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SANITARY CONDITION AND BACTERIOLOGICAL QUALITY OF ZOBO DRINK SOLD IN SELECTED AREAS IN MAIDUGURI

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Abstract: *This study was conducted to access the sanitary condition and bacteriological quality of zobo drink sold in selected areas in Maiduguri. Twelve samples of zobo drink were collected from different vendors within Ramat Polytechnic and University of Maiduguri and were analysed. Of these samples, 75% of the zobo drinks showed growth presumed to be pathogenic bacteria. The bacterial isolates are with their occurrence frequencies and percentage shows Escherichia coli having the Highest percentage occurrence frequency of (9/75%) followed by Enterobacter, (8/66.6%), Salmonella, (7/58/3%), Klebsiella and Staphylococcus, (4/33.3%) and Streptococcus, (3/25%) respectively. Antimicrobial susceptibility test results revealed Klebsiella had a high resistance to chloramphenicol 71%, followed by Sulfadiazine and trimethoprim 66.2% and then Ciprofloxacin, 45%. E. coli on the other hand had high resistance to Sulfadiazine and trimethoprim with percentage rate of 67.2%, followed by Gentamicin with 65.5% and Chloramphenicol with 62.9%. Salmonella had high resistance to Sulfadiazine and trimethoprim, followed by chloramphenicol with 50% and amoxicillin at 47%. Staphylococcus, Streptococcus and Enterobacter had low resistance rate to the antibiotics. However, Klebsiella had high sensitivity Imipenem, 92%, Gentamicin, 88.12%, Amoxicillin, 88% and Cefotaxime, 75.35%. On the other hand, E.coli showed high sensitivity to Augmentin 88%, Imipenem 75%, and Ceftriaxone 71%. Streptococcus specie were the most susceptible organisms which had very high sensitivity|(100%) to seven(7) antibiotics (Sulfadiazine, Chloramphenicol, Imipenem, Amoxicillin, Augmentin, Gentamicin and Cefotaxime) (8.2% sensitive to Doxycycline, 97% to Ceftriaxone and 66.7% to Ciprofloxacin. The presence of these bacteria is of public health importance since most of them are implicated with food borne gastroenteritis in humans. The contamination was mainly post processing due to environmental, faecal and human handlers as a result of unhygienic practices during packaging and sales. Proper hygienic and sanitary measures are recommended during processing to prevent the contamination of these drinks.*

Keywords: *Zobo, Sanitary, Food borne & Bacteria.*

Introduction

Zobo drink is a locally made alcoholic beverage drink prepared from dried calyces of Rosselle (*hibiscus sabadariffa*). It is widely produced and consumed in large quantities in the Northen part of Nigeria, it is also consumed in the Southern part of the country (Gaffa *et al*., 2002; Oyewo *et al.,* 2019). The production of the zobo involves boiling of the calyces in water with addition of ginger, gloves, black paper, cinnamons and other desired spices and this is followed

by the addition of sugar, flavour to enhance the aroma and finally chilling in the refrigerator before serving (Adegoke and Skura, 1994; Abdulmumini, 2018). Zobo drinks is usually consumed in various social gatherings, sold in schools, parks and market places (Aliyu *et al.,* 2000; Udensi *et al.,* 2020). In recent times, Zobo drinks has gained rapid demand. This is due to its low price, nutritional and medicinal properties. The recent economic situation in the country has also contributed to Zobo drink gain more acceptance (Abdulmumini, 2018; Idowo-Adebayo, 2022). Zobo drink is now consumed by millions of people from different socio-economic classes and background.

Zobo drink is said to have high nutritional value this is because raw materials used in its production are essentially agricultural products (Abdulmumini, 2018; Idowu-Adebayo, 2022; Yinusa *et al.,* 2024) the main nutritional components of zobo are carbohydrates, proteins, vitamins and minerals, while the main product of fermentation is lactic acid which is responsible for the low pH.

Zobo drink are produced locally on daily bases for sales in schools, markets, offices motor parks, restaurants and Super markets. Sometimes, zobo drinks are prepared during weddings, naming ceremonies, and festivities to supplement and compliment soft drink. It has become an important source of income in many homes both in rural and urban communities thereby elevating poverty amongst people (Essien *et al.*, 2011; Chukwu*et al.,* 2018; Idowu-Adebayo, 2022).

Preparation of these drink (zobo) varies from one locality to another, the production procedure and sales of the zobo may be carried out under unhygienic condition leading to variation in quality attributes as well as microbial qualities which might predispose them to many pathogens of public health importance Makinde *et al*., 2020; Nwaiwu *et al.,* 2020; Idowu-Adebayo *et al.,* 2022). Therefore, it is against background that this research will be conducted to a certain the hygiene status and bacteriological quality of Zobo drinks sold and consumed in Maiduguri.

Materials and Methods

The Study Area and Period

The study area (Maiduguri) is located in the semi-arid zone of Borno state and situated in north eastern part of Nigeria, with an area of about 69,436 km^2 and lie within latitude 10-13^0N and longitude 12-15^{o}E. It lies within the savannah and Sahel vegetation and receives little rainfall. The area falls within the tropical continental north, with dry season of between four to seven months (November to May), followed by a short wet season from early June to late October. The state shares boundary with Chad to the north east, Cameroon to the east and Adamawa state to the south west fig 3.1. Agricultural activities, mainly livestock production are their main source of income. (Gisilambe, 1990).

The study was conducted at Maiduguri from August, 2024 to February, 2025. Maiduguri which is located in the capital city of Borno stated.

Sampling Method

A non-probability (convenient) of sampling method was used.

Sample Collection

Twelve different samples from two different schools (tertiary institutions) namely University of Maiduguri (Unimaid) and Ramat Polytechnic (Rampoly) which are within Maiduguri metropolis. Two samples of zobo drink was bought from different sellers at three different locations of the schools. Each sample were labelled with a masking tape. Each sample was labelled with an Alphabets and a number indicating the place of sample collection for easy identification such as sample UM1a and b (Unimaid motor Park) UM2a and b (Unimaid Academic Area) and UM3a and b (Unimaid Complex Area) respectively. While RP1and b stands for Rampoly Exit gate, RP2a and b (Rampoly Central Mosque Area) and RP3a and b (Rampoly ICT area) respectively. All samples were transported immediately to the laboratory on an ice pack to avoid degeneration prior to analysis.

Identification Bacterial and Microbial Analysis

Resulting bacteria isolates were transferred onto fresh nutrient agar, medium and incubated at 30^0C for 24hours. Pure colonies of bacteria were maintained on nutrient agar slant and stored at 4^0C until needed. The identification of bacteria isolates was by morphological examination under the microscope and biochemical test following the methods of (Hamgan and McCane 1976).

Microbial analyses of the Zobo drinks were performed using the pour plating and streaking method, a protocol performed by Bharath *et al.* (2003) and Eaton *et al.* (2005). To a test tube containing 9ml each of Tryptone Soya Broth (TSB), one mililitre of each of the samples were inoculated for enrichment. The test tubes were then incubated at 37°C overnight. The inoculated broth were sub cultured onto Baird Parker supplemented with egg yolk and Potassium Tellurite for the isolation of *Staphylococci*, *Salmonella-Shigella* agar for *Salmonella*, Blood agar for *Streptococci*, Eosin Methylene Blue (EMB) agar for *Escherichiacoli*and MacConkey agar for *Klebsiella* (Egwu *et al*., 1995; Roberts and Greenwood, 2003; Oranusi et al., 2007; Kishere, 2012). All the plates were incubated overnight at 37°C. Isolates showing growth were identified using biochemical tests, which included; Gram staining, catalase, coagulase, motility, Indole, Triptone sugar Iron as described by Ameh and Abubakar (2002), Bharat *et al*. (2003) and Eaton *et al*. (2005).The isolates were identified by referring to the Bergey's Manual of systematic Bacteriology (Holt *et al.,* 2000).

Total Bacteria Count

Total bacteria count was evaluated using standard plate count method and according to the method of Edem and Elijah, (2016). The standard plate count method is based on the assumption that each bacterial cell is separate from all others and will develop into a single discrete colony The (CFU), the final plates should have 30-300 colonies to obtain acceptable accuracy.

Method of Edem and Elijah , (2016) involves subjecting the samples to 10fold serial dilution aliquots (0.1ml) of appropriate dilution for sample were plated by the pour plate method into two plates of, nutrients agar and MacConkey agar respectively. For total heterotrophic bacteria count, and total coliform count. These were incubated at 30^0C for 24hours. Resulting colonies were counted with aid of a colony counter and the colonial morphologies noted.

Data Analysis

Anova with Tukey Kramer (HSD) test, discriminant test of canonical plot and graph builder were employed in analysing the data obtained using JMP version 11 (SAS Institute Inc., Cary NC). Analyses were considered significant at $p<0.05$.

Result

Table1: The table shows the total bacterial count of Zobo drink sold at the different location in University of Maiduguri and Ramat Polytechnic of Maiduguri Metropolis. The bacterial count ranges from $8.6*10^8$cfu/ml (which was the highest recorded) and $3.0*10^8$cfu/ml (which was recorded the lowest). The bacterial count of the Zobo drink sold at the different locations of the tertiary institution varied significantly ($P<0.05$).

Table 1: Total Bacteria counts (CFU/ml) of Zobo drinks sold in different location in the two tertiary institution in Maiduguri)

Location	Colony forming unit (cfu/ml)
UM1a	**4.2×10^8**
UM1b	**$3.5 \times 10^{8.a}$**
UM2a	**8.2×10^8**
UM2b	**4.5×10^8**
UM3a	**5.5×10^8**
UM3b	**3.0×10^{8a}**
RP1a	**7.2×10^8**
RP1b	**4.4×10^8**
RP2a	**6.9×10^8**
RP2b	**3.2×10^8**
RP3a	**3.5×10^{8b}**
RP3b	**3.0×10^{8b}**

Table 2: The table shows the bacterial species isolated from samples of Zobo drinks sold at the different locations of University of Maiduguri and Ramat Polytechnic of Maiduguri Metropolis. The bacterial isolates are with their occurrence frequencies and percentage shows *Escherichia coli* having the Highest percentage occurrence frequency of (9/75%) followed by *Enterobacter*, having occurrence frequency (8/66.6%), *Salmonella* having occurrence frequency of (7/58/3%), *Klebsiella* and *Staphylococcus* having occurrence frequencies of (4/33.3%) and *Streptococcus* having the lowest occurrence frequency of (3/25%) respectively.

Table 2: Bacterial isolates from samples of Zobo sold in different locations

Bacterial isolates	U1	U2	U3	U4	U5	U6	R1	R2	R3	R14	R15	R16	Occurrence frequency
Escherichia coli	+	+	+	+	+	-	-	+	+	-	-	+	**9**
Klebsiella	-	-	+	-	-	+	+	_	+	-	-	-	**4**
Staphylococcus aureus	+	-	-	+	-	-	-	+	_	+	-	-	**4**
Streptococcus spp	+	-	-	-	-	-	+	-	-	-	-	+	**3**
Salmonella	+	-	+	-	+	-	+	-	+	+	-	+	**7**
Enterobacter aerogenes	+	-	+	+	+	+	-	+	+	-	+	-	**8**

Table 3: The table shows the morphological characteristics of the isolated organisms from Zobo drink sold in different locations of University of Maiduguri and Ramat Polytechnic Maiduguri. Highlighting on the colour, shape, and distinctive biochemical test results of each of the isolated organism.

Table 3: Morphological and Biological Characteristics of the Isolated organism from Zobo

Colour	Shape	Gram stain	Catalase	Indole	Methyl red	V Proskous	Citrate	Oxidase	Urease	Suspected organism
Black spot	**Circular mucoid**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**	**-ve**	**Salmonella**
Pinkish colony	**Large, smooth and glistering colonies**	**-ve**	**-ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**-ve**	**E. coli**
Pinkish colonies	**Large, raised, circular mucoid colonies**	**-ve**	**+ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**	**+ve**	**Klebsiella**
White grayish coccoid	**Large circular mucoid colonies**	**+ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**Strep**
Lactose fermenter	**Circular convex**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**	**+ve**	**Enterobacter**
Golden colony	**Colonies arrange in clusters**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**S. aurues**

Table 4: the table shows the result of the percentage susceptibility of the six (6) of Bacteria isolated from the different antibiotics tested. Klebsiella had a high resistance to chloramphenicol 71%, followed by Sulfadiazine and trimethoprim 66.2% and then Ciprofloxacin, 45%. *E. coli* on the other hand had high resistance to Sulfadiazine and trimethoprim with percentage rate of 67.2%, followed by Gentamicin with 65.5% and Chloramphenicol with 62.9%. *Salmonella* had high resistance to Sulfadiazine and trimethoprim, followed by chloramphenicol with 50% and amoxicillin at 47%.*Staphylococcus, Streptococcus* and *Enterobacter* had low resistance rate to the antibiotics. However, *Klebsiella* had high sensitivity Imipenem, 92%, Gentamicin, 88.12%, Amoxicillin, 88% and Cefotaxime, 75.35%.On the other hand*, E.coli* showed high sensitivity to Augmentin 88%, Imipenem 75%, and Ceftriaxone 71%. *Streptococcus specie* was the most susceptible organisms which had very high sensitivity (100%) to seven (7) antibiotics (Sulfadiazine, Chloramphenicol, Imipenem, Amoxicillin, Augmentin, Gentamicin and Cefotaxime) (8.2% sensitive to Doxycycline, 97% to Ceftriaxone and 66.7% to Ciprofloxacin

Table 4: Antibiotic susceptibility pattern of bacterial isolates from Zobo drinks sold in Maiduguri

Antibiotics	***Microorganisms Isolated***					
Ciprofloxacin	***E. coli***	***Klebsiella***	***Staph***	***Strep***	***Enterobacter***	***Salmonella***
S	**53.0**	**55.0**	**70.10**	**66.7**	**90.0**	**66.7**
R	**47.0**	**45.0**	**22.90**	**33.3**	**10.0**	**33.3**
Sulfadiazine and trimethoprim						
S	**32.8**	**33.8**	**83.3**	**100**	**85.5**	**32.8**
R	**67.2**	**66.2**	**16.7**	**0.0**	**14.5**	**67.2**
Chloramphenicol						
S	**27.1**	**29.0**	**100.0**	**100.0**	**76.5**	**50.0**
R	**62.9**	**71.0**	**0.0**	**0.0**	**23.5**	**50.0**
Imipenem						
S	**73.5**	**92.0**	**83.3**	**100.0**	**85.5**	**70.10**
R	**26.5**	**8.0**	**16.7**	**0.0**	**14.5**	**22.90**
Ceftriaxone						
S	**71.8**	**57.1**	**57.1**	**97.3**	**63.7**	**73.5**
R	**28.2**	**42.9**	**42.2**	**3.0**	**36.3**	**26.5**
Amoxicillin						
S	**50.0**	**88.0**	**64.3**	**100.0**	**85.5**	**53.0**
R	**50.0**	**22.0**	**35.7**	**0.0**	**14.5**	**47.0**
Augmentin						
S	**88.0**	**60.0**	**66.6**	**100.0**	**85.5**	**100**
R	**12.0**	**40.0**	**33.4**	**0.0**	**14.5**	**0.0**
Gentamicin						
S	**35.0**	**88.12**	**64.3**	**100.0**	**85.5**	**50.0**
R	**65.5**	**12.0**	**35.7**	**0.0**	**14.5**	**50.0**
Cefotaxime						
S	**67.2**	**75.35**	**66.7**	**100.0**	**70.0**	**65.5**
R	**32.8**	**24.65**	**38.3**	**0.0**	**30.0**	**34.5**
Doxycycline						
S	**68.7**	**55.33**	**55.7**	**98.2**	**50.0**	**60.0**
R	**31.3**	**44.67**	**44.3**	**2.0**	**50.0**	**40.0**

Discussion

Zobo drink is a non-alcoholic beverage drink and consume in large quantity with a Maiduguri Metropolis and its environs. Zobo drink is a well-accepted drink which is prepared in homes, accepted by all religions and ethnic groups. It is usually a supplement of soft drinks and are widely used as refreshment in weddings, naming ceremonies, and in festive seasons like Eid celebrations, Easter and Christmas.

Microorganism are said to be present everywhere in nature (Hobbs and Gilbert 1978; Cockell, 2024). There isolation in locally prepared drinks in this study is predominant some of these microorganisms are potential pathogens. The production of this local drinks are sometimes done under non hygienic conditions (Etang *et al*., 2017; Nwaiwu *et al.,* 2020) with no authority to monitor or check their microbial safety and safety. This study examines the presence of microflora which can probably be transmitted to humans. and could be infectious. The relative microbial count recorded were indicative of high level of microbial contamination. The Zobo

drink sold at University of Maiduguri (Um2a) Academic area had the highest count of 8.2 *10^8cfu/ml while University complex (UM3b) and Ramat Polytechnic ICT area(RP3b) had the lowest count of 3.0*10^8 cfu/ml.

The result revealed that the Zobo drink is highly contaminated with bacteria which may be potentially pathogenic to humans. The occurrence of *E.coli*, *S. aureus*, *Streptococcus spp, Klebsiella, Salmonella, and Enterobacter* are pointer to the fact that the Zobo drinks sold in the different points of the selected tertiary institution within the metropolis are contaminated with potential pathogenic bacteria and this may have come from the bottles used as most bottles are recycled or the water used for domestic purpose or even the human handling the processing and sales of the drinks respectively. In addition, most of the hawkers of such product (Zobo drinks) are young children of unknown health status. They could serve as potential source of contamination at the point of sales after processing apart from the contamination that could occur before or during processing.

However, from investigation, the temperatures at which Zobo drinks were subjected are up to 100^0C (boiling point). These temperatures are enough to eliminate most of theseorganism isolated. The contamination could be after processing during packaging (Nerin *et al.,* 2016). Other factors such as poor hygiene (improper washing of working items such as strainers), dirty environment, the presence of domestic animals in the processing premises, and source of water maybe a contributing factor to the contamination of these drink (Oranusi *et al*., 2007; Umaru *et al.,* 2014; Danladi *et al.,* 2014; Jebichi, 2018). This is also in accord the study of Oranusi *et al.*, (2003) on the hazards and critical control points of Kununzaki in Northern Nigeria. Similar to this Study, the study of Orunusi *et al.,* (2003) revealed the presence of *Staphylococcus, E. coli,* could be an indication of faecal and environmental contamination which could be a sign for the presence of other enteric bacteria. Therefore, there occurrence maybe linked to faecal as well as environmental and human contamination (Ameh and Abubakar 2002; Harwood *et al.,* 2014). This may probably occur through the use of water or during the handling of product to the respective vendor. The contamination of these water could be as a result of improper hygiene of the water as most house hold in the metropolis rely om water supply from water vendors known as “Mai Moya” these vendors source is usually wash boreholes mostly surrounded by domestic animals. More so, hygiene and sanitary measures are compromised as there is no supervision or routine inspection of their Gallon on the hygiene status of such containers used for fetching and selling water.

The sensitivity of these isolates to the antibiotics used are similar to earlier studies by Rab *et al.*, 1989; Akhta *et al.,* 1997; Iyang, 2009; Danladi *et al*., 2014; Afusat, 2022. The prevalence of resistance strains of *E. coli,Enterobacter aerogenes*, *Streptococcus spp*, *Klebsiella spp and Salmonella* in Zobo is the indication of the use and misuse in the society. This is because the general populace has access to various drugs including antibiotics at any patent store or pharmacy even without any given prescription from a medical practitioner. This implies that antimicrobial resistant strains of pathogenic bacteria may colonize the human through consumption of Zobo drink or any locally prepared beverage drink of its kind and these could lead to chemotherapeutic failures among the consumers.

Conclusion

The finding of this study showed that Zobo drink prepared and sold in various point in the University of Maiduguri and Ramat Polytechnic of Maiduguri, are contaminated with microflora which could be potentially pathogenic to human. Most of the contamination are

possibly from the environment, containers used for packaging (recycled) food contacts, as well as human handlers post production.

There is need to enlighten the general public especially producers on the dangers associated with improper hygiene and on the dangers associated with the consumption of such product.

It is important to create awareness to the producers of such product that is Zobo drink on the importance of practicing good hygiene in the various stages of their production such as knowing the source of the bottles used, washing and storage of such bottles, and storage of all utensil used in the production up to the point of sale. By so doing, it will improve the quality of the product and minimise the rate of food poisoning caused by consuming contaminated food product.

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