

# Bacteriological Examination of Sorrel Beverage (Zobo) Drinks Sold in Awka Metropolis

**Obasi Chinelo Jane, Obasi Innocent, Okafor Ugochukwu Chukwuma\*, Umeh Sophina Ogonna, Okeke Oluebube**

Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

## Abstract

Zobo drinks are made from *Hibiscus sabdariffa* and have been shown to be very nutritive with medicinal properties. This work was done to determine the bacteriological quality of zobo drinks sold in Awka metropolis. Fifteen zobo samples were purchased from different locations. Nutrient agar and pour plate method was employed for bacteria isolation. Different microscopic and biochemical test were employed to further identify the bacterial isolates. After the analysis the organisms were identified as *Staphylococcus epidermidis*, *Corynebacterium spp*, *Klebsiella spp*, *Pseudomonas spp*, *Escherchia coli* and *Proteus spp*. These organisms identified have been implicated in several gastrointestinal diseases such as dysentery, diarrhoea and many others. The result implies that precautions were not taken by the manufacturers and handlers of zobo drink hence the bacteriological contamination which could have been caused by the manufacturers and handlers themselves or gotten from the unhygienic environment or materials used in the production and packaging of the drinks. However, education of the manufacturers and handlers is necessary to avoid possible health hazard.

## Keywords

Zobo, *Staphylococcus epidermidis*, Bacteriological, Diarrhoea and Awka

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## 1. Introduction

Zobo drink is a local, cheaper soft drink made from *Hibiscus sabdariffa* calyx which is a herbaceous medicinal plant grown in the tropics [1]. The demand for zobo drinks is due to its low prices, nutritional and medicinal properties [2], [3]. It is served indoors or at special occasion to people of various tribes and tradition, in Nigeria.

The concentrated zobo is a dehydrated water extract of the dried petals of the Rosselle plant (*H. sabdariffa*). It is red in colour and is a rich source of vitamins and minerals such as calcium and iron. It is prepared by first boiling the leaves of the Rosselle (that is the dried leaves), spiced up with ginger and cloves, sweetened with sugar to produce the sorrel red drink. This is then dehydrated to concentration (67%). This

reddish liquid extract can be reconstituted as a drink. Zobo drink is relished for its colour and flavor and is increasingly becoming a substitute for the more expensively produced soft drinks and juices [4]. Studies have shown that the colour of the drink influence consumer preference for the drink and the variety used for the production of zobo drink [5].

Ginger (*Zingiber officinale*), one of the ingredients used in zobo production, contain antioxidative substances that can reduce oxidation process of colour in foods [6]. The calyces of *H. sabdariffa* have been found to be rich in vitamins, natural carbohydrates, proteins, vitamin C and other antioxidants [7] and also minerals [7],[8]. According to the work of [9], the proximate composition of zobo drink is; 88.88% water, 0.046% protein, 0.16% fat, 0.16% crude fibre, 0.21% ash and 10.64% carbohydrates.

\* Corresponding author

E-mail address: [uc.okafor@unizik.edu.ng](mailto:uc.okafor@unizik.edu.ng) (O. U. Chukwuma)

Furthermore, zobo drink if well prepared and packaged as a concentrate 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 [10]. The bacterial species commonly found in zobo include; *Pseudomonas specie*, *Escherichia coli* *Staphylococcus specie*, *Streptococcus specie* and *Bacillus species* [11].

This work is aimed at investigating the bacterial load of zobo drink sold in Awka metropolis.

## 2. Materials and Methods

### 2.1. Study Area

The study area is Awka metropolis, Anambra state in the South-East geopolitical zone of Nigeria.

### 2.2. Source of Sample

The samples were purchased from fifteen different areas in Awka metropolis. These sites include Eke-awka round-about, Tempsite, Eke-awka Timber shed, Regina, Aroma, Secretarate road, First market, Second market, shop in Unizik girl's hostel, Ifite gate, NkwoAmaenyi, Government house, Book foundation, Ukwu Orji and Quarter bus stop. These sites were chosen because they are highly patronized by consumers.

### 2.3. Sample Collection

Fifteen zobo samples were purchased from different locations mentioned above and was transferred immediately to the Laboratory for bacteriological analysis. The samples collected were for bacteriological and biochemical analysis.

### 2.4. Media Preparation

The media used for the isolation was Nutrient agar (NA). The media was prepared according to the manufacturer's instruction and sterilized.

### 2.5. Microbiological Analysis of Sample

A 10 fold serial dilution of the samples was carried out by pipetting 1ml of each sample (1-10) into 9ml of the distilled water (diluent). After performing the serial dilution, 1ml of  $10^2$  of the solution was pipette into an empty petri dish. The prepared culture media was allowed to cool and about 20ml of nutrient agar were poured into a well labelled petri dish containing 1ml of the aliquot of each sample.

The plates were swirled to mix them thoroughly and left to solidify. The plates were incubated at 37°C for 24 hours.

The cultures that developed were counted and subcultured to

obtain pure cultures which were stored on agar slants [12].

### 2.6. Identification of Isolates

Pure cultures of bacterial isolates were identified using cultural, morphological and biochemical characterization. Colonies identifiable as discrete on nutrient agar were carefully examined macroscopically for cultural characteristics such as the form, shape, colour and size. Gram staining and biochemical test was further carried out. For morphological characteristics, a small portion of the discrete colonies on each plate was smeared on a microscope slide with a drop of distilled water added. The smear was gently fixed by heat and immersion oil was dropped on the surfaces and then viewed under x 100 objective lens of the microscope [12].

### 2.7. Biochemical Analysis

The following biochemical test was carried out; Catalase test, Coagulase test, Indole test, Oxidase test, Citrate utilization test, Methylred test, Voges proskauer test and Sugar fermentation tests.

## 3. Results

The biochemical characteristics of the bacterial isolates are shown in Table 1, the isolates are *Staphylococcus epidermidis*, *Corynebacterium spp*, *Klebsiella spp*, *Pseudomonas spp*, *Escherichia coli*, and *Proteus spp*. Figure 1 shows the total count of bacterial Isolates from various samples. The morphological characteristics of bacterial isolates from the zobo samples are shown in Table 2. These morphological characteristics can be known by looking out for the form, elevation, texture, margin and pigmentation of the colonies seen on the plate. This aids in the identification and characterization of the colonies after which gram stain and biochemical tests are carried out to further identify the organisms.

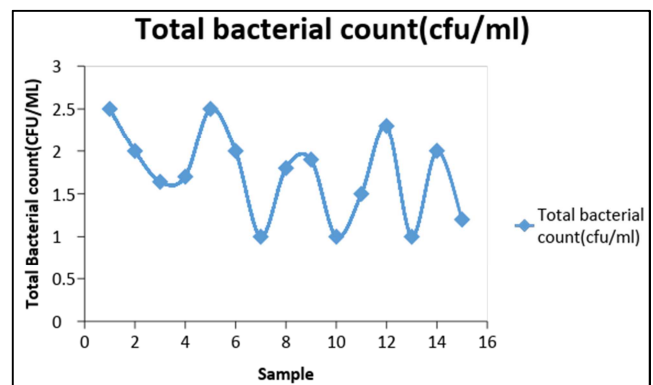


Figure 1. Total Bacterial count of the isolates.

**Table 1.** Biochemical characteristics of bacteria isolates.

| Iso | G.S | Form  | Cat. | Coa. | Ind. | M.R | V.P | Mot. | Cit. | Oxi. | G | S | L | M | Probable Org                       |
|-----|-----|-------|------|------|------|-----|-----|------|------|------|---|---|---|---|------------------------------------|
| 1   | +   | cocci | +    | -    | ND   | ND  | ND  | -    | -    | -    | + | + | + | + | <i>Staphylococcus. epidermidis</i> |
| 2   | +   | rod   | +    | +    | ND   | ND  | ND  | -    | +    | -    | A | A | A | A | <i>Corynebacteria.spp</i>          |
| 3   | -   | rod   | +    | -    | -    | -   | +   | -    | +    | -    | + | + | + | + | <i>Klebsiella spp</i>              |
| 4   | -   | rod   | +    | -    | -    | -   | -   | +    | +    | +    | - | - | - | - | <i>Pseudomonas spp</i>             |
| 5   | -   | rod.  | +    | -    | +    | +   | -   | +    | -    | -    | + | + | + | + | <i>Escherichia. Coli</i>           |
| 6   | -   | rod   | +    | -    | -    | +   | -   | +    | +    | -    | + | - | - | - | <i>Proteus spp</i>                 |

Iso: Isolate, G.S: Gram Stain, Cat: Catalase, Coa: Coagulase, Ind: Indole, M.R: Methylred,

V.P: Vogesproskauer, Mot: Motility, Cit: Citrate, Oxi: Oxidase, G: Glucose, S: Sucrose,

L: Lactose, M: Maltose, Probable Org.: Probable organism, ND: Not determined,

+: Positive, -: Negative, A: Acid production, S. epidermidis: *Staphylococcus epidermidis*, Cory. Spp: *Corynebacterium spp*, Pseudo spp.: *Pseudomonas spp*, E. coli: *Escherichia coli*

**Table 2.** The morphological characteristics of the bacterial isolates.

| Organism                          | Morphology Characteristic  |
|-----------------------------------|--|
| <i>Staphylococcus epidermidis</i> | Round, raised, smooth, shiny, with gray-white colour               |
| <i>Corynebacterium spp.</i>       | Round, convex, dull, with grayish colour                           |
| <i>Klebsiella spp.</i>            | Round, raised, shiny, with pink colour                             |
| <i>Pseudomonas spp.</i>           | Mucoid, umbonate elevation with a diffusible green pigment         |
| <i>Escherichia coli</i>           | Very large, flat and milky colony, with a smooth edge              |
| <i>Proteus spp.</i>               | Large colourless colony with a swarming motion on the agar Surface |

## 4. Discussion

The bacterial species isolated from zobo samples include species of *Staphylococcus*, *Corynebacterium*, *Klebsiella*, *Pseudomonas*, *Escherichia*, and *Proteus* with the plate count ranging from  $1.00 \times 10^2$  cfu/ml to  $2.50 \times 10^2$  cfu/ml and the highest bacterial load seen in samples 1, 5, and 12 and the least in samples 7, 10, 13, and 15. Most strains of these isolates are toxic and their detection in zobo drink poses a serious health hazard to the consumers. These microorganisms have been implicated in outbreak of dysentery, diarrhoea and other gastrointestinal diseases. However among the fifteen samples analysed, samples 1, 5, and 12 (which were purchased from Eke-Awka timber shed, Unizik girl's hostel and Government house respectively) has the highest bacterial load which might be as a result of inadequate ascetic condition during their preparation and processing.

*Escherichia coli* that was isolated from the zobo drink is an indication of a feacally contaminated drink that must have been from the water (feacally contaminated) during the processing of the zobo drink. This is because most vendors are admitted to using water to dilute zobo syrup after boiling and this is a possible source of bacterial contamination to the already boiled zobo. *Staphylococcus specie* in zobo drink could possibly be through the processing methods which usually involve the use of hands since the organism is a common flora of the skin.

Besides, other sources of contamination might be the

packaging materials or containers which are not properly washed and sterilized. This work corresponds to the work of previous author [13] who reported that hawked zobo drink harboured bacteria (*E. coli*). [14] suggested that the presence of microorganisms in zobo drinks produced with a boiling method is indicative of post-production contamination resulting from the addition of water, sugars, preservatives and other additives like pineapple flavouring. This work also corresponds to the work of previous authors [15, 16], who reported that zobo drink harboured *Proteus spp* and *Escherichia coli*, which indicates the presence of contaminants from water and humans.

## 5. Conclusion

The various bacteria genera isolated and identified from the samples includes; *Staphylococcus*, *Corynebacterium*, *Klebsiella*, *Pseudomonas*, *Escherichia* and *Proteus*. The result revealed that the samples of zobo drink purchased from fifteen different locations in Awka metropolis were directly and indirectly contaminated with high levels of pathogenic bacteria. However the occurrence of these pathogens can essentially be reduced or prevented by employing the good manufacturing practices (GMP).

From this research, the issue of food safety is of paramount importance in developing countries especially in Nigeria. Food borne illness is really preventable by good hygiene and standard food handling techniques. Thus to safe guard against the risk of food borne illness, there is need to educate the producers on good manufacturing practices (GMP).

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