



## Potential Spread of Pathogens by Consumption of Locally Produced Zobo and Soya Milk Drinks in Awka Metropolis, Nigeria

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### Authors' contributions

This work was carried out in collaboration between all authors. Author CE designed the study. Author EO wrote the protocol. Author LA wrote the first draft of the manuscript, authors EO and LA managed literature searches. Authors MI, MU, LA and AO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** To determine the presence of resistant pathogens in locally produced drinks consumed in Akwa, Nigeria.

**Study Design:** To determine type of bacteria contaminants, level of contamination from and presence of resistant pathogens in the drinks.

**Place and Duration of Study:** The study was conducted in the department of Pharmaceutical microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, between May 2012 and June

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**Methodology:** 10 each of locally prepared Zobo and Soya drinks were purchased in Akwa city and analysed using standard microbiological procedures.

**Results:** The microbial quality of the Zobo drinks is good but its safety is questionable as it contains indicator organisms like *Escherichia coli* (10%), *Bacillus cereus* (80%), *Staphylococcus aureus* (50%), *Corynebacterium* spp. (10%) and *Clostridium* spp. (10%). 40 % of the soya milk drink had an unsatisfactory microbial quality and 50 % are unsuitable for consumption as they contain *E. coli* and *S. aureus*. The antibiogram showed that most strains of the isolated organisms were susceptible to all the antibiotics with each organism having almost 100 % susceptibility. However, *Clostridium* spp. were completely resistant to amoxicillin and *Corynebacterium* spp. were completely resistant to Rifampicin. Also one strain of *E. coli* was multi-drug resistant.

**Conclusion:** The bacterial spectrum isolated from the locally prepared drinks is indicative of exogenous contamination which could cause food poisoning and lead to the spread of bacteria resistance.

**Keywords:** Microbial contamination; resistance; ready to eat food drinks; *E. coli*; *Staph. aureus*.

## 1. INTRODUCTION

Local drinks consumed in Nigeria include burukutu (sorghum beer), kunnu zaki (millet food drink), palm wine, zobo (extract of calyx of *Hibiscus sabdariffa*) and soybean drinks. These local drinks are either alcoholic or non-alcoholic beverages. The diets of people in many developing countries like Nigeria comprise mainly of starchy roots, cereals and few legumes. They lack the capability of purchasing food rich in animal proteins like meat and thus they depend on drinks from legumes like soya milk to provide themselves with proteins. This increase in this demand has led to corresponding increase in the production and supply of these drinks [1].

Zobo, burukutu and soya milk are consumed across all the ethnic groups in Nigeria. Apart from their thirst quenching ability and low cost, such drinks have documented nutritional and medicinal values [1]. Zobo and soya milk drinks are non-alcoholic local beverages that usually contain no preservative and are stored in refrigerator or freezer for as long as possible. Zobo, also known by various names in different locality [2], is a red traditional drink consumed mostly in northern part of Nigeria, basically an aqueous extract from the dried reddish purple calyxes of the plant *Hibiscus sabdariffa*. It tastes like fruit punch and serves as a source of vitamin A, Niacin, Calcium, Iron and antioxidants [3,4,5]. Other additives like ginger, sugar, honey, fruits and artificial flavours, may be added to boost its nutritional value. Its sweetness may diminish with time due to microbial spoilage when it is left unrefrigerated. In addition to its use as beverage,

various parts of the plant have different medicinal properties [6]. A recent review stated that specific extracts of *H. sabdariffa* exhibit activities against atherosclerosis, liver disease, cancer, diabetes and other metabolic syndromes [7]. The plant also has in vitro antimicrobial activity against *E. coli* [8].

Soya milk (or soy bean milk) is the water extract of grounded soybeans (*Glycine max*) or just dry grounded, roasted soy beans, which is also used to wean infants [9]. The liquid drink is a stable white or creamy emulsion of water, oil, proteins and little carbohydrate. The drink has numerous health benefits including no lactose, lower fat, carbohydrate, calcium and phosphorus, more iron, similar protein; as cow milk, and dietary fiber [10]. The medicinal properties of soy bean milk include lowering of serum cholesterol and low density lipoproteins [11], thus reducing the risk of heart disease.

Some local beverage drinks are often prepared in poor sanitary conditions usually resulting in their contamination [6]. This raising public concerns as the unsuspecting community including our school children consume such contaminated beverages. The resulting food poisoning may over shadow the health benefits of consuming such locally prepared beverages. Even though the food poisoning does not occur, the contaminating microbes can harbor resistant genes that they can transfer to opportunistic pathogens. Thus this study determined the microbiological quality of zobo and soya milk drinks sold around Awka metropolis of Anambra State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study area is Awka town, the capital of Anambra State located in the South-East region of Nigeria.

### 2.2 Test Samples

Ten samples each of Zobo and Soya milk unbranded locally produced drinks were obtained from around Awka metropolis at five different strategic locations, two samples per location. These locations were selected because they were public places where foods are sold to the unsuspecting community. The drinks were all freshly prepared. Oral consent were gotten from the sellers of these drinks after we had explained what we intended to use the drinks for. The samples were labeled properly and stored in the fridge in its original container. The five different locations were: 1) School of Pharmacy canteen, Agulu; 2) Nwagu market, Agulu; 3) Eke Awka market; 4) Igwebuike Grammar School, Awka and 5) Nnamdi Azikiwe University (NAU), Awka for the Zobo samples; and 1) St. Paul's University Nise campus; 2) Nwagu market, Agulu; 3) Eke Awka market; 4) Igwebuike Grammar School, Awka and 5) Nnamdi Azikiwe University, Awka for the Soya milk samples.

### 2.3 Enumeration of Bacteria in the Test Samples

This was carried out using the single agar layer plate count technique [12]. Briefly, 0.1 ml of  $10^{-1}$  to  $10^{-5}$  dilutions of the original drink samples were aseptically transferred using sterile syringes into sterile Petri dishes. An aliquot of 15 ml of molten Nutrient agar cooled to 45-50°C was poured aseptically into the plates containing the samples swirled and allowed to solidify. Petri dishes were inverted and incubated at 37°C for 24 hours. Petri dishes showing discrete colonies were counted after 24 hours with a colony counter.

### 2.4 Isolation and Characterization of Bacteria in the Test Samples

Bacterial colonies were identified by colonial morphology, gram reaction and biochemical characterization [13]. Each characterized microbe was transferred aseptically to nutrient agar slants. The agar slants were incubated at

37°C for 24 hours after which they were stored in the refrigerator at a temperature of 4°C. All characterized isolates were standardized to McFarland's 0.5 turbidity standard prior to carrying out any microbiological assay.

### 2.5 Antibiotic Sensitivity Screening of Bacterial Isolates

This was carried out by using the disk diffusion method [14]. Briefly, the Petri dishes were divided into four sections, two of which were labeled according to the isolates (2 plates for each isolate). Aliquot of 20 ml of sterile Mueller-Hinton agar was poured into the plates and allowed to solidify. A sterile swab stick was used to streak the surface of the agar in the plates with the suspension of the isolates. The agar plates were allowed to stand for seven minutes ajar and then a pair of sterile forceps was used to place the separated discs (Optundisc, Optun Laboratories Nigeria Ltd., Enugu, Nigeria) into each of the sections of the plates, one section for one antibiotic disc. Gram-positive antibiotic sensitivity discs containing Ciprofloxacin-10 µg, Norfloxacin-10 µg, Gentamicin-10 µg, Amoxicillin-20 µg, Streptomycin-30 µg, Rifampicin-20 µg, Erythromycin-30 µg, Chloramphenicol-30 µg, Ampiclox-20 µg, and Levofloxacin-20 µg; and Gram negative antibiotic sensitivity discs containing Amoxicillin/clavulanate-30 µg, Ampicillin-30 µg, Trimethoprim/Sulphamethoxazole-30 µg, Ofloxacin-10 µg, Pefloxacin-10 µg, Cefalexin-10 µg, Gentamicin-10 µg and Nalidixic acid-30 µg were used here. This process was repeated for all the isolates. All the plates were allowed to pre-diffuse for about 15 minutes. Thereafter the plates were incubated at 37°C for 18 hours and the resultant inhibition zone diameters were measured and recorded.

## 3. RESULTS AND DISCUSSION

### 3.1 Bioburden in Test Samples

The mean aerobic plate counts (APC) of Zobo drinks sold at different locations in Awka metropolis is shown in Table 1. APC, also called aerobic colony count, underscores the microbial quality of ready to eat food. It is not an indicator of the safety of any ready to eat food or drink. One of the samples from Igwebike Grammar School, Awka, had the highest bacteria count ( $1.0 \times 10^5$  cfu/ml) while the second sample from School of Pharmacy, Agulu, had the lowest bacteria count of ( $3 \times 10^2$  cfu/ml).

All the samples of the zobo showed a fair level of contamination but this is within the limit ( $10^6$ ) permissible for liquids ready for consumption [15], based on the APC. The zobo drinks usually undergo some processing like sieving and mixing, after it has been boiled for about 10 minutes during preparation. The location had no impact on the suitability of the Zobo drink for consumption, but generally the contamination of the Zobo drinks from the School of Pharmacy, Agulu was lower and may be attributed to their level of awareness on the sources of contamination via their interaction with students and staff. Contamination of these drinks may be due to the source water used for processing and improper handling during processing [16,17]. According to Adesokan et al. [18], the total viable count increases on storage in the refrigerator but for freshly prepared zobo drink, ie within 24 hrs, the TVC was similar to those in this study.

The mean APC of soya bean milk drinks sold in Awka metropolis is also shown in Table 1. One of the soya milk from Nwagu Market had the highest bacteria load of  $2.2 \times 10^5$  cfu/ml while the first sample from Igwebike Grammer School had the least count of  $4 \times 10^3$  cfu/ml.

**Table 1. Aerobic plate count (APC) of Zobo and soya Milk drinks**

Location	Zobo	Soya
Nwagu	$7 \times 10^4$	$2.2 \times 10^5$
	$1 \times 10^4$	$1.9 \times 10^5$
School of Pharmacy	$5 \times 10^3$	-
	$3 \times 10^2$	-
NAU	$2 \times 10^3$	$7 \times 10^4$
	$3 \times 10^4$	$9 \times 10^2$
Igwebike	$1 \times 10^5$	$2 \times 10^3$
	$2 \times 10^4$	$4 \times 10^4$
Eke Awka	$6 \times 10^4$	$4 \times 10^3$
	$8 \times 10^4$	$1 \times 10^4$
St. Paul's Uni.	-	$2 \times 10^4$
	-	$1 \times 10^4$

-; the respective samples were not collected at these locations, APC limit for zobo drink is  $10^6$  cfu/ml [15], while that for soya milk drink is APC limit is  $2.0 \times 10^4$  cfu/ml [19]

The APC of about half of the soya milk drink samples were above the acceptable limit of  $2.0 \times 10^4$  cfu/g recommended for general bacterial count by the Soy Foods Association of America [19] except for the samples from Eke Awka Market, one of the samples from St. Paul's University, Igwebike Grammer School and NAU, Awka. 40 % of the Soya Milk drinks are thus unacceptable for consumption. The soya

milk samples were generally slightly more contaminated than the zobo samples because Zobo is acidic in nature [20, 21] and hence under normal conditions it will not favour the proliferation of most bacteria. Soya milk drinks have a pH of around 7 [10] and this pH favours bacteria growth. However, pH alone is not a sufficient parameter to predict the survival and proliferation of bacteria in zobo drinks, the antimicrobial activity of *Hibiscus sabdriffa* may also provide a preservative effect [8]. Moreso, soya milk is boiled for 10-15 minutes at  $100^\circ\text{C}$  prior to addition of sweeteners and packaging. Accidental contamination can also arise from the use of contaminated sweeteners and flavouring agents prior to packaging. Addition of these sweeteners and flavours before filtration can reduce the sweetening and flavouring profile that a manufacturer intends to achieve.

### 3.2 Prevalence of Isolates in the Zobo and Soya Milk Drinks

A total of 8 different bacteria within seven genera were isolated from all the zobo or soya bean milk test samples. The different isolates and their prevalence is shown in Table 2. Prevalence being the number of times they occur in the different test samples expressed as a percentage.

**Table 2. Prevalence of potential bacterial pathogens or their indicators isolated in Zobo and soya milk**

Bacterial pathogens	Zobo (%)	Soya milk (%)
<i>Bacillus cereus</i>	80	30
<i>Bacillus subtilis</i>	40	30
<i>Staphylococcus aureus</i>	50	50
<i>Corynebacterium</i> spp.	10	0
<i>Clostridium</i> spp.	10	0
<i>Escherichia coli</i>	10	50
<i>Lactobacillus</i> spp.	0	60
<i>Streptococcus mitis</i>	0	20

*Bacillus cereus* was encountered most frequently in the zobo drinks with a percentage occurrence of 80% while *Escherichia coli*, *Corynebacterium* spp. and *Clostridium* spp. were the least occurring organism with a prevalence of 10%. The presence of *B. cereus* in the Zobo drinks is indicative of contamination from the environment during preparation. It causes *B. cereus* food poisoning which is mainly manifest as diarrhea type illness, emesis type illness, which can lead to liver failure or even death, due to the presence of its pH and heat-stable toxins in the

contaminated food drink. However, greater than  $10^4$  of the organism/g are associated with human infection [22]. All the other organisms are associated with food poisoning except *B. subtilis* and are thus all indicator organisms. *B. subtilis*

Table 2 also shows the percentage occurrence of bacteria isolated from soya milk drinks. *Staph. aureus*, *E. coli* and *B. cereus* are indicator organisms in accessing the safety of ready to eat foods. Soy Food Association of America (SFAA) suggests that *Staph. aureus* and *E. coli* should be absent otherwise such soya milk are unsuitable for consumption [19]. The presence of *Staph. aureus* indicates poor hygiene during production of this drink as it is a normal flora of the skin, while the presence of *E. coli* indicates fecal contamination of the source water. It has been suggested by Adesokan et al. [18] that proper hygiene and use of potable water in the production of locally produced drinks will improve the microbial quality of such product. But this is hardly achievable in Nigeria as people resident in rural areas and some resident in urban areas still make use of well water or the popular 'tanker water supply' as a source of water for cooking and cleaning. More than 10 years ago and recently, it was discovered in Nigeria that *E. coli* and *Staph. aureus* were commonly isolated in both branded and unbranded soya milk drinks and the necessary authorities were called to action [24,25]. Since then there has been an improvement as only 10% and 50% of the samples were contaminated with *E. coli* and *Staph. aureus* respectively as compared to 100%. But more still needs to be done.

*Lactobacillus* spp. is the predominant organism found with a percentage occurrence of 60% while *Streptococcus mitis* is the least occurring with a prevalence of 20%. The presence of *Lactobacillus* spp. in most of the samples indicates that the milk will probably undergo fermentation in a favorable condition, and probably could have. Such fermentation will lead to the production of Soya Milk Yogurt. Species participated in the fermentation include *Lactobacillus bulgaricus*, and are probiotics with health benefits [26]. Some other species are associated with dental caries and tooth decay.

The detection of *S. aureus* in half (50 %) of the zobo and soymilk samples poses a serious health hazard to the consumers. Pathogenic *Staph. aureus* is known to possess heat-stable enterotoxin [27], the production of which increases as their population increases in

has been discovered in some other studies [23]. *E. coli* occurred in such low frequency in Zobo than in Soya Milk drink (Table 2), probably because the *Hibiscus sabdariffa* plant has antimicrobial activity against this organism [10], whatever favorable medium [28]. Emphasis has been made on the increasing incidence of Methicillin Resistant *Staph. aureus* (MRSA) [29,30]. It is worthy of note that *Staph. aureus* was not detected in any of the samples purchased from NAU, Awka.

*Streptococcus mitis*, though isolated in only 20 % of the soya milk, is an  $\alpha$ -hemolytic specie of streptococcus that can cause scarlet fever-like pharyngitis when ingested orally in large quantities. Severe complications arising from such infection include; tonsillitis and bacteremia [31].

### 3.3 Percentage of Susceptibility Profile of Isolated Organisms

Table 3 shows the Percentage susceptibility to antibiotics of Gram-positive bacteria isolates to different antibiotics in a Gram-positive antibiotic disc. Ciprofloxacin, Levofloxacin and erythromycin recorded 100% susceptibility and would be ideal for empirical therapy of food poisoning or complication of such arising from consumption of these contaminated zobo or soya milk.

One strain of *Staph. aureus* isolated from soya milk drink purchased from Nwagu market was resistant to at least one antibiotics. The percentage susceptibility of the *E. coli* isolate in Zobo and Soya Milk drinks is also shown in Table 3. *E. coli* had a 100 % susceptibility to Ofloxacin, Pefloxacin, Ciprofloxacin and Augmentin. The only isolate of *E. coli* originated from the Zobo drink was multi-drug resistant, which was purchased from NAU, Awka. 50 % of the *E. coli* strains were resistant to at least one antibiotic, similar to a previous study [32], with resistance to mainly older drugs; streptomycin, ampicillin, nalidixic acid and cotrimoxazole.

The resistance profile of some of the isolated organisms using Clinical and Laboratories Standards [33] is shown in Table 4. Other organisms had no criteria, e.g *Bacillus* spp., or the amount of antibiotics in the antibiotic disc was higher than what is recommended by CLSI. *E. coli* has a 100% resistance to Nalidixic acid, one of the first quinolones to be used in clinical practice.

**Table 3. Antibiotic susceptibility pattern of bacterial isolates (percentage susceptibility\*)**

Antibiotic	Bacterial isolates*							
	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>L. spp.</i>	<i>Strep. mitis</i>	<i>C. spp.</i>	<i>Cl. spp.</i>
CIP	100	100	100	100	100	100	100	100
NOR	91.7	90	-	85.7	100	100	100	100
GEN	100	90	100	100	100	50	100	100
AMX	91.7	100	-	85.7	83.3	100	100	0
STR	91.7	80	83.3	85.7	100	100	100	100
RIF	91.7	90	-	100	100	50	0	100
ERY	91.7	100	-	100	100	100	100	100
CHL	100	90	-	100	100	100	100	100
AMP/CLOX	100	80	-	100	100	50	100	100
LVX	100	100	-	100	100	100	100	100
OFX	-	-	100	-	-	-	-	-
PEF	-	-	100	-	-	-	-	-
AMC	-	-	100	-	-	-	-	-
LEX	-	-	66.6	-	-	-	-	-
NAL	-	-	33.3	-	-	-	-	-
SXT	-	-	66.6	-	-	-	-	-
AMP	-	-	83.3	-	-	-	-	-

\*a= using 1 mm IZD for susceptibility, -; various antibiotics were not used for evaluating the susceptibility of the respective microorganisms. Abbreviations: *B.*: *Bacillus*; *S.*: *Staphylococcus*; *E.*: *Escherichia*; *L.*: *Lactobacillus*; *Strep.*: *Streptococcus*; *C.*: *Corynebacterium*; *Cl.*: *Clostridium*.

**Table 4. Resistance pattern of some of the isolates using CLSI standards**

Organism	Antibiotic (break point IZD in mm)	Percentage resistance (%)
<i>E. coli</i>	Augmentin ( $\leq 13$ )	66.67
	Gentamicin ( $\leq 12$ )	50.00
	Cotrimoxazole ( $\leq 10$ )	66.67
	Nalidixic acid ( $\leq 13$ )	100.00
<i>Staph. aureus</i>	Gentamicin ( $\leq 12$ )	50.00
	Norfloxacin ( $\leq 12$ )	60.00
	Chloramphenicol ( $\leq 12$ )	30.00
<i>Strep. mitis</i>	Chloramphenicol ( $\leq 17$ )	50.00

#### 4. CONCLUSION

The prevention of the spread of disease through the consumption of commercially sold locally prepared food and drinks has been a lost battle by the National Agency for Food and Drug Administration (NAFDAC), the FDA of Nigeria. The microbial quality of Zobo drinks sold in Awka metropolis is satisfactory but its safety cannot be assured due to the isolation of highly pathogenic organisms like *Bacillus cereus*, *Escherichia coli*, *Corynebacterium* spp. and *Clostridium* spp. 40 % of the soya milk drink had poor microbiological quality and some contained indicator organisms like *Staph. aureus*, *Bacillus cereus* and *Escherichia coli*; all which present potential health hazard to consumers. *Lactobacillus* spp. were isolated from the soya milk drink, although it was not fermented. *Staph. aureus*, *Strep. mitis* and *Escherichia coli* were highly resistant to some of the antibiotics commonly used in the treatment of infections caused by oral consumption of large quantities of these

pathogenic organisms. These contaminated unsafe drinks can serve as a source of spread of pathogenic bacteria and bacteria resistance, and this could present a public health crisis.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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