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Antibiotic susceptibility pattern of bacteria isolated from Zobo drinks sold in Keffi, Nigeria

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Aims: This study is aimed to determine the susceptibility pattern of some bacteria isolated from Zobo drink sold in Keffi Metropolis, Nasarawa State, Nigeria. Zobo drink is a locally produced indigenous non-alcoholic beverage that is prepared by boiling the flower calyx of *Hibiscus sabdarifa*.
Methodology and results: Standard microbiological methods were employed to isolate bacteria from Zobo drink sold in Keffi metropolis, Nasarawa State, Nigeria. Samples of Zobo drink were collected from ten (10) different locations and their total bacterial counts were determined using standard methods involving CFU count. The antibiotic susceptibility pattern of the bacterial isolates was determined using the Kirby-Bauer disc diffusion method. The bacterial counts of the Zobo in the ten different locations range from 3.0 x 10^8 to 8.6 x 10^8 CFU/mL. Four species of bacteria were isolated and identified by standard microbiological methods. The isolates with their percentage occurrence frequencies were *Enterobacter aerogenes* (70%), *Escherichia coli* (60%), *Staphylococcus aureus* (30%) and *Streptococcus* spp. (20%) respectively.The antibiotic susceptibility pattern revealed that *Escherichia coli* had high resistance to Chloramphenicol (75%), followed by Septrin (68.7%) and Sparfloxacin (68.7%), while *Enterobacter aerogenes, Streptococcus* spp. and *Staphylococcus aureus* had low resistance to all the antibiotics tested. *E. coli* had very high sensitivity to Pefloxacin (100%), Gentamicin (88%), Amoxicillin (88%), Augmentin (75%), Tarivid (68.7%) and Streptomycin (68.7%). *Streptococus* spp. are the most susceptible isolates which had 100% sensitivity to Septrin, Chloramphenicol, Amoxicillin, Gentamicin and Pefloxacinrespctively; and this was followed by *Staphylococcus aureus* which had 100% sensitivity to Chloramphenicol, Amoxicillin, Augmentin and Tarivid respectively.
Conclusion, significance and impact study: The antibiotic resistance pattern exhibited by *E. coli*, *Enterobacter aerogenes*, *Streptococcus* spp. and *Staphylococcus aureus* isolated from the Zobo sold in Keffi are indicative of possible abuse of the use of antibiotics, and this has serious health implications. The results further demonstrated that Zobo sold in Keffi within the period of this study had contaminant bacteria including potentially pathogenic species and this can lead to failures in antibiotic chemotherapy among consumers of Zobo.

Keywords: Antibiotics, susceptibility, resistance, Zobo, Keffi

INTRODUCTION

Zobo drink is locally made indigenous non-alcoholic beverages (Gaffa *et al.*, 2002) that is widely produced and consumed in large quantity at the northern part of Nigeria, but consumed in small quantities in the southern part of the country. This local beverage is consumed both in the wet and dry seasons because of its optimal thirst quenching properties (Makut *et al.*, 2010).

Zobo drink is prepared or produced from boiled *Hibiscus sabdarifa* calyx. Additional ingredients used include sugar, black pepper and ginger, which are added to enhance taste and aroma (Adegoke and Skura, 1994).

The method of its production does not meet the standards of production of other beverage drinks because most of the ingredients used are usually not quantified as recommended for industrial food production (Nester *et al.*, 2004). In some families, Zobo drink is consumed at household levels, whereas in many families it is being sold as a major source of income for meeting their daily needs. Zobo drinks is very unique because the raw materials as well as the additives used in its production are locally sourced as they are grown throughout the savannah belt of West Africa which is characterized by moderate rainfall. The methods involved in its production require boiling the calyx for about 30 min, adding with some local spices and allowing it to stay at room temperature for between 18-24 h for fermentation to occur. It is then filtered using a sieve, after which a taste inducing agent like sugar or honey is added to the filtrate

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to produce the desired taste which is ready for consumption (Onurah *et al.*, 1987; Asiedu, 1989; Akoma *et al.*, 2006). There are some variations that occur using this procedure which depends on taste expected and this is usually based on the cultural habits of the different local communities. Some individuals and communities add some pepper and sugar (Adeyemi and Umar, 1994).

This popular drink is usually packaged and sold in 50 mL to 1 L plastic bottles and at times sold tied in disposable polythene bags. The drink is mostly consumed within 20-35 h of its production due to its relatively poor keeping qualities (Adeyemi and Umar, 1994). Its water content coupled with the crude method of production and packaging under improper sanitary conditions often predisposes this drink to sudden contamination by bacteria (McLauchlin and Riege, 2002). The ubiquitous nature of microorganism guarantees them the opportunity to be found in this locally made beverage drink and possibly in the water used for their preparation, during storage and other processes involved in its preparation. Some of the bacterial species that are associated with this locally produced beverage drink include spoilage species, pathogenic species, coliforms and lactic acid bacteria (Onourah *et al.*, 1987).

Zobo drink is said to have high nutritional value due to the raw materials used in its production which are essentially agricultural products. Nkama (1993) reported that the main nutritional components of Zobo are carbohydrates, proteins, vitamins and minerals, while the main products of fermentation is lactic acid which is responsible for the low pH values of this non-alcoholic beverage. The spices usually added being agricultural commodities may contain high level of microbial impurities (Adeyemi and Umar, 1994), which can serve as a source of pathogenic microorganisms (Bibek, 2001). Though the pH values of Zobo are too low to allow the growth of pathogenic microorganism, the presence of contaminant organisms do cause serious problems which can lead to fast deterioration of the drink or become a vehicle for transmission of infection (Alice, 1976). The presence of pathogens even in small numbers could render a beverage unsuitable for human consumption (Obi and Chizea, 1990). It is very possible that the contamination by any pathogen may occur during packaging and hawking of the products due to improper or careless handling of the product.

This study was aimed at determining the susceptibility pattern of some bacteria isolated from Zobo drink sold in Keffi Metropolis, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Study area

The study area is Keffi town, an ancient town in Nasarawa State located in the northern part of Nigeria. Keffi is about 58 km away from the Federal Capital Territory (FCT) Abuja, and 129 km away from Lafia, the capital of Nasarawa state. Keffi is situated on latitude 8°5'north and longitude 7°5'. It is situated on an altitude of about 850 m above sea level (Akwa *et al.*, 2007).

Sample collection

Ten different locations where Zobo sold in Keffi metropolis were randomly selected for the purposes of sample collection. Samples of the Zobo were aseptically collected in triplicates into sterile corked plastic tubes from the ten different locations. The samples were labeled appropriately, placed into separate plastic bags, and conveyed in an ice packed cooler to the Microbiology laboratory of the Nasarawa State University, Keffi. The ten different locations of sample collection were Angwan Lambu, Angwan Tiv, Angwan Waje, Angwan Nepa, Area Commander, Convocation Square of Nasarawa State University, CRDP, SohonKasuwa, High Court and Locust

Determination of aerobic plate count

Standard Plate Count method proposed by Andrew (1994) was used to determine the Total Aerobic Colony count of the samples. A seven-fold serial dilution of each sample was made and plated out on Plate Count Agar (Casein Peptone, 5.0 g/L; Yeast Extract, 2.5 g/L; Dextrose, 1.0 g/L ; Agar, 15.0 g/L) using spread plate technique. The plates were incubated at 37 °C for 24 h. The average microbial load of the samples obtained from the different locations were expressed as Colony Forming Units per milliliter (CFU/mL) of Zobo.

Isolation and identification of bacteria isolated from Zobo samples

McConkey agar, Chocolate agar, Eosin Methylene Blue agar, Mannitol Salt agar and Salmonella-Shigella agar were employed for the isolation of bacteria for the purposes of identification. McConkey agar was used to isolate lactose fermenting gram negative bacteria, Chocolate agar was used to isolate fastidious bacteria, Eosin Methylene Blue was used for the selective isolation of enteric coliforms, Manitol Salt agar was for the selective isolation of salt-tolerant bacteria, and Salmonella-Shigella agar was used for the isolation of enteric bacilli particularly *Salmonella* and *Shigella* species. All plates were incubated at 37 °C for 24 h. Identification of bacterial isolates was based on the standard cultural, morphological and biochemical methods (Buchanan and Gibbons, 1974; Cheesbrough, 2004).

Antibiotic susceptibility test

The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disk diffusion method (Cheesbrough, 2004). A suspension of each isolate was prepared in peptone water to match 0.5 McFarland turbidity standards in order to standardize the inoculum. The standardized inoculum of each isolate was inoculated in triplicates onto the surfaces of plain Mueller-Hinton

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agar plates, and Septrin (30 µg), Chloramphenicol (30µg), Sparfloxacin (5 µg), Amoxycillin (30 µg), Ciprofloxacin (5 µg), Augmentin (30 µg), Gentamicin (10 µg), Pefloxacin (10 µg), Tarivid (30 µg) and Streptomycin (10 µg) discs were placed and incubated at 37°C for 24 h. The zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009).

Statistical analyses

The data obtained were subjected to statistical analyses involving means, Least Significant Difference test for separation of means as well as proportions using percentages (Bailey, 1994)

RESULTS

Table 1 shows the total aerobic bacterial counts of Zobo sold at the different locations in Keffi metropolis. The counts range from was $8.6x10^8$ CFU/mL (which was highest recorded in AngwanWaje) and $3.0x10^8$ CFU/mL (which was the lowest recorded for Convocation square). The bacterial counts of Zobo sold at the different locations varied significantly ($p< 0.05$).

Table 2 shows the bacterial species isolated from samples of Zobo drinks sold at the different locations in Keffi. The bacterial isolates with their respective occurrence frequencies were *Escherichia coli* (60%), *Staphylococcus aureus* (30%)*, Streptococcus* spp. (20%) and *Enterobacter aerogenes* (70%), respectively. *Enterobacteraerogenes* was therefore most predominant isolate, followed by *Escherichia coli*, while *Streptococcus* spp. was the leastin terms of occurrence in the Zobo drinks sold in Keffi.

Table 3 shows the results of percentage susceptibility the four species of bacteria isolated to the different antibiotics tested. *E. coli* had a high resistance to chloramphenicol (75%), followed by Septrin (68.7%) and Spafloxacin (68.7%), while *Enterobacter aerogenes*, *Streptococcus* spp. and *S. aureus* had low rates of resistance to all the antibiotics tested. However, *E. coli* had very high sensitivity to Pefloxacin (100%), followed by Gentamicin (88%), Augmentin (75%), Tarivid (68.7%) and Streptomycin (68.7%). *Streptococcus* spp. were the most susceptible isolates which had very high sensitivity (100%) to five of the antibiotics tested, namely, Septrin, Chloramphenicol, Amoxicillin, Gentamicin and Perfloxacin, respectively. *S. aureus* was also very sensitive (100%) to Chloramphenicol, Amoxicillin, Augmentin and Tarivid, respectively.

Table 1: Total bacterial counts (CFU/mL) of drinks sold in different location in Keffi metropolis.

Location	CFU/mL
A	$4.2 \times 10^{8\,b}$
B	$3.5 \times 10^{8\,a}$
C	$8.6 \times 10^{8\,e}$
D	$4.5 \times 10^{8\,b}$
E	$5.5 \times 10^{8\,bc}$
F	$3.0 \times 10^{8\,a}$
G	$7.2 \times 10^{8\,d}$
H	$4.4 \times 10^{8\,b}$
I	$6.9 \times 10^{8\,d}$
J	$3.2 \times 10^{8\,a}$

Least significant difference (LSD) at $p<0.05 = 1.08$

A, Angwan Lambu; B, Angwan Tiv; C, Angwan Waje; D, Angan Nepa; E, Area Command; F, Convocation Square; G, CRDP; H, Sohon Kasuwa; I, High Court; J, Locust.

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

Bacterial isolates	Locations										Occurrence frequency (%)
	A	B	C	D	E	F	G	H	I	J	
Escherichia coli	+	+	+	-	-	+	-	+	-	+	60
Staphylococcus aureus	-	-	-	+	+	-	-	+	-	-	30
Streptococcus spp.	+	-	-	-	-	-	-	-	+	-	20
Enterobacter aerogenes	+	+	+	-	+	+	+	-	-	+	70

A, Angwan Lambu; B, Angwan Tiv; C, Angwan Waje; D, Angwan Nepa; E, Area Command; F, Convocation Square; G, CRDP; H, SohonKasuwa; I, High Court; J, Locust.

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Table 3: Antibiotic susceptibility pattern of bacterial isolates from Zobo drinks sold in Keffi.

Antibiotics	Sensitivity/ Resistance	Bacterial isolates			
		Escherichia coli	*Enterobacter aerogenes*	*Streptococcus* spp.	*Staphylococcus aureus*
Septrin (SXT)	Sensitivity	31.3%	85.7%	100.0%	83.3%
	Resistance	68.7%	14.3%	0.0%	16.7%
Chloramphenicol (CH)	Sensitivity	25.0%	78.6%	100.0%	100.0%
	Resistance	75.0%	21.4%	0.0%	0.0%
Sparfloxacin (SP)	Sensitivity	31.3%	64.3%	66.7%	83.3%
	Resistance	68.7%	35.7%	33.3%	16.7%
Ciprofloxacin (CPX)	Sensitivity	50.0%	92.9%	66.7%	83.3%
	Resistance	50.0%	7.1%	33.3%	16.7%
Amoxicillin (AM)	Sensitivity	88.0%	64.3%	100.0%	100.0%
	Resistance	12.0%	35.7%	0.0%	0.0%
Augmentin (AU)	Sensitivity	75.0%	50.0%	66.7%	100%
	Resistance	25.0%	50.0%	33.3%	0.0%
Gentamicin (CN)	Sensitivity	88.0%	85.7%	100.0%	66.6%
	Resistance	12.0%	14.3%	0.0%	33.4%
Pefloxacin (PEF)	Sensitivity	100.0%	57.1%	100.0%	66.6%
	Resistance	0.0%	42.9%	0.0%	33.4%
Tarivid (OFX)	Sensitivity	68.7%	71.2%	66.7%	100.0%
	Resistance	31.3%	28.8%	38.3%	0.0%
Streptomycin (S)	Sensitivity	68.7%	85.7%	66.7%	83.3%
	Resistance	31.3%	14.3%	33.3%	16.7%

DISCUSSIONS

The relative microbial counts recorded were indicative of high level of microbial contamination. Zobo sold at Angwan Waje had the highest counts of $8.6x10^8$ CFU/mL, while the Convocation Square location had the lowest counts of $3.0x10^8$ CFU/mL. However, there was no significant difference ($p>0.05$) in the microbial counts of the Zobo sold at the different locations of the metropolis which is indicative that the beverage sold at the different locations most likely had similar microbial quality. This may be due to the fact that similar handling procedures are employed during processing and marketing of the beverage. The high microbial counts may to a large extent be attributed to lack of effective precautions on hygiene practice in handling procedures during processing of the beverage. The practice of addition of some quantity of water to Zobo after fermentation may also be a source of introducing microbial contaminants which may have come from the water itself or from the utensils used for such purposes.

The percentage occurrence of *E. coli*, *S. aureus*, *Streptococcus* spp. and *Enterobacter aerogenes* are pointer to the fact that the Zobo drinks sold in the different locations in Keffi are contaminated with potentially pathogenic bacteria, and this may have come from the water used for domestic purposes, or the human handling during processing and sales of the product, respectively. This is in agreement with McLauchlin and Riege (2002) who reported earlier that water used for production coupled with the crude method of production and packaging under improper sanitary conditions predisposes Kunun-zaki drink to microbial contamination by an array of both Gram negative and gram positive bacteria. There is therefore need for surveillance by Public Health officials to ensure safety of the Zobo being sold in Keffi for public consumption. There is need to also ensure that the water used for production especially post-heating processing of the Zobo is safe and free from microbial contaminants. The source of contamination may also have come from the spices used additives (Adeyemi and Umar, 1994; Bibek, 2001).

The sensitivity of these isolates to the antibiotics used are comparable to earlier reports (Rab*et al.*, 1989; Akhtar*et al.*, 1997; Inyang, 2009). The prevalence of resistant strains of *E. coli*, *Enterobacter aerogenes*, *Streptococcus* spp. and *S. aureus* in Zobo is a reflection of the use and misuse of the antibiotics in the society. This is not surprising because outside the hospital environment, the general populace have access to various kinds of antibiotics at any drug store even without any prescription from a medical practitioner.

The Public Health implication of this study is that antimicrobial resistant strains of pathogenic bacteria may colonize the human population through consumption of contaminated Zobo, and this would lead to

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chemotherapeutic failures among the human consumers of this popular beverage in the Keffi metropolis.

CONCLUSION

The presence of resistant strains of *E. coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*and *Streptococcus* spp. in Zobo sold in Keffi suggests that consumption of this beverage has potential health hazard to the consumers in Keffi, Nasarawa state, Nigeria. The consumers of this popular drink were therefore placed at health risk which may culminate into failures of commonly used clinical antibiotics for the treatment of infections.

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