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Bacterial Quality of Locally Produced Zobo Ginger and Tamarind Drinks in Birnin Kebbi Nigeria

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ABSTRACT
Locally produced beverages can acquire microorganisms during preparation, cooling, bottling and storage. This study assessed bacterial counts and presumptive bacterial isolates in zobo, ginger and tamarind drinks prepared in Birnin Kebbi, Nigeria. Twenty-seven drink formulations were analyzed: 12 zobo, 12 tamarind and three ginger formulations, including spice-treated and control samples. Total viable counts were determined by serial dilution and pour plating. Colonies were purified and characterized using cultural, microscopic and biochemical procedures. Mean counts are expressed as $\times 10^5$ CFU/mL. Zobo counts ranged from 162.00 ± 3.46 in ZG1 to 222.00 ± 14.11 in ZC3; tamarind counts ranged from 105.33 ± 10.07 in TX to 247.00 ± 17.09 in T+; ginger counts ranged from 108.33 ± 14.01 in GL to 292.33 ± 31.86 in G+. The study recorded 91 isolates comprising 12 reported taxa in 11 genera. Staphylococcus aureus was the most frequent taxon (21/91; 23.1%), followed by Bacillus subtilis and Shigella spp. (11/91 each; 12.1%). The results characterize formulation-level bacterial counts and the distribution of culturable isolates recovered in the study.
Keywords: bacterial quality; local beverages; presumptive isolates; zobo; tamarind; ginger

INTRODUCTION
Non-alcoholic plant-based drinks form part of the informal food economy in many Nigerian communities. Zobo, ginger and tamarind beverages are prepared from locally available materials and are often produced in small batches. Their accessibility and cultural familiarity make them important products for consumers and vendors. At the same time, steps such as washing, extraction, cooling, addition of sweeteners, bottling and storage provide opportunities for microbial contamination if water, utensils, containers or handling practices are inadequate.

The microbiological quality of a finished drink is influenced by the quality of raw ingredients and water, sanitation of equipment, handling during cooling and the condition of containers used for packaging.

Heating during extraction may lower vegetative bacterial populations, while contamination can be introduced again after heating through hands, utensils, bottles or storage conditions. Studies of zobo and related beverages have discussed these processing stages as important points for maintaining product quality (Egbere et al., 2007; Omeremu et al., 2019; Yusuf et al., 2024).

The three beverage matrices in this study are prepared from different plant materials and formulations. Zobo is based on roselle calyces, ginger drinks on fresh ginger extract, and tamarind beverages on tamarind pulp. Spices, lemon, sweeteners and preservative treatments further distinguish the formulations. Enumeration across these products therefore provides a formulation-specific description of culturable bacterial loads rather than a single value applicable to every beverage sold under the same common name.

Bacterial quality assessment of beverages usually combines enumeration of viable organisms with isolation and identification of selected colonies. Earlier investigations of zobo and other locally prepared drinks have reported diverse bacterial flora and have highlighted the importance of hygienic processing and storage (Amusa et al., 2005; Omemu et al., 2006; Egbere et al., 2007). Studies in other Nigerian settings have also reported bacterial contamination in vended drinks (Ayandele, 2015; Bello et al., 2014; Uwandu et al., 2022). These reports provide context, but results from different recipes, vendors and analytical protocols are not directly interchangeable.

A total viable count is commonly used to describe the number of colonies recovered under defined culture conditions. It provides a practical summary of the culturable fraction in a sample, while selective and differential media support recovery of colonies with particular growth or appearance characteristics. Isolate frequency tables can complement counts by describing the organisms identified among the colonies selected for further examination.

Food microbiology studies of ready-to-drink products have used different sampling schemes, culture media, incubation periods and identification methods. These protocol differences affect which organisms are recovered and how results can be compared. Accordingly, counts are most informative when their dilution factors, plated volumes, incubation conditions and replicate structure are documented along with the product formulation.

Counts and isolate identities answer different questions. A viable count summarizes the culturable bacterial burden under the selected conditions but does not identify all organisms or establish the presence of a particular pathogen. Phenotypic culture, microscopy and biochemical tests can provide presumptive identification, yet closely related organisms may share test reactions. Molecular confirmation is needed where species-level or strain-level conclusions are important. Likewise, a single sampling time cannot establish shelf life or demonstrate microbial growth kinetics.

This study compared bacterial counts in 27 prepared beverage formulations and summarized the frequency of presumptive isolates recovered from the products tested in Birnin Kebbi.

MATERIALS & METHODS

Sample design and preparation. Raw ingredients were obtained from Birnin Kebbi Central Market. The study describes 27 samples consisting of 12 zobo, 12 tamarind and three ginger formulations. Zobo and tamarind drinks were prepared as traditional or improved formulations using clove, dried ginger, dried pepper, combined spices or sodium benzoate in designated treatments. Ginger formulations were ginger with lemon (GL), ginger with sodium benzoate (G+) and ginger without additives (G−). Drinks were placed in clean bottles and stored at room temperature for analysis.

Enumeration. The beverage samples were serially diluted in sterile diluent and plated by the pour-plate method. The methods describe incubation at 37°C for 24 h and counting colonies as CFU/mL. The results tables report means and standard deviations from three replicate values, expressed as $\times 10^5$ CFU/mL.

For the reported serial dilution, 1 mL of beverage was transferred into 9 mL of sterile diluent and mixed before subsequent tenfold dilutions were prepared to 10^{-3}. A 1 mL aliquot from each selected dilution was plated using the pour-plate method. The inoculated plates were incubated at 37°C for 24 h, and colonies were enumerated to calculate CFU/mL. Distinct colonies were then selected from the culture plates for purification and phenotypic characterization.

Isolation and presumptive characterization. Distinct colonies from culture media were subcultured to obtain purified isolates. Gram staining and biochemical reactions, including catalase, oxidase, citrate, coagulase, urease, indole, methyl red, Voges–Proskauer and motility tests, were used for phenotypic characterization. The study lists nutrient agar, MacConkey agar, mannitol salt agar and Salmonella–Shigella agar among the media used. Taxonomic assignments are reported as presumptive identifications based on these phenotypic tests.

Statistical analysis. One-way ANOVA was performed, with statistical significance set at $p < 0.05$.

RESULTS

The reported bacterial counts differed among formulations in each drink group (Table 1). In zobo, ZG1 had the lowest reported mean (162.00 ± 3.46 ×10⁵ CFU/mL) and ZC3 the highest (222.00 ± 14.11 ×10⁵ CFU/mL). In tamarind, TX had the lowest mean (105.33 ± 10.07 ×10⁵ CFU/mL) and T+ the highest (247.00 ± 17.09 ×10⁵ CFU/mL). In ginger, GL had the lowest mean (108.33 ± 14.01 ×10⁵ CFU/mL), while G+ had the highest (292.33 ± 31.86 ×10⁵ CFU/mL). The study reported significant differences within each beverage matrix.

Table 1. Reported total bacterial counts by formulation (mean ± SD; ×10⁵ CFU/mL)

Drink	Formulation	Mean ± SD
Zobo	ZC1	199.33 ± 7.02
Zobo	ZC2	205.67 ± 5.69
Zobo	ZC3	222.00 ± 14.11
Zobo	ZG1	162.00 ± 3.46
Zobo	ZG2	209.33 ± 9.02
Zobo	ZG3	212.00 ± 7.55
Zobo	ZP1	192.67 ± 8.33
Zobo	ZP2	216.67 ± 14.47
Zobo	ZP3	216.00 ± 2.00
Zobo	ZX	179.33 ± 17.93
Zobo	Z+	213.33 ± 15.28
Zobo	Z−	213.33 ± 16.07
Tamarind	TC1	140.67 ± 19.01
Tamarind	TC2	114.67 ± 15.53
Tamarind	TC3	182.67 ± 3.06
Tamarind	TG1	146.67 ± 21.39
Tamarind	TG2	174.00 ± 12.17
Tamarind	TG3	185.67 ± 30.89
Tamarind	TP1	117.33 ± 2.08
Tamarind	TP2	138.33 ± 11.72
Tamarind	TP3	140.00 ± 19.08
Tamarind	TX	105.33 ± 10.07
Tamarind	T+	247.00 ± 17.09
Tamarind	T−	213.33 ± 11.55
Ginger	GL	108.33 ± 14.01

Drink	Formulation	Mean ± SD
Ginger	G−	211.33 ± 7.02
Ginger	G+	292.33 ± 31.86

A total of 91 bacterial isolates were recorded. The frequency table contains 12 named taxa representing 11 genera. Staphylococcus aureus was the most frequent taxon (21 isolates; 23.1%). Bacillus subtilis and Shigella spp. each accounted for 11 isolates (12.1%), followed by Staphylococcus epidermidis (10; 10.9%). Serratia marcescens was least frequent (2; 2.2%). Together, the taxa sum to 91 isolates (Table 2).

Table 2. Frequency of presumptive bacterial isolates (n = 91; percentages calculated from isolate counts)

Taxon	Isolates	Occurrence (%)
Aerobacter aerogenes	7	7.7
Bacillus subtilis	11	12.1
Enterococcus spp.	3	3.3
Escherichia coli	3	3.3
Micrococcus luteus	7	7.7
Proteus vulgaris	4	4.4
Salmonella spp.	5	5.5
Serratia marcescens	2	2.2
Shigella spp.	11	12.1
Staphylococcus aureus	21	23.1
Staphylococcus epidermidis	10	10.9
Streptococcus spp.	7	7.7

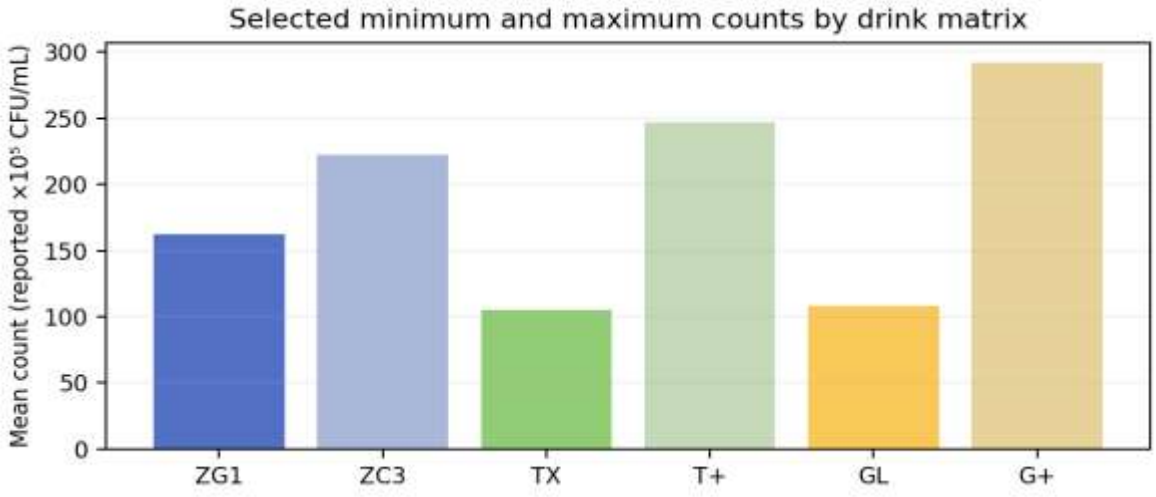

Figure 1. Minimum and maximum bacterial counts for each beverage matrix (×10⁵ CFU/mL).

DISCUSSION

The tested formulations varied in their reported viable counts, with the lowest values recorded for ZG1, TX and GL in their respective matrices and the highest values observed for ZC3, T+ and G+. Differences in microbial quality among locally prepared and vended beverages have also been reported (Amusa et al., 2005; Omemu et al., 2006; Bello et al., 2014).

The variation was observed within each drink type as well as between drink types. Among zobo formulations, the reported means covered a range of 60×10^5 CFU/mL between the lowest and highest entries. Tamarind showed the broadest numerical spread, with the TX value at the lower end and the sodium-benzoate formulation T+ at the upper end. Among the three ginger formulations, the GL count was below those of G− and G+. The associated standard deviations are shown in Table 1.

These formulation-level differences provide a descriptive account of the prepared batches. The count pattern can be considered alongside the distribution of isolates in Table 2: enumeration summarizes the recovered bacterial burden, while the frequency table describes the composition of the isolate collection. Neither measure alone describes the full microbial community of a beverage.

Staphylococcus aureus was the most frequently reported presumptive taxon, followed by Bacillus subtilis and Shigella spp. Previous surveys of zobo and other local drinks have reported Staphylococcus, Bacillus, enteric bacteria and other environmental taxa (Omemu et al., 2006; Ayandele, 2015; Uwandu et al., 2022). Differences among studies may reflect ingredient sources, water, handling, processing, sample selection, culture media and identification methods.

Staphylococcus aureus is commonly associated with human handling, while Bacillus species may occur in raw plant materials and the surrounding environment. Enteric bacteria among the reported taxa can also signal contamination pathways involving water, equipment or handling. These associations provide context for beverage hygiene, but the present sampling did not directly assess the source of contamination for individual isolates.

Isolates were assigned phenotypically using cultural, microscopic and biochemical characteristics. The most frequently reported taxa were Staphylococcus aureus, Bacillus subtilis and Shigella spp.

Previous investigations have emphasized sanitation and safe handling in local beverage quality (Egbere et al., 2007; Omeremu et al., 2019; Yusuf et al., 2024).

CONCLUSIONS

Reported bacterial counts varied across zobo, tamarind and ginger formulations. Staphylococcus aureus was the most frequently reported presumptive taxon, followed by Bacillus subtilis and Shigella spp. Good hygiene, potable water, clean equipment and suitable packaging are recommended during local beverage preparation.

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