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Isolation Of Bacteria from Sampled Street-Vended Food in Jos South Local Government, Plateau State of Nigeria

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Abstract- ***Food safety is a major public health concern as improper food handling could result to food borne illness. This aimed to isolate and identify bacteria and determined microbial load in some selected cooked foods sold by street food vendors in the study area. A Descriptive Cross-Sectional Study Design was adopted and a total of fifteen (15) portion of Jollof rice were randomly purchased from different vendors in Jos South LGA of Plateau State. The samples were transported in ice to the laboratory. The samples were bacteriologically analyzed using spread plate technique and sub-culture. Serial dilution of the sample was carried out after which the first to the eight tube were picked and 1 ml of each sample was pipette into a Nutrient agar. The plates were then incubated for 24hours at 370C, after which they were examined for growth. Sub culture was done using bacteriological agar. All the screened food samples had varying levels of bacterial growth ranging from 297 X 105 to 3.0 X 106 cfu/ml. Ninety percent of the sampled foods had bacterial counts above the acceptable limits (104cfu/ml). Four bacterial species - Staphylococcus aureus, Bacillus cereus, Klebsiella aerogenes and CoNS (Coagulase Negative Staphylococci were isolated from the foods sampled. The findings revealed that street vended foods in the area were unhygienically prepared, handled in manner that exposed to contamination, and were potential vehicles for transmitting food borne illnesses. Therefore, there is need to educate food vendors on practical steps in food handling and hygiene practices to promote food safety, while the local environmental health practitioners should enforce relevant food safety regulations especially the National Environmental Health Practice Regulations, 2024.***

Index Terms- ***Food Safety, Bacteria, Food Borne Illness, Street food vendors.***

I. INTRODUCTION

Food safety has posed a major public health concern for centuries because of the devastating effects of the consumption of contaminated food, (Zahir, Anis and Jauhar, 2025) The Centers for Disease Control and Prevention (CDC) estimates that approximately 48 million people in the United States contract foodborne illnesses each year, resulting in 128,000 hospitalizations and 3,000 deaths (CDC, 2022). Recent data globally indicates that, in 2020, there were 9,761 reported cases of foodborne illness caused by bacterial pathogens, including 2,835 cases attributed to Salmonella, 2,546 to Campylobacter, 1,071 to Shigella, and 595 to Escherichia coli (CDC, 2023). Most foodborne illnesses are classified as "acute": they are usually self-limiting and of short duration with symptoms including acute gastroenteritis. However, some illnesses can progress to severe conditions such as neurological or renal syndromes, known as sequelae (Scallan *et al.,* 2019). Poor sanitary condition in most of the local markets and in the open environment being highly polluted and charged with spoilage and pathogenic flora is likely the source of contamination of food items sold by such vendors (Nathan, Adebayo and Samuel, 2025).

In Nigeria, consumption of street vended food has witnessed a phenomenal growth over the years. This has been due to increasing population, urbanization and increasing human mobility across a wide spectrum of activities, and most people unable to create enough time to prepare or eat proper meal

along their daily schedules (Makinde, et al, 2023; Oyedeji, et al, 2023).

According to, Etinosa and Emmanueal, (2016) and Bintsis, (2017), yearly, more than 90% of the cases of food poisoning are caused by *Staphylococcus aureus,* Salmonella, *Clostridium perfringes*, *Clostridium botulinum*, Campylobacter, *vibro parahaemolyticus*, *Bacillus cerus* and *Entropathogenic Escherichia coli*, These bacteria are commonly found on many raw foods and some in cooked food. De Boer and Beuner (2011) stated that food poisoning can be prevented by controlling initial bacterial growth and multiplication and inhibiting growth through proper cooking and avoiding re-contamination. Key practices include keeping food out of the 40°F–140°F (5°C–60°C) "danger zone" to manage pathogens like *Salmonella* and *Bacillus cereus*. Apparently, contamination occurs when personal hygiene and sanitary standards are compromised, allowing bacteria to grow on raw and cooked food products.

II. MATERIALS AND METHODS

Study design

This study employed a descriptive cross-sectional design combined with experimental laboratory analysis to determine the bacterial load and quality of street-vended foods within Jos South LGA. Samples were analyzed using standard microbiological techniques for bacterial isolation and identification.

A cross-sectional study was conducted on ready-to-eat (RTE) Jollof rice sold across five popular vending districts: Zawan, Kuru, Vom, Vwang, and Gyel. Using a purposive sampling approach, fifteen (15) samples were collected—three from each location—aseptically stored in sterile containers, and transported immediately with ice to the National Institute for Veterinary Research (NIVR) laboratory in Vom for microbial analysis to assess contamination levels and consumer safety

Materials

The following materials and chemicals were used for laboratory analysis:

Electric thermostatic incubator for culturing bacteria, fungi, and microorganisms.

Autoclave for sterilizing scientific instruments used for the experiment.

Microscope for examination of objects (*enlarged image of a small object).*

Refrigerator for preserving the food to slow down bacterial growth, keeping food, produce, fresh at 0–4°C.

Electronic scale for digital weight measurements

Micro pipette used to accurately and precisely measure and transfer tiny volumes of liquid, typically in the range of to (microliters).

Masking tape for labelling the samples collected.

Petri dishes used to culture the microorganism

Marker was for labeling samples of Jollof Rice collected from different location

Sterilized glass containers for collecting the Jollof Rice samples,

Clinical hand gloves used to avoid cross contamination of the Jollof Rice Samples, other materials used in the experiment,

Nutrient Agar; mac Conkey Agar used as food sources for culturing microorganism present in the Isolated Vented Jollof Rice

Crystal violet stain used as Gram staining for differentiating bacteria into Gram-positive (purple) and Gram-negative.Acetone.

Safranin for biological stain used primarily in microbiology and histology to color cell nuclei, cartilage, mast cell granules, and bacteria red. It is most famously used as a counterstain in Gram staining to identify Gram-negative bacteria and as a stain for lignin and secondary cell walls in plant tissues

Lugols iodine primarily used for medical, antiseptic, and laboratory purposes

Procedures

Sterile sample bottles were used to collect and transport the purchased Jollof rice to the department's laboratory and stored in refrigerator to allow for preparation of media. One gram (1g) portion of each sample was macerated and use for analysis.

The nutrient agar and mac conkey agar media were weighed appropriately into petri dishes, and prepared according to manufactures instruction by Neogen, formerly LabM. The media was prepared by dissolving 28g of nutrient agar in one liter of distilled water. The mixture was dissolved on the hot plate to achieve the total dissolution of the nutrient agar. It

was then collected with cotton wool and aluminum foil, and was sterilized in the autoclaved at 121°C for 15 minutes. The media was allowed to cool to 45°C and was dispense into a different sterile petri dish and allowed to solidify. Also, 51.5g of the powder was suspended in 1liter of distilled water. It was mixed well and heated to boil for a minute and was shaken frequently until completely dissolved. It was then sterilized in the autoclave at 121ºC for 15 minutes, 1 ml of each macerated sample was added into the test tube containing 9 ml of sterile distilled water and finally, fold serial dilutions was made from 10^{-1} to 10^{6} and was examined by means of spread plate method.

III. SERIAL DILUTION, INOCULATION AND INCUBATION

A series of dilution bottles were labeled with the dilution factors (10^-2, 10^-3, 10^-4, 10^-5, 10^-6.). 1 ml of the homogenized sample (10^-1 dilution) was transferred into a dilution bottle containing 9 ml. The bottle was vortexed to mix well, resulting in the 10^-2 dilution. The process was repeated to create further serial dilutions (e.g., 10^-3, 10^-4, up to the 6th dilution level). Sterile petri dishes were labeled with the corresponding dilution factors. Using a sterile pipette, 0.1 ml from each dilution was transferred onto separate labeled agar plates. The inoculum was spread evenly over the surface of the agar using a sterile wire loop. The plates were incubated upside down at 37°C for 24-48 hours.

Colonies count

After incubation, the plates were examined, and the number of colonies formed on each plate were counted. Plates with 30-300 colonies and those below 30 were chosen for accurate counts. The number of colony-forming units (CFUs) per gram of the original sample were calculated using the following formula: CFU/ml (Number of colonies X dilution factor) / Volume of cultured plate).

Gram Staining and examination

A smear of colonies isolate was made on a glass slide using a sterile wire loop. It was dried and heat-fixed. Then, the fixed smear was flooded with crystal violet solution for 60 seconds and washed. This was later flooded with Lugol's iodine for 60 seconds, then washed off and decolorized with ethanol 70% concentration. The smear was then flooded with safranin solution for 60 seconds and then rinsed with water and air-dried. Thereafter, microscopy examination was carried out using 40x and 100x objectives following conventional microbiological protocols for bacteria. A standardized step-by-step approach was maintained to ensure accurate identification and visualization of specimen bacteria, focusing on morphology and Gram reaction.

Biochemical reaction

Catalase test was carried out mostly on gram-positive cocci to test their ability to produce the catalase enzyme. In this case, it differentiates between staphylococcus which is catalase-positive and *Streptococcus,* which is catalase negative. Catalase test was carried out also in both gram-positive and gram-negative bacilli and cocci. A colony of culture was emulsified in a drop of hydrogen peroxide on a clean glass slide. The presence of oxygen bubbies indicated positives result of a catalase test, while the absence of oxygen bubbles indicated a negative result of a catalase test, (Agbaje *et al*, 2017). Coagulase test was used to differentiate between *Staphylococcus aureus* from other staphylococcus species, due to their production of the enzyme coagulase by the *S. aureus* only. A looped of the isolate was emulsified in a drop of normal saline and a drop of citrated plasma was added and mixed. The slides were rocked gently for 2 minutes observing for coagulate reaction or dumping positive isolate to produce agglutination reaction with the plasma (Wuletaw *et al*. 2018).

Urease test was applied for bacteria species that can decompose urea by an enzymatic reaction to produce ammonia. After solidification of the urea medium, the inoculums was inoculated into the slant bottles and incubate at 37°C for 24 hours. A positive test was indicated by purple-pink colour and for a negative test there is no change in colour, (Odeyemi *et al*. 2017). Koser's citrates medium was inoculated with the isolate and incubate at 37°C for 48 hours. It was examine after two days. The presence of growth leads to an increase in pH resulting to change in colour table for a positive test and initial green colour for a negative test. For Indole test colonies were picked and inoculated into the test tube containing the Indole medium and finally incubated at 37°C for 48 hours

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was required. 0.5 ml of Kovac's reagents was added drop-wise to the test tubes and was shaken gently. This production of indole is confirmed by the formation of red ring colorations on the surface of the medium, which indicated a positive reaction, while in negative reaction red colorations was not produce (Sarkar *et al*. 2017).

Standard Incubation (24–48 hours): Most clinical isolates produce enough tryptophanase in 24–48 hours for detection.

Rapid Spot Test (Seconds to Minutes): Using high-concentration media (like tryptone broth or agar), a result can be obtained within 18–24 hours, followed by a rapid reaction (3 minutes) using Ehrlich's or Kovac's reagent, especially for spotting colonies.
Extended Incubation (up to 7 days): Some weak indole-producing bacteria may require longer, up to 7 days, to produce a positive result.
Optimal Timing: 18–24 hour old cultures are generally best for inoculation, with the test read after a further 24 or 48 hours of incubation.

Finally, motility test, aimed at identifying motile bacteria was carried out. A drop of normal saline was place on a sterile slide and a colony of test organism was suspended and emulsified and then covered with a coverslip. The prepared slides were examined with a light microscope by microscopically using 10x and 40x objective lens. Movement in different directions gave a positive test (Lee *et al*. 2017).
The data obtained from microbial load determination, Gram staining, and biochemical tests were analyzed using a combination of descriptive and inferential statistical methods to determine the frequency, diversity, and significance of microbial contamination.

IV. RESULTS

Table 1: Total bacteria count in food sold by Vendors in Jos south LGA

Sample code	No. of colonies	Bacterial counts (g/cfu)
ZD 1	278	2.78×10^5
ZD 2	223	2.23×10^5
ZD 3	300	3.0×10^6
DD 1	15	1.5×10^5
DD 2	67	6.7×10^4
DD 3	92	9.2×10^4
VD 1	206	2.06×10^5
VD 2	287	2.87×10^5
VD 3	290	2.90×10^5
GD 1	295	2.95×10^5
GD 2	234	2.34×10^5
GD 3	98	9.8×10^4
KD 1	296	296×10^5
KD 2	86	8.6×10^5
KD 3	297	2.97×10^5

Keys: ZD (Zawan district), KD (Kuru district), GD (Gyel district), DD (Du district), VD (Vwang district), g (Gram), CFU (Colony, Forming Unit).

This table shows the bacterial load of the isolated bacterial species from the food samples analyzed. The result reveals that Du district has the lowest bacterial counts, while Gyel district Kuru district, Vwang district and Zawan district have higher distribution of bacterial counts respectively. Du district indicates that bacterial counts is significantly different from others, while Zawan, Kuru, Vwang and Gyel district's bacterial counts were not significantly different from each other, rather they were significantly higher than Du district.

Table 2: Biochemical Characterization of the Bacteria

Biochemical Test	Bacterial isolate: *Bacillus species*	*Klebsiella Aerogenes*	*Staphylococcus aureus*	CoNS
Gram stain	+ Rods	+ Rods	+ Cocci	+ Cocci
Catalase	+	+	+	+
Coagulase	NA		+	-
Citrate	+	+	+	-
Gelatin hydrolys	+	-		-

is				
Hemolysis	+	-		-
TSIA	NR	AAG		NA
H_2S	-	-		
Indole	-	-	-	
Motility	+	-	-	-
Oxidase	-	-	-	-
Urease	+	-	+	+
Adonitol	-	+		
Arabinose	+	+		
Cellobiose	+	+		
Dulcitol	-	+		
Glucose	+	+	+	
Inositol	+	+		
Lactose	+	+	+	+
Maltose	+	+		+
Mannitol	+	+		-
Mannose	+	+		+
Melibiose	-	+		
Raffinose	-	+		
Rhamnose	+	+		
Salicin	-	+		-
Sorbitol	+	+		
Sucrose	-	+	+	
Trehalose	+	+		-
Xylose	+	+		

Keys: NA (Not Applicable), NR (No Reaction), AAG (Acid/ Acid/ Gas), TSIA (Tripple Sugar Ion Agar), CoNS (Coagulase Negative Staphylococcus species), H2S (Hydrogen sulphide production).

The results in table 2 shows that the characteristics of the isolated bacterial species using biochemical tests such as gram stain, TSIA, catalase, coagulase, citrate, indole, urease, motility, H_2S, Arabinose, Maltose, Raffinose Glucose etc. The identified species includes; *Staphylococcus aureus*, *Bacillus spp*, *Klebsiella aerogenes* and CoNs (Coagulase Negative *Staphylococcus*).

The table shows the identification of the suspected isolated organisms from different biochemical tests. All the samples were identified to be contaminated with the suspected target organisms. The biochemical test results showed similarities with the standard biochemical test results

Table 3: Susceptibility of the Bacteria isolate

Bacteria Susceptible	No. Susceptible (%)	P-value
Bacillus spp	8 (38.1%)	2.810[a]
Klebsiella aerogenes	6 (28.6%)	
Staphylococcus aureus	4 (19.0%)	
CoNS	3 (14.3%)	
Total	21 (100.0)	

Percentages shown in parentheses; "S" denotes statistical significance at the level ($P < 0.005$).

This table shows the susceptibility of bacterial isolates from the sampled foods sold by vendors. The result revealed varying levels of susceptibility across different bacterial species. Specifically, the results indicated that *Klebsiella aerogenes* has a susceptible rate of 28.6%, *Staphylococcus aureus* has a susceptibility rate of 19.0%. *Bacillus spp* indicates a higher susceptibility rate of 38.1%. While Coagulase-negative *Staphylococci* (CoNS) showed a susceptibility rate of 14.3%.

V. DISCUSSION

The study reveals that *Klebsiella aurogenes* (28.6%) is an opportunistic pathogen known for causing foodborne illnesses. According to a study by Olaitan *et al.* (2019), the presence of Klebsiella in food products signifies potential health risks, especially in immune-compromised individuals. *Staphylococcus aureus* (19.0%), found in the samples, is a known cause of food poisoning. It's presence in food sold indicates potential contamination from handlers, as supported by findings from Asghar *et al.* (2018).

Coagulase-Negative Staphylococci (CONS), which are part of the normal human skin flora, are recognized as opportunistic pathogens, which are sometime responsible for multi-drug resistance (Chen, et al, 2025). While generally less pathogenic than *Staphylococcus aureus* (low virulence in healthy populations), they can cause serious, often nosocomial infections in specific conditions. Isolation at high rates (ranging from 14.3% or more in clinical samples), predominantly cause infections from foreign body and devices, and in immune-compromised individuals. The occurrence of CoNS in food sold to public was also noted in some studies including that of Peterson *et al.* (2020), which highlighted the need for adequate hygiene measures in food handling.

In this study, *Bacillus spp* showed a susceptibility rate of 38.1%, which is relatively low. This finding is consistent with research conducted by Osman *et al.* (2018), which found that *Bacillus* species from various food sources, including food sold to public, often exhibit low susceptibility to antibiotics due to their ability to form resistant spores. Similarly, a study by Abriouel *et al.* (2019) reported that *Bacillus spp* isolated from food products showed significant resistance to multiple antibiotics, which poses a public health concern due to their potential pathogenicity and possible trigger for anti-microbial resistant (AMR) as noted by Chen, et al (2025). A high percentage of *Bacillus spp* isolated could be explained by their spore forming ability which makes them able to resist harsh environmental condition, withstand dry heat and certain chemical disinfectants for a considerable long period. *Bacillus* spp. were isolated from environmental sites in Mecca City, as reported by (Samy et al, 2012), *several* other Bacillus species have also been implicated in human pathogenesis and as food spoilage organisms (Collins and Lyne 2019).

The susceptibility rate for *Klebsiella aerogenes* was also 28.6%. Park *et al.* (2020) reported similar findings, indicating that Klebsiella species isolated from food items such as food sold in public places exhibit considerable antibiotic resistance. This resistance is often attributed to the production of extended-spectrum beta-lactamases (ESBLs), which degrade many beta-lactam antibiotics. Eze *et al.* (2020) further supported these findings, highlighting the fact that Klebsiella spp in food products show increased resistance, which complicates treatment options for infections caused by these bacteria.

The susceptibility rate for *Staphylococcus aureus* was 19.0%, which is notably low. Holmes *et al.* (2018) reported that *Staphylococcus aureus*, especially methicillin-resistant *Staphylococcus aureus* (MRSA), particularly the drug-resistant strain – MRSA, are major food safety issue. Because MRSA resists many standard antibiotics, it is very difficult to treat when it causes illness, making its presence in food (due to poor hygiene or handling) a serious health risk, and it is a common concern in food safety due to its high resistance to many antibiotics. Ali *et al.* (2019) similarly found that S. aureus isolated from food sources frequently exhibit significant resistance, posing challenges for treatment and control.

Coagulase-negative staphylococci (CoNS) showed 14.3% susceptibility in this study. Brown *et al.* (2018) observed that CoNS isolated from food sources often exhibit high levels of resistance to antibiotics commonly used in clinical settings. Green *et al.* (2020) reported that CoNS isolated from food products also show significant resistance, which aligns with the findings of this study. This resistance can be attributed to the frequent use of antibiotics in food production, leading to the emergence of resistant strains.

CONCLUSION

The study highlights the diverse bacterial contamination in food sold by food vendors, with *Bacillus spp* being the most prevalent. The study aligns with other research indicating that foodborne pathogens such as Klebsiella, Staphylococcus, and CoNs are common food contaminants of serious public health concern in food safety. Also the findings of this study have confirmed that pathogenic bacteria can exist in cooked foods even though they may physically appear to be quite wholesome. We therefore conclude that pathogenic bacteria capable of causing food poisoning can be isolated from cooked foods using microbial analysis. Based on these findings and conclusion it is hereby recommended that Environmental Health Officers

should educate food vendors practical steps in food handling and food practices to promote food safety, while the local environmental health practitioners should enforce relevant food safety regulations especially the National Environmental Health Practice Regulations, 2024.

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