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Bacteriological assessment of selected locally processed beverages vended within a tertiary institution in Ogun State

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Abstract

Beverages are food grade liquids commonly processed from plants or animal sources, and they constitute a major diet in African households. The aim of this study was to determine the bacteriological safety of selected locally processed beverages vended within a tertiary institution in Ilishan Remo, Ogun State. Twenty-two beverage samples comprising of *Hibiscus sabdariffa* drink (*zobo*; n=8), ginger drink (n=7) and *kunu zaki* (n=7) were purchased from different sales points on the campus of a tertiary institution in Ogun state. Bacterial contaminants in the samples were isolated on a set of bacteriological agar and potential pathogenic species were presumptively identified using the selective-differential media approach. A total of 57 enterobacteriaceae isolates were obtained from the samples and identified as *Escherichia coli* (n=29) and *Klebsiella* spp. (n=28) The presence of these enterobacteriaceae reveals the potential food safety risks associated with the consumption of the poorly processed and handled beverages. Large scale surveillance studies are thus imperative for these widely consumed locally processed beverages. The processors of these beverages require urgent food processing and personal hygiene training in other to reduce potential risks such contamination may pose to consumer health.

Keywords: *Escherichia coli*, food safety, ginger drink, *kunu zaki*, *zobo*.

Introduction

Beverages are food-grade liquids commonly processed from plants or animal sources (Ezekiel *et al*., 2018). They are widely consumed in African households and could be categorized into industrially or traditionally processed depending on the type of processing methods adopted in their production. They can also be classified as alcoholic or non-alcoholic (Tafere, 2015; Ezekiel *et al*., 2018).

In Nigeria, traditional beverages are becoming the choice drinks over commercial soft drinks for individuals from the low-income settings due to lower price, higher nutritional contents, perceived

health benefits, and thirst-quenching properties (Ezekiel *et al*., 2015; Nworie *et al*., 2016). Ginger drink, *kunu zaki* and *zobo* are three commonly vended non-alcoholic traditionally processed beverages in Southwest, Nigeria due to their ease of production and acceptability by consumers. However, these beverages are prone to microbial contamination by foodborne pathogens because they are usually processed and packaged in rural households and often by individuals who lack knowledge about good hygiene and standard food processing practices. There have been reports on contamination of non-alcoholic beverages by potential foodborne pathogens such as *Escherichia coli*, and species of *Shigella* and *Salmonella* (Oshoma *et al*., 2009; Sharma, 2013). Although studies have assessed the microbiological quality of locally made beverages (*kunu zaki* and *zobo*) in Anambra and Oyo states of Nigeria (Amusa *et al*., 2005; Onuorah *et al*., 2014), there are sparse reports on the bacteriological safety assessment of ginger drink and the other two locally made beverages consumed in Ogun state.

Considering the high demand for the traditional beverages and the potential public health risk that could arise if foodborne pathogenic bacteria are ingested *via* these drinks, there is a need to routinely monitor and assess the safety of ginger drinks, *kunu zaki* and *zobo* in order to ensure consumer safety. This study therefore aimed to assess the bacteriological safety of selected locally processed beverages vended within a tertiary institution marked with high consumer population in Ilishan Remo, Ogun state.

Materials and methods

Beverage sample collection

A total of 22 samples of ginger drink (n=7), *kunu zaki* (n=7) and sorrel drink (*zobo*; n=8) were purchased from various food retail outlets situated on the main campus of a tertiary institution in Ogun state. All the samples were purchased cold (~10ºC) as packaged in 50cl plastic bottles in the month of February 2018. The samples were properly labelled and immediately transported in cold ice boxes to the Microbiology laboratory, Babcock University for analysis. Samples were analysed after bringing them to ambient temperature.

Bacteriological examination of the beverage samples

Isolation of bacteria

The serial dilution method of the American Public Health Association (APHA) (1992) was adopted in the isolation of foodborne bacteria from the beverages. Briefly, each beverage sample was carefully agitated by hand shaking and 1 ml of the beverage was added to 9 ml of sterile peptone water. The mixture was then homogenized using a vortex mixer and serially diluted 10-fold. Aliquots (1 ml) from the dilutions were pour-plated in Petri dishes and carefully overlaid separately with molten nutrient agar (Lab M Ltd, Heywood, Lancashire, United Kingdom) and MacConkey agar (Lab M Ltd, Heywood, Lancashire, United Kingdom). The inoculated plates were incubated at 37 ˚C for 24 hours (APHA, 1992).

Enumeration and identification of bacteria

The total aerobic counts (TAC) of the beverage samples were enumerated using the 24 hour-incubated nutrient agar plates while the total enterobacteria counts (TEC) were scored using 24 hour-incubated plates of MacConkey agar. All colonies of lactose fermenters on the enumerated MacConkey agar plates were purified on freshly prepared eosin methylene blue (EMB) agar (Lab M Ltd, Heywood, Lancashire, United Kingdom) by a repeated sub culturing. All EMB agar plates were incubated at 37 ˚C for 24 hours (APHA, 1992). Colonies with characteristic metallic green sheen on EMB were identified as *E. coli* while all other colonies were further subjected to biochemical tests for presumptive identification (Barrow and Feltham, 1993). All the isolates belonging to *E. coli* were transferred to freshly prepared Sorbitol MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, England) and incubated at 37 ˚C for 24 hours in order to presumptively identify the O157 strain (Hessain *et al*., 2015).

Enzyme and haemolytic assays of the foodborne bacteria

Amylase test

The method of Clark and Cowan (1952) was adopted in determining the ability of foodborne bacteria to utilize starch via the production of the enzyme amylase on starch agar. The presence of a zone of clearing after incubation of inoculated starch agar plates at 37 ˚C for 24 hours and consequent flooding with iodine solution revealed the ability of the isolate to utilize starch.

Casease test

The method of Larone (1993) was adopted in determining the ability of bacteria to hydrolyse caesin. Nutrient agar supplemented with 10g of skimmed milk (Marvel dried skimmed milk) was prepared according to manufacturer's instruction. The presence of a zone of clearing after incubation of inoculated agar plates at 37 ˚C for 24 hours revealed the ability of the isolate to utilize caesin.

Haemolytic assay

All isolated bacteria were screened for hemolytic potential on blood agar (2.8 g/100 mL Nutrient agar and 5 mL/100 mL human blood free from antibiotics for 60 days) incubated aerobically at 37^{o}C for 24 hours for haemolysis.

Results and discussion

A total of 57 isolates (45.2%) belonging to the enterobacteriaceae were obtained from the beverage samples. In all the beverage types, the TAC (1.0×10^5 – 10.0×10^5 CFU mL^{-1}) were higher than the TEC (1.4×10^4 – 2.0×10^4 CFU mL^{-1}) (Table 1). This finding agrees with the reports of Ayandele (2015) and Asuquo *et al*. (2017) who reported higher TBCs than TECs in *kunu zaki* and *zobo* drinks. Specifically, the range of TBC reported by Ayandele (2015) in *zobo* (1.5×10^5 – 10.0×10^5 CFU mL^{-1}) vended on the campus of a tertiary institution in Oyo state, was similar to our findings for the same beverage.

The TBC and TEC observed in the three types of beverages studied exceeded the stipulated local and international standards of 10^2 CFU mL^{-1} (NAFDAC, 2004; WHO, 2006). This suggests poor food processing and handling, both of which could be traced to possible use of faecally-contaminated water in the production of the beverages as well as poor hygienic practices of producers and perhaps vendors (Clayton *et al*., 2002; Ojokoh *et al*., 2002; Agwa *et al.*, 2014; Ayandele, 2015).

Figure 1 shows the percentage occurrence of *E. coli* and *Klebsiella* spp obtained from the beverages. Overall, *E. coli* and *Klebsiella* spp were recovered from the three beverages. All the *E. coli* colonies transferred to Sorbitol MacConkey agar appeared bluish purple indicating that they don't belong to *E. coli* 0157:H7. Although, the percentage occurrences of the recovered isolates vary across beverage type, their recovery from all the beverages calls for concern, because these bacteria can cause gastrointestinal tract related diseases when ingested via contaminated food (Mensah *et al*., 2002; Yeboah-Manu *et al*., 2010).

The percentage occurrence of *E. coli* was observed to be higher in *zobo* samples but lower in *kunu zaki* samples when compared with the findings of Zumbes *et al*., (2014) and Nwachukwu *et al.* (2007) who reported a percentage occurrence of 20% and 40% respectively from *zobo* samples with the former reporting 50% occurrence in *kunu zaki* samples. The percentage occurrence of *Klebsiella* species was also found to be higher in *zobo* drink when compared to the reports of Nwachukwu *et al*. (2007) who reported a percentage occurrence of 26%. Also, the percentage occurrence of *Klebsiella* species observed was lower when compared to the findings of Aboh and Oladosu, (2014) who reported a percentage occurrence of 22% in *kunu zaki*

The lower percentage occurrence of isolates identified as *Klebsiella* spp and *E. coli* in *kunu-zaki* and ginger drink when compared to *zobo* drink could be attributed to food processing techniques and/or nutrient contents of the beverages. *Kunu-zaki* is produced by spontaneous fermentation (Gaffa *et al*., 2002) and fermenters (e.g. *Lactobacillus*) present during *kunu* processing could have produced metabolites, which had bacteriostatic and/or bacteriocidal effects on other potential pathogens including *E. coli* and *Klebsiella* spp (Chelule *et al*., 2010). Similarly, ginger is known to inhibit the growth of some bacterial species (Hasan *et al*., 2012). Thus, high ginger contents could have been responsible for the low occurrence of the bacterial species in the ginger drink. In view of the findings in this study, proper hygienic practice is of great importance in ensuring the safety of these beverages for consumption (Wonang *et al*., 2001; Elmahmood and Doughari, 2007; Umaru *et al*., 2014).

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Table 1: Bacterial distribution in locally processed beverages in Ilishan Remo, Ogun state.

Sample type	No of samples	Mean Total Aerobic Count cfu/ml	Mean Total Enterobacterial count (cfu/ml)	Number of species		Total isolates recovered
				Escherichia coli	*Klebsiella* sp.	
Zobo	8	$10.0x10^5$	$1.4x10^4$	26	22	48
Ginger drink	7	$0.2x10^5$	$1.7 x10^4$	1	2	3
Kunu zaki	7	$1.0 x10^5$	$2.0 x10^4$	2	4	6
TOTAL	22			29	28	57

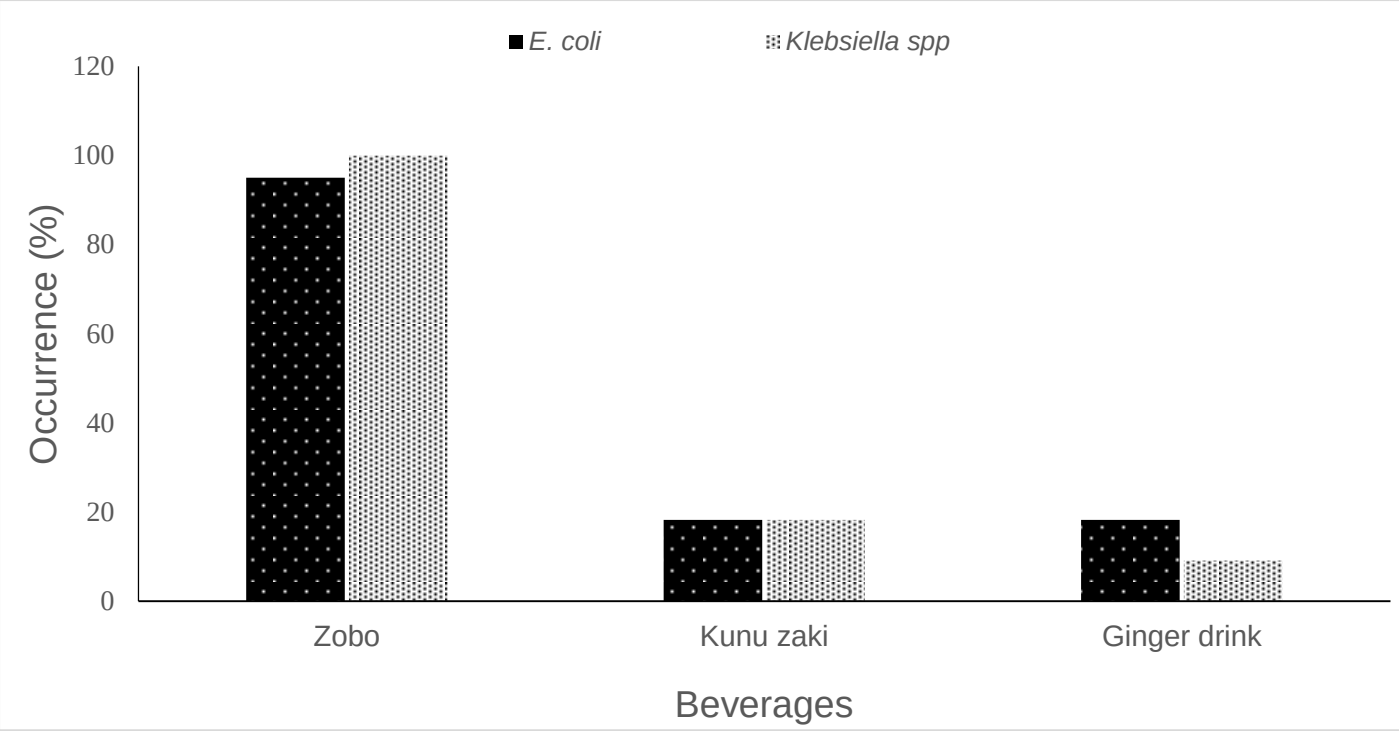

Figure 1: Occurrence of enterobacteriaceae in locally processed beverages from Ilishan Remo, Ogun state.

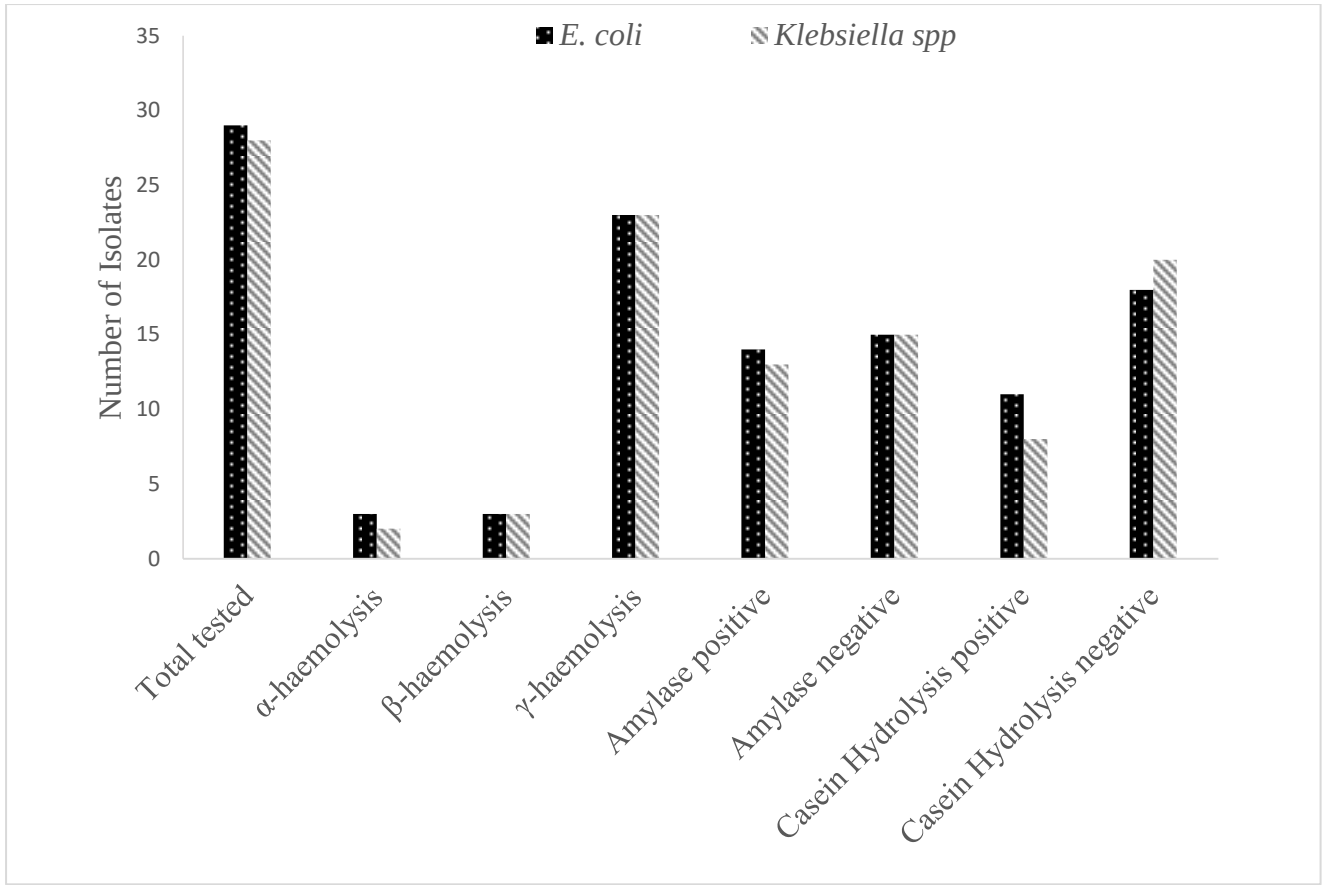

Figure 2: Haemolytic and enzyme (amylase and casein) production potential of *E. coli* and *Klebsiella* spp. from locally processed beverages in Ilishan Remo, Ogun State.

Figure 2 shows the haemolytic and enzyme (amylase and casein) production potential of *E. coli* and *Klebsiella* spp. recovered from the beverages investigated. Of the 50.9% *E. coli* isolates, 5.3(%), 5.3(%) and 40.4(%) exhibited beta, alpha and gamma haemolysis respectively. Similarly, 3.5(%), 5.3(%) and 40.4(%) of the 49.1% *Klebsiella* spp. identified in this study, exhibited alpha, beta and gamma haemolysis respectively. According to Cheung *et al*. (2012), haemolytic bacteria produces haemolysin enzyme that is capable of hydrolysing red blood cells, which can pose serious health risk to humans. Thus, the recovery of potential haemolytic *E. coli* and *Klebsiella* sp from the beverages suggests that frequent consumers of these beverages may be at risk.

With respect to enzyme production, 24.6(%) and 19.3(%) of the *E. coli* isolates were capable of producing amylase and casease enzyme respectively while 26.3(%) and 31.6(%) were non-amylase and casease producers. Similarly, 22.8(%) and 14.3(%) of the *Klebsiella* spp isolates could produce amylase and casease while 26.3(%) and 35.1(%) were non-amylase and casease producers. The recovery of amylase and casease producing bacteria from the beverage suggest potential rapid deterioration of the beverage that could cause poor product palatability.

Conclusion

This study has shown that potentially pathogenic bacterial species are present in locally processed beverages and could threaten the safety and health of consumers. Obviously, the presence of the enterobacteriacae in the beverages analyzed is a food safety challenge considering the high demand in the consumption of these beverages and its preference to carbonated drinks. It is therefore pertinent that awareness should be created within the vendors by relevant agencies of government, to highlight the necessity to maintain proper hygiene in order to avoid outbreaks associated with these microorganisms.

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