

Molecular Characterization of Bacteria Isolated from Cooked Food from Restaurants and Local Eateries (Buckas) in Samaru Zaria, Kaduna State, Nigeria

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Abstract

Food contains the nutrients that humans and animals need to be healthy, it is eaten to provide energy and nutrition. The etiological factors contributing to foodborne illnesses are the consumption of microbial pathogens, biochemical substances, or biotoxins generated by the microorganisms. The study aimed to investigate the isolation and molecular characterization of bacteria isolates from cooked foods from restaurants and local eateries (Buckas) in Samaru Zaria, Kaduna State. A total of twenty samples (ten samples each for Buckas and Restaurants) were collected from various sites within Samaru Zaria for the analysis. The bacteriological analysis was carried out by isolation and then molecular characterization (using DNA extraction kit, PCR technique and 16SrRNA Sequence identity of the selected bacteria isolates). The growth of the microorganisms was observed and counted per mL. Four species of bacteria were isolated from all the samples which includes: *Staphylococcus aureus*, *Bacillus* spp, *Proteus* spp, and *Klebsiella pneumoniae*. The food analyzed revealed that Mesophilic count had the highest bacterial load as 7.8×10^6 CFU/g and 8.8×10^6 CFU/g, followed by staphylococcal count of 7.9×10^6 CFU/g with *Staphylococcus aureus* having the highest frequency of occurrence as 11(55%), followed by *Bacillus* spp 7(35%) then *Klebsiella pneumonia* 1(5%), and *Proteus* spp 1(5%). Molecular characterization showed the band sizes of the subjected isolated bacterial colonies and sequencing testified the specified bacteria species as *S. saprophyticus* and *B. paramycoides*. further studies with the sequencing of *Proteus* containing isolates obtained from cooked food should be performed to determine their sequence variation and relatedness and also its mechanism of action can be very significant and exploited for further research.

Keywords: Bacterial species; Foods; Bucka; Restaurant; Polymerase Chain Reaction;

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Salihu S. et al, DUJOPAS 11 (2d): 1-10, 2025

INTRODUCTION

Foodborne illnesses remain a significant global public health challenge. Through a comprehensive assessment of 31 major foodborne pathogens, the World Health Organization (WHO) estimated that 26 prioritized hazards account for over 33 million Disability-Adjusted Life Years (DALYs), with a disproportionately high burden – approximately 40% – affecting children under five years of age (Havelaar, 2015). Vulnerable populations, particularly young children, the elderly, and individuals with underlying health conditions, are more susceptible to severe outcomes from foodborne illnesses (Lund *et al.*, 2019).

The burden of foodborne diseases is disproportionately high in low- and middle-income countries (LMICs), particularly in Sub-Saharan Africa, due to inadequate food safety regulations, limited access to clean water, and poor hygiene practices. Food handlers and vendors have been implicated in the microbial contamination of food. Due to widespread poverty, scarcity of resources, and lack of awareness in the region, many individuals consume readily available food and drinks regardless of their safety or hygiene (Akther *et al.*, 2021). In Nigeria the most populous country in Africa – foodborne illnesses are often underreported, and outbreaks are typically recognized only after widespread transmission. For example, a cholera outbreak in 2024 led to 30 deaths across multiple states, underscoring persistent challenges in ensuring food and water safety (Premium Times Nigeria, 2024). Similarly, in January 2018, the Nigerian Centre for Disease Control (NCDC) responded to a botulism outbreak in Abuja linked to the consumption of contaminated food (Okunromade *et al.*, 2020).

According to the World Health Organization (WHO, 2020), approximately 550 million people fall ill and 230,000 die each year from diarrheal diseases linked to the consumption of food contaminated by microbial pathogens. Additionally, a report by the World Bank (2018) estimates that food-borne diseases result in a \$95.2 billion annual loss in productivity in developing countries, with an additional \$15 billion spent yearly on treatment. Food-borne illnesses have posed a significant global health challenge throughout history and continue to affect populations worldwide (WHO, 2020).

Timely diagnosis of foodborne diseases depends on both laboratory detection of pathogens or toxins and a thorough assessment of recent food intake (Andargie *et al.*, 2008).

Food plays a fundamental role in human health by providing energy, sustaining life, and supporting growth. Different populations and regions display unique dietary patterns and food handling practices that reflect cultural, environmental, and socio-economic factors (Sapea, 2020). However, in Nigeria and many other LMICs, there is a notable regulatory gap between Restaurants and Buckas. Despite this, no direct comparative study has been conducted on the microbial contamination levels between these two food sources in Samaru, Zaria.

This gap highlights the urgent need for enhanced hygiene regulations, public health education, and monitoring policies, especially in the Buckas food sector – an area often overlooked in national food safety strategies. Notably, this study introduces a novel aspect by identifying *Staphylococcus saprophyticus* and *Bacillus paramycoides* through 16S rRNA gene sequencing, contributing new insights into the microbial profile of cooked foods in the region.

MATERIALS AND METHODS

The study area was Samaru community Market Zaria, Sabon Gari local government area, Kaduna State-Nigeria. Samaru is located on latitude 11°09'30.99"N and longitude 7°38'15.68 to 7°39'20.68"E at an altitude of 550-700 m. It lies within the Northern Guinea savanna

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SAMPLE PREPARATION, INOCULATION AND INCUBATION

Ten grams (10g) of each of these food sample (Semolina, Amala, Moi-Moi, Tofu, Golden yam, Salad, Fried rice, Spaghetti, Jollof rice, Peppered chicken, Spaghetti, Boiled yam, Jollof rice, Boiled beand, Rice and beans, Moi-Moi, Fried potatoes, Dan-wake Dumplings, pounded yam and Semolina swallow) was weighted and added to 90ml of 0.1% sterile peptone water. This was the stock solution and was allowed to settle for few minutes. However, 9ml of 0.1% sterile peptone water was measured and taken in 4 bottles, exactly 1ml was taken from the stock using a sterile pipette and transferred aseptically into the first bottle containing 9ml of 0.1% sterile peptone water diluents to obtain a (10^{-1}) homogenate. Samples in tubes were thoroughly mixed for 30 second using vortex mixer to make initial dilutions. These dilutions were serially diluted into the factor of 10^{-4} . Each suspension was further treated on duplicate plates by spread plate method using 0.1 ml and spread on sterile Mannitol Salt Agar & Plate Count Agar and incubated at 37°C for 24hours. For the analysis of desired microorganisms according to the ISO suggested protocols. (ISO, 21528-2, 2004). All media and reagents for analysis were of Oxoid (Hampshire, UK) brand.

Isolation, Counting and Identification of Colonies

After completion of specified incubation, counting of colonies were performed as CFU/gm of the samples according to the ISO protocol (ISO 18593, 2004). Identification and further confirmation of isolated bacterial species were performed through Gram staining and biochemical tests which involve: catalase, coagulase, Citrate, Indole, Motility, Methyl-Red Voges-Proskauer (MRVP), and Urease test as mentioned in the respective protocols (Cheesbrough, 2006). Nutrient Agar, MSA, Nutrient agar Slants, PCA, Mueller-Hinton agar, deoxyribonuclease agar (DNase agar), and buffered peptone water were prepared according to the manufacturers' instructions.

Extraction of genomic DNA from bacteria isolates

DNA was extracted at African Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria Kaduna State, Nigeria using Zymo research extraction kit following manufacturer protocol. The quality and concentration of the extracted DNA was determined using a Nanodrop spectrophotometer and the DNA was stored at -20°C for further downstream application.

Amplification of 16S rRNA of bacteria isolates

Extracted DNA at African Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria Kaduna State, Nigeria was used as template to amplify the 16S rRNA using the universal primers. PCR was performed in a final reaction volume of 25 μL in a PCR tube, containing 12.5 μL of master mix (mixture of dNTP, taq polymerase, MgCl_2 and PCR buffer), 2 μL forward primer (AGAGTTTGATCCTGGCTCAG), 2 μL reverse primer (GGTACCTTGTTACGACTT), 2 μL of extracted DNA and 6.5 μL of nuclease-free water. Thermal cycles for the amplification were set as: initial denaturation for 5min at 94°C , 30 cycles of 40 s at 94°C , 30s at 50°C , and 1 min at 72°C and final extension for 5 min at 72°C . Thereafter, 5 μL of each PCR product was analyzed on 1 % (w/v) agarose gel supplemented with ethidium bromide. The amplicons were then visualized using gel documentation system (Nupur *et al.*, 2020).

Nucleotide Sequencing and Alignment

16S rDNA sequencing of the isolated strain was carried out by Dye terminator cycle sequencing (Quick start kit). The gene sequences of each isolate obtained in this study was compared with known nucleotide database at the National Center for Biotechnology

Information (NCBI) by using their worldwide website, and the BLAST (Basic Local Alignment Search Tool) algorithm (Jyothi *et al.*, 2012).

RESULTS AND DISCUSSIONS

A list of food samples was compiled to show the variety of foods collected from both Bucka's and various restaurants in Samaru, Zaria, Kaduna State. The aerobic mesophilic and Staphylococcal counts of these food samples were determined. These counts were used to calculate the mean, standard deviation, and colony-forming units per gram (CFU/g), as presented in Table 2. The table also displays the number and range of bacterial growth observed on the two media used: Plate Count Agar (PCA) for total aerobic mesophilic bacteria and Mannitol Salt Agar (MSA) for Staphylococci.

Biochemical Characterization of Isolates

Table 1 showed the characteristics of the isolated bacterial species using gram staining and biochemical tests from which the identified species includes; *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus spp* and *Bacillus spp*. It also showed that two of the organisms (*Proteus* & *Klebsiella*) were gram-negative and the remaining two are gram-positive (*Bacillus* & *Staph*) with *Staphylococcus aureus* being positive for all but Cit and M, *Bacillus spp* being positive for all but Cat, Coa, I, MR and VP, *Proteus spp* being positive for all but Coa, M, VP and I. And then *Klebsiella pneumonia* being positive for all but VP and Cit. 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.

The mesophilic bacterial count was consistently higher than the staphylococcal count across all samples. Samples collected from Bucka's recorded the highest mean counts for both groups, with mesophilic counts of 7.8×10^6 CFU/g and 8.8×10^6 CFU/g, and a staphylococcal count of 7.9×10^6 CFU/g. (moimoi, fried potatoes and dan-wake dumplings) and 5.0×10^5 CFU/g and 5.0×10^5 CFU/g (Rice and beans jollof and semolina swallow) whereas, the least mean counts are 1.6×10^6 CFU/g and 1.0×10^6 CFU/g (Boiled beans and Jollof rice) respectively (Table 2).

The highest mean count for mesophilic and staphylococcal restaurant food samples were 5.9×10^6 CFU/g, 7.0×10^4 CFU/g and 7.0×10^4 CFU/g (Salad, Amala and Tofu) and the least mean counts are 1.5×10^6 CFU/g and 1.1×10^6 CFU/g (Golden yam and Jollof rice).

Table 1. Gram's Reaction, Cell Morphology and Biochemical Characterization of Bacterial Isolates

Organism Identified	Gram's Rxn	Morphology	CAT	COA	IND	MR	VP	CIT	URE	MOT
<i>Staphylococcus aureus</i>	+	Cocci	+	+	-	+	-	-	-	-
<i>Bacillus spp</i>	+	Long-rod	+	-	-	+	+	+	-	+
<i>Proteus spp</i>	-	Short-rod	+	-	+	+	-	+	+	+
<i>Klebsiella pneumoniae</i>	-	Short-rod	-	-	-	-	+	+	-	-

Key to Abbreviations:

- **Gram Rxn:** Gram Reaction (+ = Gram-positive, - = Gram-negative), **CAT:** Catalase, **COA:** Coagulase, **IND:** Indole, **MR:** Methyl Red, **VP:** Voges-Proskauer, **CIT:** Citrate, **URE:** Urease and **MOT:** Motility

Table 2. Summary of CFU Counts from Food Samples Samaru Zaria

Sample Label	Source	Food Item	Aerobic Mesophilic CFU/g	Staphylococcal CFU/g	CFU Above Permissible Limit?
A	Restaurant	Semolina	5.0×10^5	2.4×10^5	✓ Yes
B	Restaurant	Amala	3.3×10^6	7.0×10^4	✓ Yes
C	Restaurant	Moimoi	7.8×10^6	2.0×10^4	✓ Yes
D	Restaurant	Tofu	2.7×10^6	7.0×10^4	✓ Yes
E	Restaurant	Golden Yam	1.5×10^6	6.0×10^4	✓ Yes
F	Restaurant	Salad	5.9×10^6	3.0×10^4	✓ Yes
G	Restaurant	Fried Rice	1.7×10^6	4.0×10^4	✓ Yes
H	Restaurant	Spaghetti	2.6×10^6	5.0×10^4	✓ Yes
I	Restaurant	Jollof Rice	2.3×10^6	1.1×10^6	✓ Yes
J	Restaurant	Peppered Chicken	2.2×10^6	6.0×10^5	✓ Yes
1	Bucka	Spaghetti	2.0×10^6	2.0×10^5	✓ Yes
2	Bucka	Boiled Yam	2.1×10^6	2.0×10^5	✓ Yes
3	Bucka	Jollof Rice	2.3×10^6	1.0×10^6	✓ Yes
4	Bucka	Boiled Beans	1.6×10^6	2.0×10^5	✓ Yes
5	Bucka	Rice & Beans Jollof	3.0×10^6	5.0×10^5	✓ Yes
6	Bucka	Moimoi	8.4×10^6	2.0×10^5	✓ Yes
7	Bucka	Fried Potatoes	8.8×10^6	1.58×10^7	✓ Yes
8	Bucka	Dan-wake Dumplings	7.9×10^6	3.0×10^5	✓ Yes
9	Bucka	Pounded Yam	2.24×10^7	3.0×10^5	✓ Yes (Extreme outlier)
10	Bucka	Semolina Swallow	6.1×10^6	5.0×10^5	✓ Yes

The mesophilic count was larger than the staphylococcal count for both collecting groups, with samples collected from Bucka's having the highest mean count for both mesophilic and staphylococcal count as 7.8×10^6 CFU/g, 8.8×10^6 CFU/g and 7.9×10^6 CFU/g (moimoi, fried potatoes and dan-wake dumplings) and 5.0×10^5 CFU/g and 5.0×10^5 CFU/g (Rice and beans jollof and semolina swallow) whereas, the least mean counts are 1.6×10^6 CFU/g and 1.0×10^6 CFU/g (Boiled beans and Jollof rice) respectively (Table 2). The highest mean count for mesophilic and staphylococcal restaurant food samples are 5.9×10^6 CFU/g, 7.0×10^4 CFU/g and 7.0×10^4 CFU/g (Salad, Amala and Tofu) and the least mean counts are 1.5×10^6 CFU/g and 1.1×10^6 CFU/g (Golden yam and Jollof rice).

Frequency Occurrence of the Isolated Bacterial Species

The study revealed that *Staphylococcus aureus* and *Bacillus spp* were the predominant bacteria species isolated from cooked foods (55% and 35%) followed by *Proteus spp* (5%) and *Klebsiella pneumonia* (5%). It was observed that *Staphylococcus aureus* had the highest incidence of occurrence, followed by *Bacillus spp* and then *Proteus spp* and *klebsiella pneumonia* which have equal and the least incidence of occurrence as summarized in Table 3

Table 3: Frequency Occurrence of the Isolated Bacterial Species

Organism	Occurrence	Frequency (%)
<i>Staphylococcus aureus</i>	11	55
<i>Bacillus spp</i>	7	35
<i>Proteus spp</i>	1	5
<i>Klebsiella pneumonia</i>	1	5
TOTAL	20	100

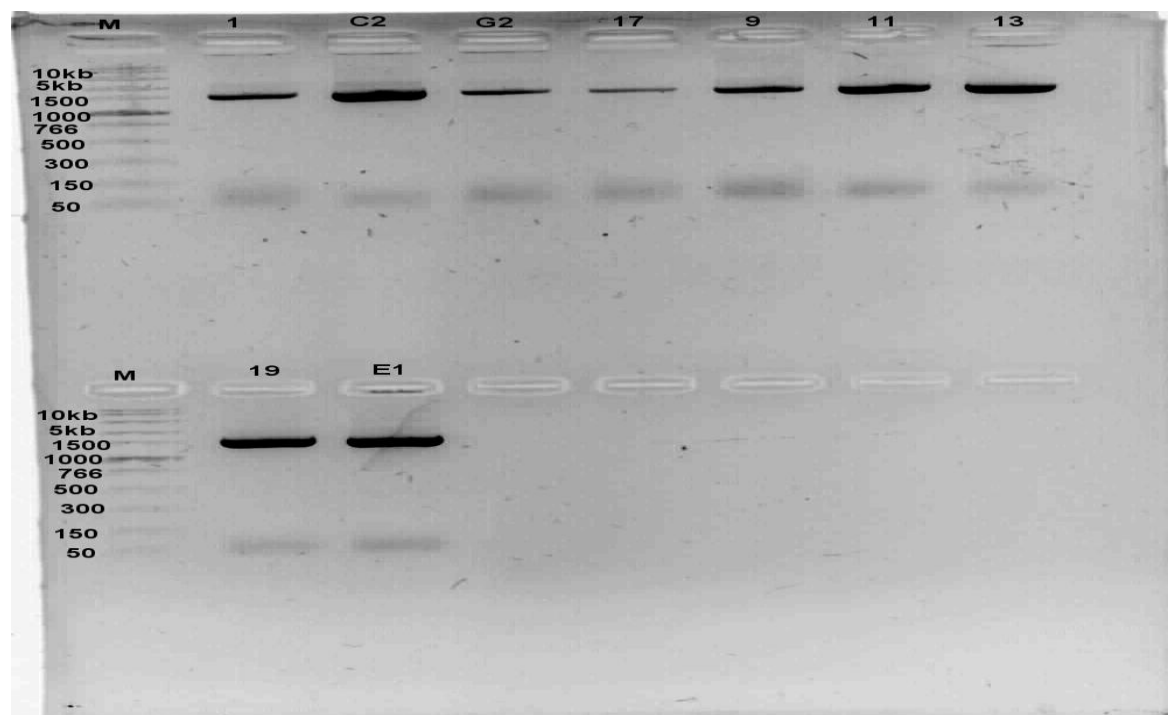


Plate 1: Agarose Gel image of the amplicons detected by PCR

Lane M= Molecular ladder (100bp)

Lanes 1, C2, G2, 17, 9, 11, 13, 19 & E1 are positive for bacterial strains (bands of 1500bp)

KEY

1, C2, G2 and 17-*staphylococcus aureus* ,9,11,19 and 13-*Bacillus spp* then E1-*Proteus spp*.

Table 4: 16SrRNA Sequence identity of Selected Bacteria Isolated from Cooked Food Samples.

Sample code	Identity	% Identity	Query Cover %	Accession Number
C2	<i>S. saprophyticus</i>	92.18	96	MT256266.1
19	<i>B. paramycoides</i>	96.96	95	CP035294.1

The AMCs were nearly similar to that recorded by Amany *et al.* (2015) which found that the total aerobic plate count ranged from 6×10^3 to 3.4×10^5 with a mean value of $4.8 \times 10^4 \pm 3.6 \times 10^3$ in fast food, while Nimri *et al.* (2014) and Odu and Akano (2012) found that APCs for shawarma food samples were in the range of 2.0×10^3 to 1.8×10^3 CFU/g. Samples collected from Buckas were in range with the work of Lambu *et al.* (2022), who reported high bacterial counts of 7.6×10^4 CFU/mL for Rice and stew, and nearly similar for least mean counts as 1.7×10^1 CFU/mL in jollof Rice. Despite the use of heat in food preparation, certain harmful organisms remain present during sample analysis. This could be because some of the enumerated bacteria such as *Bacillus spp* can survive the high cooking temperature that Shawarma items were exposed to, which is insufficient to eliminate hazardous microorganisms Abdelhai *et al.* (2015).

The isolated bacteria from the food samples comprise some members from the family Enterobacteriaceae known to be associated with food borne illness. This agrees with the findings of Mengistu *et al.* (2022) who isolated *Escherichia coli*, *Staphylococcus aureus*, *Bacillus specie*, *Micrococcus luteus*, *Salmonella enterica* and *Citrobacter freundii*, from jollof Rice, Beans and Moin moin samples in Kumasi Ghana and with the work of Amadi *et al.* (2018) who isolated *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus* and *Citrobacter freundii* from the different Rice dishes analyzed.

In this study, *Staphylococcus aureus* was detected in 55% of the samples collected. This prevalence is lower than the studies reported by Bantawa *et al.* (2018), they showed *S. aureus* in 68% samples. While, the prevalence of *S. aureus* in meat was lower than the study of Rong *et al.* (2017), the results of them showed 37.2% of samples were positive for *S. aureus* (Rong *et al.*, 2017).

The observed variation in microbial load between food samples collected from restaurants and local eateries (buckas) can be scientifically attributed to differences in hygiene practices, infrastructure, and environmental exposure. Restaurants typically operate within enclosed environments equipped with better sanitation facilities, access to clean water, refrigeration, and designated food preparation zones. These factors collectively reduce the likelihood of cross-contamination and microbial proliferation. Moreover, restaurant staff are more likely to receive food safety training and are subject to periodic inspections, which may encourage adherence to proper handling protocols. In contrast, buckas often function in open-air settings, where food is exposed to dust, insects, and high human traffic, significantly increasing the risk of contamination. Additionally, inadequate temperature control, limited access to cleaning supplies, and prolonged holding times of cooked foods create favorable conditions for bacterial growth, particularly for mesophilic and Staphylococcal species. These disparities highlight the need for stricter food safety regulations and awareness campaigns targeted at informal food vendors (Buckas) to mitigate the risk of foodborne illnesses in such settings.

Molecular identification via 16S rRNA gene sequencing yielded definitive taxonomic resolution of selected bacterial isolates, corroborating the limitations of conventional biochemical assays in differentiating phylogenetically proximate taxa. Amplified sequences aligned with reference strains in the NCBI GenBank database confirmed the presence of *Staphylococcus saprophyticus*, a coagulase-negative staphylococcus frequently implicated in opportunistic infections, and *Bacillus paramycoides*, a spore-forming bacillus with notable thermotolerance. The precision afforded by PCR-based molecular diagnostics affirms its utility in foodborne pathogen surveillance, particularly in resource-limited contexts where phenotypic plasticity may obscure accurate microbial characterization.

CONCLUSION

Thirty-six (36) bacteria were isolated in replicates from cooked foods between two categories within Samaru, Zaria metropolis. Fifty-five (55) percent of the isolates was *S. aureus*, *Bacillus spp* was thirty-five (35) percent of the isolates and *Proteus spp* was five percent (5). In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) oversees food safety regulations. While specific microbial thresholds like the $<10^3$ CFU/g standard set by Food Standards Australia New Zealand (FSANZ) are not explicitly detailed in publicly available NAFDAC guidelines, the agency emphasizes adherence to international best practices, such as those from the Codex Alimentarius Commission, in its food hygiene protocols. The percentage of isolates with bacterial counts exceeding the acceptable threshold of $<10^3$ CFU/g, as set by Food Standards Australia New Zealand (2018), suggests that the food samples may serve as vehicles for the transmission of potentially pathogenic bacteria. Using 16S rRNA and the NCBI BLAST search, the isolates were sequenced to identify the tested organisms under the genera *Bacillus* and *Staphylococcus*. The results showed a close resemblance to *B. paramycoides* and *S. saprophyticus*. It can be suggested that further studies with the sequencing of *Proteus* containing isolates obtained from cooked food should be performed to determine their sequence variation and relatedness. Corrective action needs to be employed to minimize the risk of consuming contaminated food and the need for regular

surveillance by public health regulatory bodies with World Health Organization (WHO) and International Organization for Standardization (ISO) standards for food safety.

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