



Bacteriology Quality of Zobo Drinks Consumed in Some Parts of Osun State, Nigeria

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ABSTRACT: The bacteriology of Zobo drink prepared and sold at nine market; Alamisi market, Ikirun; Oluode market, Osogbo; Oja obi market, Ila-Orangun, Ede; Sabo market, Ile-ife; Ilesha market, Owena market; Timi market; Orile owu market, Ikire market in Osun state Nigeria were assessed. Zobo drinks were purchased from these markets on twelve different occasions for a period of six month. The samples were bacteriologically analyzed using standard methods. All the screened drink samples had varying levels of bacterial contamination ranging from 1.2×10^2 to 1.2×10^6 CFU/ml for a total viable count. 58.3% of the total sampled drinks (n=108) i.e (63/108) had bacterial count above the acceptable limit $< 10^4$ CFU/ml. While all the drinks sampled had total coliform count exceeding the recommended safe level of zero organisms detectable per 100ml. seven bacteria species were isolated from the zobo drinks sampled. The bacteria isolated were *E coli*, *Bacillus species*, *S aureus*, *S faecalis*, *proteus species*, *Enterobacter species*, and *Klebsiella species*. *E coli*, *Bacillus species*, *Enterobacter species* and *S aureus* were isolated from all the samples. The present finding revealed that zobo drinks retailed and sold in these markets are potential vehicles for transmitting food borne illness, thus the need to develop Good Manufacturing Process (GMP) and Post Production Preservation and Packaging technique. © JASEM

Zobo drinks are traditional non- alcoholic beverage which is consumed in most part of Nigeria, mostly in northern part of Nigeria (Osuntogun, 2004). The economic and religious situation in Nigeria has made the zobo drink gain wide acceptance in different occasions. It's used as refreshment, entertainment in parties or as appetizers before the main dish is served and it is also sold in market to various consumers (Onuorah *et al* 1987). The zobo drink is a red liquid drink and taste like fruit punch, served as a fair source of vitamin A, riboflavin, niacin, calcium and iron (Qi *et al* 2005), and is low in sugar content. This drink also contains anthocyanins and Vitamins C, among others and it is used in curing minor stomach ailments, sore throat and strengthening the heart among other uses. www.magdalin.com (2004), Olawale (2011). Zobo drink is extracted from the dried reddish purple calyces of the plant *Hibiscus sabdriffa* (Scott, 2003). The calyces are used to produce herbal teas and other food products Akanya *et al* (1997).The juice drink which is usually obtained by extraction of the calyx of Hibiscus contains about 1% solid. The drink contains some microorganisms which can cause food spoilage Omemu *et al* (2006). At present, the production processes in neither mechanized nor standardized. Consequently, the shelf life of the drink is less than two days Samy (1980). Furthermore, the mode of packaging or dispensing of the juice in nylon or

plastic container before retailing, that is taken as Zobo i.e the largely unregulated nature of the trade, and poor hygienic practices as well as lack of running water, toilet, proper storage and waste disposal facilities at preparation and services point has resulted in poor unsanitary conditions exposure to potential contaminants and an increased risk to public health (Omemu and Aderoju 2008). Consequently, street drinks and foods safety has remained a major public health concern globally, and more importantly in Nigeria were the regulation of this critical sector is virtually non-existent or inadequate, making street foods and drinks hazardous source of nutrition (Oyeyi and Lum-nwi,2008; Wada kura *et al* 2009). Foods frequently serve as vehicle for spreading of several organisms some of which are pathogenic. Singleton (1999).Many picnic suppers and banquets have come to a disastrous end which home prepared foods and drinks serves not only as food and drinks for guest, but also as the vehicle for transmitting staphylococcus food poisoning. Alabi and Akinsinyun (1996). In view of the facts, that Zobo is never subjected to any form of post production treatment that can eliminate or at least reduce the bacteria load in the drink, it could be a potential source of health hazard. Also the activities involved in the cooling and subsequent dispensing of the drink into containers also represent potential source of health hazard. Some researchers Cruck and Shank

(1984) have reported that some gastro intestinal illness characterized by diarrhea, abdominal cramps, and vomiting which may be assumed as been of unknown aetiology may arise from drinking drinks contaminated with microorganisms. In this study, retailed Zobo drinks were purchased from various locations within Osun State, they were analyzed for their bacteriological quality as indicated by the number (counts) and kinds of bacteria they harbor

MATERIALS AND METHODS

Study location: Osun state is located in the south western part of Nigeria. It covers an area of approximately 14,875 square kilometers lies between longitude 04 00E and latitude 05 55S and its total population is approximately 3,416,959.

Sampling: The state was divided into three zones using the senatorial district: CENTRAL, WEST, AND EAST to ensure adequate representative sampling. Following this procedure, nine major towns were chosen for sample collection and analysis in Osun State Nigeria. Three towns from each senatorial (Osun central senatorial district: Alamisi market Ikirun, Olu ode market Osogbo, Oja Obi market Ila Orangun. Osun East senatorial district: Sabo market Ile Ife, Ilesa market, Owena market. Osun West senatorial district: Timi market, Orile owu market, Ikire market). All samples were collected in sterile containers held at 4°C and analyzed within three hours, a total of 108 zobo drinks, 12 zobo drinks samples from each market and 36 samples per senatorial district were analysed. The study lasted April 2012 to September 2012.

Samples Analysis: 1ml of zobo drink were measured into 9ml of sterile 0.1% peptone water as diluents to make a 1:10 dilution, further ten fold serial dilution were made and examined by means of the pour plate method (yeboah manuu *et al* 2010). The plates were marked for easy identification and 1ml of the dilution used for the inoculation and incubation.

Bacterial Enumeration and Isolation: Total bacterial count was determined by pour plate

techniques using standard methods. Nutrient agar medium was used for the enumeration of bacteria in the samples. The total bacteria count was obtained by incubation aerobically at 37°C for 24 hours. Total coliform count was determined by MPN index method using 3-3-3 regimen. MacConkey broth was used and positive result was associated with acid and gas production on incubation at 37°C for 48 hours. (fawole and Oso 2001). Morphological features and biochemical reaction patterns were used for the identification of bacterial isolates.

RESULTS AND DISCUSSION

A total of 108 zobo drink samples were examined. The result shows that the pH of the samples range from 2.9-4.3 (Table 1). The mean pH value was 3.04. All the zobo drinks sampled were contaminated with varying levels of bacterial count ranging from 1.2×10^2 to 1.2×10^6 CFU/ml. 58.3% (63/108) of the screened zobo drink samples have total bacteria counts of $> 10^4$ CFU/ml and were classified as unsatisfactory while 41.7% were classified as satisfactory and had total bacteria counts of $< 10^4$ CFU/ml.

The total coliform count in the zobo drink sampled ranged from 3 MPN/ml to 60 MPN/ml 108/108 i.e 100% of zobo drink samples examined had coliform count which is well above the zero value recommended for safe water by Saskatchewan drinking water quality standard and health Canada's Guidelines for Canadian drinking water quality state that the maximum acceptable concentration (MAC) for coliform in drinking water is zero organisms detectable

Per 100ml (<http://www.health.gov.sk.ca/lab>). Seven bacteria genera were isolated from the zobo drink samples. This includes *Escherichia coli*, *Bacillus species*, *Staphylococcus aureus*, *Enterobacter species*, *Proteus species*, *Klebsiella species* and *Streptococcus faecalis*. *Escherichia coli*, *Streptococcus faecalis* were found in 58.3% (63/108) of samples examined

Table 1 pH values for zobo drinks

SAMPLE AREA	pH VALUES											
Alamisi market	3.0	3.2	3.3	3.3	3.1	2.9	3.1	3.1	3.3	3.0	2.9	3.0
Olu ode market	3.3	3.2	4.3	3.2	3.1	3.0	3.1	3.0	3.3	3.2	3.1	3.2
Oja Obi market	3.0	3.1	3.3	3.3	3.2	3.1	3.1	3.0	3.2	3.1	3.1	3.1
Sabo market ile-ife	3.0	3.1	3.1	3.1	3.3	2.9	3.2	3.1	3.0	3.1	3.1	3.0
Ilesha market	3.0	2.9	2.9	3.2	3.0	3.1	3.1	3.2	3.1	3.1	3.0	3.1
Owena market	3.2	3.0	2.9	3.0	3.1	2.9	3.0	3.2	3.1	3.2	3.1	3.0
Timi market	3.0	3.0	3.1	3.2	3.0	3.2	3.0	3.0	2.9	3.1	3.1	3.0
Orile owu market	3.1	3.2	3.1	3.0	3.0	2.9	3.0	2.9	3.1	3.0	3.0	3.0
Ikire market	2.9	2.9	3.0	3.1	3.1	3.1	3.2	3.3	3.2	3.0	2.9	3.0

Table 2 Bacterial count (CFU/ml) of zobo drink samples

Alamisi market	2.1x10 ⁴	4.0x10 ⁴	2.2x10 ³	3.5x10 ²	1.0x10 ⁴	2.1x10 ⁴	2.2x10 ²	2.1x10 ⁴	2.3x10 ²	1.2x10 ⁴	1.1x10 ⁴	1.1x10 ⁵
Olu ode market	1.0x10 ⁴	1.3x10 ⁴	1.1x10 ³	1.6x10 ⁵	3.5x10 ²	2.2x10 ⁴	1.3x10 ⁵	2.5x10 ²	1.8x10 ⁴	1.5x10 ⁴	2.2x10 ⁵	2.1x10 ²
Oja Obi market	2.6x10 ⁴	1.5x10 ³	1.3x10 ⁴	3.1x10 ³	1.0x10 ⁵	2.7x10 ⁴	2.5x10 ³	1.2x10 ³	2.7x10 ⁴	2.4x10 ⁵	5.0x10 ³	2.6x10 ⁴
Sabo market ile-ife	2.1x10 ⁴	1.2x10 ³	1.8x10 ⁴	1.0x10 ⁴	3.1x10 ⁴	2.7x10 ³	4.1x10 ³	1.4x10 ²	5.1x10 ²	2.5x10 ³	2.1x10 ⁵	4.1x10 ³
Ilesha market	2.0x10 ⁵	2.1x10 ⁴	1.0x10 ²	2.5x10 ³	2.1x10 ⁴	3.6x10 ⁴	5.1x10 ³	1.2x10 ⁴	5.1x10 ³	2.0x10 ⁴	1.2x10 ⁴	1.8x10 ³
Owena market	1.0x10 ⁴	1.7x10 ³	3.1x10 ⁴	1.2x10 ⁴	1.2x10 ⁶	3.2x10 ²	4.2x10 ³	2.0x10 ⁵	3.2x10 ⁴	1.2x10 ²	1.0x10 ⁴	1.3x10 ⁴
Timi market	1.3x10 ⁴	3.0x10 ⁴	1.0x10 ⁴	5.1x10 ⁵	3.1x10 ³	4.2x10 ²	2.3x10 ³	1.2x10 ²	1.1x10 ⁶	3.6x10 ⁴	5.6x10 ³	2.1x10 ⁴
Orile owu market	1.2x10 ⁴	1.6x10 ⁴	3.5x10 ³	3.5x10 ²	2.1x10 ⁴	1.6x10 ³	1.0x10 ⁴	1.6x10 ³	2.1x10 ³	2.9x10 ⁴	3.0x10 ²	3.1x10 ⁴
Ikire market	3.0x10 ⁴	2.0x10 ⁴	1.0x10 ⁴	2.5x10 ²	2.1x10 ³	3.2x10 ⁴	1.6x10 ⁴	3.1x10 ³	1.1x10 ⁵	2.7x10 ²	1.7x10 ⁴	2.6x10 ³

Table 3 Total coliform count (MPN) of zobo drinks samples

Alamisi market	10	41	21	7	9	11	21	3	3	4	21	10
Olu ode market	9	31	10	17	57	21	43	43	20	7	3	11
Oja Obi market	10	12	8	41	8	13	43	41	8	9	20	9
Sabo market ile-ife	7	30	3	50	8	25	7	22	41	5	7	5
Ilesha market	28	7	9	13	12	19	31	21	60	25	9	21
Owena market	7	20	7	7	31	7	51	9	41	21	7	13
Timi market	2	41	10	7	14	8	4	14	8	6	43	7
Orile owu market	6	41	9	7	22	11	9	32	8	19	20	9
Ikire market	52	15	3	7	7	3	8	22	41	3	2	4

Epidemiology investigation, although continue to be extremely difficult in Nigeria and outbreak of food and drinks borne diseases generally under reported.

Gastro enteritis has remained a major health care problem in Nigeria both in terms of human suffering and economic cost. The isolation of bacterial in all

the zobo drinks samples (n=108) and the unacceptable total bacteria count of $> 10^4$ CFU/ml established in 58.3% (63/108) of screened zobo drink samples implies extreme contamination and potential health risk of these zobo drink samples. The findings corrected with similar earlier (olukoya *et al* 1991: Mensah *et al* 2002: Yeboah – manu *et al*, 2010). The high incidence of bacterial contamination encountered in this study are mainly due to unsanitary and largely unhygienic nature of the drink preparations and areas as food and drinks are good indicator of the state of environment in which they are prepared or served (Ehiri *et al* 2001): Omemu and Aderoju, 2008).

The Presence of bacteria is divers majority of post heating contamination. Similar post treatment contamination of water has been reported. Contamination of zobo drink can occur during cooling of the hot extract, addition of flavours and sweetner, or dispensing of the extract into nylons and bottles. Utensils and water used during the post heating stages can also serves as source of contamination. Water used in processing has been identified as the major source of contamination of locally made drinks (Okeke *et al* 2000). The presence of *Escherichia coli*, *Klebsiella*, *Enterobacter* and *Proteus* in this project indicate faecal contamination, while the presence of *Streptococcus faecalis* is particularly indicate a fairly recent faecal contamination. The isolation of coliform bacteria in all the zobo drinks samples. The samples count exceeding the recommended limit of zero coliform/ml makes these drinks hazardous for human consumption. Coliform bacteria chiefly faecal coliform is *Enterobacter* whose natural habitat is the intestinal tract of human and animals (pelczar *et al* 2005). They are faecal indicators, and their isolation in zobo drinks indicates the presence of faecal or sewage contaminants introduced into the food via the use of contaminated water or contamination from the unsanitary environment and equipment or via human handler or operators (Pelczar *et al* 2005). The isolated enteric bacteria are known pathogens responsible for millions of cases of infection gastrointestinal diseases and death each year. The seven genera of microorganism encountered in this study correlated with earlier reports (Chumber *et al* 2007; Yeboah-Manu *et al* 2010). Their detection in the zobo drink samples suggest the possibility that other intestinal pathogens like Entero pathogenic strain of *E coli* (0157:H7), *Campylobacter species*, *Listeria*, *Entamoeba histolytica*, *Giardia Lamblia*, etc and enteric viruses like *Hepatitis A virus*, *rotavirus*,

Raimi Olayinka Risiquat

astrovirus etc may also be present in the food samples (James 2005).

The diverse groups of pathogenic microorganism are transmitted by faecal oral route, often by the ingestion of contaminated foods, drinks and water. The isolation of unacceptable level of *S aureus*, an enterotoxins producer responsible for *staphylococcal* food poisoning in zobo drinks. Samples evidently revealed that zobo drinks can be sources of bio toxins that may make food injurious to health on an acute or chronic basis (Achi and Madubuike, 2007). The isolation of *S aureus* in zobo drinks is a pointer to largely poor personal hygienic, improper storage facilities use of low quality raw materials and unhygienic environment.

Conclusion

The bacteria load and coliform count were high, which indicates a high level of contamination. This is very dangerous to the public, as these contaminants has ability to cause varying level of diseases, ranging from food borne illness and food poisoning due to *staphylococcus aureus*. Presence of *E coli* can be responsible for prevalence of diarrhea, fever, nausea, and cramps in children and adult exposed to contaminated drinks (Nweze 2010). Therefore drinks and beverages should be regulated in Nigeria by NAFDAC and other food regulatory bodies, as drinks of low and below minimum safety standard is injurious to health on acute or chronic basis. Consequently education of the manufacturers and provision of basic facilities will greatly improve zobo drink quality and safety. To the effect, regulation, effective monitoring and enforcement of the existing punitive measures is therefore recommended.

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