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Original Research Article

Public Health implications of the microbiological analysis of Zobo (*Hibiscus sabdariffa*) drink produced and hawked in Eket, South-South Nigeria

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Abstract

Purpose*:* Zobo drink is a local, cheaper soft drink made from *Hibiscus sabdariffa* calyx, which is an herbaceous medicinal plant grown in the tropics. The study was designed to assess the Public Health implications of the microbiological analysis of Zobo (*Hibiscus sabdariffa*) drink produced and hawked in Eket, South-South Nigeria.
Methods*:* Hawked Zobo drinks packaged in plastic bottles were purchased from ten different hawkers at different locations. Nutrient Agar (NA), MacConkey Agar (MCA), *Salmonella Shigella* Agar (SSA) and Sabouraud Dextrose Agar (SDA) were used for analysis of bacteria and fungi. Different microscopic and biochemical tests were employed to further identify the bacterial and fungal isolates.
Results*:* The bacteria isolated from the samples analyzed include *Staphylococcus aureus, Salmonella spp, Pseudomonas spp*., *Escherichia coli, Streptococcus spp*., *Campylobacter spp*., *Lactobacillus spp*., and *Klebsiella spp.*, with the highest percentage of occurrence being *S. aureus.* The total bacterial count ranged from 2.6 to 6.6 x10^3 cfu/ml in Nutrient agar, 1.0 to 2.5 x 10^3 cfu/ml in MacConkey agar and 2.4 to 7.3 x 10^3 cfu/ml in *Salmonella-shigella* agar. The fungi isolated from the samples were yeast (*Saccharomyces cerevisiae), Aspergillus sp, Fusarium sp, Penicillium sp and Rhizopus sp.,* with a total count ranging from 5.4 to 9.1 x 10^4 cfu/ml in Sabouraud dextrose agar.
Conclusion: As Pubic Health implication, the likely sources of contamination of the zobo drinks analyzed are from the handlers, equipment and utensils used for processing, the water source and the method of dispensing into nylon or plastic bottles.

Keywords: Zobo drink, Public Health implications, Microbiological analysis, Bacteria, Fungi

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INTRODUCTION

Hibiscus sabdariffa are species of hibiscus native to West Africa. It is commonly known as 'Roselle'. It is also known as 'Belchanda' among Nepalese, 'Bissap' in Senegal, Ghana and Niger, 'Zobo', 'Zoborodo' or 'Isapa' in Nigeria. Fresh or dried calyces of *H. sabdariffa* are used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectioneries, ice cream, chocolates, flavouring agents, puddings and cakes.[1] With the rapidly growing and changing food demands by urban dwellers and the dwindling economy, there has been a noticeable increase in food vendors in Nigeria. Types of vending sites encompass stalls, a variety of push-carts, roadside stands and hawkers, depending upon the ingenuity of the individual, resources available, type of food sold and the availability of other facilities. In spite of the numerous advantages offered by street foods, there are several Public Health implications and hazards associated with this sector of the economy. Multiple lines of evidence reveal that foods exposed for sale on the roadsides may become contaminated either by spoilage or pathogenic microorganisms.[1-5]

Many studies indicate that most outbreaks of foodborne acute gastroenteritis occur in places of mass production and distribution.[2-4] In developing Countries like Nigeria, it has not been possible to have control over the processing of hawked foods. Also, since most of the vendors lack adequate knowledge of food processing and handling practices, there is likely to be a high risk of chemical and microbial contamination. Currently, the production process of Zobo drink is neither mechanized nor standardized; hence, the mode of packaging or dispensing in nylon or plastic containers is fraught with exposure to potential contaminants and an increased risk to public health. The study was therefore carried out to assess locally prepared and hawked Zobo drinks for microbial quality and its Pubic Health implications.

MATERIALS AND METHODS

Sample collection[18]

Hawked Zobo drinks packaged in plastic bottles were purchased from ten different hawkers at different locations in Eket, Akwa Ibom State (Figure 1). These areas included Nka Market, Affiong Etok Market, Eket Oron Road, Heritage Polytechnic Mini-mart and a Motor park along Atabong Road. The samples were taken to the laboratory in the same plastic containers used in hawk them. While in the laboratory, they were aseptically transferred into sterile specimen bottles and stored in the refrigerator prior to microbiological investigation.

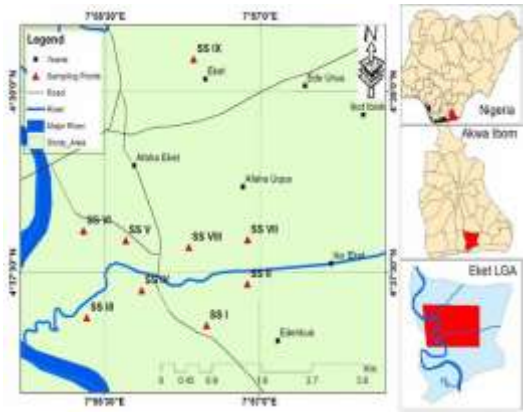

Figure 1: Map of study location (Eket)

Media preparation[18]

The media used for isolation and enumeration of bacteria and fungi (yeast and mould) included Nutrient Agar (NA), MacConkey Agar (MCA), *Salmonella shigella* Agar (SSA) and Sabouraud Dextroxe Agar (SDA). The composition and preparation of these media were according to the manufacturer's instructions.

Determination of the pH

The pH of the various samples of Zobo drink was determined using a pH meter (3505 pH meter, Jenway) by first preparing a standard buffer solution in a clean conical flask. One gram of standard buffer was dissolved in 100ml of distilled water. The pH probe was inserted into the standard buffer solution and calibrated to pH 4. The pH probe was then cleaned with distilled water and wiped dry before being inserted into each Zobo drink sample measured into sterile beakers. The reading was taken when the pH meter read to the highest point and remained constant.

Determination of Total Bacterial Count

Each sample was serially diluted with sterile distilled water before inoculation. The media used for inoculation were NA, MCA and SSA using the pour plate method. 1ml of the appropriate dilutions (at 10^{-3}) of each sample was inoculated on each of the media in separate sterile petri dishes. Two sterile petri dishes were provided for each dilution. One set of plates of the dilution was incubated for 24 hours at 37^0C for bacteriological investigation.

Colonies were counted after incubation, and the number of colonies obtained was multiplied by the dilution and plating factors. The means of duplicate results were then calculated and recorded.[14]

Determination of Total Fungi Count

This was carried out on a plate of Sabouraud Dextrose Agar (SDA) using the pour plate method. A ten-fold serial dilution of the sample was carried out by transferring 1ml of the Zobo drink sample with a sterile pipette into 9mls of distilled water in test tubes. After dilution, 1ml of the serially diluted sample at 10^{-3} dilutions was transferred into sterile petri dishes before pouring the media that was previously prepared into plates in duplicate. The plates were swirled gently in different directions and allowed to solidify. The plates were then incubated at room temperature for 48 hours.[14]

Subculturing of Isolates

After the primary isolation of colonies, plates were further cultured to get a pure isolate of bacteria and fungi. This was done first by preparing a fresh plate of NA, MCA and SDA. The media were then poured into various plates and allowed to set. The plates were streaked with a loopful of distinct colonies using a sterile wire loop. Plates of NA and MCA were incubated at 37 °C for 24 hours for bacteria, while SDA plates were incubated at room temperature for 48 hours for fungal growth. After incubation, the pure isolates were stocked in various McCartney bottles. The stock cultures were preserved in a refrigerator at 4 ºC and used for subsequent identification of the organisms.[14]

Characterization and identification of Fungal Isolates

The characterization and identification of fungi isolates were based on cultural and microscopic characteristics. The microscopic identification was done by preparing a net number of isolated colonies. 1 drop of Lactophenol was placed on a clean slide. A sterile inoculating needle was then used to pick a colony of the fungi from the subculture plate and smear on the slide. The slides were covered with cover slips and observed under a light microscope using the X40 objective lens. The Burnette and Pankhurst yeast identification scheme was used to identify the moulds.[14, 15]

Characterization and identification of Bacterial Isolates

The characterization and identification of bacterial isolates were based on morphological, cultural and biochemical characteristics using the gram staining method and then identified using Bergey's Manual of Systemic Bacteriology. The biochemical tests for identification of the isolates were Citrate Utilization, Catalase, Methyl Red-Voges Proskauer, Coagulase, Triple Sugar Iron (TSI), Oxidase and Motility Indole Ornithine (M.I. O.) tests.[14-17]

RESULT AND DISCUSSION

pH and Total Bacterial and Fungal Counts of Zobo Drink Hawked in Eket

Results of the pH of samples of Zobo drink purchased from various parts of Eket metropolis, as indicated in Table 1 showed that all samples were acidic. The number of colonies varied based on the type of media used in the cultivation and isolation. As shown in Table 1, the average number of colonies on Nutrient Agar plates for total heterotrophic bacteria ranged from 2.6×10^3 to 6.6×10^3 cfu/ml at 10^{-3} dilution. On MacConkey Agar, the average number of colonies for total coliform ranged from 1.0×10^3 to 2.5×10^3 cfu/ml at 10^{-3} dilution. On Sabouraud Dextrose Agar, the average number of colonies for fungi ranged from 5.4×10^4 to 9.1×10^4 cfu/ml at 10^{-3} dilution.

Table 1: Mean Values of pH and Total Bacterial and Fungal Counts

		Colony forming unit/ml (mean)			
SAMPLE AREA	**pH**	**NA**	**MCA**	**SSA**	**SDA**
A (Affiong Etok Market)	3.52	6.4×10^3	2.0×10^3	7.3×10^3	8.2×10^4
B (Nka Market)	3.98	3.3×10^3	1.5×10^3	2.4×10^3	7.3×10^4
C (Heritage Polytechnic mini-mart)	4.20	6.6×10^3	2.5×10^3	3.8×10^3	9.1×10^4
D (Eket-Oron Road)	3.80	2.9×10^3	1.5×10^3	5.5×10^3	6.2×10^4
E (Atabong Motor park)	4.12	2.6×10^3	1.0×10^3	4.3×10^3	5.4×10^4

Values are mean values of duplicate samples. **Key:** NA = Nutrient Agar**,** MCA = MacConkey Agar**,** SSA = Salmonella Shigella Agar **,** SDA = Sabouraud Dextrose Agar

Occurrence of Bacterial Isolates

Eight different types of bacteria occurred at varying frequencies in the Zobo drink samples purchased from the five locations in Eket. Among the bacteria isolates, *Staphylococcus aureus*

(24.53%) has the highest percentage frequency of occurrence, followed by *Salmonella spp* (18.87%), with the lowest being the *Klebsiella spp* (3.77%) (Figure 2).

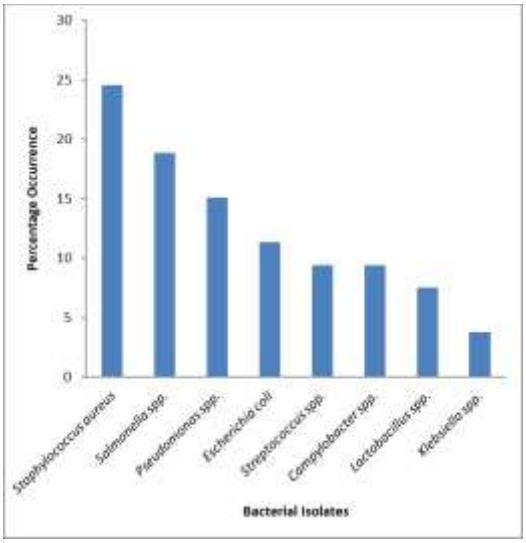

Figure 2: Percentage Frequency of Occurrence of Bacterial Isolates

Occurrence of Fungal Isolates

Aspergillus spp, *Yeast*, *Fusarium spp*, *Penicillium spp* and *Rhizopus spp* were some of the identified fungal isolates in the samples of Zobo drink examined. *Aspergillus spp* occurred most with a frequency of 42.50% while *Rhizopus spp* was the least found (7.50%). Figure 3 shows the percentage frequency of occurrence of the fungi.

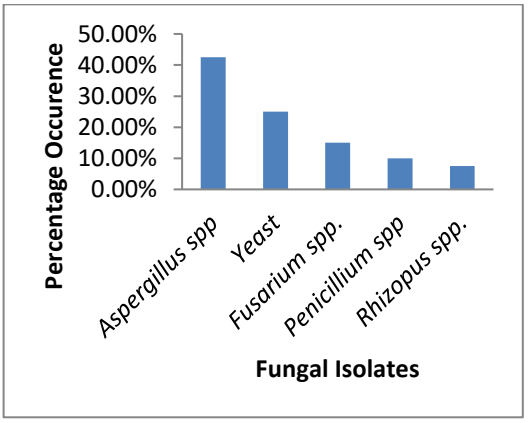

Figure 3: Percentage Frequency of Occurrence of Fungal Isolates

The pH of the samples was low. This agrees with the observation of a previous study that the pH was on the low side indicating and confirming the high acidity usually noticed in Zobo drinks.[6,13] It is found to be a naturally acidic fruit rich in organic acids like ascorbic, oxalic, tartaric, malic and succinic acid. The high acid level may also inhibit the growth of some microorganisms that are not tolerant to it.[7]

All the Zobo drink samples examined had coliform count well above the zero-value recommended for safe water. Eight bacteria were isolated from the Zobo drink samples. Amongst these, *Salmonella spp*, *E. coli* and *S. auerus* were found in all ten samples examined. Although epidemiology investigation has been difficult in Nigeria and outbreaks of food and drink-borne diseases are generally under-reported, gastroenteritis has remained a major health care problem in the country. The isolation of bacteria in all the Zobo drink samples analyzed and the unacceptable total bacterial count of $>10^3$CFU/ml established in all the samples implies extreme contamination and potential health risks of these drinks. These findings correlate with earlier studies by different researchers.[8, 9]

The presence of *E.coli, Streptococcus sp* and *Klebsiella* in this study indicates faecal or sewage contamination introduced via the use of contaminated water or contamination from the unsanitary environment and utensils or via human handlers or operators.[10] The isolated enteric bacteria are known pathogens responsible for millions of cases of infection, gastrointestinal diseases and death. *S. aureus* and *Pseudomonas sp* are possible contaminants from handlers and utensils used, especially after the processing, as they are mesophiles, though some *Pseudomonas* are spoilage organisms at refrigerated temperatures.[13]

Staphylococcus aureus is a normal flora of the skin, nose, mucous membrane, throat, palms, hairs and a common etiological agent of septic arthritis. It is a ubiquitous microorganism that can enter foods from many sources, such as handlers with acute pyogenic infections or healthy carriers who harbour the organism in their nose or throat. It is commonly implicated in water and food contamination. The detection of *S. aureus* is of serious public health concern because of its ability to cause a wide range of infections, especially food-borne intoxication. This organism was equally isolated by different studies.[11,12]

The increasing frequency of food-borne *Salmonella* has been causing recurring outbreaks, sometimes with fatal infections, which has been linked to the unsanitary practices of food and beverage processes leading to contamination of

foods by *Salmonella*.[12] The routine detection of *Salmonella* in the environment, including in foods and beverages, is a necessary component of public health programs.

The fungi isolation and identification revealed the presence of yeast and moulds. Moulds, even in small quantity, contribute to the spoilage and contamination of foods. The presence of fungi may be attributed to the acidic nature of the samples, since it has been observed that yeasts and moulds are capable of utilizing organic acids. Also, the presence of fungi in the drinks may lead to poisoning and production of undesirable odour, colour and taste changes. The moulds isolated include *Aspergillus sp*., *Fusarium sp*., *Penicillium sp*., and *Rhizopus nigrigans*. The fungi species isolated are capable of producing toxins. *Aspergillus sp*, for instance, are potential carcinogens. *Aspergillus* and *Penicillium* are common spoilage organisms of carbohydrate foods and storage microflora of many foods. The high survival rate of their spores could also explain their role. The high incidence of contamination encountered in this study is mainly due to the unsanitary and unhygienic nature of the drink preparations and the water used. Contamination may have occurred during cooling of the hot extract, addition of flavours and sweeteners, dispensing of extract into bottles, utensils and water used during the post-heating stages. Water used in processing has been identified as the major source of contamination of locally made drinks.[4, 13]

CONCLUSION

It is obvious that Zobo drink is one of the locally made drinks consumed by many people, not only for its low price but also for its nutritional composition. If well prepared and packaged as a concentrate, it can compete favorably with most of the imported non-alcoholic beverages available in the country, considering the increasing acceptance, socio-economic potentials, vitamin C and other minerals content. However, they are prepared in homes usually under unhygienic conditions and are prone to contamination by the microflora of the raw materials and of the utensils as observed through this research. Most of these organisms isolated from the Zobo drink are known to be the causative agents of food-borne gastroenteritis; therefore, adequate hygienic practices must be observed during the preparation and handling of the Zobo drink.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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