ISOLATION OF MULTIDRUG RESISTANT BACTERIA FROM TWO LOCALLY PRODUCED DRINKS SOLD IN NORTH CENTRAL, NIGERIA

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ABSTRACT

The predominance of multidrug resistant (MDR) bacteria among the populace, edible foods and drinks is fast becoming the major concern in most communities. Six (6) locally prepared drinks from three locations were aseptically collected and transported to the Microbiology Laboratory of Federal University of Technology, Minna. Samples were serially diluted and were inoculated on various media through the spread plate method. The bacterial isolates were identified based on their Gram reaction and other biochemical tests. The antibiotic susceptibility tests were carried out for the bacterial isolates using the disc diffusion method on Muller Hinton agar. The result revealed that out of all the locally prepared drinks sampled tiger-nut drink (Kunuaya) (3.9×10^3) and Zobo (3.4×10^3) from Federal University of Technology, Minna, Bosso campus had the highest microbial count. Various bacterial pathogens were isolated and identified with *Salmonella* sp having the highest frequency of occurrence (30.7%) and *Klebsiella* sp having the lowest frequency (7.7%). The antibiotic susceptibility tests revealed that all bacterial isolates were Multidrug resistant and as such are a great threat to the health of the general public especially the regular consumers of these locally prepared drinks. Hence, there is a need for adequate and continuous surveillance by food regulatory bodies in Nigeria, to curtail the spread and infections associated with Multidrug resistant bacteria.

Keywords: Locally prepared drinks; Bacteria; Multi-drug resistant bacteria; Zobo; Kunuaya

Introduction

Locally produced drinks are liquids mainly processed from animal or plant sources. They may be regarded as stimulants such as tea, as refreshers such as soft drinks, juices or as nutritional drinks such as milk. The processing of locally produced drinks could either be by simple non-microbial processes or physical techniques (such as malting, boiling, pasteurization and distillation) or may involve microbial process such as fermentation and/or enzyme clarification (Koketso *et al*., 2018; Onuoha & Fatokun, 2014; Umar *et al.,* 2014).

Fermentation by microorganisms, mainly involves the breakdown of sugars to yield acids and then the acids are converted to alcohol. Fermentation is the major processing technique employed in the preparation of over 90% of the diverse locally produced drinks across Africa (Umar *et al.,* 2014). The fermenters and saccharifying enzymes are usually intrinsic to the grains and other ingredients (Koketso *et al*., 2018; Umar *et al.,* 2014).

Locally produced drinks could be classified as either alcoholic (such as burukutu and pito) or non alcoholic (such as kunuaya (tiger-nut milk), kunu-samiya, kunu-zaki, zobo and palm wine) and based on the process involved they could either be regarded as industrially processed beverages or traditionally processed beverages (Kigigha *et al*., 2018).

Locally produced drinks are usually known for their nutritional and therapeutic benefits (Onuoha & Fatokun, 2014), they are basically rich in Vitamins, Minerals and carbohydrates (Umar *et al*., 2014). The additional supplements such as Nuts, spices, Tubers, have

tremendously boosted the protein content and antioxidants properties of most consumed locally produced drinks (Kigigha *et al*., 2018).

Most of these indigenous drinks are usually exposed to certain pathogenic microbes "especially the resistant strains" (Kigigha *et al*., 2018) during the production and packaging of these products. Based on the fact that, these pathogenic organisms are usually associated with the spoilage of the drinks and food borne diseases which lead to severe diseases and deaths, there is need to continuously examine the resistant microbial burden associated with most food and locally produced drinks commonly consumed. Thus this study is therefore said to determine the multi drug resistant (MDR) bacteria associated with locally prepared drinks that are commonly sold in Minna.

MATERIALS AND METHODS

Study Area

The study area was Minna, Niger State. The state is located in the North Central geopolitical zone of Nigeria and covers a landmass of 76,363 square kilometers. It lies between latitude 8°.00-11° .30'N and Longitude 4°.00-8 °.00'E (Kigigha *et al*., 2017).

Sample Collection

A total of six samples for each drink namely: kunuaya and zobo were purchased from three different vendors in three different locations (namely: Bosso market, Federal University of Technology Minna, Bosso campus and El-waziri) in Minna, North central region of Nigeria. The samples were taken to the Microbiology laboratory, for further analysis.

Microbiological Analysis of Sample

Serial dilution of the each drink sample was carried out by suspending one mililitre of the kunuaya and zobo samples into 9mL of sterile distilled water in the test tube. The mixture was shaken thoroughly to ensure proper dissolution of the sample. Spread plate method was employed to inoculate the media. Aliquot of 1mL of the sample was pipetted each from 10^{-4} dilution tubes into well labeled Petri dishes containing a 20mL of molten nutrient agar (this was done in triplicate) and was swirled gently to allow proper mixing. The Petri dishes were later incubated at 37°C for 24hrs. The colonies formed were counted and expressed as colony forming unit per millilitre (cfu/ml). The colonies that grow on the growth media that are different in size, shape and color were picked and sub-cultured on MacConkey agar and SSA (*Salmonella Shigella* agar) to determine the cultural characteristics of the organisms. The pure isolates were preserved on agar slant bottle for further investigations.

Identification of Bacteria

The isolated bacteria were identified via Gram staining and other conventional biochemical tests such as: Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar test as described by Cheesbrough, (2010).

Antibiotic Sensitivity Test

The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disc diffusion method .The colony of each organism was transferred into sterile Mueller-Hilton broth and incubated at 37°C for 24hrs.The overnight culture was adjusted to the turbidity equivalent to 0.5 Mcfarland standard by adding 0.85% sterile normal saline to the overnight culture. The adjusted innocula was subcultured on the surface of Mueller-Hilton agar (MHA) and the antibiotic discs such as: Penicillin G (10µg), Augmentin (30µg), Streptomycin(10µg), Ciprofloxacin(5µg), Nalidixic acid(30µg), Gentamycin (10µg), Ofloxacin (5µg), Chloramphenicol(10µg) and so on (Spencer *et al.,* 2014), were aseptically placed at the center of the MHA plate and incubated at 37°C for 24hours. The zones of inhibition of the bacterial isolates were measured using a transparent ruler as described by Clinical and Laboratory Standard Institute (CLSI), (2016).

Results

Out of all the locally prepared drinks sampled, Kunuaya (3.9×10^3) and Zobo (3.4×10^3) drinks obtained from Federal University of Technology Minna, Bosso Campus had the highest microbial count as seen in Table 1.

Table 1: Microbial count of 2 locally prepared drinks from 3 locations in Bosso Minna.

Locations	Sample	Point A	Point B	Point C
El-Waziri	Kunu aya	2.0×10^3	2.9×10^3	3.0×10^3
	Zobo	3.0×10^3	2.8×10^3	2.5×10^3
Federal University of Technology,Minna, Bosso campus	Kunu aya	3.9×10^3	3.0×10^3	3.1×10^3
	Zobo	3.4×10^3	2.9×10^3	3.0×10^3
Bosso market	Kunu aya	2.9×10^3	2.7×10^3	2.6×10^3
	Zobo	2.7×10^3	2.1×10^3	2.8×10^3

Table 2: Biochemical characteristics of the isolated bacteria

Code	GR	Sh	Coa	Cit	Ure	Oxi	Ct	MR	VP	H2S	Ind	Starch	Suspected Organisms
1	+	C	+	+	+	-	+	+	-	-	-	-	*Staphylococcus* sp
2	-	R	-	+	-	+	+	-	+	NA	+	+	*Pseudomonas* sp
3	+	R	-	+	-	-	+	-	+	-	-	+	*Bacillus* sp
4	+	C	+	+	+	-	+	+	-	-	-	-	*Staphylococcus* sp
5	-	R	-	+	+	-	+	-	+	-	-	+	*Klebsiella* sp
6	-	R	-	-	-	-	+	+	-	+	+	-	*Escherichia coli*
7	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp
8	+	C	+	+	+	-	+	+	-	-	-	-	*Staphylococcus* sp
9	+	R	-	+	-	-	+	-	+	-	-	+	*Bacillus* sp
10	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp
11	-	R	-	-	-	-	+	+	-	+	+	-	*Escherichia coli*
12	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp
13	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp

Key: Isc (Isolate code), GR (Gram Reaction), Sh (Shape), Ct (catalase), Cit (Citrate), H2S (Hydrogen Sulphide), MR (Methyl Red), VP(Voges Proskauer),Ure (Urease), Oxi (Oxidase), Ind (Indole), Glu (Glucose), Coa (Coagulase), C (Cocci), R (Rod), + (Positive), - (Negative) , NA (Not applicable).

Six (6) bacterial isolates were obtained with *Salmonella sp* having the highest frequency of occurrence of 30.7% while *Pseudomonas aeruginosa* and *Klebsiella sp* had the least frequency of occurrence of 7.7% respectively (as seen in Table 3).

Table 3: Frequency of occurrence of bacterial isolate

Organisms	Frequency of occurrence	Percentage of occurrence
Staphylococcus sp	3	23.1
Salmonella sp	4	30.7
Pseudomonas sp	1	7.7
Escherichia coli	2	15.4
Bacillus sp	2	15.4
Klebsiella sp	1	7.7
Total	13	100

Eight (8) bacterial isolates out of thirteen (13) bacteria isolates were obtained from kunuaya, while five (5) bacteria isolates were obtained from Zobo. Samples from Elwaziri area had the highest bacterial contamination with 6 bacterial isolates followed by Samples from FUT Minna, Bosso campus with 4 bacterial isolates and then samples from Bosso Market with 3 bacterial isolates (as seen in Table 4).

Table 4: Sources and location of the bacterial isolates

Bacterial isolates	Number of isolates	Sources of isolates	Location
Staphylococcus sp	3	Kunuaya (2 isolates)	El-waziri
		Zobo(1 isolate)	Bosso campus
Salmonella sp	4	Kunuaya (2 isolates)	Bosso market
		Zobo(2isolate)	El-waziri
Pseudomonas sp	1	Zobo	Bosso campus
Escherichia coli	2	Kunuaya (2 isolates)	Bosso campus
			El-waziri
Bacillus sp	2	Kunuaya (1 isolates)	Bosso market
		Zobo(1 isolate)	El-waziri
Klebsiella sp	1	Kunuaya	Bosso campus

Table 5.1: Susceptibility profile for Gram positive bacteria

Bacterial isolate	Number of isolate	Z(%)	AMP(%)	R(%)	PEF(%)	CN(%)	CPX(%)	APX(%)	SXT(%)	E(%)	S(%)
Staphylococcus aureus											
S	3	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)
I		0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	0(0)	2(66.6)	0(0)	0(0)
R		3(100)	3(100)	2(66.6)	2(66.6)	3(100)	3(100)	3(100)	0(0)	3(100)	3(100)
Bacillus sp											
S	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)
I		0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	1(50)	0(0)	0(0)
R		2(100)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	0(0)	2(100)	2(100)

Key: R= resistance, S=susceptible, I=intermediate, CN=Gentamycin, CPX=Ciprofloxacin, APX=Ampiclox, SXT=Septrin, PEF=Perfloxacin, R=Rifampicin, AMP=Ampicillin, E=Ethromycin,S=Streptomycin,Z=Zithromax

Table 5.2: Susceptibility profile for Gram negative bacteria

Bacterial isolate	Number of isolate		SP(%)	PEF(%)	OFX(%)	CPX(%)	CH(%)	S(%)	AU(%)	CN(%)	AM(%)	SXT(%)
Pseudomonas sp	1	S	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		I	0(0)	1(100)	(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	(0)
		R	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Escherichia coli	2	S	0(0)	0 (0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		I	0(0)	1(50)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)
		R	2(100)	1(50)	0(0)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)
Klebsiella sp	1	S	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		I	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)
		R	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)
Salmonella sp	4	S	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		I	0(0)	0(0)	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)	2(50)
		R	4(100)	4(100)	3(75)	4(100)	2(75)	4(100)	4(100)	4(100)	4(100)	2(50)

Key: R= resistance, S=susceptible, I=intermediate, CN=Gentamycin, CPX=Ciprofloxacin, CH=Chloramphenicol, SXT=Septrin, PEF=Perfloxacin,OFX=Ofloxacin, AM=Amoxacillin, SP=Sparfloxacin,S=Streptomycin,AU=Augmentin

DISCUSSION

This study reveal that kunuaya (3.9×10^3) and zobo (3.4×10^3) from F.U.T Minna, Bosso campus are highly contaminated with disease causing microorganisms, as seen in Table 1 . This could be based on the unhygienic packaging associated with most of these drinks, which introduces high microbial contaminants to the drinks before they are consumed. Similarly poor or inadequate use of personal protective wears by the manufacturers of these drinks exposes these locally prepared drinks to high microbial contamination. This result agrees with the findings of (Adesakin and Obiekezie, 2020; Ayandele, 2015) who revealed that improper use of protective wears by manufacturers enhances the introduction of microbial contaminants into the drinks.

The study also revealed that *Salmonella sp.* had the highest frequency of occurrence (30.7%) followed by *Staphylococcus aureus* (23.1%). This could be due to the fact that certain production materials of these locally prepared drinks such as water usually harbor large populations of faecal coliforms (from either human or animal sources) and environmental wastes. This finding agrees with (Musa *et al.*, 2018) who revealed that most locally prepared drinks analysed, were highly contaminated by faecal coliforms.

The high bacterial contamination recorded in kununaya compared to zobo drink (as seen in Table 4) could be based on the fact that the raw materials for kunuaya, which are tiger nuts are easily prone to microbial contamination during their growth and harvest in the fields and this usually exposes the tiger nut milk (kunuaya) to heavy contamination with various microbes. Similarly milling machine used to mill the tiger nuts are usually for commercial purpose and in most cases are usually unclean and heavily contaminated with bacteria, which in turn contaminates the tiger nut milk (kunuaya) after the tiger nuts have been milled. However the high contamination of various bacteria observed in locally prepared drinks obtained in Elwaziri area (as seen in Table 4) could be attributed to certain factors such as low personal hygiene of the populace in that area and improper disposal of waste by the populace in that area. This agrees with the finding of (Ayandele, 2015) who revealed that most raw materials used for local drinks are edible roots of crops and hence are prone to microbial contamination.

This study revealed that all bacterial isolates (namely the Gram positive and Gram negative) were all multidrug resistant (Table 5 and 6). This could be attributed to the fact that most bacterial prevalent among the populace or within the study area exhibited multidrug resistant due to the rapid dissemination of the resistant gene's through various genetic transfer material such as plasmids.

Conclusion and Recommendation

This study revealed that two commonly consumed local drinks, namely; kunuaya (tiger nut milk) and zobo are highly contaminated with various bacteria such as: *Staphylococcus sp*, *Salmonella sp*, *Pseudomonas sp*, *Escherichia coli*, *Bacillus sp* and *Klebsiella sp*. However all these bacterial contaminants are multi drug resistant, thus there is an eminent need for Government and food monitoring agencies to enlighten and encourage producers and vendors of these locally prepared drinks, on the importance to employ adequate hygienic standards in the production and packaging of these locally prepared drinks to ensure that bacterial contaminants, especially multi drug resistant bacteria are curtailed and controlled.

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