randomly chosen suya vendors and carefully wrapped in newspaper and sterile foil paper. Bacteriological analysis was performed using the pour plate method with nutrient agar and mannitol salt agar media. The isolates virulence factors and antibiotic susceptibility profile were analyzed. The results showed that samples obtained from Adolor wrapped with Newspaper had the highest bacterial counts of 4.00×10^9 cfu/g. The highest *Staphylococci* count of $3.10 \pm 0.7 \times 10^4$ cfu/g was from a sample wrapped in Newspaper. The identified bacterial isolates were *Escherichia coli, Listeria monocytogenes, Streptococcus sp., Bacillus cereus, Bacillus subtilis, Clostridium sp, Staphylococcus aureus* and *Staphylococcus epidermis*. *Bacillus subtilis* had the highest occurrence, while *Staphylococcus epidermidis* had the least occurrence, with cumulative values of 29.26 and 2.44 %, respectively. The isolates were resistant to most of the antibiotics tested, with an average antibiotic resistance index of above 50 %, which poses a serious public health concern. This study shows that packaging material and handlers are major sources of contamination in suya. It is recommended that suya be sold in more sterile packaging materials. The handlers should be trained on safe hygiene practices for handling suya

DOI: https://dx.doi.org/10.4314/jasem.v28i5.19

Cite this Article as: OSUNDE, D. O; ISOAH, G. O; OBASUYI, C; AKPOGHELIE, P. O; OSHOMA, C. E (2024). Bacteriological Analysis of Ready to Eat Suya Meat Sold In Adolor and Oluku Area of Benin City, Nigeria. *J. Appl. Sci. Environ. Manage.* 28 (5) 1477-1483

Dates: Received: 21 February 2024; Revised: 22 March 2024; Accepted: 20 April 2024 Published: 19 May 2024

Keywords: Suya; Bacteria load; packaging materials; roasted meat; *Escherichia coli*

Ready to eat food sold on the street is an old practice common in most developing countries as a way of providing income (Falegan *et al*., 2017). Such food is an inexpensive meals accessed by the populace and can also represent the culture of local communities (Oshoma *et al*., 2019). Suya is one of the ready to eat food traded on the street. It is a boneless lean meat of sheep, cow, goat or chicken that is staked on sticks, covered with sauces, oiled and then roasted over wood using a fire from charcoal (Falegan *et al*., 2017). It is served hot and sold in public places including bars, parks, and restaurants as well as on the streets (Nwakanma *et al.*, 2015). The preparation of Suya and related items are typically processed and packaged without strictly adhering to hygienic practices, resulting to food spoilage poisoning (Oshoma *et al*., 2019). According to Falegan et al. (2017) Meat is a highly perishable food product making it a medium for

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microbial growth causing visual, textural and organoleptic changes when they release metabolite (Orpin *et al*., 2019). Suya spoilage is mainly caused by invasion of microorganism such as mould, yeast and bacteria. Bacterial contamination is of great concern because very often the food does not look bad even though severely infected, it may appear quite normal. The presence of highly dangerous toxins and bacterial spores is often not detected until after an outbreak of food poisoning, laboratory examination uncovers the infecting agent (Rawat, 2015). This can be prevented by Food rotation system, addition of preservatives, refrigeration, canning. The wide spectrum of microorganisms such as *Bacillus* sp, *Enterobacter* sp, *Escherichia* sp, *Clostridium* sp, *Klebsiella* sp, *Pseudomonas* sp, *Salmonella* sp, *Shigella* sp, *Micrococcus* sp, *Proteus* sp, *Staphylococcus* sp and *Streptococcus* species have been implicated in Suya eaten from various parts of Nigeria (Samuel *et al*., 2015; Falegan *et al*., 2017; Agade *et al* 2019). Sources of microbial contamination of suya have been linked to poor handling, unsanitary circumstances during preparation, nonsterile packaging materials, tools, and utensils, exposure to air microflora, and flies (Chukwura and Mojekwu, 2002; Egbebi and Muhammad, 2016). Consumption of suya contaminated with microorganisms can result to foodborne infection thus constituting food hazard risk (Bello and Bello, 2020). However, one of the food safety measures is the treatment of recommended antibiotics. The inappropriate usage of antibiotics has led to antibiotic resistance – a serious global health challenge (Akinjogunla *et al*., 2022). Appropriate information on the current bacteriological quality of street vended Suya meat is an essential factor in the identification and assessment of safety issues pertaining to the production of wholesome/safe ready to eat foods (Falegan *et al*., 2017). Results of laboratory studies on the evaluation of bacteriological profile and their antimicrobial susceptibility pattern is critical in the development of effective intervention strategies which would address the contamination of these food sources by potentially pathogenic bacteria or its toxic metabolites such as enterotoxins (Bello and Bello, 2020; Yandev *et al*., 2021). Hence, the objective of this paper was to determine the bacteriological analysis of readyto-eat suya meat sold in Adolor and Oluku areas of Benin City, Nigeria.

MATERIALS AND METHODS

Sample collection: Suya samples were collected from two (2) different suya hot spots; Adolor and Oluku, Benin City. One set of the randomly purchased samples was wrapped with Newspaper used by the Vendors, while the other was wrapped with sterile foil paper. The samples were transported to the Microbiology laboratory of University of Benin for analysis. The samples were then labeled sample AFP (Adolor foil paper), sample ANP (Adolor newspaper), sample OFP (Oluku foil paper), and sample ONP (Oluku newspaper). Swabs of both packaging materials were also taken for microbiological analysis. They were labeled as Sample SNP (Swab of newspaper) and Sample SFP (swab of foil paper) for both locations. Swab samples were collected from the different wrappers used. Swabbing was carried out aseptically using sterile swab sticks moistened with peptone water spanning 5cm (length) by 2cm (breadth) in area. The swabs were taken to the laboratory for immediate culturing and incubation within 2 hours according to the method described by Public Health England (2014).

Enumeration and Characterization of Bacterial Isolates: From the Suya samples 5 g was weighed and serially diluted (ten-fold) using sterile distilled peptone water. Aliquots of 1ml of all the diluents above were pour-plated on nutrient agar and mannitol salt agar for total bacterial and Staphylococcal counts respectively. Plates were incubated at 37 °C for 24 h, after which discrete colonies were counted according to Abdullah *et al*. (2020). The swab sticks were dipped into 10 ml of sterile distilled water in test tubes and allowed to stand for 30 mins after being slightly whisked. From this stock, a 10-fold dilution was carried out, there after 1.0 ml was transferred using spread plated method, onto plates containing Nutrient Agar and Mannitol Salt Agar respectively. All the plates were incubated at temperature of 37 ^{o}C for 24 h. The method described by Public Health England (2014) for estimating bacterial counts was used to enumerate the total viable counts of the isolates. Colonial growths were sub-cultured for purification and purified bacterial isolates were preserved in Nutrient agar (NA) slants. The isolates were characterized by morphology, Gram's reaction and biochemical test using the scheme in Bergey's manual of determinative bacteriology (Holt *et al*., 1994; Cheesbrough, 2000).

Detection of Virulence factor tests

Lipase Activity: The isolates lipase activity was measured on tryptone soy agar (TSA) plates supplemented with Tween 80 (1 %v/v). The isolates were spot inoculated onto plates of TSA and then incubated for 24 h at 37 ^{o}C. Clear halo surrounds the regions where the lipase producing bacteria has proliferated indicates lipase production.

Protease Activity: The activity of bacterial isolates extracellular protease was assayed on TSA plates that had been supplemented with 1% casein (v/v). The

isolates incubated for 24 – 48 h at 37ºC after being inoculated on 1% casein supplemented TSA plates. Clear zones surrounding the colonies as a result of casein hydrolysis indicated positive result, whereas no clearing was regarded a negative result.

DNA Degrading Activity: The capacity of bacterial isolates to degrade DNA was tested on DNase agar plates. The bacterial isolates were inoculated on DNase agar plates and incubated for 24 – 48 h at 37 °C. After incubation, methyl green is released during DNA hydrolysis, turning the medium around the test organism colourless. When DNA degradation does not occur, the medium remains green (Edward *et al*., 2023).

Gelatinase Production: The ability of bacterial isolates to produce gelatinase was measured in a nutrient gelatin medium. The bacterial isolates were inoculated on gelatin medium and incubated for 24 – 48 h at 37 °C. Zones of clearance in the media indicated the presence of gelatin-liquefying bacteria. If there is no zone of clearance, the result is negative (Edward *et al*., 2023).

Antibiotic susceptibility test: Antibiotic sensitivity of identified bacterial isolates were assayed using Kirby-Bauer disk diffusion susceptibility test using the following antibiotics: Septrin (30µg), Amoxillin (30µg), Gentamycin (10µg), Ciprofloxacin (10µg), Pefloxacin (10µg), Ampiclox (30µg), Zinnacef (20µg), Rocephin (25 µg), Septrin (30µg), Erythromycin (10µg), Sparfloxacin (10µg), Ofloxacin (10µg).

The test bacteria were inoculated into sterile nutrient broth in a test tube and incubated for 24 h at 37 ºC . From the pure culture, 0.1 ml was transferred into solidified Nutrient agar (NA) in a petri-dish and a sterile spreader was used to guarantee uniform distribution on the agar plate. The plates were allowed to air dry for 5 min thereafter the standard antibiotics discs were laid on the inoculated agar. The plates were incubated for 24 h at 37 ºC.

Clear zones around the discs were measured and interpreted as either susceptible, intermediate or resistant. Zones of inhibition less than or equal to 14mm were recorded as Resistant, 15mm-17mm as Intermediate and greater than or equal to 18mm as Susceptible (Bauer *et al.*, 1996).

The bacterial isolate's Multiple Antibiotic Resistance (MAR) index is defined as x/y, where 'x' denotes the number of antibiotics to which the test isolate showed resistance and 'y' denotes the number of antibiotics to which the isolate was exposed (Odonkor and Addo 2018).

RESULTS AND DISCUSSION

The heterotrophic bacterial count obtained from suya sample and it's packaging materials is presented in Table 1. The heterotrophic bacterial count in suya samples ranged from $5.00 \pm 1.4 \times 10^2$ to 4.00×10^9 cfu/g on Nutrient agar. Sample with the highest count was suya wrapped with Newspaper from Adolor while the least was sample wrapped with foil paper from Oluku. With values 4.00×10^9 and 2.00×10^6 cfu/g. Table 2 showed *Staphylococci* count of suya samples collected from Adolor and Oluku, wrapped with Newspaper and foil paper. The Suya sample, wrapped in newspaper and purchased in Oluku, had the highest count of $3.10 \pm 0.7 \times 10^4$ cfu/g, while the swab sample from the foil paper used to wrap the Suya sample obtained from Oluku and Adolor showed no count. The results obtained from the cultural, morphological and biochemical identification of bacterial isolates is shown in Figure 1. The isolates identified were *Escherichia coli, Listeria monocytogenes, Streptococcus* sp, *Bacillus cereus, Clostridium sp., Bacillus subtilis, Staphylococcus aureus,* and *Staphylococcus epidermidis.* The frequency of occurrence of isolates in suya samples are shown that *Bacillus subtilis* had the highest occurrence while *Staphylococcus epidermidis* had the least occurrence with cumulative values of 24.38 and 2.44 % respectively.

Table 1: Heterotrophic bacterial Count of Suya obtained from Adolor and Oluku with Different Packaging Material.

Sample	Location	
	Oluku	**Adolor**
FP (cfu/g)	2.00×10^6	5.00×10^6
NP (cfu/g)	5.00×10^6	4.00×10^9
SNP (cfu/cm^2)	$1.65 \pm 2.1 \times 10^3$	$9.50 \pm 4.9 \times 10^4$
SFP (cfu/cm^2)	NG	$5.00 \pm 1.4 \times 10^2$

FP- Foil paper, NP – Newspaper. SNP- Swab of newspaper, SFP- Swab of foil paper and NG- No Growth

Table 2: Staphylococci Count of suya obtained from Adolor and Oluku.

Sample	Location	
	Oluku	**Adolor**
FP (cfu/g)	$1.10 \pm 1.4 \times 10^4$	$9.00 \pm 1.4 \times 10^3$
NP (cfu/g)	$3.10 \pm 0.7 \times 10^4$	$9.50 \pm 0.7 \times 10^3$
SNP (cfu/cm^2)	2.00×10^4	2.00×10^2
SFP (cfu/cm^2)	NG	NG

FP- Foil paper, NP – Newspaper. SNP- Swab of newspaper, SFP- Swab of foil paper and NG- No Growth

Suya is rich in nutrients for both humans and microbes, making it susceptible to rapid deterioration due to microbial growth. Bacterial load was found to vary between Suya samples from different locations, with the highest load found in Adolor and the lowest in Oluku. This finding may be explained by the fact

that the suya vendor in Oluku maintains better personal hygiene practices than the one in Adolor (Apata *et al*., 2013). According to bacterial guidelines for processed foods, the plate count must be less than 10^6cfu/g and staphylococcal count less than 10^4 cfu/g (Centre for food Safety, 2007). The suya sample bacterial load was observed to be higher than the permissible limit, hence, their existence poses a health risk (Oranusi *et al*., 2013; Madueke *et al*., 2014). The high bacteria load (above the acceptable limit) obtained in all the sampled food items are indicative of post-treatment contamination due to the heating applied during suya preparation (Ogugbue *et al*., 2011) thus, making the food unfit for human consumption. The majority of ready-to-eat foods have been found to be primarily contaminated by microorganisms during cooling and air exposure (Madueke *et al.*, 2014). A high viable bacterial count suggests that the vendors in the various locations may have been handling their products improperly, not practicing proper hygiene, and maintaining unclean conditions.

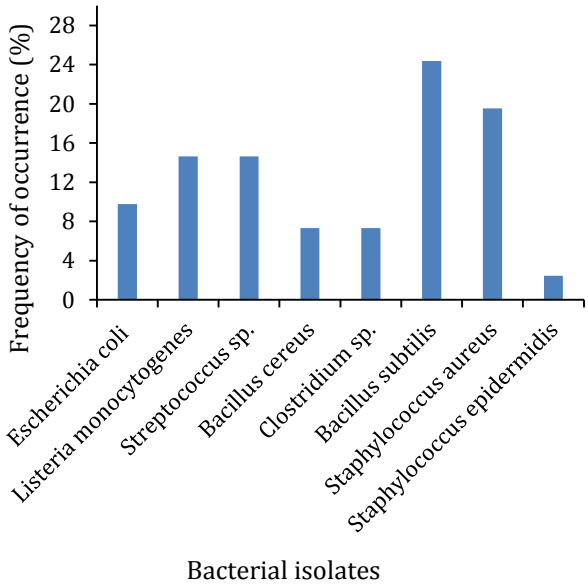

Fig 1: Identified bacterial isolates their percentage frequency of occurrence

Newspaper wrapped suya from Adolor had the highest heterotrophic bacterial and Staphylococcal counts. Possible explanation could be the unclean packing materials, which will increase the bacterial burden. These packaging materials could be a source of microbiological contamination (Owuna *et al*., 2015). Considering the processing preparation method of suya, there could involve a lot of handling during and after preparations. Each of them increased the possibility of microbial contamination of suya. (Agbo *et al*., 2016). Furthermore, it was observed that Adolor is a busier neighbourhood than Oluku, so the increased traffic in Adolor may have contributed significantly in the contamination of the suya meat (Okhuebor and Izevbuwa, 2020). The bacterial species identified in this investigation have been linked to issues like food deterioration, foodborne infections, and food intoxications. These problems can result to abrupt declines in food quality, modifications in flavor and appearance health risks and monetary l losses (Oshoma *et al.*, 2019). The presence of indicator organism such as *E. coli* suggests contamination by fecal matter which could possibly be acquired from the gut of the slaughtered animal or polluted water during cleaning by vendors (Alonge *et al.*, 2017). Contamination by *Staphylococcus aureus* and *Staphylococcus epidermis* indicates bacteria of the normal skin flora (Oshoma and Dijeh, 2017) that may have been transferred from direct contact with the meat handlers and vendors as it is a normal flora of the skin (Nwakanma *et al*, 2015). *Bacillus subtilis* was predominant with 24.39 % which may have been caused by airborne and heat-resistant spores, and decomposing plant material from onions mixed with spices (Roberts *et al*., 2010). *Clostridium perfringens* was present in 7.32 % suggesting that the meat was improperly cooked. *C. perfringens* are found in dust, soils, vegetation among other environmental media. Its presence could be attributed to growth parameters like favourable temperature (Bello and Bello, 2020). The isolation of these spores forming bacteria not surprising due to their ability to survive the heat during roasting (Hammuel *et al*., 2022). Since *Streptococcus* sp is a common normal microflora of the nasopharynx, the presence of the bacterium may be related to sneezing and more of the personal hygiene of the handler. In general, all the isolated bacteria found in the meat sample have the potential to seriously endanger human lives and cause severe health problem (Alonge *et al.*, 2017). It is important to note that majority t of the bacteria isolated in Suya are of public health significance capable of causing food-borne illnesses and intoxication such as travellers' diarrhea, gastroenteritis, abdominal disorder, Staphyloenterotoxemia, mild fever and other conditions (Egbebi and Muhammad, 2016; El-Hassan *et al.*, 2018). *Bacillus cereus* and few other bacteria identified from the Suya sample were also isolated in the work carried out by Egbebi and Seidu, (2011). This investigation agrees with the study of Bello and Bello (2020) who isolated same strains of bacteria from suya sample. Table 3 shows the virulence factors of isolated suya samples obtained from Oluku and Adolor in Benin City. The results showed that *Escherichia coli, Listeria monocytogenes, and Bacillus cereus* had positive results for all the tests. *Staphylococcus epidermidis* was least percentage of 25 % for all the tests.

Table 3: Virulence Factors of Isolated Bacteria from Suya Sample collected from Adolor and Oluku in Benin City.

Isolates	Virulence Factors				%
	Protease	DNase	Gelatinase	Lipase	
Escherichia coli	+	+	+	+	100
Listeria monocytogenes	+	+	+	+	100
Streptococcus sp	+	+	-	+	75
Bacillus cereus	+	+	+	+	100
Clostridium sp	+	+	-	-	50
Bacillus subtilis	-	+	-	+	50
Staphylococcus aureus	+	+	-	+	75
Staphylococcus epidermidis	-	-	+	-	25

Table 4: Antibiotic Sensitivity Test for Bacteria Isolated from Suya Sample Collected from Adolor and Oluku in Benin City, Nigeria

Isolates	Antibiotic Standards and Zones of Inhibition											
	APX (30µg)	CPX (10µg)	CN (10µg)	E (10µg)	PEF (10µg)	S (30µg)	SXT (30µg)	R (25µg)	AM (30µg)	SP (10µg)	OFP (10µg)	Z (20µg)
E. coli	R	I	R	S	S	R	R	R	R	R	R	R
L. monocytogenes	R	S	R	R	S	R	R	R	R	R	R	R
Streptococcus sp	R	R	R	R	R	R	R	R	R	R	R	R
B. cereus	R	R	R	R	S	R	R	R	R	I	S	R
Clostridium sp	R	S	R	R	S	R	R	R	R	R	R	R
B. subtilis	R	I	R	R	I	S	R	R	R	R	R	R
S. aureus	S	S	S	S	S	S	S	S	S	S	S	S
S. epidermidis	R	S	S	S	S	S	I	S	R	R	R	R

Key: *I- Intermediate, R- Resistant and S-Susceptible. KEY: SXT- Septrin, CH- Chloraphenicol, SP- Sparfloxacin, CPX- Ciprofloxacin, AM- Amoxacillin, AU- Augmentin, CN- Gentamycin, PEF- Pefloxacin, OFX- Ofloxacin, S- Streptomycin, APX- Ampiclox, Z- Zinnacef, R- Rocephin, SXT- Septrin, E- Erythromycin*

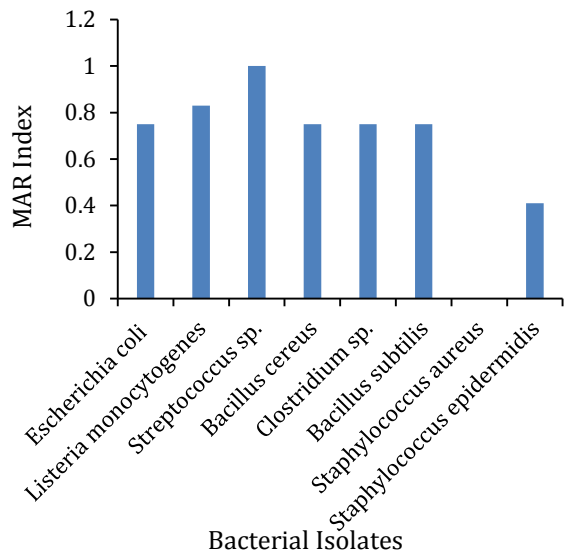

Fig 2: Multiple Antibiotic Resistance Index of bacterial isolates

The results of antibiotic tests carried out on all the bacterial isolates are shown in Table 4. *Streptococcus sp*. was completely resistant to all the antibiotics used. *Staphylococcus aureus* was susceptible to all of the antibiotics tested. Figure 2 shows the multiple antibiotic resistance (MAR) index of the bacteria isolates. *Staphylococcus aureus* had a MAR index of 0 while *Streptococcus sp*., had a MAR index of 1.

The bacterial isolates displayed varying levels of antibiotic resistance, with most showing resistance to multiple antibiotics. Due to their broad spectrum bactericidal action and exerts rapid antibacterial action, Pefloxacin and Ciprofloxacin were the most effective antibiotics against bacterial isolates, exhibiting antimicrobial potency against 84% and 53% of the bacterial isolates (Amadi *et al*, 2016). Majority of the bacterial isolates had a MAR index score above 0.3, indicating high resistance to all the antibiotics tested. The high MAR index observed in the isolates from the suya samples suggests that the meat suppliers may have abused antibiotics (Datok et al., 2021). According to Odonhor and Addo (2018) stated that bacteria isolated from samples where antibiotics are used indiscrimately the MAR index is expected to be higher than the allowable limit. On the other hand, these bacterial isolates with MAR index above 0.2 is an indication of high risk of contamination. This resistance presents a public health problem as it limits antibiotic treatment options for infections.

Conclusion: The bacterial load of the suya sampled in this study was above the stipulated bacteriological limits, their presence constituted a health risk. These findings emphasize the importance of suya vendors enforcing stronger quality control procedures, improving hygiene standards, and increasing public knowledge of the possible health hazards connected to consuming contaminated suya. Public health considerations should direct efforts to guarantee the safety of this popular delicacy. Furthermore, the choice of suya packaging material significantly influences its microbial content, making it essential to select the right packaging material and handle it

correctly to maintain the safety and quality of suya for consumers.

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