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BACTERIOLOGICAL ASSESSMENT OF KUNU-ZAKI SOLD IN SELECTED COMMUNITIES IN PORT HARCOURT, NIGERIA

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ABSTRACT

Kunu-zaki is a fermented beverage sold in Nigeria; often prepared under unsafe conditions, predisposing it to pathogen contaminations. The bacteria associated with Kunu-zaki sold in the different area of Port Harcourt and an antibiogram of the pathogens was determined using standard methods involving nutrient agar, Salmonella-Shigella agar, Thiosulphate citrate bile salt sucrose agar, MacConkey agar, Mannitol salt agar, Eosin-Methylene blue agar and Mueller Hinton agar. The counts for kunu-zaki obtained from Choba, Mile 1, Rumuodara and Rumuokoro ranged from 2.87±0.11 to 5.39±0.19, 4.50±0.27 to 5.46±0.38, 4.02±0.54 to 5.96±0.27 and 4.24±1.13 to 5.94±0.22 log_{10}cfu/ml, respectively. The confirmation of the isolates as: Staphylococcus spp. (27%), Lactobacillus spp. (20%), Streptococcus spp. (17%), Bacillus spp. (17%), E. coli (10%), and Shigella at (10%) was based on cultural and biochemical characteristics. The Gram-negative bacteria were 100% susceptible to pefloxacin, gentamycin, ciprofloxacin and tarivid while the Gram-positive bacteria isolates showed varying resistance to the antibiotics with Staphylococcus being the most susceptible. The occurrence of the antibiotic resistance isolates possess danger for potential consumers, hence the need to improve the production process.

Keywords: Antibiogram, beverage, Kunu-zaki, *Lactococcus* spp.
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INTRODUCTION

Kunu-zaki an indigenous fermented non-alcoholic beverage, with characteristic sweet-sour taste sold and widely consumed in majorly in northern Nigeria extensively during the dry season (Amusa & Odunbaku, 2009; Edobor & Emmanuel-Akerele, 2021) but has received wide acceptance among people of other parts of the country as well as neighbouring North African countries.The beverage is consumed as breakfast snack,weaning food and as a food supplement/appetizer (Oranusi,Umoh and Kwaga, 2003). Kunu-zaki is traditionally prepared using millet (*Pennisetum glaucum)*, maize (*Zea mays*), wheat (*Triticum aestivum*) or sorghum (*Sorghum bicolor*) and flavoured with ginger (Efiuvwevwere & Akoma, 1995; Gaffa et al., 2002; Umaru et al., 2014; Ndukwe et al., 2023). It is also produced from tiger nuts (Belewu & Abodunrin, 2006), guinea corn or rice (Umaru et al., 2014). The grains are known to contain

potassium, zinc, vitamins A, B, and C, as well as anti-diabetic, anti-diuretic, and anti-cancerous compounds that can be used to treat urogenital tract infections, cancer, and diabetes. (Amusa & Odunbaku, 2008). According to taste and cultural customs, there is a great deal of diversity in the preparation process, which accounts for the inconsistent quality of the product (Adeyemi & Umar, 1994). For example, some people prefer their kuni prepared with a lot more sugar or pepper than others. Kunu has a milky cream appearance and is often consumed a few hours after it is produced (Adeleke & Abiodun, 2010). It is prepared using conventional method; hence the constituents' concentrations are neither quantified nor standardized (Aboh & Oladosu, 2014). The production procedure varies depending on household, taste and cultural habits of the consumers resulting in variation in the taste, quality and specifications of the product. Nutritionally, Kunu is made up of 87-92% moisture, 3.19-7.86% crude protein, 0.37- 0.75% crude fat, 0.93-1.20% ash and 2.69-5.84% carbohydrate as reported by Gaffa et al., (2002). In addition to its nutritional advantages, kunu has been demonstrated to have other advantages such as lowering blood cholesterol, reducing the risk of diabetes, and preventing blood clot formation. (Ofudje et al., 2016).

Microorganisms may thrive in the unsanitary conditions, filthy surroundings, and inadequate storage conditions utilized in the manufacturing of kunu, as well as in the lack of regulations or standardization of ingredients used.The organisms isolated and characterized consisted mainly of lactic acid bacteria and notable pathogens and spoilage bacteria including *Lactobacillus delbrueckii, L. fermentum, L. plantarum, L. casei*, *Corynebacterium, Lactococcus lactis*, *Saccharomyces cerevisiae, Streptococcus* spp., *Bacillus brevis, B. Badius, B. polymyxa, B. macquariensis, B. pantothenticus, Staphylococcus aureus, S. epidermidis, Serratia marcescens* and *Leuconostoc* spp. (Osuntogun & Aboabo, 2006; Adeniran et al., 2020). Kunu-zaki has a short shelf life because of its high moisture content, which enhances microbial activities making it very prone to spoilage. (Adeyemi & Umar ,1994).The aim of the study was to investigate the microbial quality of Kunu-zaki sold in selected communities in Port Harcourt and the antibiogram of the pathogens.

MATERIALS AND METHODS

Sources of Sample

Freshly prepared Kunu-zaki samples were purchased from Mile one, Choba, Rumudara, Rumuokoro areas in Port Harcourt. Ten samples were purchased from each vendor from the four locations and taken to the University of Port Harcourt laboratory for bacteriological assessment.

Isolation and Enumeration

Ten millilitres of each Kunu-zaki sample were added to 90 ml of sterile peptone water. Ten-fold serial dilution of the samples was carried out and 0.1 ml of suitable dilutions were plated in duplicate in Nutrient agar (NA), thiosulfate citrate bile salt agar (TCBSA), eosin methylene blue agar (EMBA), *Salmonella-Shigella* agar (SSA), MacConkey agar and Mannitol salt agar (MSA).

The plates were incubated at room temperature (29±2^{o}C) for 24 to 48 h. The average colony counts for duplicate plates were obtained and expressed as colony forming unit per millilitre (cfu/ml) of sample. Characteristic colonies on the differential and selective media were purified and stored in Nutrient agar slants in refrigerator pending for confirmation and further analysis.

Confirmation of Bacteria Isolates

Characteristic discrete colonies on the differential and selective media were confirmed on the basis of their cultural morphology, physiological (Gram's staining) and biochemical characteristics [catalase, indole, citrate oxidase, TSIA, methyl-red, Vague Proskauer, motility and utilization of sugars (glucose, lactose, mannose, fructose)] (Holt, et al., 2000).

Antibiotic Sensitivity Test

The CLSI (2009) suggested techniques were employed for the standard disk diffusion method on Mueller-Hinton agar to determine the antibiotic sensitivity patterns of all the identified bacterial taxa. Ten commonly used antibiotics (µg/disc) each for Gram-positive and Gram-negative viz. pefloxacin (PEF) 10, gentamycin (GEN) 10, ampiclox (APX) 30, zinnacef (ZNF) 20, amoxacillin (AMX) 30, Rocephin (RCP) 25, ciprofloxacin (CPX) 10, streptomycin (STP) 30, septrin (SPT) 30 and erythromycin (ERY) 10, and augumentin (AUG) 30, tarivid (TRV) 10, reflacine (PEF) 10, ciprofloxacin (CPX) 10, gentamycin (AUG) 30, streptomycin (STP) 30, ceporex (CEP) 10, nalidixic acid (NAD), septrin (SPT) 30 and ampicillin (APM) 30, respectively were tested. A 10^{8} cell/ml (0.5 MacFarland turbidity standards) bacterial culture was prepared in sterile saline from an overnight culture in brain heart infusion broth. Subsequently, 0.1ml was inoculated onto Mueller Hinton agar, after which antibiotic discs were aseptically placed on the surface of the agar. The plates were incubated at 29±2^{o}C for 24h. Zone of inhibition was measured in millimeter.

Statisticalanalysis

The statistical analysis was conducted using the IBM, New York, USA program, Statistical Package for the Social Sciences (SPSS) ver. 25.0.

RESULTS AND DISCUSSION

Total heterotrophic bacterial count of Kunu-zaki

The total heterotrophic bacterial count (THBC) from the four communities sampled are presented in Table 1. The counts for Choba, mile 1, Rumuodara and Rumuokoro ranged from 2.87±0.11 to 5.39±0.19, 4.50±0.27 to 5.46±0.38, 4.02±0.54 to 5.96±0.27 and 4.24±1.13 to 5.94±0.22 $\log_{10}$cfu/ml, respectively. These results showed that the higher limits of all counts were comparable unlike the lower limits of the average counts. The values recorded in this study is comparable to the 3.00 to 4.26, 3.15 to 4.65, 3.62 to 4.18, 4.54 to 4.92 and 4.69 to 8.76 $\log_{10}$cfu/ml in Kunu-zaki vended on the streets of Girei, Adamawa State, Owerri metropolis, Imo State, selected towns in Osun State, Keffi metropolis, Nasarawa State and Kunu-zaki drink

enriched with cocoa powder reported by Elmahmood and Doughari (2015), Anumudu and Anumudu (2019), Imoukhuede et al. (2018), Gyar et al. (2014) and Adeniran et al. (2020), respectively.

The counts recorded in this study were below the range of 6.20 to 6.76, 7.18 to 7.98 and 8.56 to 8.96 $\log_{10}$cfu/ml reported by Edem et al. (2017), Braide et al. (2018) and Makut et al. (2013), respectively in Kunu-zaki vended in Owerri Metropolis, Imo State and Keffi metropolis, Nasarawa State. The counts from all the locations in this present study, except Choba were higher than the range of 3.48 to 3.76 $\log_{10}$cfu/ml reported by Etang et al. (2017). This is an indication that Kunu-zaki, irrespective of the substrates and location are generally highly contaminated with fermentative beneficial bacteria as well as spoilage and pathogenic bacteria since it a spontaneous process.

Detected bacteria

The bacteria detected in the examined Kunu-zaki samples were: *Staphylococcus*spp. (27%), *Lactobacillus*spp. (20%), S*treptococcus*spp. (17%), *Bacillus* spp. (17%), *E. coli* and *Shigella* (10%) (Table 2). As expected, *Lactobacillus* was dominant since its activity lowers the pH thereby limiting the presence of other bacteria. These bacterial and several others as well as fungi have been reported in Kunu-zaki products from all parts of Nigeria basically because of contaminated by the raw materials, chance fermentation production process, unhygienic processing environment and lack of good manufacturing practices (GMP) by producers. (Efiuvwevwere & Akoma, 1995; Umoh & Odoba, 1999; Edward & Ohaegbu, 2012; Gyar et al., 2014; Elmahmood & Doughari, 2015; Braide et al., 2018; Imoukhuede et al., 2018; Edobor & Emmanuel-Akerele, 2021). According to Elmahmood and Doughari (2015), the pH of *Kunu-zaki* is usually too low to allow the growth of pathogenic microorganisms, but the presence of *E. coli, S. aureus* and *Streptococcus* spp. could be a matter of serious concern.According to Elmahmood and Doughari (2015), the majority of individuals involved in the manufacture, packing, and hawking do not take the required precautions, therefore contamination might have happened during sieving and packaging. As a result, contamination could have been highly noticeable. More so *S. aureus*being normal flora of the human skin, nose, throat, palms, hairs and mucus membrane and a common etiological agent of septic arthritis (Ariahu *et al.,* 2005) while *Streptococcus* spp. is also normal flora of the throat and the buccal activity (Elmahmood & Doughari, 2015).

Bacillus and *Staphylococcus* spp. accounted for 15 and 20%, respectively of the total isolates and have been reported as major bacteria of food, including Kunu-zaki in street foods sold in Zaria, Nigeria (Umoh and Odoba, 1999). *Bacillus* spp. is a widely dispersed bacterium that is frequently isolated from soils and a wide range of foods in various countries (Okechukwu, et al., 2011). *Bacillus* spp. are spore formers whose spores could survive high temperature of processing and low acid content like juices and beverages in which they produce organic acids. *Vibrio* and *Shigella* species were not detected in the samples examined.

Antibiotics Sensitivity

The antibiotic resistance of the Gram-negative (*Shigella* and *Escherichia coli*) and Gram-positive (*Bacillus, Staphylococcus* and *Streptococcus* species) isolates are presented in Table 3 and Table 4, respectively. The two Gram-negative bacteria were 100% susceptible to pefloxacin, gentamycin, ciprofloxacin and tarivid. They however, showed varying resistance to the other antibiotics. There is paucity of report on antibiogram of bacterial isolates from Kunu-zaki in literatures at the disposal of the authors of this study. The resistant pattern of *E. coli* in this study agrees with the 100% susceptibility to pefloxacin and, gentamycin, ciprofloxacin and ofloxacin reported by Makut et al. (2013) and Braid et al. (2018), respectively of *E. coli* from Kunu-zaki sold in Keffi metropolis, Nasarawa State, Nigeria and Owerri, Imo State, Nigeria. Makut et al. (2013) also reported varying resistance against septrin (68.7%), tarivid (31.3%) and streptomycin (31.3%).

The Gram-positive isolates showed varying resistance to the selected antibiotics with the most sensitivity observed with *Staphylococcus* spp. which were 100% sensitive to pefloxacin, gentamycin, rocephin ciprofloxacin streptomycin and erythromycin. This agrees with the 100% sensitivity reported by Braid et al. (2018) of *Staphylococcus* against ciprofloxacin and gentamycin. On their part, Makut et al. (2013) reported varying resistance of staphylococcus against ciprofloxacin (16.7%), gentamycin (33.4%), pefloxacin (16.7%). In this study, 25% resistance of *Staphylococcus* spp was observed against ampicillin whereas, Makut et al. (2013) and Braid et al. (2018) reported 0% resistance of *Staphylococcus* also isolated from Kunu-zaki. *Streptococcus* spp. in this study showed varying resistance against pefloxacin (33.3%), gentamycin (33.3%), septrin (66.7%) and amoxicillin (33.3%) whereas Makut et al. (2013) reported a 0% resistance against these same antibiotics. Braid et al. (2013) reported 100% resistance of *Bacillus* spp. against gentamycin and amoxicillin whereas in this present study, they showed a resistance of 33.35 and 66.7% against gentamycin and amoxicillin, respectively.

CONCLUSION

The continued production of Kunu-zaki using the traditional methods involving chance inoculation, rudimentary equipment and poor sanitary conditions if not abated will continue to predispose this nutritious drink to contamination by pathogens and spoilage organisms thereby posing health threat to consumers and short shelf-life of the product now substituted for or used to complement soft drinks and wines at social gatherings.The Nigerian Government through the oversight organizationslike National Agency for Food and Drug Administration Control (NAFDAC) and Standard Organization of Nigeria (SON) should set standards for raw materials, production processes and finish product inspection for kunu-zaki.

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APPENDICES

Table 1. Total heterotrophic bacterial count

Sample number	Average counts (Log_{10}cfu/ml) from the different locations			
	Choba	Mile 1	Rumuodara	Rumuokoro
1	2.87±0.11	5.28±1.05	4.83±0.51	5.35±1.03
2	2.80±1.02	5.15±0.48	4.37±1.01	5.10±1.11
3	5.37±0.27	5.46±0.38	5.17±1.13	4.89±0.26
4	5.39±0.19	5.35±0.23	5.34±0.19	5.23±0.16
5	5.37±0.13	5.15±0.53	5.51±0.24	5.38±0.25
6	4.98±0.25	5.02±0.42	4.06±0.35	5.21±1.01
7	4.19±1.20	4.99±1.15	4.02±0.54	4.24±1.13
8	5.15±1.14	5.05±0.09	5.96±0.27	5.94±0.22
9	4.99±0.39	4.89±0.25	4.93±1.12	4.43±0.26
10	5.30±0.14	4.50±0.27	4.97±0.53	4.28±0.17

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Table 2. Physiological and biochemical characteristics of isolates

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Organism	Number	Cell morphology	Gram reaction	Oxidase	Catalase	Coagulase	Glucose	Lactose	Fructose	Acid	Alkaline	H_2S	Gas	Indole	MR	VP	Citrate	Motility
Shigella spp.	3	Rod	-	-	+	ND	A	A	-	A	B	-	-	-	+	-	-	-
Bacillus spp.	5	Rod	+	+	-	ND	A	A	-	A	B	-	-	-	-	-	-	+
Staphylococcus spp.	8	Cocci	+	+	+	+	AG	AG		A	B	-	-	-	-	-	-	+
Lactobacillus spp.	6	Cocco bacilli	+	-	-	ND	A	A	A	B	B	-	-	-	+	+/-	-	+/-
Escherichia coli	3	Rod	-	-	+	ND	A	-	A	B	B	-	-	+	+	+	-	+
Streptococcus spp.	5	Cocci	+	-	+	ND	A	A	-	A	B	-	-	-	-	-	-	-

+ =Positive, - = Negative, A = Acidic, B = alkaline, AG= Acid and gas, ND= Not determined

Table 3. Resistance of the Gram-negative bacteria isolates

Organism	No	Number of resistant isolates to the selected antibiotics									
		PEF	GEN	AUG	CPX	SPT	STP	APM	CEP	TRV	NAD
Shigella spp.	2	0	0	1	0	1	1	2	2	0	0
Escherichia coli	2	0	0	1	0	0	0	1	1	0	2

PEF=pefloxacin, GEN=gentamycin, AUG=augumentin, CPX=ciproflox, SPT=septrin, STP=streptomycin, APM=ampicillin, CEP=ceporex, TRV= tarivid and NAD=nalidixic acid.

Table 4. Resistance of the Gram-positive bacteria isolates

Organism	No	Number of resistant isolates to the selected antibiotics									
		PEF	GEN	APX	ZNF	AMX	RCP	CPX	STP	SPT	ERY
Bacillus spp.	3	1	1	3	2	2	1	1	1	2	1
Staphylococcus spp.	4	0	0	3	2	1	0	0	0	1	0
Streptococcus spp.	3	1	1	3	3	1	1	1	1	2	1

PEF= pefloxacin, GEN= gentamycin, APX= ampiclox, ZNF= zinnacef, AMX= amoxacillin,RCP= rocephin, CPX= ciprofloxacin, STP= streptomycin, SPT= septrin and ERY= erythromycin