



## Research Article

# Antimicrobial Susceptibility Test of *Salmonella* Species Isolated from Two Drinks (*Zobo* and *Kunun-Aya*) Sold at University of Maiduguri, Campus, Borno State, Nigeria

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### ABSTRACT

The research was conducted to isolate and conduct an antimicrobial susceptibility test of *Salmonella species* from *Zobo* and *Kunun-aya* drinks sold at the University of Maiduguri, Borno State, Nigeria. A total of 150 samples were collected from different sales points using a systematic sampling technique. The samples were analysed using standard bacteriological procedures and antimicrobial susceptibility tests using the Kirby-Bauer disc diffusion technique. The results showed that 38(25.3%) samples were positive for *Salmonella*. *Kunun-aya* drink has 28(37%), and *Zobo* has 5(6.7%) of the *Salmonella* isolates, which indicated that *Kunun-aya* had the highest level of contamination than *Zobo* drink. The *Salmonella* isolates were sensitive to Tarivid 18(24%), Ciprofloxacin 18(24%), Gentamycin 18(24%), and Ampillicin 15(20%). It was found resistant to Nalidixic acid 14(19%), Augmentin 14(19%) and Seprin 9(12%). The contamination observed in the *Kunun-aya*, and less contamination in *Zobo* drinks may be due to the product used and improper sanitary conditions during its processing. The presence of *Salmonella* in the beverages may pose serious public health concerns as some of this bacterium is implicated in causing foodborne diseases, thus the need to develop Good Manufacturing Processes (GMP) and post-production preservation and packaging techniques.

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## INTRODUCTION

A homemade drink has become part of the indigenous drinks in every society worldwide. They are produced in different ways and served on special occasions to people of various tribes and traditions in Nigeria (Umar *et al.*, 2016; Jane *et al.*, 2018)

Some of these drinks are prepared by fermentation, a widely practised ancient technology. These fermented foods are an essential part of the diet in all regions of the world (Umar *et al.*, 2016; Jane *et al.*, 2018). Non-alcoholic drinks such as *Zobo* and *Kunun-aya* (tiger nut drinks) are among the recent most popular traditional food drinks which are very important in the dietary patterns of people in the Northern part of Nigeria, especially Maiduguri, Borno State (Adejuyitan, 2011; Umar *et al.*, 2016). The non-alcoholic nature of the drinks makes them readily consumed by all religions and cultures as a substitute for an alcoholic drink (Adegaz, 2002). *Zobo* drink is a local, cheaper soft drink made from the calyx of Roselle (*Hibiscus sabdariffa*). The drinks have become a household name in Nigeria since it is affordable among low-income earners. Other people use it during social occasions such as naming ceremonies, weddings, and birthdays as refreshing drinks (Umar *et al.*, 2016; Jane *et al.*, 2018).

It is prepared in small quantities for family use as a source of energy and quenches thirst. *Zobo* is thus a drink available in many local stores, marketplace, campuses, and local soft drinks. Since it does not require special procedures, it is obtained from a local water source such as boreholes and sometimes well water is used in the preparation (Umar *et al.*, 2016; Jane *et al.*, 2018). The condition results in microbial contamination of the product coupled with the contamination that may result from handling (Umar *et al.*, 2016; Jane *et al.*, 2018). The bacterial species commonly found in *Zobo* drinks include *pseudomonas* species, *Escherichia coli*, *Staphylococcus* species and *Bacillus* species (Jane *et al.*, 2018; Dulo, 2017). *Kunun-aya* (tiger nut drink) in Nigeria and

(Horchata de chufa) in Spain are naturally sweet, creamy, and offer a luxuriously rich and nutty flavour. Nigeria and Spain generally love tiger nut drinks or tiger nut milk. Tiger nut drink is rich in minerals, including calcium, iron, magnesium, potassium, and phosphorus. They are rich in vitamin C and E and also rich in monounsaturated fat (Ameya, 2019).

Tiger nut drink and *Zobo* drink are traditional foods in Nigeria and are a substrate for microbe growth and proliferation (Umar *et al.*, 2016; Ameya, 2019). The types of microorganisms present in food and the environmental conditions are also very important considerations of food quality. However, foods determine what microbe can or cannot grow. Nutrients of food play an important role in the kinds and number of microorganisms that grow on them because of the great organic compound in food and the numerous kinds of microorganisms that decompose the drinks (Onvo and Ogaraku, 2007; Umar *et al.*, 2016)

However, like any other non-alcoholic locally brewed drink, microbial contaminants are not always underestimated due to the unhygienic condition of preparation. Hence, microbial isolates reported associated with those drinks include *Lactobacillus* species, *Bacillus* species, and *Candida* species and include *Salmonella* species, *Escherichia coli*, *Clostridium* and *Enterobacteria* species (Risiquat, 2013; Jane *et al.*, 2018).

Foodborne salmonellosis has remained a neglected zoonosis in Nigeria and other developing countries, despite an upsurge of cases reported in *Kunun-aya* and *Zobo* drinks which are, increasingly becoming important sources of human infection with *Salmonella* (Umar *et al.*, 2016) lot vehicles implicated in most foodborne disease outbreaks are often consumed or discarded before clinical symptoms develop in an exposed individual (Karshima *et al.*, 2013). The screening of *Kunun-aya* and *Zobo* drinks against pathogenic organisms will play a role in curtailing human infections. This study determined the presence of *Salmonella* and antimicrobial susceptibility test of isolates in *Kunun-aya* and *Zobo* drinks.

## MATERIALS AND METHODS

### Study area

The study was conducted at the University of Maiduguri campus, Borno state.

### Sample collection:

A total of 150 Zobo and *Kunun-aya* (tiger nut) drinks samples were collected from the University of Maiduguri campus. All the samples were purchased from sellers and transferred into sterile containers, labelled and placed in an ice block cooler before being transported to Veterinary Microbiology Laboratory, Department of Veterinary Microbiology, University of Maiduguri and analysed within three hours.

### Isolation and identification of *salmonella*

The isolation of *Salmonella* was carried out using the method described by Quin *et al.* (2002). One millimetre each of Zobo and tiger nut drinks were enriched into 9ml of Rappaport-Vassiliadis soya peptone broth and incubated at 37°C for 24 hrs. A loopful of Rappaport-Vassiliadis broth was streaked onto Xylose Lysine Desoxycholate agar (XLD) at 37°C for 24 hrs. The colonies were examined for the characteristics and colour of *Salmonella* with or without hydrogen sulphide. Suspected colonies are subjected to gram staining and biochemical tests.

### Gram staining

The method used was described by (Cheesbrough, 2006); a smear of the isolate was prepared and heat-fixed on a clean, grease-free slide. The smear was stained for 1 minute with crystal violet. This was washed out with running tap water. The slide was flooded with dilute iodine solution. This was washed off with water and the smear was decolourized with 95% alcohol till the blue colour no more dripped out, the smear was then counterstained and stained with safranin solution for about 10 seconds. Finally, it was washed with gentle running water, air-dried and observed under oil immersion objective.

### Biochemical test of *salmonella*

The following biochemical tests were employed to characterize the suspected *Salmonella* isolates further.

#### Citrate utilization Test

An inoculation needle was used to inoculate Simmons citrate agar slant prepared in a bijou bottle and incubated at 37°C for 24 to 72 hours. The development of deep blue colour indicated a positive reaction, while negative results indicate no change in colour (Cheesebrough, 2000)

#### Triple Sugar Iron Test

The triple sugar iron test was made in such a way that there are slants and the butts with a sterilized straight inoculation needle, the isolated colony was picked from the solid media and stabbed the TSI up to the butt and then streaked the surface of the agar slant. The cap was loosed and incubated at a temperature of 37°C for 24 hours. After the incubation, the black precipitate was observed on the TSI and also reddish or yellow colouration signifies acidic or basic utilization. The black colour on the butt indicates H<sub>2</sub>S was produced. The development of black precipitate and gas at the butt indicated the presence of *Salmonella* species (Cheesbrough, 2006; Umar *et al.*, 2016)

#### Motility Test

The inoculation needle was used to inoculate a motility agar by stabbing about two-thirds down and then pulled the needle up in the same path. It was incubated at 37°C for 24-48 hours. The appearance of the cloudy tube with organisms spread over the top of the media indicates motile, while non-motile organisms grew along the streak line only, the media showed no cloudy appearance (Cheesbrough, 2006, Umar *et al.*, 2016, Nworie *et al.*, 2016)

#### Catalase Test

A loopful of pure colony was transferred into a plain, clean glass slide; the sample was then mixed with a drop of 3% v/v hydrogen peroxide. The reaction was observed immediately. The gas product indicated by the

production of gas bubbles confirmed the presence of catalase (Nworie *et al.*, 2016)

#### **Oxidase Test**

A freshly prepared reagent of 0.2g of tetraphenylenediamine hydrochlorides in 20 ml of distilled water was poured over inoculated agar plate and incubated for 24 hours, the excess was immediately poured off, and positive colonies turned bluish-purple (Nworie *et al.*, 2016)

#### **Antibiotic Susceptibility Test of *Salmonella* isolates**

The sensitivity of the pure culture of bacterial isolates to different antibiotics was determined

## **RESULTS**

The results from the antimicrobial susceptibility test of *Salmonella* isolates from two drinks (Zobo and kunun-aya) are shown in Table 1, which indicates that out of one hundred and fifty samples analysed for the presence of *Salmonella*, only 38(25.3%) were positive using biochemical tests that revealed that the isolates were catalase positive, oxidase negative, citrate positive, triple sugar iron (TSI) positive and motility positive. The typical colonial morphology of *Salmonella* on XLD agar showed red colonies with black centres (Fig1). Developing a deep blue colour indicated a positive citrate reaction, while the negative result showed no green colour change (Figure 2). The appearance of cloudiness and thin spreading filaments showed or confirmed the motility of the organism, and black strands or

using the Kirby-Bauer diffusion technique and interpreted based on the guidelines of Clinical and Laboratory Standard (Stella *et al.*, 2018). Commercially prepared antimicrobial disc (Optum Diagnostic, Nigeria) was used in the Muller-Hinton agar plates after seeding the media with suspected *Salmonella* isolates. These were incubated at 37°C for 24 hours, after which zones of clearance or inhibition were read (Adenaike *et al.*, 2016; Stella *et al.*, 2018)

filaments also confirmed that some *Salmonella* could produce hydrogen sulphide (H<sub>2</sub>S)(Fig3). Figure 4 showed a test on triple sugar iron, which indicated reddish or yellow colouration signifies acidic or basic utilization, whereas the gap spacing of the medium signifies gas formation. The clear zone of inhibition is indicated in Figure 5, while no zone of inhibition is shown in Figure 6.

Table 2 showed the inhibition zone diameter of the *Salmonella* isolated from *Kunun-aya*, where most of the antibiotics tested against them were susceptible, with little resistance to Ciprofloxacin and Gentamycin.

Table 3 shows the inhibition zone diameter of *Salmonella* isolates susceptible to Pefloxacin and Cephrorex while it is relatively resistant to Gentamycin and Augmentin.



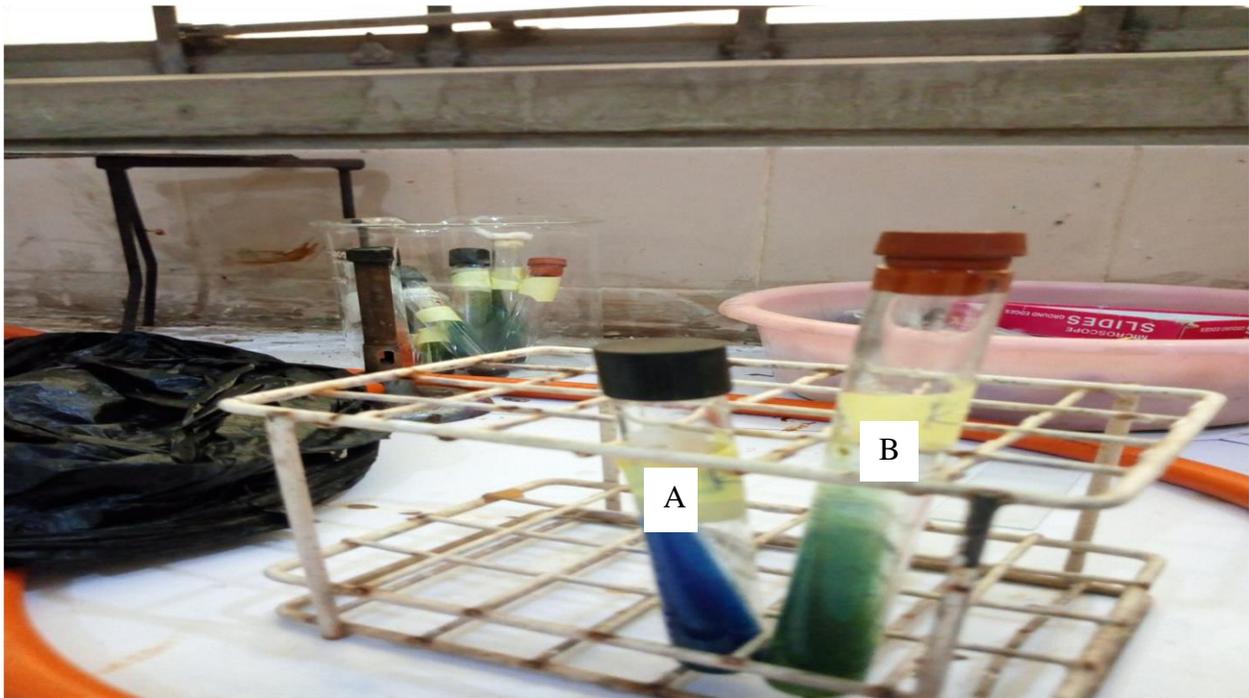
**Figure 1:** shows colonial morphology of *salmonella* on XLD agar

**Table 1:** Biochemical characterization of salmonella isolates from both *Kunun-aya* and *Zobo* drinks (n = 38)

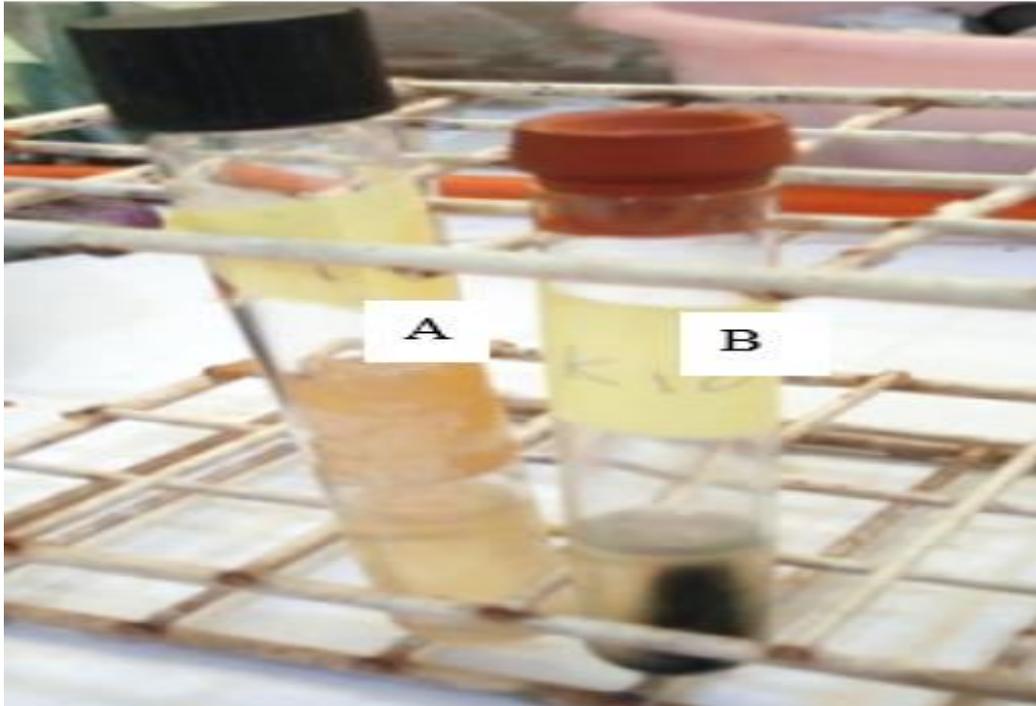
Samples	Gram stain	CatOxi	Citrate	TSI	Motility	Remarks
K1	-ve	+	+	+	M	<i>Salmonella</i> isolated
K2	-ve	+	+	+	M	<i>Salmonella</i> isolated
K3	-ve	+	+	+	M	<i>Salmonella</i> isolated
K4	-ve	+	+	+	M	<i>Salmonella</i> isolated
K5	-ve	+	+	+	M	<i>Salmonella</i> isolated
K6	-ve	+	+	+	M	<i>Salmonella</i> isolated
K7	-ve	+	+	+	M	<i>Salmonella</i> isolated
K8	-ve	+	+	+	M	<i>Salmonella</i> isolated
K9	-ve	+	+	+	Nm	<i>Salmonella</i> suspected
K10	-ve	+	+	+	M	<i>Salmonella</i> isolated
K11	-ve	+	+	+	M	<i>Salmonella</i> isolated
K12	-ve	+	+	+	M	<i>Salmonella</i> isolated
K13	-ve	+	+	+	M	<i>Salmonella</i> isolated
K14	-ve	+	+	+	Nm	<i>Salmonella</i> suspected
K15	-ve	+	+	+	Nm	<i>Salmonella</i> suspected
K16	-ve	+	+	+	M	<i>Salmonella</i> isolated
K17	-ve	+	+	+	M	<i>Salmonella</i> isolated
K18	-ve	+	+	+	M	<i>Salmonella</i> isolated
K19	-ve	+	+	+	M	<i>Salmonella</i> isolated
K20	-ve	+	+	+	M	<i>Salmonella</i> isolated
K21	-ve	+	+	+	M	<i>Salmonella</i> isolated
K22	-ve	+	+	+	M	<i>Salmonella</i> isolated
K23	-ve	+	+	+	M	<i>Salmonella</i> isolated
K24	-ve	+	+	+	M	<i>Salmonella</i> isolated
K25	-ve	+	+	+	M	<i>Salmonella</i> isolated
K51	-ve	+	+	+	M	<i>Salmonella</i> isolated
K52	-ve	+	+	+	Nm	<i>Salmonella</i> suspected

K54	-ve	+	+	+	+	Nm	<i>Salmonella</i> isolated
K57	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
K59	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
K60	-ve	+	+	+	+	Nm	<i>Salmonella</i> suspected
K64	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
K67	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
Z1	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
Z5	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
Z55	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
Z59	-ve	+	+	+	+	M	<i>Salmonella</i> isolated
Z67	-ve	+	+	+	+	M	<i>Salmonella</i> isolated

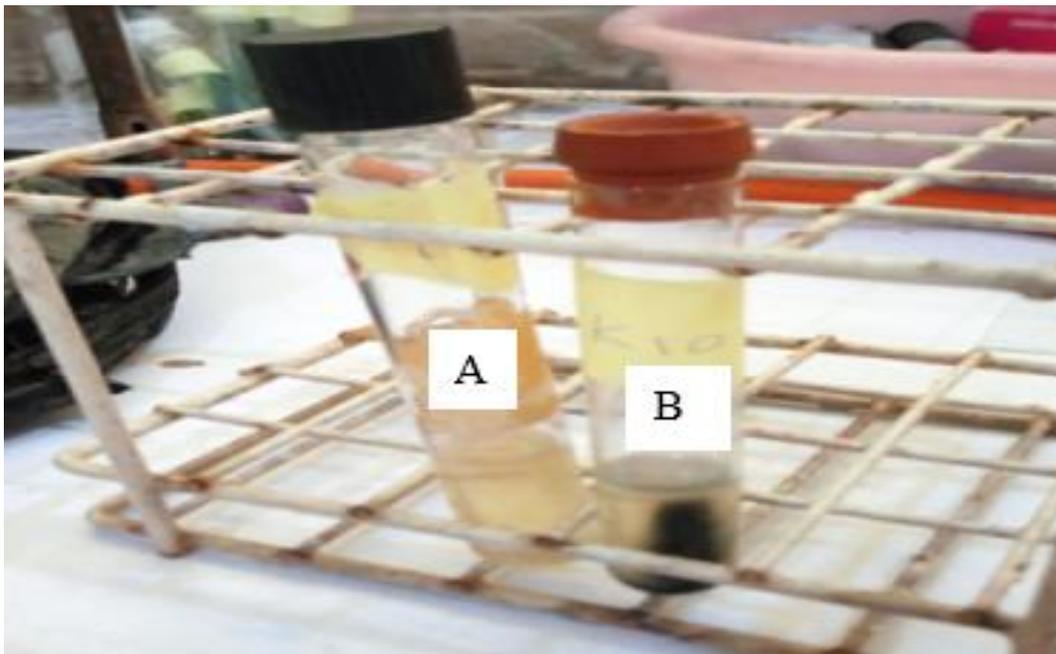
Key: GS = Gram Stain, SIm = Sulphide Indole Motility Test, Cit = Simon's citrate Test  
 TSI = Triple Sugar Iron Test, Cat = Catalase Test, Oxi = Oxidase Test; M = Motile, Nm = Non-motile



**Figure 2:** Citrate positive indicated with blue (A) and negative indicated green color with (B).



**Figure 3:** showed the cloudy appearance of the organism and the black filament indicating H<sub>2</sub>S



**Figure 4:** showed A Triple Sugar Iron negative and B is TSI positive.



**Figure 5:** shows the zone of clear inhibition.



**Figure 6:** shows no zone of inhibition.

**Table 2:** Antibiotic susceptibility patterns of the isolates from *Kunun-aya* (N = 33)

Antibiotics	Highly susceptible		Intermediate Susceptible		Resistant	
	N	%	N	%	N	%
Tarivid	18	24	7	9	7	9
Ceprrorex	9	12	8	11	11	15
Gentamycin	18	24	12	16	5	7
Augmentin	6	8	14	19	12	16
Nalidixic acid	5	7	15	20	14	19
Ciprofloxacin	18	24	10	13	3	4
Streptomycin	10	13	10	13	11	15
Peflacine	14	19	9	12	9	12
Septin	11	15	12	16	9	12
Ampicillin	15	20	11	15	14	19

Table 2 shows that majority of the isolates were susceptible to most of the antibiotics tested against them, with little resistance to Ciprofloxacin (4.0%) and Gentamycin (7.0%), as per zone of inhibition

**Table 3:** Antibiotic susceptibility pattern of isolates from *Zobo* drink (N=5)

Antibiotics	Highly susceptible		Intermediate Susceptible		Resistant	
	N	%	N	%	N	%
Tarivid	5	7	0	0	0	0
Ceprrorex	4	5	1	1	0	0
Gentamycin	3	4	1	1	1	1
Augmentin	2	3	2	3	1	1
Nalidixic acid	0	0	3	4	2	3
Ciprofloxacin	5	7	1	1	0	0
Streptomycin	2	3	2	3	1	1
Peflacine	5	7	0	0	0	0
Septin	3	4	1	1	1	1
Ampicillin	2	3	2	3	1	1

Table 3 shows that the *salmonella* isolates from *Zobo* were susceptible to the most antibiotics tested against them, high resistant to Nalidixic acid 2(3%), Ampicillin 1(1%), Gentamycin 1(1%), Septin 1(1%) and Augmentin1 (1%), as per zone of inhibition.

## DISCUSSION

*Salmonella* are a common bacterial pathogen implicated in most food-borne infections. The organism causes salmonellosis, and it is also the leading cause of salmonellosis which can be transmitted through the food chain, especially in locally prepared drinks where proper hygiene and infection control practices are not observed (Ifeanyichukwu *et al.*, 2016). Out of the 150 samples of *Kunun-aya* and *Zobo* drinks

analysed, only 38 (25.3%) were positive for *Salmonella*. *Zobo* drink has 5(6.7%), and *Kunun-aya* has 28(37%) of the *Salmonella* isolates, which indicated that *Kunun-aya* was found to record high level of contamination than the *Zobo*. This level of contamination of the drinks might be due to the addition of some ingredients and processing methods. Ingredients such as sweetening agents and spices have been added to provide flavouring

for foods and beverages worldwide (Umar *et al.*, 2016). However, the study conducted by Umar *et al.* (2016) showed a 100% contamination of the *kununzaki* and *Zobo* drinks. The contamination may develop as a result of poor handling, processing and storage, and faecal contamination. This study indicated that the tap water used, spices, and flavour may be the source of contamination as reported by Abitoye *et al.*, 2017; Mayo clinic, 2018).

The production of *Kunun-aya* and *Zobo* drinks is still at the local level, where low sanitary practice during preparation and sales accounts for poor quality and a cause for concern (Adenaike *et al.*, 2016; Risiquat, 2013). However, the most significant limitation of the production of *Kunun-aya* and *Zobo* is the rapid deterioration of the drinks, its short shelf-life allows for the proliferation of associated microorganisms (Zumbeset *et al.*, 2014; Risiquat, 2013). Furthermore, the mode of packaging and dispensing of the drinks in bottles and plastic containers before retailing and post-processing handling practices are attributed to the contamination of the drinks (Musa and Hamza, 2013). Therefore, the presence of *Salmonella* in the drinks is linked to poor sanitary conditions during preparations, storage and personal hygiene of the drink handlers (Risiquat, 2013; Ghouhan, 2015; Zumbes, 2017). The presence of *Salmonella* in the present study agrees with the findings of Amusa *et al.* (2005) reported that despite the popularity of the local drinks, it had been associated with peculiar problems such as non-consistency in quality and short shelf life. Morphologically, *salmonella* on xylose lysine deoxycholate agar (XLD) produces red colonies with a black centre. The black centre was an insoluble black precipitate of ferrous sulphide, which agrees with (Amusa *et al.*, 2005; Aryal, 2018). This showed that the majority of the isolates are *Salmonella*.

In the biochemical characterization of the isolates after preliminary identification by microscopy and cultural appearance, the isolates were citrate positive, catalase-positive, triple sugar iron (TSI) positive and sulphide indole motility (SIM) positive. The appearance of cloudiness and thin spreading filaments in the sulphide indole motility test showed that the isolates are motile; At the same time, some are non-motile with black thread, indicating that majority of the isolates were confirmed to be *Salmonella* which is known to be motile and produce hydrogen sulphide gas (Umar *et al.*, 2016; Maduka and Ire, 2019). The contamination might have developed due to poor handling, processing and storage, and faecal contamination from human and domestic origin. This is in line with the findings of (Risiquat 2013; Adenaike *et al.*, 2016).

Contamination of *Kunun-aya* may occur at any point since the drink, or the tiger nut is not boiled or heat-treated before preparation, and *Zobo* drinks may also be contaminated during cooling of the hot extract, addition of flavours and sweeteners or dispensing of the extracts into nylons and bottles (Risiquat, 2013; Ifeanyichukwu *et al.*, 2016). Water used in processing has been identified as a major source of contamination. The distribution patterns and cooling of the drinks also introduced some microbes into the drinks since ice blocks, when melted, become contaminated with microorganisms such as *Salmonella* (Umar *et al.*, 2016).

The antimicrobial susceptibility test of the *Salmonella* isolates from *Kunun-aya* drinks was found to be highly sensitive to Tarivid 18 (24%), Gentamycin 18 (24%), and Ciprofloxacin 18(24%), Ampicillin 15 (20%). In comparison, it is relatively resistant to Ceprorex 0(0%), Ciprofloxacin 3 (4%), Gentamycin 6(8), and Nalidixic acid 5(7%). The isolates from *Zobo* showed sensitivity to Tarivid 5(7%), Ciprofloxacin 5(7%), ceprorex

4(5%) and Septrin 3(4%), while resistant to Nalidixic acid 2(3%) and Ampillicin 1(1%) (Adenaiké *et al.*, 2016; Ifeanyichukwu *et al.*, 2016).

### CONCLUSION

*Salmonella* isolates obtained from the drinks suggested that *Kunun-aya* were highly contaminated by *Salmonella*, while *Zobo* drink was less contaminated. The *Salmonella* isolates from *Kunun-aya* and *Zobo* drinks were susceptible to most antibiotics such as tarivid, gentamycin, ciprofloxacin and ampicillin. At

the same time, it is resistant to ciprofloxacin, gentamycin and peflaccine. Examination of local drinks such as *Kunun-aya* and *Zobo* for human consumption on campus is critical to ensure the safety of the drinks and to safeguard students' health since the presence of *Salmonella* is involved in severe health implications causing foodborne diseases; thus, the need to develop good manufacturing process (GMP) and post-production preservation and packaging techniques.

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