

# MICROBIAL QUALITY OF HOME-MADE STREET VENDED BEVERAGES IN REUSED BOTTLES SOLD IN MAKURDI, BENUE STATE, Nigeria

## ABSTRACT

This study aimed to assess the microbial quality of home-made street vended beverages sold in reused bottles in selected locations in Makurdi, Nigeria. Standard microbiological techniques were applied to enumerate and identify the predominant pathogens in the drinks collected from different sale points. The result of the analyses revealed high total viable counts ranging from 5.66- 7.85 log CFU/ml in Kunu, Zobo, and Soymilk sold in reused bottles from different sale points within three selected locations. In addition, variable counts of *Staphylococcus aureus*, *E.coli*, *Salmonella*spp, and *Shigella*spp as the predominant bacteria were observed in Kunu, Zobo, and Soymilk samples from various locations. The pathogen counts ranged from 0.0-5.08 log CFU/ml for *Staphylococcus*spp, 4.14-5.15 log CFU/ml for *E. coli*, 0.0-5.30 log CFU/ml for *Salmonella* spp and 0.0-4.70 log CFU/ml, with *E. coli* having the highest frequency of occurrence. The pathogen counts were above the permissible levels stated by the Centre for Food Safety, therefore the use of reused plastic bottles for home-made beverages should be avoided or carried out with proper cleaning and sanitization to minimize contamination.

**Keywords:** Bacteria pathogens, home-made beverages, reusable bottles, microbial quality.

## 1.0 INTRODUCTION

Food safety has been identified as a significant barrier to social and economic development. Addressing food safety may lead to a decline in foodborne illness and promote economic gains [1]. Globally, safety issues surround the production, distribution and consumption of indigenous/ home-made street vended beverages largely due to unhygienic processing and handling occasioned by poor infrastructural development, lack of social amenities provision, poor food safety knowledge and practices among consumers and vendors of such foods [2, 3]

Homemade beverage consumption has been on the rise in Nigeria, with increased consumer awareness of the quality of food they consume and the cost of bottled drinks. Urban and rural dwellers consume these drinks along with snacks to satisfy hunger, boost nutrition and reduce the intake of preservatives and other food additives owing to their health risks[37-39]. These drinks are sold on the streets, in schools, offices, markets, and motor parks, where consumers enjoy them without knowing their safety. A common attribute of these products is that they are produced outside government regulations regarding standard food safety guidelines [4].

Common home-made beverages, including tiger nut milk, soymilk, kunu and zobo, which may be sugar-sweetened during production [5, 6], are prepared and packaged in reusable bottles that may have been used initially to package water or other carbonated drinks. These already used bottles are collected from event centres and wedding reception venues, and sometimes from refuse dumps and washed for use. The use of these bottles in packaging beverages is not hygienic and also not standard practice. Furthermore, the package could be compromised, predisposing consumers to microbial risks and other hazardous substances [7], especially since the beverage bottles usually have contact with the consumer's mouth and sometimes saliva which may be a vehicle for transmitting pathogens onto the bottles.

Microbial food-borne disease outbreaks usually pose a serious public health concern and results in economic losses and even death. Although many food-related illnesses are not reported or diagnosed, they are common worldwide, including Nigeria [8]. According to the World Health Organization (2022) as reported by Maziet *al.* [4] about 600 million (1 in 10) people fall ill, and 420,000 deaths occur each year

from consuming contaminated food, with several associated with bacteria. The burden of foodborne diseases falls disproportionately on groups in vulnerable situations and especially children under 5 years of age, with the highest burden in low- and middle-income countries. Reports by Maziet *al.* [4] showed a high burden of foodborne diseases associated with consuming contaminated home-made street-vended foods. Omidiran *et al.* [9], also stated that street-vended foods/beverages are perceived to be a major health risk; quality assessments of such foods in several countries have shown that they are positive vectors of foodborne illness. The most common foodborne pathogens associated with home-made street vended beverages include *Escherichia coli*, *Shigella* spp, *Salmonella* spp, *Clostridium perfringens*, *Campylobacter jejuni*, *Bacillus cereus* and *Staphylococcus aureus*. A concern which may lead to microbial contamination of such home-made drinks in Nigeria has been poor hygiene. Microorganisms of public health concern may occur at any point during the production, packaging or storage of finished products and public sales. Around the world, it has been established that poor hygiene in developing countries contributes to the proliferation of food pathogens in several home-made street vended beverages [10 - 11]. The prevalence of bacteria food-borne illnesses and the question of the safety of home-made drinks packaged in plastic reused bottles has led to the need for this study. This study, therefore, seeks to evaluate the microbial quality of home-made street vended beverages packed in reused bottles sold in Makurdi.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample collection

Four samples each of kununzaki, soy milk and zobo were purchased from Northbank, Wurukum and Wadata markets, located in Makurdi, Benue State, Nigeria.

### 2.2 Preparation of media

All media used were prepared according to the manufacturer's instructions.

### 2.3 Methods

#### 2.3.1 Total viable bacterial count

Total viable bacterial counts were carried out on kununzaki, soymilk and zobo sold in reusable bottles from different locations in Makurdi. 1 ml of each sample was transferred into 9 ml of sterile distilled water to make the first dilution ( $10^{-1}$ ), and the procedure was repeated up to dilution  $10^{-4}$ . Furthermore, About 0.1 ml was transferred into sterile Petri dishes with approximately 15 ml of molten Plate Count Agar (PCA) poured into the labelled Petri dishes. The plates were then gently rocked to allow even distribution of the organisms. Afterwards, the media were left to solidify, and plates were incubated at 37°C for 24 h. Bacterial counts were expressed in colony-forming units (CFU) per millilitre. All counts were carried out in duplicates. Observed colonies were subcultured repeatedly on media used for primary isolation to obtain pure cultures [12-13].

$$\text{CFU/ml} = \frac{\text{no. of colonies}}{\text{volume of inoculum} \times \text{DF}}$$
 where D.F. is the dilution factor

#### 2.3.2 Bacterial counts of predominant pathogens

##### *Escherichia coli* counts

About 0.1 ml of serially diluted samples were aseptically plated in MacConkey agar using the pour plate method. The plates were incubated at 37°C for 24 hours. The total colonies formed were counted. Pinkish red colonies with a metallic sheen were observed in the plates. Hence, the growth was streaked on Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hours. The presence of small, nucleated colonies with greenish metallic sheen were observed indicating *E. coli*. The total count was calculated and expressed in CFU/ml [12, 14].

## **Salmonella-Shigella counts**

About 0.1 of the serial diluted samples were pour plated in *Salmonella-Shigella* Agar. The Agar plates were incubated inverted at 37°C for 24 hours. The presence of black colonies indicated *Salmonella*, while the pink colonies indicated the presence of *Shigella* [12].

## **Staphylococcus aureus counts**

The pour plate method was employed for *Staphylococcus spp* counts. About 0.1 ml of serial diluted samples were aseptically plated in Mannitol Salt Agar and incubated inverted at 37°C for 24 hours. The presence of yellow-pigmented colonies, which did not show hemolytic properties on blood agar, were identified as *Staphylococcus aureus* [12].

### **2.3.3 Isolation and maintenance of pure culture**

Each distinct bacterial colony was picked using a sterilized inoculating needle and streaked as a primary inoculant on nutrient agar plate and nutrient agar slants in Bijou bottles. These culture plates were incubated at 37°C for 24 h, after which the slants were maintained inside the refrigerator at 4°C [12].

### **2.3.4 Identification/ Confirmation of bacteria**

#### **2.3.4.1 Gram staining**

A thin smear of each bacterial species was prepared on a clean glass slide, after which it was dried. The dried smear was heat fixed, covered with crystal violet for 1 minute, followed by washing with distilled water for a few seconds. The smear was covered with iodine solution for 1 min, washed, decolourized using ethyl alcohol and washed again. After draining, it was counter-stained using safranin for 30 seconds, after which it was washed and dried with absorbent paper before viewing under a microscope to determine the cell morphology [12].

#### **2.3.5. Biochemical Test**

##### **Indole Test**

This was used to determine the ability of an organism to split indole from the amino acid tryptophan using the enzyme tryptophanase. Tryptophan broth was inoculated with the test organism and incubated for 24 hours. Drops of Kovacs Reagent were then added to the broth. The formation of a red ring at the surface of the broth signified a positive result [15].

##### **Oxidase Test**

Fresh growth was removed from the agar plate using a sterile swab. The oxidase test strip was moistened slightly with an oxidase reagent, and a smear of the colony made onto the moistened paper strip. The presence of cytochrome oxidase in the organism changed it from its colourless appearance to a deep indigo blue in 10-20 seconds. [15].

##### **Coagulase Test**

A drop of distilled water was placed on two separate slides. The isolate colony was emulsified on each drop to make two thick suspensions. Next, a loopful of plasma was added to one of the suspensions and mixed gently. The formation of clumps confirmed the presence of *Staphylococcus aureus*. No plasma, however, was added to the second suspension. This was to differentiate any granular appearance of the organism from true coagulase clumping [15].

### 2.3.6 Statistical analysis

All data collected were subjected to analysis of variance (ANOVA), where the least significant difference was adopted to ascertain the difference between samples. Treatment means were compared at  $p < 0.05$ , using Genstat statistical software, version 17.1

## 3.0 RESULTS AND DISCUSSION

### 3.1 Total bacterial count of *Kunu* reused bottles sold in selected locations in Makurdi

Table 1 shows the microbial counts of *kunu* in reused bottles sold in different locations in Makurdi and the specific counts for *Staphylococcus aureus*, *E. coli*, *Salmonella* spp, and *Shigella* spp, the predominant pathogens found in the beverages. *Kunu* samples from Wurukum gave the highest total viable count of 7.85 log CFU/ml, with the least count of 6.86 log CFU/ml was recorded from Wurukum. However, there was no significant difference ( $p > 0.05$ ) in the mean total viable count of *kunu* from the different locations. Generally, higher TVC suggests poorer quality or improper handling during production, packaging and storage. Nwiyi and Elechi [2] reported lower total viable counts of 3.89 log CFU/ml in *kunu* samples from Lafia. Etanget *al.* [16] documented a viable count of 3.80 log CFU/ml, as well as Ekanamet *al.* [17] who reported a comparable viable count of 4.70 log CFU/ml, both less than that reported in this study. The count observed in this study may be due to the processing techniques used, hygiene practices and reuse of contaminated plastic bottles for bottling the beverages. The lack of variations in microbial density among the samples from the different locations suggests that the processors and handlers may have practiced similar hygiene levels and processing methods.

*E. coli*, *Salmonella*, *Shigella* and *Staphylococcus spp* were the predominant organisms identified in the *Kunu* samples. This corresponds with the previous works of Amusa and Odunbaku [18], who reported microbes associated with hawked (marketed) *Kunuzaki* in South-Western Nigeria, which include *Lactobacillus plantarum*, *Bacillus subtilis*, *Bacillus cereus*, *Streptococcus faecium*, *Streptococcus lactis*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Etanget *al.* [16] also reported the presence of *Staphylococcus aureus*, *Enterobacter aerogenes*, *E. coli*, *Bacillus spp.* and *Streptococcus spp.* from *kunu*. *Kunu* from Wadata market were shown to have the highest *Salmonella*, *Shigella* and *Staphylococcus spp* count of 4.62, 4.70 and 4.89 log CFU/ml, while Northbank market had the least *Salmonella*, *Shigella* and *Staphylococcus spp* count of 2.00, 2.78 and 0.00 log CFU/ml respectively with no significant differences among the samples from the different locations. Research by Ogbonna *et al.* [19] reported lower mean bacteria counts in *Kunuzaki* sold in Maiduguri metropolis Nigeria as  $9.01 \times 10^2$  CFU/ml for *E. coli*,  $2.6 \times 10^3$  CFU/ml for *Staphylococcus spp*,  $6.35 \times 10^2$  CFU/ml for *Salmonella* spp,  $5.01 \times 10^2$  CFU/ml for *Shigella* spp, and total viable count of  $5.654 \times 10^3$  CFU/ml. According to Benson [20] and Omidiran *et al.* [9], the presence of *Salmonella* spp can lead to serious health problems due to poor sanitary conditions and hygiene practices during processing. Salmonellosis poses a substantial public health threat, often contributing to significant global mortality rates.

High counts of *Staphylococcus spp* was observed in *Kunu* from Wadata, and there was no growth in Wurukum samples. Amusa and Odunbaku [18] reported the risks associated with consuming food contaminated with *Staphylococcus spp*. It is a possible contaminant from food handlers and utensils used in processing. Etanget *al.* [16] stated that *S. aureus* could cause food poisoning and food intoxication by producing *Staphylococci enterotoxin*, the primary cause of toxic shock syndrome (TSS) in humans. The growth of *E. coli* was highest in Wurukum market samples and least in Wadata market samples respectively. *E. coli* is regarded as the primary indicator for microbiological quality of water and food, and its presence in food is an indication that such a food is contaminated with fecal materials and as such not safe for human consumption [21]. The higher pathogen counts observed in this study may be due to contaminants from the reusable bottles, the quality of water used and poor hygiene. Foods contaminated with unsafe levels of pathogens, may pose substantial risk to consumers and place severe economic burden on communities and nations [9].

In Comparison to microbiological standards for foods, the total viable bacterial count was within the borderline, as the Centre for Food Safety [22] reported a borderline of  $10^4 - 10^7$  CFU/ml, while the *E. coli*,

*Salmonella* and *Staphylococcus* counts were higher than the borderline (*E. coli*<100 CFU/ml, *Salmonella* and *Shigella*, no detection in 25ml and *Staphylococcus aureus*<100 CFU/ml). This indicates a risk for consumers.

**Table 1: Microbial count (log CFU/ml) of Kunuin reused bottles sold in selected locations in Makurdi**

Sample source area	Total viable count	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Salmonella</i> spp	<i>Shigellaspp</i>
Northbank	6.86 <sup>a</sup> ±1.31	4.25 <sup>a</sup> ±0.47	4.14 <sup>a</sup> ±0.64	2.00 <sup>a</sup> ±1.11	2.78 <sup>a</sup> ±0.98
Wadata	7.80 <sup>a</sup> ±0.35	4.89 <sup>a</sup> ±0.52	4.47 <sup>a</sup> ±0.17	4.62 <sup>a</sup> ±1.144	4.70 <sup>a</sup> ±0.97
Wurukum	7.85 <sup>a</sup> ±1.04	N.G.	4.79 <sup>a</sup> ±0.69	3.98 <sup>a</sup> ±1.209	4.59 <sup>a</sup> ±0.24
LSD	1.92	0.86	0.89	2.83	1.70

Values are means ± standard deviations of triplicate observations. Mean values in the same column with different superscripts are significantly different ( $p > 0.05$ ).

### 3.2 Total bacterial count of Zoboin reused bottles sold in selected locations in Makurdi

The total viable bacteria count and specific pathogen counts of *Zobodrink* in reused bottles sold in Northbank, Wurukum and Wadata in Makurdi are shown in Table 2. The total viable count ranged from 5.66-6.61 log CFU/ml with the highest count of 6.61 log CFU/ml observed in zobo from Wadata, while the least count of 5.66 log CFU/ml was from Wurukum. There were no significant differences in total viable counts of samples from the selected locations. *E. coli*, *S. aureus*, *Salmonella* and *Shigellaspp* were identified in Zobo from Northbank, Wadata and Wurukum markets with no significant differences among the samples. No *Salmonella* was detected in Zobo from Northbank market. Zobo sold in Wadata market had the highest *S. aureus* and *Salmonella* spp counts while Northbank and Wurukum had the highest *E.coli* and *Shigellaspp* counts, with no significant differences between the locations. These findings are consistent with the work of Umar *et al.* [23], who reported total aerobic bacterial counts ranging from  $0.3 \times 10^6$  CFU/ml to  $4.4 \times 10^6$  CFU/ml. This result further agrees with studies carried out by Raimi [24], Adebayo-Tayo and Samuel [25], and Nwachukwu *et al.* [26]. The presence of these pathogens indicates fecal contamination possibly from inadequate sanitation practices during preparation or handling and underscores the importance of implementing stringent hygiene practices and food safety regulations in the production and sale of street-vended beverages to prevent foodborne illness outbreaks [11].

Comparing to microbiological standards, the bacterial counts are within the borderline, as the Centre for Food Safety [22] reported a borderline of  $10^4 - 10^7$  CFU/ml for total viable counts of foods, while *E. coli*, *Salmonella* and *Staphylococcus* spp counts are higher than the borderline (*E. coli*<100 CFU/ml, *Salmonella* and *Shigella* no detection in 25ml and *S. aureus*<100 CFU/ml).

**Table 2: Microbial count (log CFU/ml) of Zobo drink in reused bottles sold in selected locations in Makurdi**

Sample source area	Total viable count	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Salmonella</i> spp	<i>Shigella</i> spp
Northbank	6.36 <sup>a</sup> ±0.58	3.44 <sup>a</sup> ±0.64	5.15 <sup>a</sup> ±0.53	NG	2.00 <sup>a</sup> ±0.82
Wadata	6.61 <sup>a</sup> ±0.24	4.69 <sup>b</sup> ±0.13	4.66 <sup>a</sup> ±0.84	4.15 <sup>a</sup> ±0.95	3.92 <sup>a</sup> ±1.49
Wurukum	5.66 <sup>a</sup> ±1.34	NG	4.38 <sup>a</sup> ±0.53	3.42 <sup>a</sup> ±0.45	4.48 <sup>a</sup> ±0.67
LSD	1.33	0.83	1.14	1.29	2.77

Values are means ± standard deviations of triplicate observations. Mean values in the same column with different superscripts are significantly ( $p>0.05$ ) different

### 3.3 Total bacterial count of Soymilkin reused bottles sold in selected locations in Makurdi

Table 3 shows the microbial count of soymilkin reused bottles sold in Northbank, Wurukum and Wadata. *S. aureus*, *E.coli*, *Salmonella* and *Shigella*spp were identified in soymilk from the selected locations. Soymilk samples from Wadata had the highest total viable count of 7.76 log CFU/ml, and the highest *S. aureus*, *Salmonella* and *Shigella*spp counts with no significant difference among the locations. [Seiyabohet al. \[33\]](#) reported lower total bacteria count of 5.41 logCFU/ml in soymilk packaged in reused plastic bottles. High *E.coli* counts were recorded in soymilk from Northbank. [Ezigboet al. \[27\]](#), isolated predominantly *Bacillus* spp, *Staphylococcus* spp, *Lactobacillus* spp, *Enterobacter*spp, *Pseudomonas* spp and *E. coli* from soymilk samples collected from major markets and commercial spots in Aba, Nigeria while [Umeoduaguet al. \[28\]](#) isolated *S. aureus*, *Bacillus* spp, *E. coli*, *Klebsiella*spp, *Salmonella* spp, *Pseudomonas* spp and *Vibrio* spp from soymilk samples sold in Onitsha metropolis. This agrees with previous studies carried out by [Adelekeet al. \[29\]](#), who assessed the microbial quality of branded and unbranded soymilk samples to ascertain their hygiene practices during production. Screening for microbial contaminants revealed high bacteria counts of  $2.9 \times 10^7$  to  $1.0 \times 10^8$  CFU/ml. In addition, [Mbajiukaet al. \[30\]](#) studied the microbiological quality of locally-produced soymilk stored under ambient and refrigeration conditions and reported a bacterial count of  $2.0 \times 10^3$  CFU/ml to  $2.9 \times 10^4$  CFU/ml after six days of storage at an ambient temperature of 27°C. [Liamngeet al. \[31\]](#) conducted a microbial analysis of soymilk sold by women and children in Makurdi metropolis. The microbial load ranged from  $6.9 \times 10^7 - 7.6 \times 10^7$  CFU/ml,  $4.1 \times 10^7 - 5.6 \times 10^7$  CFU/ml,  $3.0 \times 10^7 - 4.7 \times 10^7$  CFU/ml, and  $6.0 \times 10^7 - 8.5 \times 10^7$  CFU/ml for samples from North Bank, Wurukum, High level and Wadata area respectively.

**Table 3: Microbial count (log CFU/ml) of Soymilk in reused bottles sold in selected locations in Makurdi**

Sample source area	Total viable count	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Salmonella</i> spp	<i>Shigella</i> Spp
Northbank	6.74 <sup>a</sup> ±0.65	3.84 <sup>a</sup> ±1.04	4.95 <sup>a</sup> ±0.50	3.31 <sup>a</sup> ±0.04	3.98 <sup>a</sup> ±0.21
Wadata	7.76 <sup>a</sup> ±0.43	5.08 <sup>a</sup> ±0.34	4.78 <sup>a</sup> ±0.42	5.30 <sup>c</sup> ±0.38	4.92 <sup>b</sup> ±0.40
Wurukum	7.68 <sup>a</sup> ±0.09	4.09 <sup>a</sup> ±0.77	4.38 <sup>a</sup> ±0.19	4.54 <sup>b</sup> ±0.34	NG
LSD	0.54	1.33	0.63	0.53	0.56

Values are means ± standard deviations of triplicate observations. Mean values in the same column with different superscripts are significantly ( $p>0.05$ ) different

Soy milk, according to Asogwa *et al.* [32] serves as food for many microorganisms due to its nutrient content, high moisture and neutral pH, promoting their growth. This may explain the reason for the high TVC besides contaminants from unhygienic bottles, poor sanitary practices among others.

Significant differences were observed in *Shigella* counts of samples from Northbank and Wadata, with the latter having the highest. This contrasts with the report of Ozoh and Umeaku [8], who studied the public health implication of ready-to-drink soy milk and soy milk yoghurt sold in Onitsha Urban, Anambra State, Nigeria. They reported the presence of *E. coli* in the range of  $1.1 \times 10^3 - 8.0 \times 10^3$  CFU/ml in the samples. Comparing their mean counts, there was no significant difference between the locations in *Shigella* spp count. In comparison with established microbiological standards, the bacterial enumeration falls within a marginally acceptable range, as delineated by the Center for Food Safety [22] with a threshold of  $10^4 - 10^7$  CFU/ml. Nevertheless, the quantification of *E. coli*, *Salmonella*, and *Staphylococcus* spp surpassed the designated threshold, with *E. coli* registering below 100 CFU/ml, and no detection of *Salmonella* and *Shigella* in 25 ml, while *Staphylococcus aureus* exhibited counts lower than 100 CFU/ml.

### 3.4 Frequency of occurrence of bacterial Isolates from Kunu, Zobo and Soy milk in reused bottles sold in selected locations in Makurdi

Table 4 presents the percentage frequency of occurrence of predominant bacterial isolates found in selected home-made street-vended beverages sold in Makurdi, along with their total percentage for each bacterial species. The presence of pathogens such as *S. aureus*, *E. coli*, *Salmonella* and *Shigella* spp at the levels presented poses a significant risk to public health. Similar microorganisms as isolated in this study were reported by Nwaiwu *et al.* [6] and Oduoriet *et al.* [1] in Kunu, Zobo and Soy milk. *E. coli*, *Staphylococcus*, *Salmonella* and *Klebsiella* spp were reported to be among the most common pathogens in home-made beverages [1, 11]. Seiyabohet *et al.* [33] reported similar microorganisms including *Aeromonas* spp in locally processed beverages sold in reused plastic bottles. *S. aureus* and *E. coli* were found present in all the three types of beverages analyzed (Kunu, Zobo, and Soy milk) at varying frequencies, ranging from 16.67% to 25.00%, while *Salmonella* and *Shigella* spp were found in two out of three beverages analyzed (Kunu and Soy milk) at a frequency of 25.00 % and 25.00 %, and (Kunu and Zobo) at a frequency of 25.00 % and 25.00 %, respectively.

**Table 4: Percentage frequency of occurrence of predominant bacteria isolates from Kunu, Zobo and Soy milk in reused bottles sold in selected locations in Makurdi**

Sample	Bacteria isolates				Total (%)
	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Salmonella spp</i>	<i>Shigella spp</i>	
Kunu	16.67	25.00	25.00	25.00	91.67
Zobo	16.67	25.00	16.67	25.00	83.34
Soy milk	25.00	25.00	25.00	16.67	91.67
<b>Total (%)</b>	58.34	75.00	66.67	66.67	

The percentage occurrence of these pathogens are higher than that reported by Etanget *et al.* [16], who observed a 10 % occurrence of *Staphylococcus*, 15 % occurrence of *E.coli* and 12.5 % prevalence of *Salmonella* spp in Kunu. *S. aureus* is a common foodborne pathogen known to cause food poisoning through the production of enterotoxins [2, 16]. *E. coli* (especially when they are pathogenic strains) and *Shigella* spp are also bacterial pathogens that can cause severe gastrointestinal illness, including

diarrhea, fever, and abdominal cramps. *Salmonella* is a well-known bacterial pathogen associated with foodborne illness, commonly transmitted through contaminated food and beverages. Its presence suggests a significant risk of foodborne illness if consumed [9]. As reported earlier, the burden of foodborne diseases to public health and economies has often been underestimated by consumers due to under-reporting and difficulty to establish the relationship between causative agents of food contamination and resulting illness or death.

The reuse of plastic bottles in bottling beverages could be a contributing factor to the high percentage occurrence of bacteria pathogens in beverages such as these studied in this work.

A number of earlier studies conducted to assess the microbiological quality of home-made street vended beverages have shown that the beverages are contaminated with pathogens [4, 9] because they are processed and handled under unhygienic conditions, processed using unhygienic utensils, held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings that make them prone to contamination.

In addition to the reasons noted previously, the packaging materials could be a significant source of contamination of the beverages as these reused plastic bottles may harbor bacteria and other pathogens as they may not be adequately washed and disinfected before use [34, 35, 36]. Consumption of beverages bottled in reused bottles can pose health risks to consumers, due to contamination from various sources including bacteria pathogens, previous bottle contents which may lead to allergy or cross-contamination, improper handling especially when they are not properly cleaned and sanitized. These can enhance growth of both spoilage and pathogenic bacteria leading to deterioration of beverage quality and safety. According to Maziet *al.* [4] and Adeleye *et al.* [11], producers and vendors of these home-made drinks in reused bottles often lack proper education and training in food handling and safety and are not aware of the health hazards associated with the consumption of beverages in such packages. Safe food supplies have great impact on the national economy, trade and tourism, contributes positively to food and nutrition security and enhances sustainable development. Therefore, to ensure safety of home-made street vended beverages, proper sanitation practices including thorough cleaning and sanitization of bottles be carried out before reuse. To minimize the risks of contamination, it is recommended that new bottles designed for single use be used for bottling the beverages.

#### **4.0 CONCLUSION**

Upon culmination of this investigation, the prevailing bacteria in kunu, zobo, and soymilk retailed in reused plastic bottles across Northbank, Wadata, and Wurukumwere successfully identified and quantified. The prevalent bacteria included *Salmonellaspp*, *Shigellaspp*, *E. coli*, and *Staphylococcus aureus*. While the overall bacterial count adhered to permissible levels stipulated by the Centre for Food Safety, the concentrations of *Salmonellaspp*, *Shigellaspp*, *E. coli*, and *Staphylococcus aureus* surpassed these regulatory thresholds. The reuse of plastic bottles for home-made street vended beverages, which may not be properly cleaned before use, can lead to a build-up of pathogenic bacteria posing risks to consumers for foodborne outbreaks. It is recommended that food safety campaigns should be launched regularly to educate these food producers on safe food handling practices, risks associated with reuse of beverage bottles, importance of hygienic packaging materials. The regulatory bodies should enforce the use of appropriate and hygienic packaging materials for home-made beverages.

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- 1.
- 2.
- 3.

## **REFERENCES**

- [1] Oduori, D. O., Kwoba, E., Thomas, L., Delia, G. and Mutua, F.(2022). Assessment of Foodborne Disease Hazards in Beverages Consumed in Nigeria: A systematic Literature Review. *Foodborne Pathogens and Disease*, 19(1):1-18 doi: 10.1089/fpd.2021.0043
- [2] Nwiyi, I. U. and Elechi, J. O. G. (2022). Evaluation of Food Safety and Nutritional Quality of indigenous beverages vended in informal market of Nasarawa state, North Central, Nigeria, *Eurasian Journal of Food Science and Technology*, 6(2):100-112
- [3] Owoko, M.A.,Kiptui, M. and Cheserek, G. J. (2024). Socio-Economic and Environmental Outcomes of Street food vending and its sustainability in selected urban areas of Kenya. *International Journal of Innovative Science and Research Technology*, 9(5):314-322, doi.org/10.38124/ijisrt/IJISRT24MAY140
- [4] Mazi, I. M.,Onyeaka, H. Akegbe, H., Njoagwuani, E. I., Ochulor. C.E., Oladunjoye, I. O., Omotosho, A.D., Nwaiwu, O., Tamasiga, P. and Odeyemi, O.A. (2023). Street Vended foods in Nigeria: An analysis of the current state of affairs and the way forward. *Cogent Food and Agriculture*, 9(2): 1-13, 2266194

- [5] Ajiboye, T.O.; Iliasu, G.A.; Ojewuyi, O.B.; Abdulazeez, A.T.; Muhammed, A.O.; Kolawole, F.L. (2014). Sorghum-based alcoholic beverage, Burukutu, perturbs the redox status of the liver of male rats. *Food Science and Nutrition*. 2, 591–596.
- [6] Nwaiwu, O., Aduba, C.C., Igbokwe, V.C., Sam, C.E. and Ukwuru, M.U. (2020). Traditional and Artisanal Beverages in Nigeria: Microbial Diversity and Safety Issues. *Beverages*, 6 (53):1-22, doi:10.3390/beverages6030053
- [7] Okaru, A.O.; Rehm, J.; Sommerfeld, K.; Kuballa, T.; Walch, S.G.; Lachenmeier, D.W (2019), The threat to quality of alcoholic beverages by unrecorded consumption. In : Grumezescu, A.M., Holban, A.M., (Eds) *Alcoholic Beverages*, 7th ed. Woodhead Publishing (Elsevier): Kidlington, U.K., pp. 1–34.
- [8] Ozoh, C.N. and Umeaku, C.N. (2016). Public health implication of ready-to-drink soymilk and soymilk yoghurt sold in Onitsha Urban, Anambra State, Nigeria. *Journal of Multidisciplinary Science and Technology*. 3(8): 5386 – 5393.
- [9] Omidiran, A.T., Sanni, L.O., Sobukola, O.P., Sanni, S.A., Adebawale, A.A., Obadina, A.O., Kadihausa O.E., Adegunwa M.O., Tomlins, K and Wolfgang, T. (2015). Evaluation of some properties of wheat-brewers spent cassava flour blends. *Nigerian Food Journal*. 33(2):10-18.
- [10] Omoleke, S.A.; Ajibola, O.; Ajiboye, J.O.; Raji, R.O (2018), Quagmire of epidemic disease outbreaks reporting in Nigeria. *BMJ Glob. Health*, 3
- [11] Adeleye, A.O., Akinterinwa, T.D., Amoo, F.K., Amoo, A. O., Asaju, C.I., Shiaka, P.G. and Yerima, M. B. (2024). Detection of Bacterial Contaminants in Ready-to-drink Beverage (Zobo) sold on Federal University Dutse campus, North West Nigeria. *Innovare Journal of Sciences*, 12(1):1-5 doi.org/10.22159/ijss.
- [12] Harrigan, W.F and McCance, M.E (1976). *Laboratory Methods in Food and Dairy Microbiology*. Academic press.
- [13] Fawole MO and Oso B.A. (2001) *Laboratory manual of microbiology*. 5th Edition. Ibadan: Spectrum Books Ltd. Pp 15-22.
- [14] Pepper, I.L. and Gerba, C.P. (2005). *Environmental Microbiology. A laboratory manual*. Second edition. Elsevier Academic Press
- [15] Cheesbrough, M. (2004). *District Laboratory Practice in Tropical Countries*. Low price Edition part 2. Cambridgepress, England
- [16] Etang U. E., Ikon G. M., Udofia S. M., Umo A. N., Udo E. E., Uyanga F. Z. and Ohagim P. I. (2017), Microbiological Analyses of Kunu Drinks Locally Produced and Sold in Calabar, Southern Nigeria. *Journal of Advances in Microbiology* 5(2): 1-8
- [17] Ekanem, J. O; Mensah, B.J., Marcus, N.S., Ukpe, B.A. (2018) Microbial Quality and Proximate Composition of Kunu Drinks Produced and Sold in IkotEkpene Metropolis, Akwalbom State, Nigeria. *Journal of Applied Science and Environmental Management* 22 (11): 1713–1718
- [18] Amusa, N.A. and Odunbaku, O.A. (2009). Microbiological and Nutritional Quality of Hawked Kunun (A Sorghum Based Non-Alcoholic Beverage) Widely Consumed in Nigeria. *Pakistan Journal of Nutrition* 8 (1): 20-25
- [19] Ogbonna, I.O., Opobiyi, M.Y., Katuka, B. and Waba, J.T. (2011). Microbial Evaluation and Proximate Composition of Kunuzaki, an Indigenous Fermented Food Drink Consumed Predominantly in Northern Nigeria. *Internet Journal of Food Safety*, 13: 93-97.

- [20] Benson, H.J. (2002), Microbiological applications: Laboratory manual in general microbiology. Complete version, 5th edition. McGraw-Hill, New York
- [21] Afolabi, O. R., Oloyede, A. R. and Agbaje, M. (2011). Microbiological safety of corn-based snack product, Aadun, sold in Abeokuta, south-western Nigeria. *Nigerian Food Journal*, 29(1): 36-40
- [22] Centre for Food Safety (2014), Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items) Food and Environmental Hygiene Department 43/F, Queensway Government Offices, 66 Queensway, Hong Kong.
- [23] Umar, M., Mohammed, I. B., Abdulkarim, I. M., Yusuf, G., Yaya, A.A. and Leo, G. (2016), comparative Studies on the Prevalence of Salmonella Comparative Studies on the Prevalence of Salmonella Species in Two Homemade Fermented Beverages ( Zobo and KununndKunun - Zaki) Sold At Samaru, Zaria, Kaduna Nigeria. *International Journal of Scientific and Research Publications*. 6(3):2250-3153.
- [24] Raimi OR (2013), Bacteriology quality of zobo drinks consumed in some parts of Osun State, Nigeria. *J. Appl. Sci. Environ. Manage.* 17:113-117.
- [25] Adebayo-Tayo BC, Samuel UA (2009). Microbial quality and proximate composition of dried Hibiscus sabdariffa calyxes in Uyo, Eastern Nigeria. *Malaysian Journal of Microbiology*;5 (1):13-18.
- [26] Nwachukwu E., Onovo, O.M., Ezeama C.F (2007), Effect of lime juice on the bacterial quality of zobo drinks locally produced in Nigeria. *Research Journal of Microbiology*. 2:787-791.
- [27] Ezeigbo, O.R; Ekaiko, M.U; Kalu, T. and Nwodu, J.A. (2014). Quality assessment of soymilk in Aba, Southeastern Nigeria. *International Journal of Epidemiology and Infection*. 2(4): 88 – 91.
- [28] Umeoduagu, N.D., Dimejesi, S.A., Nworie, O., Orji-Jerry, O. and Oti-Wilberforce, R. O. (2016). Microbiology Assessment of Soymilk sold in Onitsha metropolis. *African Journal of Basic and Applied Sciences*, 8(2):87-89
- [29] Adeleke, O.E; Adeniyi, B.A. and Akinrinmisi, A.A. (2000). Microbiological quality of local soymilk: a public health appraisal. *African Journal of Biomedical Research*. 3: 89 – 92.
- [30] Mbajiuka, C.S; Obeagu, E.I; Ifediora, A.C. and Ugwu, G.U. (2014). Isolation and identification of microorganisms involved in the spoilage of soymilk. *IOSR Journal of Pharmacy and Biological Science*. 9(5): 29 – 36.
- [31] Liamngee, K., Terna, T.P., Bem, A.A, Orpin, J.B., Mzungu, I., Obaje, M. and Anum, T. (2013). Microbial Analysis of Soybean milk sold in Makurdi Metropolis. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 3(3):97-104
- [32] Asogwa, I.S., Ugwu, O. L and Ugwanyi, C.A. (2018). Effect of hurdle factors on the physicochemical and sensory properties of stored soymilk. *Nigerian Food Journal*, 36(2): 25-37
- [33] Seiyaboh, E. I., Angaye, T. C. N. and Seiyaboh, Z. (2020). Assessment of Microbiological Contaminants associated with locally processed Beverages in Reused plastic containers. *Direct Research Journal of Public Health and Environmental Technology*, 5(4):49-51 doi.org/10.26765/DRJPHET8037126915
- [34] Aboagye, G., Gbolonyo-Cass, S., Kortei, N. K. and Annan, T. (2020). Microbial evaluation and some proposed good manufacturing practices of locally prepared malted corn drink ("asaana") and *Hibiscus sabdariffa* calyxes extract ("sobolo") beverages sold at a university cafeteria in Ghana. *Scientific African*, 8:1-16 doi.org/10.1016/j.sciaf.2020. e00330

- [35] Okolie, N.P., Anyiam, O. L., Idowu, O. H., Ilesanmi, Y. V. and Olawale, V. (2023). Production and storage stability of mulled Zobo drink stored in different packaging materials. *Nigerian Food Journal*, 41(2): 1-11 doi.org/10.4314/nifoj.v41i2.1
- [36] Etim, O. O., Okon, A. J., Olarewaju, O. E. and Inah, S. A. (2022). Public health risk knowledge, Awareness and Hygiene practices of producers of Artisanal drinks using previously disposed plastic bottles in Calabar municipality, Cross River State, Nigeria. *Global Journal of Pure and Applied Sciences*, 28: 201- 211 doi.org/10.4314/gjpas.v28i2.10
- [37] Ire, Francis Sopuruchukwu, Goziem Kim Benneth, and NdukweMaduka. 2020. "Microbiological Evaluation of Ready-to-Drink Tigernut Drinks Sold Within Port Harcourt Metropolis, Rivers State, Nigeria". *Asian Food Science Journal* 16 (1):45-58. <https://doi.org/10.9734/afsj/2020/v16i130164>.
- [38] Ade-Omowaye, B. I. O., T. O. Adedeji, and I. B. Oluwalana. 2015. "The Effect of Ginger Extract on the Acceptability and Storability of a Non-Alcoholic Beverage (Sorghum Stem Sheath Drink) in Nigeria". *Journal of Scientific Research and Reports* 7 (3):178-84. <https://doi.org/10.9734/JSRR/2015/10634>.
- [39] Gironés-Vilaplana A, Huertas JP, Moreno DA, Periago PM, García-Viguera C. Quality and microbial safety evaluation of new isotonic beverages upon thermal treatments. *Food Chemistry*. 2016 Mar 1;194:455-62.