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MICROBIOLOGICAL EVALUATION OF READY-TO-EAT FOODS SOLD AROUND SA'ADU ZUNGUR UNIVERSITY GADAU, BAUCHI, NIGERIA: IMPLICATIONS FOR FOOD SAFETY AND PUBLIC HEALTH

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ABSTRACT

Food safety remains a global public health concern, and the eruption of diseases caused by food contamination occurs in places where sanitation and hygiene conditions are generally poor. This study evaluated the microbiological quality and safety of ready-to-eat foods (Awara, Akara, and Masa) sold around Sa'adu Zungur University, Gadau Campus, Bauchi, Nigeria. A total of 27 samples were randomly collected from the cafeteria, Gadau Market, and off-campus sites over one month. Samples were immediately analyzed using standard microbiological assays, including serial dilution, inoculation, incubation, microscopy, and biochemical tests to identify and confirm microbial contaminants. A total of four pathogenic bacteria were isolated: *Staphylococcus aureus* (51.9 %), *Escherichia coli* (25.9 %), *Salmonella species* (18.5 %), and *Pseudomonas species* (3.7 %). Awara sample had the highest total aerobic bacteria count of $9.33 \pm 0.37 \times 10^5$ cfu/g, with total coliform count (TCC) obtained ranging from $4.07 \pm 2.8 \times 10^5$ to $7.20 \pm 0.23 \times 10^5$ cfu/g. Akara sample has the highest TCC ($7.20 \pm 0.23 \times 10^5$ cfu/g), and *Staphylococcus* count (TSC) of $5.40 \pm 0.31 \times 10^5$ cfu/g, while Masa samples have the lowest TSC of $1.13 \pm 0.03 \times 10^5$ cfu/g. The findings reveal poor hygiene among food vendors, likely due to inadequate food safety knowledge, posing health risks. Mandatory health certification, regular renewal, and food hygiene training are recommended to enhance safety and protect consumer health.

Keywords: Bacteria, Gadau, Foodborne illness, Food safety, Ready-to-eat

INTRODUCTION

The consumption of ready-to-eat foods has become increasingly prevalent in both urban and rural settings worldwide, offering convenient and time-saving meal options for individuals with busy lifestyles (Adeosun *et al.*, 2022). Within educational institutions such as Sa'adu Zungur University, Gadau Campus, Bauchi State, where students and staff often have demanding schedules, ready-to-eat foods (REFoods) like akara, awara, and masa serve as popular choices due to their affordability, accessibility, and palatability. However, the microbial safety of these foods is a growing concern, as they are susceptible to contamination during various stages of production, handling, and storage (Amoo *et al.*, 2024; Oyewole *et al.*, 2024; Muendo *et al.*, 2022; Rifat *et al.*, 2022; Mot-taleb *et al.*, 2018).

REFoods can be raw or cooked or hot or chilled foods, which can be bought directly from street vendors or hawkers, and either consumed at the point of sale or at a later time without further processing (Omemu *et al.*, 2018). Examples of such foods among others include meat pie, coleslaw, fried chicken, soya bean cake, suya meat, porridge, rice, and moi-moi (Mengistu *et al.*, 2022). Safe food is a basic human right despite the fact many foods are frequently contaminated with naturally occurring pathogenic organisms (bacteria) that cannot be detected organoleptically (seen, smelled, or tested) but can cause diseases including death especially if the way they are conserved during exposition for sale provides the condition for those bacteria to grow and reach considerable levels of contamination (WHO, 2019). There are many reasons why people eat away from home, which resulted in the transfer of food sanitary measures and proper food handling from individuals/families to the food vendors who rarely enforce such practices. It was estimated that 2.5 billion people patronize food vendors worldwide according to Lambil *et al.* (2022) and Adeosun *et al.* (2022).

Although it is extremely difficult to pinpoint the precise beginning of human awareness of the presence and role of bacteria in foods, the available evidence indicates that this knowledge preceded the establishment of bacteriology as a science (Adeosun *et al.*, 2022; NSWFA, 2019). In addition, some REFoods are also regarded as potentially hazardous. While such foods can at times support the growth of pathogenic (food poisoning) bacteria, and must be kept at certain temperatures to minimize the growth of any pathogens that may be present in the food or to prevent the formation of toxins in the food. Food-borne illness is a major international health problem with consequent economic reduction (Akther *et al*. 2021). Other findings showed that, REFoods constitute food-borne illnesses that were responsible for 691 food poisoning outbreaks and 49 deaths from 1983 to 1992 in Shandong Province of China. Another study by Igbinosa *et al.* (2020), described food-borne as a disease resulting from ingestion of bacteria, toxins, and cells produced by microorganisms present in food. According to WHO (2019), 20 % of deaths among children under five years are caused by diarrheal disease, and UNICEF estimated that about 1000 children below the age of five years die every day in India due to diarrhea. Among food-borne bacterial pathogens commonly detected in REFoods are *Bacillus cereus* which causes vomiting and diarrhea, *Staphylococcus aureus* which causes vomiting, diarrhea, loss of appetite, severe abdominal cramps, and mild fever, and *Salmonella* species which causes typhoid, food poisoning irritation, and inflammation in the gastrointestinal tract (Abebe *et al*., 2020; Adesokan *et al*., 2017; Odeyemi *et al*., 2019; Rufai & Wartu, 2023).

Despite the potential health risks associated with microbial contamination of REFoods, there is paucity of scientific information regarding the microbial quality of these foods within university campus settings in Nigeria. Several studies have investigated the microbiological status of REFoods in

other environments such as street vending sites and marketplaces (Amoo *et al.,* 2024; Olapade *et al*., 2020; Muhammad *et al.,* 2016). Limited attention has been given to the specific context of university campus like Sa'adu Zungur University Gadau, Bauchi State. The choice of selected foods such as akara, awara, and masa as the focus of this assessment is based on their popularity among the university community and their status as staple foods in Nigerian cuisine. Akara, a deep-fried bean cake, is commonly consumed as a breakfast or snack item, while awara, a tofu-like product made from soybeans, and masa, a fermented rice cake, are widely enjoyed as snacks or light meals. However, the microbial safety of these foods has not been adequately evaluated within the study area. Therefore, this study seeks to address this gap in knowledge by evaluating the microbiological quality and safety of ready-to-eat foods sold around Sa'adu Zungur University, Gadau Campus, Bauchi, Nigeria, with a focus on identifying potential microbial contaminants and assessing their implications for food safety on public health.

MATERIALS AND METHODS

Study Area

Sa'adu Zungur University-Main Campus, located in Gadau, Bauchi State, Nigeria, was established in 2011. The campus is situated at a latitude of 11.1769° N and a longitude of 10.3240° E. It serves as a vital educational hub in Bauchi State. It has a growing population of approximately 8,000 students and over 500 academic and non-academic staff. The student body comprises individuals from diverse ethnic and cultural backgrounds, offering a rich microcosm of the region's socio-demographic characteristics (NBS, 2023).

Sample Collection

A total of 27 ready-to-eat food samples (Awara, Akara, and Masa) were randomly collected from three locations within the study area: the cafeteria (X), Gadau Market (Y), and off-campus sites (Z), following the sampling approach adopted from Amoo *et al.* (2024). A simple random sampling technique was used to obtain samples from food vendors without prior notification or explicit consent to ensure unbiased data collection. Sampling was conducted three times over one month. The samples were aseptically collected, properly labeled, and immediately transported to the microbiology laboratory for analysis. Standard microbiological assays, including serial dilution, inoculation, incubation, microscopy, and biochemical tests, were performed to identify microbial contaminants (Adeleye *et al.,* 2019; Ekeleme & Mohammed, 2024).

Sterilization of Materials

All materials used were properly sterilized both before and after use to ensure aseptic conditions. Glassware such as test tubes, conical flasks, and pipettes were thoroughly cleaned with detergent, rinsed with water, and allowed to dry. They were then wrapped in aluminum foil and sterilized in a hot air oven at 170 °C for 1 hour. All media were prepared following the manufacturer's instructions, and both the prepared media and distilled water were autoclaved at 121 °C for 15 minutes. Metal instruments, such as the inoculating loop, were sterilized by heating to redness in an open flame before and after use. The laboratory bench was disinfected with 70 % alcohol before each analysis. Isolation and inoculation procedures were conducted near an open flame to minimize contamination of agar plates and tubes as reported by Adeleye *et al.* (2019); Yadav *et al.* (2012).

Bacterial Count (aerobic mesophilic count)

The pour-plate method was employed for this analysis, following the procedure outlined by the Food and Agricultural Organization (FAO, 2002) and adapted by Madgan *et al.* (2017). To begin, 25 grams of each sample were aseptically collected and placed in a sterile blender, 225 ml of buffered peptone water was added, and the mixture was homogenized for 2 minutes at a standard speed. 1 mL aliquot of the 10^{-1} dilution was transferred to 9 mL of sterilized peptone water to create subsequent dilutions up to 10^{-6}. From each serial dilution, 1 mL was pipetted into a sterile Petri dish, followed by the addition of molten agar (approximately 45 °C). The contents were mixed thoroughly by gently swirling the plate on the bench. The molten agar was allowed to solidify before incubation. Plates containing Nutrient Agar were incubated at 37 °C for 24 hours. Similarly, plates with MacConkey Agar and Mannitol Salt Agar were prepared using the same method and incubated at 37 °C for 24 - 48 hours. After the incubation period, colonies were counted using a colony counter, and the results were expressed as colony-forming units per gram (cfu/g) of the sample. Colony counts between 30 and 300 on a standard 10 cm Petri dish were considered valid. The CFU/ml was calculated using the following formula:

$$CFU/mL = \frac{No.of\ colonies \times Dilution\ factor}{volume\ plated}$$

Coliform Count

Following the method described by Odu and Assor (2013), the setup consisted of nine test tubes, with each containing 9 mL of lactose broth and an inverted Durham tube. The tubes were autoclaved to sterilize them and expel any trapped air. Serially diluted samples, ranging from 1:10 to 1:1000 dilutions, were then inoculated into the prepared tubes. The inoculated test tubes were incubated at 37 °C for 24 hours and observed for gas production. If no gas was produced, incubation continued for another 24 hours. After 24 to 48 hours, the tubes were examined for gas production as an indicator of microbial activity. The number of gas-positive tubes was compared with the Most Probable Number (MPN) table to estimate the MPN of coliforms per gram of the sample, following the procedure outlined by Madgan *et al.* (2017).

Enumeration and Identification of Bacterial Isolates

After 24 hours of incubation, bacterial colonies were enumerated using a colony counter. The number of colonies on each plate was multiplied by the corresponding dilution factor to calculate the plate count per gram of the sample. Identification of bacterial isolates was conducted by subjecting pure cultures to gram staining and a series of biochemical tests, including coagulase, catalase, indole, oxidase, citrate, methyl red, urease, Voges-Proskauer, and motility tests, to ensure accurate identification. Additionally, macroscopic and microscopic examinations of the bacterial isolates were performed following the methods described by Muhammad *et al.* (2016); Bello & Osho, (2013); Mboto *et al.* (2012); Cheesbrough (2008).

Data Collection and Analysis

Microbial data were analyzed using SPSS and Excel. Descriptive statistics were used to summarize bacterial counts across different food samples and locations. Pearson's correlation was applied to assess the relationships between microbial loads, while frequency distribution determined the prevalence of bacterial isolates. Biochemical characterization confirmed bacterial identification, with results were presented in tables and graphical representations.

RESULTS AND DISCUSSION

The mean total aerobic bacteria count (TBC) for samples collected from locations X, Y, and Z ranged from 6.22×10^5 to 9.33×10^5 CFU/g, as presented in Table 1. Among the samples, the *Awara* sample recorded the highest TBC at 9.33×10^5 CFU/g, while the *Akara* sample had the lowest TBC at 6.22×10^5 CFU/g. The total coliform count (TCC) across the same locations ranged from 4.07×10^5 to 7.20×10^5 CFU/g. The *Akara* sample exhibited the highest TCC at 7.20×10^5 CFU/g, whereas the *Masa* sample had the lowest TCC at 4.07×10^5 CFU/g. Similarly, the total *staphylococcus* count (TSC) for all locations ranged from 1.13×10^5 to 5.40×10^5 CFU/g. The *Akara* sample again had the highest TSC at 5.40×10^5 CFU/g, while the *Masa* sample recorded the lowest TSC at 1.13×10^5 CFU/g.

Table 1: Mean Total Aerobic Bacterial Counts Identified in Food Samples

FS	TBC cfu/g	Mean cfu/g	TCC cfu/g	Mean cfu/g	TSC cfu/g	Mean cfu/g
AX	9.8×10^5	6.22×10^5	7.2×10^5	7.2×10^5	5.6×10^5	5.4×10^5
AY	7.8×10^5		6.8×10^5		4.8×10^5	
AZ	1.06×10^5		7.6×10^5		5.8×10^5	
BX	9.8×10^5	9.33×10^5	4.8×10^5	5.93×10^5	4.4×10^5	4.3×10^5
BY	9.6×10^5		5.6×10^5		4.3×10^5	
BZ	8.6×10^5		7.4×10^5		4.4×10^5	
CX	9.6×10^5	9.20×10^5	1.14×10^5	4.07×10^5	1.1×10^5	1.13×10^5
CY	9.2×10^5		9.8×10^5		1.2×10^5	
CZ	8.8×10^5		1.26×10^5		1.1×10^5	

Key: FS: food sample; TBC: Total bacteria count, TCC: Total coliform count, TSC: Total Staphylococcus count. A: Akara, B: Awara; C: Masa; X: cafetaria; Y: Gadau market; Z: Off-campus

Bacterial isolates from the food samples were identified based on cultural, staining, and biochemical characteristics. The analysis revealed that samples from location X contained *Escherichia coli* and *Staphylococcus aureus*. At location Y, the identified bacteria included *Escherichia coli*, *Salmonella* sp., and *Staphylococcus aureus*. Samples from location Z exhibited a broader range of bacterial isolates, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., and *Pseudomonas* sp. These findings are summarized in Table 2.

Table 2: Biochemical Characterization of Bacterial Isolates from Locations X, Y, and Z

Location	GMR	CA	CO	CU	OX	IN	UR	MT	MR	VP	Organism present
X, Y, Z	+ve Cocci	+	+	+	-	-	+	-	+	-	*S. aureus*
X, Y, Z	-ve Rod	+	-	-	-	+	-	+	+	-	*E. coli*
Y, Z	-ve Rod	+	-	-	-	-	-	+	+	-	*Salmonella sp*
Z	-ve Rod	+	-	+	+	-	-	+	-	-	*Pseudomonas sp*

GMR: Gram staining reaction, CA: Catalase Test, CO: Coagulase Test, CU: Citrate Utilization Test, OX: Oxidase Test, IN: Indole Test, UR: Urease Test, MT: Motility Test, MR: Methyl Red Test, VP: Voges-Proskauer Test, +: Positive, -: Negative

The results presented in Table 3 reveal the frequency of bacterial occurrence across the food samples analyzed. *Staphylococcus aureus* was the most prevalent organism with 51.9 % (Figure 1) for all isolates. This was followed by *Escherichia coli* at 25.9 %, *Salmonella* sp. at 18.5 %, and *Pseudomonas* sp., which had the lowest occurrence at 3.7 % (Figure 1).

Table 3: Frequency of Bacterial Isolates from Food Samples

Organism isolated	Akara	Awara	Masa	No. of isolate
Staphylococcus aureus	5	6	3	14
Escherichia coli	3	2	2	7
Salmonella sp.	1	1	3	5
Pseudomonas sp.	-	-	1	1
Total	9	9	9	27

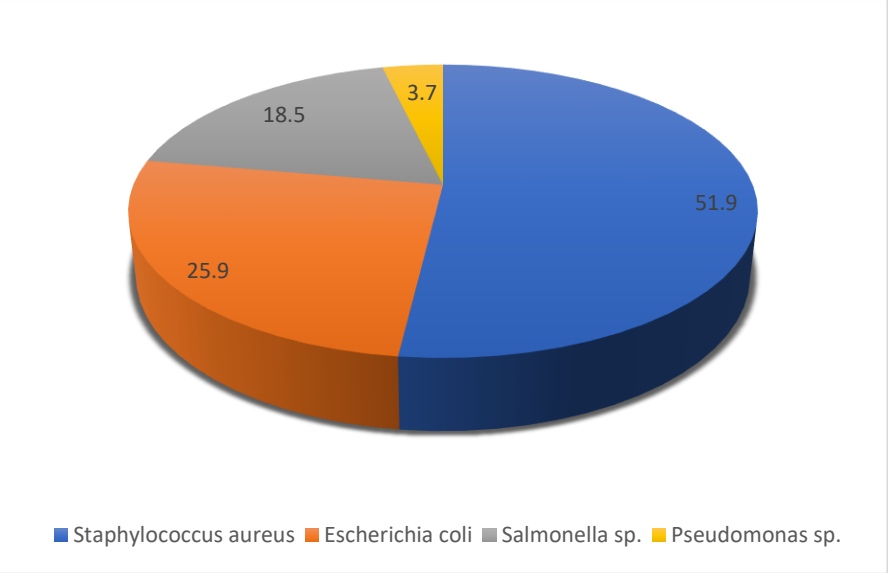

Figure 1: Percentage (%) of bacteria isolated from samples

Discussion

This study uncovered significant levels of contamination in the REFoods sold both within and around Sa'adu Zungur University, Gadau, Bauchi, which is in agreement with the submission of Amoo *et al.* (2024); Daginet *et al.* (2023); Akinyemi *et al.* (2013). According to Ajiboye *et al.* (2023) and Pepple (2017), highlighted that pathogenic bacteria, environmental pollutants, and neglect of proper production and hygiene standards contribute to the persisting relationship between food and diseases. Azounwu *et al.* (2018) observed that many food vendors often lack formal education, operate without licenses, and are untrained in proper food hygiene practices. They frequently work in unhygienic environments and have limited or no knowledge about the causes of foodborne illnesses. According to Muhammad *et al.* (2023), the bacterial load in food at any given time is influenced by factors such as handling practices, storage conditions, temperature control, and the duration of storage. In this study, the total aerobic bacteria count exceeded the food acceptable limits set by the Food and Agriculture Organization (FAO, 2002) of the United Nations, which specifies that aerobic bacteria should not exceed 1×10^4 CFU/g. Additionally, coliform bacteria should not exceed 10 CFU/g, and *Escherichia coli* and *Staphylococcus aureus* should be completely absent in food for it to be considered safe for consumption. However, the results of this study revealed that some of the sampled foods contained significantly high levels of these microorganisms. These findings are in agreement with those reported by Ajiboye *et al*. (2023).

The predominant bacterial pathogens identified in this study include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp., and *Salmonella* sp. Similar pathogens have been previously reported in various food types, including hawked, raw, and ready-to-eat foods. These findings align with those of Amoo *et al.* (2024), who isolated *E. coli*, *S. aureus*, *Streptococcus* sp., *Bacillus* sp., *Vibrio cholerae*, and *Klebsiella* sp. from ten different hawked REFoods such as *awara*, *akara*, *gurasa*, *masa*, *dan-wanke*, beans, and yam in Dutse ultra-modern market, Jigawa State. Similarly, Muhammad *et al.* (2023) reported the presence of *E. coli*, *S. aureus*, and *Salmonella* sp. in soybean cake (*awara*) in their study. These findings suggest poor hygienic practices as a significant contributing factor to contamination in the study area.

As shown in Tables 2, 3, and Figure 1, *Staphylococcus aureus* was the most frequently isolated organism, accounting for 51.9 % of all bacteria identified in the food samples. This well-known foodborne pathogen is often introduced through human handling of raw food and food products. Its presence in the samples likely reflects inadequate sanitary practices during food preparation and handling. *Staphylococcus aureus* is a major cause of bacterial food poisoning, frequently associated with improper hygiene by food handlers (Igbinosa *et al.*, 2020; Oranusi & Olorunfemi, 2011; Rufai & Wartu, 2023). However, proper precautions can effectively prevent contamination, growth, and enterotoxin production of *S. aureus* in food products (Nwiyi *et al.*, 2022; Amusan *et al.*, 2010). Muhammad *et al.* (2016) identified *Staphylococcus aureus* as the most prevalent organism in their study, representing 43.5 % of all isolates from food samples, in which the report is in align with the submission from this study. The contamination in REFoods was attributed to human handling of raw food and related products. For instance, the use of latex gloves during food preparation can minimize direct hand contact, significantly reducing the risk of contamination. Open-air markets have been identified as hotspots for the direct transmission of *S. aureus*, particularly during the handling of REFoods such as cooked, smoked, dried, or fried fish and shellfish by traders and customers (Azanaw *et al.*, 2019).

Escherichia coli was present in all the food samples, amounting to 25.9 % (Figure 1) of the isolates. As a member of the *Enterobacteriaceae* family, *E. coli* is widely found in the environment, with contaminated food and water serving as the primary routes of transmission. Certain strains of this bacterium are capable of causing a range of infections both in healthcare settings and within the community (Hariri *et al.*, 2022; Oranusi & Braide, 2012). These infections include diarrhea, urinary tract infections, meningitis, sepsis, wound infections, nosocomial pneumonia, and dysentery. In Nigeria, several foods have been reported to have a high prevalence of *E. coli* contamination (Akinnibosun *et al.*, 2015; Okonko *et al.*, 2009).

Salmonella sp. was identified in 18.5% of the bacterial isolates in this study, aligning with the findings of Ajiboye *et al.* (2023). As noted by Temesgen *et al.* (2016) that *Salmonella* species are commonly linked to fecal coliform contamination, indicating possible fecal contamination of

food. The presence of *Salmonella* in food can be attributed to various factors, including poor sanitation, unclean utensils, contaminated water, improper food handling and processing, inadequate storage conditions, and unhygienic food display practices (Bristone *et al.*, 2019). *Pseudomonas* sp. with 3.7 % of the samples is an opportunistic pathogen known to cause bacteremia and gastrointestinal infections. Preventing foodborne illnesses caused by such pathogens requires strict personal hygiene, proper food handling, regular cleaning and disinfection of equipment, use of clean water, and the correct storage of food products after processing. The results of this study confirm that pathogenic bacteria can be present in cooked foods that appear to be safe and wholesome, so it is important to take the right preventive measures to limit the presence of these harmful microorganisms in food. Even a small number of microbes may rapidly spread into harmful colonies if foods are not preserved properly. The stomach acidic conditions and the immune system of the body can often combat minor contamination, but as long as the contamination is not severe, it is still possible to eat food containing pathogenic bacteria without get sick (Bristone *et al.*, 2019).

CONCLUSION

The findings of this study revealed that the ready-to-eat food samples examined contained bacterial pathogens that pose significant public health risks to students, staff, and the university community. These microorganisms have been linked to foodborne illnesses and diarrheal diseases. The bacterial counts exceeded the safety limits set by food safety agencies, indicating that the food did not meet the required quality standards. Some of the isolated bacteria, particularly *Staphylococcus aureus* and *Escherichia coli*, are known enteric pathogens that can cause gastroenteritis. This suggests poor food handling and management practices, which likely led to cross-contamination, as *S. aureus* is a common skin flora and *E. coli* is associated with fecal contamination. Based on these findings, it is recommended that students, staff, and the university environs need to be educated about the health risks of consuming contaminated ready-to-eat, street food/hawked food, and that food producers and vendors receive training on proper hygiene and food safety practices. Relevant health agencies should emphasize the importance of hygiene and food safety to food vendors and a regular monitoring and evaluation of handling processes should be done by environmental or public health professionals. However, this study has some limitations. The reliance on culture-based methods may have missed viable but non-culturable (VBNC) bacteria, and the study focused on only three locations, limiting broader contamination trends. Additionally, variations in food handling and hygiene practices were not fully assessed, and antibiotic resistance profiles were not examined. The lack of molecular confirmation and bacterial toxin analysis may also affect the accuracy of findings. Future research should address these limitations by incorporating molecular techniques, expanding the study scope, and evaluating antibiotic resistance patterns to provide a more comprehensive assessment of food safety risks.

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