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Bacterial contaminants reducing quality of food drinks: A case study of Zobo drink (a non-alcoholic beverage from *Hibiscus sabdariffa*) sold in Abakaliki metropolis, Ebonyi state, Nigeria

OnyinychiEsther Udu-ibiam [1,*] Akindele Kayoede [2] and Ikemesit, Udeme Peter [3]

[1] *Department of Applied MIicrobiology, Faculty of Science, Ebonyi State University, Abakaliki. Ebonyi State. Nigeria.*
[2] *Department of Applied Microbiology, Faculty of Biological Sciences, Alex Ekwueme Federal University Ndufu Alike Ikwo. Ebonyi State. Nigeria.*
[3] *Department of Applied Microbiology, [3] Federal College of dental technology and therapy Trans ekulu, Enugu, Enugu State. Nigeria.*

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Abstract

Non-alcoholic beverages, Zobo drink important among the dietary pattern of people in the northern and southern part of Nigeria were analyzed. Four different locations where ready-to-drink zobo is sold within Abakaliki were randomly selected. Three samples of ready-to-drink zobo were collected each from the following locations: Presco market, international market, kpirikpiri market and Spera-in-deo. The total aerobic and coliform bacterial counts of Zobo drinks sold at different locations within Abakaliki ranges from 0.9 x 10 cfu/ml (the lowest) to 0.3 xl0^5 cfu/ml (the highest) count for the total coliform count and 0.8 x 10^8 (the lowest) to 5.8 x 10^8 (the highest) for the Aerobic bacteria Count. *Staphyloccus* spp was isolated from all the twelve samples (100 %), *Escherichia coli* was isolated from Eight samples (75 %), *Pseudomonas* species from Nine samples (83.33 %), *Klebsiella pneumoniae* (75.00 %) and *Shigella* sp with the least occurrence (33.33 %). All the bacteria isolates were susceptible to ciprofloxacin, gentamycin and chloramphenicol except *Pseudomonas aeruginosa* with the least sensitivity to chloramphenicol (43.8 %), *Klebsiella pneumonia* and *Staphylococcus aureus* were resistant to Ampicillin while *Pseudomonas aeruginosa* was resistant to erythromycin. None of the bacteria isolates was resistant to all the antibiotics; however, they exhibited high level of resistance against erythromycin, ampicillin and sulphamethazole with the least resistance percentage of 92.5 %, 57.5 % and 81.25 % respectively. It is important to monitor food items before releasing to the consumers to avoid food related illness.

Keywords: *Hibiscus Sabdariffa*; Non - Alcoholic Beverage Bacterial Contamination; Bacteriological Food Quality; Antibiotics Susceptibility

1. Introduction

In every society, drinks of indigenous origin are produced in different ways and served as beverages at home and sometimes in occasions (Abdullahi and yakubu, 2013). Non-alcoholic beverages rich as Zobo drink (sorrel drink) are of recent among the popular traditional food drinks which are very important among the dietary pattern of people in the northern and southern part of Nigeria (Yang *et al*, 1999), there is an increase in the demand of zobo drinks due to its low price, nutritional and medicinal properties. A Zobo drink is prepared by boiling the dry calyces (sepals) of *Hibiscus sabdariffa* in water for about 10-15 minutes from which the pigment embedded in the flower is extracted. The sharp sour taste of the raw extract is usually sweetened with sugar cane granulated sugar, pineapple/e orange or other fruits depending on choice. Zobo in found to be rich in various amino acids, proteins, carbohydrate, vitamins fat and other anti-oxidant (Adebayo,2010; Adeneye, 1991; Ajmair and Akhtar,2012; Amao et al,2006; Bamidele et al, 2010;Emodi and

* Corresponding author: Udu-Ibiam, Onyinyechi. E.

Madukwe,2011;Flake and Nzeka,2009). The leaves are source of vegetable and the seeds as sources of oil (Gujurati,2004). Hibiscus especially Roselle is associated with traditional medicines and used for treatment of several diseases such as hypertension and urinary tract infections (Jabbar and Domenico, 2010). A recent review stated that specific extract of *Hibiscus sabdariffa* exhibits activities against athescieriosis, liver disease, cancer, diabetes and other metabolic syndromes (Lin and Hgu, 2002).

Zobo drink if not properly refrigerated can detoriate rapidly within 24hrs. The packaging and poor hygienic condition as well of lave of potable water in some area have resulted to potential contamination which in turn leads to. Contamination as well as pose serious health hazard to the public (Jansen,1992).

Food spoilage by bacteria and the grooving resistance to antibiotics are of public health concern in both developed and developing countries (Kim et al; 2011). These have posed a serious problem in the treatment of infectious diseases in the area of health care delivery.

2. Methodology

2.1. Study Area

The study area is Abakaliki town in Ebonyi State located in south eastern part of Nigeria. It is located 64 kilometers southeast of Enugu. Abakaliki is situated on latitude $6^0$20'N and longitude $8^0$06E (NPC, 2010)

2.2. Sample Collection

Four different locations where ready-to-drink zobo is sold within Abakaliki were randomly selected for the purposes of sample collection. Three samples of ready-to-drink zobo were collected each from the following locations: Presco market, meat market, kpirikpiri market and Spera-in-deo. The samples were transported to the microbiology laboratory of EBSU, Abakaliki in transport media where they were analyzed following standard techniques in Microbiology.

2.3. Analysis of Samples

Samples gotten from each location were used for the test to represent the four locations of sample collection. Each of the samples obtained from the four points were macerated using a sterile mortar. Five milliliter (5ml) each of the samples was dissolved into 25ml of distilled water contained in a test tube labeled according to the locations of collection. A tenfold serial dilution was carried out on each of the labeled samples. Exactly 0.5ml of the suspension was collected each from 10^{-1} to 10^{-5} serial dilution of the different samples were inoculated into a nutrient agar plate using pour plate method. They were all incubated for 18 to 24 hours at 37 ℃.

2.4. Determination of Aerobic Plate Count

Standard plate count method proposed by Cheesebrough (2002) was used to determine the total aerobic colony count of the samples. Only plates with moderate growth were counted. The average microbial loads of the samples obtained from the different location were expressed as colony forming units per Milliliter (Cfu/ml).

2.5. Isolation and Identification of Bacteria Isolated from Zobo Samples.

Mac Conkey agar, Eosin methylene Blue agar, mannitol salt and salmonella – shigella agar were employed for the isolation of bacteria for the purpose of identification. Mac Conkey agar was used to isolate lactose fermenting gram negative bacteria, Eosin methylene Blue agar was used for the selective isolation of enteric coliforms, mannitol salt agar was used 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 ℃ for 24 hours. Identification of bacteria isolates was based on the standard culture, morphological and biochemical methods (Cheesbrough, 2002).

2.6. Antibiotic Susceptibility Test.

The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disk diffusion method (Cheesbrough, 2002). A suspension of each isolates was prepared in peptone water to match 0.5 Mcfarland turbidity standards in order to standardize the inoculums. The standardized inoculums of each isolate were inoculated onto the surface of plain Mueller- Hinton agar plates.

The tested antibiotics include; Erythromycin (15 μg), Ciprofloxacin (5 μg), Ampicilin (10 μg), Clindamycin(2 μg), Gentamycin (30 μg), Sulfamethoxazole (25 μg), Tetracycline (30 μg), Nitrofuratin (200μg), Vancomycin (30μg)Cefoxitin

(30μg) discs were placed and incubated at 37^0c for24 hours. The zones of inhibition were measured with meter rule and compared with the clinical and laboratory standards institute (CLSI) guidelines (Melesse and Beyene, 2009; Mullins et al.,1994).

3. Results

One hundred and thirty three (133) Isolates were obtained from the twelve Zobo drink samples; out of these isolates five different bacteria general were identified. Gram reaction showed four (4) species to be Gram negative while the other one is gram positive.

3.1. Total Viable Count

The total aerobic and coliform bacterial counts of Zobo drinks sold at different locations within Abakaliki (table 1). The counts range from 0.9 x 10 Cfu/ml (the lowest) to 0.3 xl0^5 Cfu/ml (the highest) count for the total coliform count and 0.8 x 10^8 (the lowest) to 5.8 x 10^8 (the highest) for the Aerobic bacteria Count.

Table 1 Total bacterial counts (cfu/ml) of Zobo drinks sold in different locations within Abakaliki**.**

S/N	Sample code	Total Coliform Count (Cfu/ml)	Aerobic Bacteria Count(Cfu/ml)
1	SI	0.3 xl0^4	2.0 X 10^8
2	S2	0,1 Xl0^5	3. 5 Xl0^8
3	S3	0.4 Xl0^4	4.3 X 10^8
4	S4	1.3 Xl0^5	5.8 X 10^8
5	S5	0.3 Xl0^5	3.2 Xl0^8
6	S6	0.1 Xl0^3	2.6 X 10^8
7	S7	2.0 Xl0^4	2.8 Xl0^8
8	S8	0.2 Xl0^4	3.4 Xl0^8
9	S9	0.9 X 10^3	2.7 Xl0^8
10	S10	0.1 Xl0^4	0.8 X 10^8
11	Sll	0.2 Xl0^4	1.4 Xl0^8
12	S12	0.3 X 10^5	2.4 X 10^8

3.2. Identification of the isolates

The microorganisms isolated from the Zobo drink were identified based on their cultural, morphological and biochemical characteristics (Tabje^. A total of five (5) bacteria species were isolated and identified from all the samples. These include; *Escherichia coli, Pseudomonas species, Staphyloccus aureus, Shigella sp, and Klebsiella pneumonia.*

Table 2 Cultural, Morphological and Biochemical Characteristics of the Bacteria Isolates.

Cultural characteristics Red testorganism	**Morphology**	**Gram Reaction**	**Catalase**	**Oxidase**	**Citrate**	**Coagulase**	**Indole**	**V-P**	**Methyl**	**Probable**
Greenish metallic Rod shines on EMB	Rod	-	+	+	-	-	+	-	+	*E. coli*
Creamy Yellow Colonies on Mannitol salt Agar	Cocci	+	+	+	+	+	-	+	+	*S. aureus*
Mucoid Colourless Colonies on salmonella-shigella Agar	Rod	-	+	+	-	-	-	-	+	*Shigella sp*
Mucoid Pink to purple colonies.	Rod	-	+	+	-	-	-	-	+	*Klebsiella sp*
Green to blue colonies on cetrimide Agar	Rod	-	+	-	+	-	-		+	*Pseudomonas*

Keys: + positive - Negative

3.3. Distribution of the bacterial isolates in the Zobo drink samples

The distribution of the bacteria isolates in the Zobo drink samples base on their occurrence in each sample is shown in table. Sample S4 had the highest number of isolates while sample S8 had the least.

Table 3 Distribution of the bacteria isolates in the Zobo drink samples.

Sample Code	***E, coli***	***Staphylococcus***	***Shigella sp Aureus***	***Klebsiella pneumoniae***	***Pseudomon asSp***	**Total**
SI	5	5	-	1	1	12
S2	5	4	1	1	2	13
S3	5	3	-	2	-	12
S4	-	10	1	-	3	14
S5	5	4	-	1	1	11
S6	3	4	-	1	1	09
S7	3	5	-	2	1	12
S8	-	3	1	-	1	05
S9	5	5	-	2	-	10
S10	4	4	-	2	2	12
S11	-	5	-	-	3	09
S12	5	5	I	2	1	12
Total	**40**	**57**	**4**	**14**	**16**	**131**

3.4. Frequency of occurrence of bacterial species in the Zobo drink Samples

The distribution of each bacteria specie in the Zobo drink samples base on their percentage occurrence is shown in the Table 5. *Staphyloccus spp* was isolated from all the twelve samples (100 %), *Escherichia coli* was isolated from Eight samples (75 %), *Pseudomonas species* from Nine samples (83.33 %), *Klebsiella pneumoniae* (75.00 %) and *shigella sp* with the least occurrence (33.33 %).

Table 4 Occurrence of microbial isolates in the Zobo drinks Sample Isolates

	Samples												**Percentage Occurrence**
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	
Escherichia coli	+	+	+	+	+	+	+	+	+	+	-	+	75.00 %
Shigella sp	+	+	-	+	-	-	-	+	-	-	-	+	33.33%
Staphylococcu	+	+	+	+	+	+	+	+	+	+	+	+	100.0%
Klebsiell Pneumonia	+	+	+	-	-	+	+	+	-	+	+	+	75.00%
Pseudomonas Aeruginosa	+	+	-	+	+	+	+	+	-	+	+	+	83.33%

Keys

SI = Sample 1 from presco market, S2 = sample 2 from presco market, S3 = sample 3 from Presco market.

S4 = Sample 1 from Meat market, S5 = Sample 2 from Meat market, S6 = Sample 3 from Meat market.

S7 = Sample 1 from Kpirikpiri market, S8 = Sample 2 from Kpirikpiri market, S9 = Sample 3 from Kpirikpiri market and

S10 = Sample 1 from Spera-in-deo, S11 = Sample 2 from Spera-in-deo, S12 = Sample 3 from Spera-in-deo.

3.5. Antibiotic susceptibility profiles of the isolates

The sensitivity or resistance of the isolates is shown in table 6 below. All the bacteria isolates were susceptible to ciprofloxacin, gentamycin and chloramphenicol except *Pseudomonas aeruginosa* with the least sensitivity to chloramphenicol (43.8 %), *Klebsiella pneumonia* and *Staphylococcus aureus* are resistant to Ampicillin while *Pseudomonas aeruginosa* is resistant to erytromycin. None of the bacteria isolates was resistant to all the antibiotics; however, they show high level of resistance against erythromycin, ampicillin and sulphamethazole with the least resistance percentage of 92.5 %, 57.5 % and 81.25 % respectively. Most prominent among them is *Pseudomonas aeruginosa* with highest value of resistant to most of the antibiotics tested.

Table 5 antibiotics susceptibility pattern of bacterial isolates from zobo drinks sold within abakaliki.

Antibiotics	**Sensitivity/ Resistance**		**Bacterial**	**Isolates**		
		Escherichia coll (n =40)	***Shigella Spp (n=4)***	***Staphylococcus aureus (n=57)***	***Klebsiella pneumoniae* (n=14)**	***Pseudomonas aeruginosa* (n=16)**
Ciprofloxacin (5 *^g)*	Sensitivity	37(92.5 %)	3(75.0 %)	51(89.5%)	13(92.9%)	14(87.5%}
	Resistance	3(7.5 %)	1(25.0%)	6(10.5%)	1(7.1 %)	2(12.5%)
Gentamicin (30 *pg)*	Sensitivity	35(87.5 %)	4(100%)	49(85.9 %)	12(85.7%)	15(93.8%)
	Resistance	5(12.5%)	0(0.0 %)	8(14.1%)	2(14.3%)	1(6.2%)
Chloramphenicol (10 *fig)*	Sensitivity	31(77.5%)	4(100%)	54(94.7 %)	11(78.5%)	7(43.8 %)
	Resistance	9(22.5 %)	0(0.0 %)	3(5.3 %)	3(21.5 %)	9(56.3 %)

Tetracycline (30 M#)	Sensitivity	29 (72.5 %)	3(75.0 %)	30(52.6 %)	ND	5(31.25%)
	Resistance	11(27.5%)	1(75.0%)	27(47.4 %)		11(68.75%)
Clindamycin, (2 *i*g)*	Sensitivity	33(82.5%)	0	51(89.5%)	0	0
	Resistance	7(17.5 %)		6(10.5%)		
Erythromycin (15 M#)	Sensitivity	3(7.5 %)	0	14(24.6%)	0	0(0.0 %)
	Resistance	37(92.5 %)		43(75.4 %)		36(100%)
Nitrofuratoin (200 jig)	Sensitivity	32(80 %)	0	0	14(100%)	0
	Resistance	8(20 %)			0(0.0 %)	
Ampicillin(lO^)	Sensitivity	17(42.5%)	2(50.0 %)	0	0(0.0 %)	2(12.5%)
	Resistance	23(57.5 %)	2(50.0%)	57(100%)	14(100%)	14(87.5%)
Sulphametazole(25 *frg)*	Sensitivity	1(2.5 %)	3(75.0 %)	11(19.3%)	11(78.6%)	3(18.75%)
	Resistance	39(97.5 %)	1(25.0%)	46(81.5%)	2(21.4%)	13(81.25%)
Ofloxacin (30 ug)	Sensitivity	32(80 %)	4(100%)	18(31.6%)	13(92.9 %)	0
	Resistance	8(20 %)	0(0.0 %)	39(88.1%)	1(7.1%)	
Vancomycin (30 Mg),	Sensitivity	0	0	54(94.7)	0	2(12.5)
	Resistance			3(5.3)		14(87.5)

Keys; n= number of isolates S - Sensitivity R - Resistance 0= not tested

3.6. Multiple Antibiotic Resistance (MAR) Index of the Bacterial isolates from Zobo Drinks

The multiple antibiotic resistance indexing of the bacteria isolates (table 7) indicated that *Pseudomonas aeruginosa* had the highest MAR index of 0.70, followed by *Staphylococcus aureus* with MAR index of 0.40, *Escherichia coli* 0.37, *Klebsiella pneumonia* 0.19 and *Shigella sp* had the least MAR index of 0.13 respectively

Table 6 Multiple Antibiotic Resistance (MAR) Index of the Bacterial isolates from Zobo Drinks

S/N	Organisms	MAR (Multiple Drug Resistance) Index
1	Escherichia coli	0.37
2	*Shigella Spp*	0.13
3	*Staphylococcus aureus*	0.40
4	*Klebsiella pneumoniae*	0.19
5	*Pseudomonas aeruginosa*	0.70

4. Discussion

Zobo drink is a non-alcoholic beverage prepared and consumed in large quantities in Abakaliki and its environs. The drinks is well accepted by many in these areas, and hence are being produced as supplements and complements to soft drinks in schools and during occasions like birthday celebrations, weddings and naming ceremonies. The production systems are sometimes done under unhygienic conditions (Nwachukwu *et al,* 2011) with no authority to monitor their microbial quality and safety.

In this study, the hawked Zobo drinks were examined for the presence of microbes which can probably cause infections in humans. The results revealed that Hawked Zobo drinks are highly contaminated with bacteria species and that bacteria isolated from the sampled Zobo drinks include *E. coli, Staphylococcus* spp, *Shigella sp, Klebsiella pneumonia* and *Pseudomonas* spp. *E. coli,* and *Staphylococcus aureus* occurred in most of the samples. This result agrees with the findings of some authors (Annie et al., 2022; Onyemauwa,2010 ; Philip *et al,* 2013; Pindyck and Rubinfield, 1998,Popkin et al., 1989). Also from the analysis it was revealed that *Staphylococcus aureus* was the most prevalent (most occurred) bacteria isolated, this was closely followed by *E.coli* (an uropathogen that indicates feacal contamination), *Pseudomonas aeruginosa, Klebsiella pneumonia and Shigella sp* accordingly, this is line with the report of some authors (Popkin et al., 1989; Ezeigbo *et al.,* 2015) but none of them reported isolation of *shigella sp* which its presence might be as a result of fecal contamination of the samples.

The relative microbial counts recorded were indicative of high level of microbial contamination. Zobo drink with code S4 sold in meat Market had the highest microbial counts of 5.8 x 10 Cfu/ml while sample S10 from Spera-in-deo area of Abakaliki had the lowest microbial counts of 0.8 x 10^8 Cfu/ml. The coliform count indicated that sample S2 and S12 from presco area and Spera-in-deo area had the highest coliform count with load of 0.3 x 10^5 Cfu/ml, sample S6 from Meat market had the lowest coliform bacteria with count 0.l x l0^3 Cfu/ml and this findings is comparable to previous results (Ruel *et al,* 2005). However, there was no significant difference *(p* > 0.05) in the microbial counts of the Zobo drinks sold at different locations within Abakaliki metropolis which indicates that the beverages had similar microbial quantity. This may be due to the fact that similar handling procedures are employed during processing and marketing of the food and due to the fact that some of the microbial species are mesophilic, they can cause spoilage even at refrigeration temperatures (Wojcicki and heyman, 2010). This could be the reason why the bacteria growth in refrigerated zobo drink is often high as reported by many authors (Popkin et al., 1989). Also the range of bacterial count is above the acceptable limit of > 10^4 and the average coliform count is well above the zero value recommended for safety. All the sampled Zobo drinks were contaminated with varying levels of bacterial counts that can be classified as unsatisfactory.

The high microbial counts may be due to a large extent attributed to lack of effective processing of the Zobo drinks, for example contaminants may be due to inadequate access to portable water which is one of the major problems in Abakaliki as a whole or from the utensils used for such purposes. Studies both within and outside Nigeria have shown that *E.coli* and other enteric pathogens including *Salmonella typhi, Shigella* spp and the non-enteric organism; *Staphylococcus aureus* are responsible for many of the global cases of food poisoning (Popkin et al., 1989; Ade-omowaye *et al,* 2006; Armstrong and Kotler, 2009; Ayo and Madaki, 2005).

The percentage occurrence of *E.coli* (75.00 %), *Staphylococcus aureus* (100.0 %), *Pseudomonas Spp ($3.33* %), *Klebsiella pneumonia* (75.00 %) and *Shigella spp* (33.33 %) are pointer to the fact that Zobo drinks sold at different locations within Abakaliki are highly contaminated with potentially pathogenic bacteria and this may be as a result of the type of water used for domestic purposes or the human handling during processing, packaging and sales of the product respectively. This is in agreement with Baker and Riley (1995) who reported earlier that water used for production coupled with the crude method of production and packaging under improper sanitary conditions pre-disposes drinks and foods to microbial contamination by an array of both gram negative and gram positive bacteria. The sources of contamination may also have come from the spices used i.e additives (Collis and Hussey, 2003; Dana, 1999).

There is therefore need for surveillance by public health officials to ensure safety of the Zobo drinks being sold within Abakaliki for public consumption. There is need also to ensure that the water used for the preparation and processing of Zobo is safe and free from microbial contaminants.

The occurrence of antimicrobial resistance bacteria in local beverages is a major public health concern as they could be transmitted to humans through ingestion of contaminated drinks which then contributes to the spread and persistence of antimicrobial resistance bacteria in general population and environment with its associated potential health threats (Dibb and Simkin, 1997). The current investigation also assessed antimicrobial susceptibility of the five bacteria species isolated from Zobo drinks to some selected conventional antibiotics (Table 6). Most of the isolates exhibited varied levels of susceptibility and resistance to the antibiotics. All the isolates were susceptible to Gentamycin, Ciprofloxaxin and Ofloxacin and all the isolates were resistance to Erythromycin, this is in line with the report of (Galguera etal., 2006; Ganguly, 1995*).* E. coli was sensitive to clindamycin, chloramphenicol and tetracycline but resistant to sulphamethazole. *Staphylococcus aureus* showed high sensitivity to vancomycin, clindamycin and chloramphenicol but completely resistance to ampicillin, and had low resistance to tetracycline, and sulfamethazole. *Klebsiella pneumoniae* was completely susceptible to nitrofuratine, high sensitively to sulfamathoxazole but completely resistant to tetracycline. *Shigella spp* is also susceptible to chloramphenicol, equally showing high sensitivity to sulphamethazole and tetracycline. *Pseudomonas aeruginosa* was resistance to erythromycin and sulphamethazole. The investigation conforms to the findings of Gbadamosi, (2007), where similar antibiotics were used.

The presence of isolates that are resistant to more than three antibiotics in the Zobo indicates multiple antibiotics resistances and highlights the potential risk to succesful treatment against infectious diseases associated with the consumption of such drink.

The prevalence of resistant strains of *E. coli,* enteric bacteria such as *(Klebsiella pneumoniae* and *Shigella* spp), *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Zobo drinks with MARI of 0.37, 0.19, 0.13, 0.40, 0.70 respectively was observed. He and Mukherjee, (2007), reported highest MARI of 0.75 and least of 0.55 for various strains of *E. coli*, also this is in agreement with previous report by Hemmington and King (2000). This is a reflection of the use and misuse of the antibiotics in the society. This is not surprising because outside the hospital environment, the global population have access to various kinds of antibiotics at any drug store with or without any prescription from a medical practitioner.

Therefore, regular monitoring of the quality of foods and drinks sold to students and other un-suspected members of the public in this region is required to forestall any imminent health danger. The public health implication of this study is that antimicrobial resistant strains of pathogenic bacteria may colonize the human population through consumption of contaminated ready local beverages and this would lead to chemotherapeutic failures among the human consumers. Food handlers should also be educated and be observant to current public health guidelines in their profession so as to minimize food- borne related illnesses.

5. Conclusion

The presence of pathogenic organism in the food drink, zobo sold at different locations within Abakaliki was significantly high and the isolates exhibited considerable resistance to some tested antibiotics. The public health implication is that antimicrobial resistant strains of pathogenic bacteria may spread amongst the populace through consumption of such contaminated food drinks and this would lead to increase in antibiotics resistance among the human consumers which can in turn spread to non-consumers with time within Abakaliki. Food handlers must be properly guided to minimize such imminent health danger.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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