"Bacteriological Quality Assessment of Zobo Drink Sold in Bayelsa State Nigeria.

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ABSTRACT

Introduction: Zobo is a non-alcoholic locally produced beverage from dried petals, acid-succulent calyxes of *Hibiscus subdariffa* by boiling and filtration. Zobo is rich in carbohydrates, proteins, calcium, vitamins, minerals, iron and antioxidant. The consumption of zobo may be associated with food infection and/ or food borne illness arising from unhygienic processes.
Aim: The aim of this study is to determine the bacterial quality of zobo sold in Bayelsa, identify the bacteria isolated and determine the enterotoxin producing ability of some strains.
Materials and Methods: A total of 150 bottles of zobo were examined, 50 were purchased from each zone (Yenagoa, Sagbama and Ogbia). Each bottle of zobo was well mixed by gentle inversion and 1mL of the zobo was added to 9mL of sterile peptone water in a test tube. Serial dilution was made to 10^5 and 0.1mL of the last dilution (10^5) was inoculated on already prepared and dried media (nutrient, MacConkey and salmonella/shegella agar) in duplicate and spread evenly with sterile glass rod. The plates were incubated at 37°C for 18-24 hours and examined for growth. Commercial purchased kits were used to test for enterotoxin production of some isolated strains.
Results: Out of the 150 zobo samples examined, the bacteria isolated were *S. aureus* 120 (25%), *Coagulase negative Staphylococci sp.*120 (25%), *Bacillus sp*. 150 (31.3%) and *Salmonella sp*. 90 (18%) respectively. Out of 120 *S. aureus* isolated, 18 (15%) produced enterotoxin.
Conclusion: Regulatory Agencies should as a matter of urgency consider the regulation of zobo production for public consumption and producers should be instructed on the principles of food preservation, sanitation and hygiene. The consumption of locally produced zobo is a public health concern in Nigeria.

Keywords:

1. INTRODUCTION

Zobo is a non-alcoholic local beverage made from dried petals, acid-succulent calyxes of *Hibiscus sabdariffa* by boiling and filtration [1, 2]. *Hibiscus sabdariffa* is an annual erect, herbaceous shrub with smooth or almost smooth, cylindrical and typically red stem. The flower is mostly cultivated in northern Nigeria and zobo is now popular in West African Sub Region, especially among the youths who see zobo as an alternative, cheap, relaxing non-alcoholic drink in social gatherings [3].
Zobo is a red coloured non-alcoholic local beverage made from different varieties of dried, succulent aqueous acid extract of Roselle carlyx [4]. This beverage has soured taste but often sweetened. The name zobo is derived from zoborodo in Hausa, goneura in Hindi, krajeab in Thailand, bissap in Senegal and sorrel in Caribbean [5]. Zobo as non-alcoholic beverage is quite popular in northern part of Nigeria.[6].
Religious and health campaigns against alcoholic beverages in Nigeria and the subsequent decrease in intake of alcoholic beverages in some areas have made zobo drink an alternative to alcoholic beverages. Zobo is known to be rich in carbohydrate, protein, calcium, vitamin, minerals, iron and antioxidants. Aside this, it was used in folk medicine as diuretic, mild laxative, treatment for cardiac and nerve diseases and management of cancer. It was reported that zobo is a good traditional medicine for the treatment of diseases such as hypertension, UTI etc.[7] The danger of food infections

and food borne illness that may be associated with zobo outweighs the benefits derived. Bacteria isolated from zobo drink includes, *S. aureus, Bacillus* sp, *Lactobacillus* sp, *Escherichia coli, Pseudomonas* sp, *Enterobacter* sp [8]. Other researchers isolated *S. aureus, Streptococci* sp *and Proteus* sp. [9] Bacteria isolates from Zobo include *S. aureus, Bacillus sp, Micrococcus, Proteus* sp, *E. coli* [10]. It was noted that the consumption of zobo might serve as vehicle for food borne disease agents[9,11]. In a study conducted on the microbiological quality of zobo in Aba South East Nigeria, the bacteria isolated were *Staphylococcus* sp, *E. coli, Lactobacilus, Bacillus* sp *and Pseudomonas* sp. The level of contamination was attributed to lack of personal and environmental hygiene in the processing, packaging and preservation [12]. Looking at the nutritional, sensory and microbiological assessment of zobo, the following bacteria were isolated, *E. coli. Klebsiella* sp *and Bacillus* sp. which was linked to contamination from vendors and materials used for production [13]. In assessing the microbial quality of zobo sold in Yenagoa metropolis the bacteria isolated were *E. coli, S. aureus, Enterobacter* sp. *Micrococcus and Proteus* sp.[14]. The poor bacteriological quality was attributed to poor handling, and poor quality materials used for production, unhygienic processing and vendors. Other researchers also isolated similar organisms [15,11]. It was noted that lack of proper hygiene and sanitary measures in processing and packaging of kunu were responsible for contamination.

The aim of this research is to determine the bacteriological quality of zobo drinks marketed in Bayelsa State, Nigeria, identify bacteria isolated and the enterotoxin producing ability of some strains.

2. MATERIAL AND METHODS

2.1 Study Area

The study was conducted in Bayelsa State, Nigeria. The samples of Zobo drink were purchased from the three (3) senatorial district headquarters of Bayelsa State, namely; Yenagoa (capital), Sagbama and Ogbia town. Bayelsa state was carved out of River State in 1996. Bayelsa is located in latitude 4°15' North, latitude 5°23' South and longitude 5°22' West and longitude 6°45' East. It is bound by Delta State on the North, River State on the East and Atlantic Ocean on the West and South. Bayelsa has the largest wetland in West African Sub-Region. It has a population of about 1.7 million.

2.2 Collection of Samples

The method of sampling was random collection using convenient sampling method and the statistical analysis were done with Graph Pad Prison version 5.03.

A total of 150 bottles of zobo were purchased, out of which fifty (50) were bought from each of the senatorial headquarters. The samples of zobo were transported to the laboratory in a cooler of ice-packs for examination. Zobo drinks were packaged and sold in recycled coke, fanta, sprite or medium water bottles of 35cl at 50 naira each.

2.3 Bacteriological Examination of Samples

Each sample of Zobo was mixed properly by gentle inversion several times and 1ml of the sample (neat) was pipetted and transferred to 9mL of sterile normal saline (sterilized by autoclaving at 121° C for 15 minutes). Subsequent serial dilutions were made up to 10^5 and 0.1ml of the last dilution (10^5) was placed on already prepared and dried nutrient, MacConkey and *Salmonella/Shigella* agar plates in duplicates. These were spread evenly with the aid of sterile glass rod (sterilized by dipping in absolute alcohol and flaming in Bunsen flame).The inoculated plates were incubated at 37° C for 18 - 24 hours and examined for growth.

2.5 Identification of Isolated Bacteria

The bacteria isolated were identified using morphology, cultural, Grain's stain reaction, chemical and biochemical reactions such as citrate, VP, Methyl red, indole, catalase, coagulase and carbohydrates fermentation etc.

2.5 Test for Bacterial Load in stored Zobo

A set of purchased zobo were kept at room temperature and another in the refrigerator at 4°C after the initial determination of the bacterial counts in CFU/mL. The counts from the preserved zobo at ambient and refrigerator temperature were determined on the second and third day respectively.

2.6 Test for Toxigenicity of *S. aureus*

The ProlexTM Staph Latex Kit is used to detect S. aureus that produce enterotoxin. The Kit uses blue polystyrene latex particles that had been sensitized with fibrinogen and IgG.

2.6.1 Procedure

The latex kit were removed from refrigerator and allowed to attain room temperature before use. The reagent was re-suspended by gentle inversion several times. A drop of the Staph test Latex reagent was placed in a circle on test card and colony of Staphylococcus aureus was mixed with the reagent on the entire circle. This was rocked gently by allowing the mixture flow the entire area. Strong agglutination within 20 seconds was regarded as positive. Negative control Latex reagent was included as quality control.

3 RESULTS

3.1 Percentage occurrences of bacteria isolated from zobo

Out of the 150 samples of zobo purchased, 50 were purchased from Yenegoa, Sagbama and Ogbia town respectively. In zobo from Yenegoa *S. aureus* were 40 (26.7%), coagulase negative *Staphylococci* were 35 (23.3%), *Bacillus* sp.50 (33.3%) and *Salmonella* sp. 25 (16.7%). In Sagbama *S. aureus* 4.5 (25%), *Bacillus* sp. 50 (28.6%), Coagulase negative *Staphylococci* 45 (25.7%) and *Salmonella* sp. 35 (20.0%) respectively. In Ogbia town *S. aureus* 35 (22.6%), coagulase negative *Staphylococci* 40 (32.3%), *Bacillus* sp. 50 (32.3%) and *Salmonella* sp.30 (19.4%) respectively. The overall percentage occurrences of *S. aureus* was 120 (25.0%), coagulase negative *Staphylococci* 120 (25%), *Bacillus* sp. 150 (31.3%) and *Salmonella* sp. 90 (18.6%) respectively.

Table 3.1. Percentage occurrence of Bacteria Isolated from Zobo in Yenegoa, Sagbama and Ogbia Town

Location	*S. aureus*	Coagulase negative *Staph.*	*Bacillus sp.*	*Salmonella sp.*	Total
Yenegoa	40 (26.7)	35 (23.3)	50 (33.3)	25 (16.7)	150 (31.3)
Sagbama	45 (25.7)	45 (25.7)	50 (28.6)	35 (20.0)	175 (36.6)
Ogbia	35 (22.6)	40 (25.8)	50 (32.3)	30 (19.4)	155 (32.3)
Total	120 (25.0)	120 (25.0)	150 (31.3)	90 (18.6)	480

Numbers in parenthesis = percentages

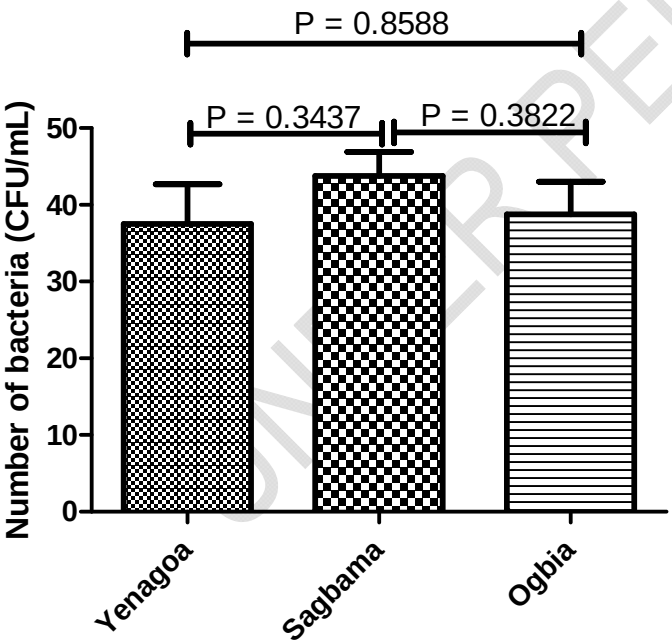

Figure3.1 Difference in the counts of bacteria isolated from different locations.

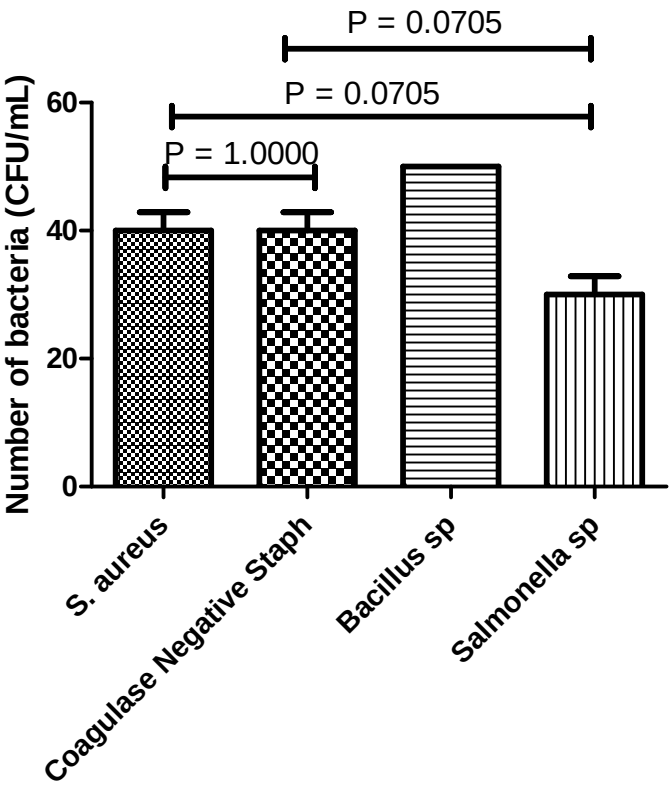

Figure 3.2 The difference in the counts of bacteria species isolated from zobo.

3.1 The percentage of enterotoxin producing *S. aureus*

Out of the 40 isolates of *S. aureus* obtained from zobo drinks, 4 (10%) produced enterotoxin from Yenegoa zone, out of 45 *S. aureus* isolated from zobo purchase in Sagbama, 6 (13.3%) produced enterotoxin, while in Ogbia, 35 *S. aureus* were isolated and 8 (22.8%) were capable of producing enterotoxin respectively.

Table 3.2. Percentage occurrence of enterotoxin producing *S. aureus* isolated from zobo drink from each zone.

Location	No. Of *S. aureus* Isolated	No. Positive
Yenegoa	40	4 (10.0)
Sagbama	45	6 (13.3)
Ogbia	35	8 (22.8)
Total	120	18 (15.0)

Numbers in parenthesis = percentages

3.3 Bacterial counts in preserved zobo for 24Hrs and 48Hrs at ambient and refrigeration (4^{O}C).

The total count of bacteria isolated from zobo sample immediately after purchase was;1.14×10^5 CFU/mL (Day 1), 1.56×10^5 CFU/mL on Day 2 and 1.60×10^5 CFU/mL on Day 3 respectively. The counts obtained at ambient temperature storage were; 1.14×10^5 CFU/Ml on Day 1, 2.34×10^5 CFU/mL on Day 2 and 2.54×10^5 CFU/mL on Day 3 respectively.

Table3.3 Bacteria counts in zobo preserved at room and refrigeration temperature for 3 days.

Temperature	Day 1 Counts in CFU/Ml	Day 2 Counts in CFU/Ml	Day 3 Counts in CFU/Ml
Refrigeration	1.14×10^5	1.5×10^5	1.6×10^5
Ambient	1.14×10^5	2.34×10^5	2.54×10^5

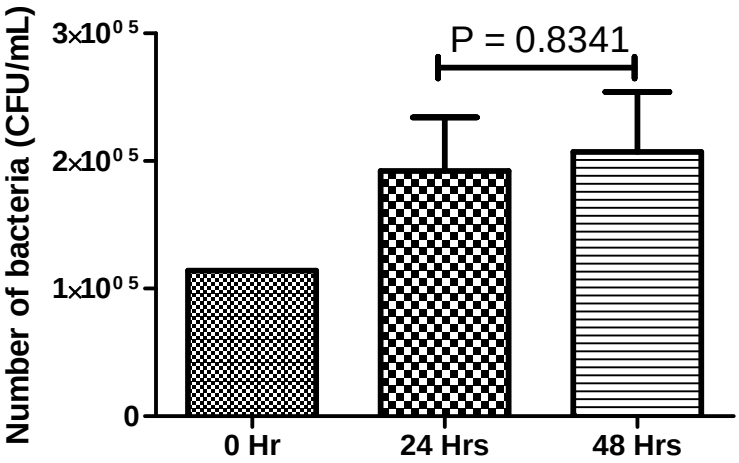

Figure 3.3: Mean values of bacterial counts in zobo.

Figure3.4: Identification of isolated bacteria from zobo

S/No	Colour	Surface	Edge	Translucency	Texture	Gram Rxn	Size	Shape	Motility	Methyl Red	Voges Proskauer	Oxidase	H2S Production	Indole	Coagulase	Catalase	Citrate	Urease	Starch Hydrolysis	Glucose	Lactose	Sucrose	Maltose	Galactose	Mannitol	Arabinose	Oxidative	Fermentative	Bacteria
1	M	R	E	C	D	+	Md	Rd	+	-	+	-	-	-	-	+	-	-	+	A	A	A	A	A	±	-	+	+	***Bacillus sp.***
3	M	S	E	C	Mt	-	Md	Rd	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	-	+	+	***Salmonella sp.***
4	Cr	S	E	C	Mt		Md	Cc	-	+	+	-	-	-	-	+	N	N		A	A	A	-	A	-	-	+	+	***Coagulase –ve Staphylococci***
5	Cr	S	E	C	Mt	+	Md	Cg	-	+	+	-	-	-	+	+	N	N	-	A	A	A	A	A	A	-	+	+	***S. aureus***

Key: M =Milky, Cr =Creamy, R =Rough, S =Smooth, E =Entire, C =Clear, D =Dry, Mt =Moist, Md = Moderate, Cc =Cocci in chain,, Cg = Grape like cocci Rd =Rod, N = Not determined

4. DISCUSSION

The most prevalent bacteria isolated from zobo were *Staphylococci* followed by *Bacillus sp.* and *Salmonella sp. Staphylococcus* is a common contaminant of foods and other similar preparations if good hygienic practices are not employed. *Staphylococci* as normal flora of human inhabit the nostrils, hands, skin, mouth and dresses etc. They might easily gain access to zobo without good sanitary practices. Those who prepare zobo locally do not consider the use of good water as a means of reducing contamination therefore water may also serve as source of contamination introducing *Staphylococcus* and other bacteria into zobo, especially water used for washing and rinsing of recycled containers for packaging. *Bacillus* sp. were the second most prevalent bacteria, the calyxes may have come in contact with soil during harvesting and the dusts blown by the wind during drying in the sun might deposit both bacteria and/ or their spores on the calyxes. *Bacillus* is geophilic and the above process may favour their deposition on the calyxes. Some of the spores may survive the only Critical Control Point (CCP) in the preparation of zobo (boilling at about 100° C for about 30 minutes).The spores might germinate and re-contaminate zobo. *Salmonella sp.* might be present as a result of the handling processes from contaminated hands and water. These bacteria were also isolated by other researchers from zobo drink as contaminants with similar prevalence [12]. Zobo drinks were marketed on recycled bottles of water, fanta, coke, sprite etc. These bottles were only washed or rinsed without any sterilization process. The sterility of the water used for washing or rinsing is questionable. The ready to drink zobo were just transferred directly into the bottle for sale. This process might aids contamination of zobo drinks. The percentage occurrence of bacteria isolated from zobo is an indication of poor hygienic handling [16].

Other workers also isolated *Escherichia coli* from zobo drinks, but from our study, *E. coli* was not isolated. It might be possible that the concentration of extracted *Hibiscus* calyxes used for zobo preparation might possess antimicrobial

activity against *E. coli* or the phytochemical substances in the extracted calyxes and/or other spices used with the calyxes may have inhibitory action on *E. coli*.

Zobo had been noted to harbour bacteria associated with food borne illness and the health implication of consuming contaminated zobo outweighs the benefits.[17,18] The spices added to zobo were mainly agricultural produce which had been noted to contain heavy microbial load.[11,19] The consumption of local drinks such as zobo is of public health concern. Zobo might serve as a vehicle of trasfering bacteria associated with food borne illness.[9,20].

One of the challenges of zobo is the preservation of finished product. The total bacterial counts from freshly prepared zobo were 1.41×10^5 CFU/ mL and after 18 – 24 hours the count rose to 2.34×10^5 CFU/ mL and on the third day 2.54×10^5 CFU/mL. Freshly prepared zobo should be consumed the same day, except when preserved in refrigerator at a temperature of 4°C to control the proliferation of contaminating bacteria. In a similar estimation of total heterotrophic counts in zobo, a count of 4.02×10^5 CFU/mL was obtained [10]. Post preparation contamination from personnel and packaging might play major role in contamination of zobo.

The test for the production of enterotoxin by *S. aureus* isolated from zobo showed that 15% of *S. aureus* were capable of producing enterotoxin. This clearly showed that the consumption of locally produced zobo drink is a public health concern because the bacteria isolated were associated with food infections and food poisoning (foodborne illnesses).

4. CONCLUSION

With the increasing interest in consumption zobo drink, there is need for regulatory agencies to regulate the production zobo. The isolation of enterotoxin producing *S. aureus* is of major public health concern associated with the consumption of zobo drinks. Left over zobo from previous day sale should be confiscated, unless preserved at 4° C.

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21.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

Here is the Definitions section. This is an optional section.

Term: Definition for the term