

# Bacteriological Quality of Kunu Drink Sold In Bayelsa State Nigeria and the Pathogenic Potential of Some Isolates.

## ABSTRACT

**Introduction:** Kunu or kununzaki is a beverage drink made from grains such as millet, sorghum and maize or other combinations. It is a non-alcoholic beverage marketed in public places such as offices, markets, schools, motor parks and used in festivities such as weddings, birthday celebration, naming ceremonies etc. The high bacterial content of kunu calls for investigation.

**Aim:** The aim of this research is to isolate, identify bacterial contaminants in kunu and determine enterotoxin producing abilities of some isolates.

**Methodology:** A total of 150 bottles of kunu were purchased, 50 each from Yenagoa, Sagbama and Ogbia respectively. Each bottle of kunu was properly mixed by gentle inversion several times and 1mL of the kunu was pipetted and added to 9mL sterile peptone water. Subsequent serial dilution was made to  $10^5$ . Then 0.1mL was placed on agar media in duplicate. The plates were incubated at 37°C for 18-24 hours and examined for growth.

**Results:** The bacteria isolated from Kunu were *S. aureus* 150 (27.8%), *E. coli* 150 (27.8%), *Bacillus* sp. 150 (27.8%) and *Staphylococci* sp. 90 (16.7%) respectively. Out of the *S. aureus* isolated, 25 (16.6%) produced enterotoxin and *E. coli* isolated, 19 (13%) produced enterotoxin respectively.

**Conclusions:** The contamination of kunu occurs during processing, packaging and by vendors. Improved personal hygiene of the producers, environment and proper preservation methods will reduce microbial proliferation and spoilage of kunu. The consumption of kunu is of public health interest.

**Keywords:** Bacteria, Contamination, Kunu, Enterotoxin, Strains

## 1. INTRODUCTION

Kunu or kununzaki is a beverage drink made from grains such as millet, sorghum and maize or their combinations. It is a popular drink in northern parts of Nigeria. Kunu made from sorghum is milky light-brown in colour, while that made from maize is whitish in colour [1,2].

The grain seeds used for the production of kunu drink were allowed to germinate while steeped in water for few days and after which blended with sweet potatoes and ginger or pepper to form a smooth paste. The paste is divided into two, one part is placed in a vessel and boiled water is added to it to form a thick mixture. The unheated half is added to the previous and stirred to give a thick mixture. The mixture is left for a day or two for the grain husk to settle. The husk and other sediments are filtered out of the mixture and the filtrate is boiled for consumption.

Kunu is a non-alcoholic beverage marketed in several public places such as offices, markets, schools, motor parks and a very common consumed beverage in occasions such as weddings, naming ceremonies, birthday celebrations, burials etc.[3] Kunu is an appetizer,

**Comment [WU1]:** Lines 17-18: Authors should provide information from literature about other similar fermented cereal based beverages from Europe.

Citation: [Pasqualone A, Summo C, Laddomada B, Mudura E, Coldea TE, 2018. Effect of processing variables on the physico-chemical characteristics and aroma of bors, a traditional beverage derived from wheat bran., Food Chemistry, 265:242-252. doi: 10.1016/j.foodchem.2018.05.095](#)

**Comment [WU2]:** Line 28: Authors should check English grammar. English proofing is needed in entire document.

food complement and refresher to quench thirst [4,5,6] The proximate analysis of kunu was determined and the content includes; protein 2.31 – 3.63%, fats 3.35 – 3.65%, ash content 1.16 – 1.21% and carbohydrates 82.92 – 83.55% [2]

There are varieties of Kunu depending on the feed stock used for processing, they are; kunuzaki, kunugyada, kunusamiya, kunubaule, kununjiko and kunugayamba [1,7] Out of these, kunuzaki is most widely produced and consumed [8,9] Some of the microorganisms involved in the fermentation of kunu were *Lactobacilli*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Penicillium* and *Sacharomyces* sp.[10] The high bacterial content of kunu may be an indicator of poor hygiene, poor quality cereals and water used in preparation and packaging processes [11] The bacteria isolated from kunu were *E. coli* 33.3%, *S. aureus* 26.7%, *Streptococcus* sp. 23.3%, *Pseudomonas* sp. 10% and *Bacillus* sp. 6.7% [11]. In a study to determine the microbiological quality of kunu in Yenagoa, Bayelsa State, the bacteria isolated were *E. coli*, *Enterobacter* sp, *Bacillus* sp, *Salmonella* sp, *Micrococcus* and *Streptococcus* sp. It was noted that most of the bacteria isolated were of public health importance and they were introduced during processing and handling due to poor hygiene [12] In another research investigating microbial quality of locally produced kunu in Calabar the bacteria isolated were *Bacillus* sp. 15%, *E. coli* 15%, *Salmonella* sp. 12.5%, *Streptococcus* sp.10%, *Pseudomonas* sp. 7.5%, *Proteus* sp. 7.5%, *Lactobacillus* 22.5% and *S. aureus* 10% respectively [13]. The microbiological qualities of kunu sold in Calabar were below acceptable standard and unfit for human consumption sought It was noted that the contamination of kunu could come from different source, the bacteria isolated may present health risk to consumers [14]. Bacteria isolated from kunu might be associated with food spoilage, food infections and poisons [15]. Similar organisms were isolated by other researches and they attributed the contamination of kunu to processing and handling. The processing and handling of kunu should be improved for consumers wellbeing [16]. Kunuzaki contamination with pathogenic bacteria is of public health importance and might cause diverse food related illnesses and infection to consumers [17]. The aim of this work is to determine the bacteriological quality of kunu sold in Bayelsa, identify isolated bacteria and the enterotoxin producing strains of some isolates.

## 2. MATERIAL AND METHODS

### 2.1 Study Area

The study was conducted in Bayelsa State, Nigeria. The samples of Zobo drink were purchased from the three (3) senatorial district headquarters of Bayelsa, namely; Yenagoa (the capital), Sagbama and Ogbia town. Bayelsa state was carved out of River State in 1996. Bayelsa is located in latitude 4°(15)' North, latitude 5°23' South and longitude 5°22' west and longitude 6°45' east. It is bound by Delta State on the North, River State on the East and Atlantic Ocean on the West and South. Bayelsa has the largest wetland in West African sub-region. It has a population of about 1.7 million people.

### 2.2 Collection of Samples

The organism used for the experiment is *P. aeruginosa*. It was molecularly identified at Lahor Research Laboratories, Benin, Edo State, Nigeria. The organism was stored in 50% glycerol and kept at -20°C.

### 2.3 Bacteriological Examination of Samples

Each sample of kunu was gently mixed by inversion several times and 1mL of the sample (neat) was added to 9ml of sterile peptone water (sterilized by autoclaving at 121° C for 15 minutes). Subsequent serial dilutions were made up to 105 and 0.mL of the last dilution

**Comment [WU3]:** The collection of samples is not clearly described. Authors should specify if beverage samples were provided from local stores, markets, storage temperatures is the store, their shelf life, storage conditions until the moment of analyze. Volume of collect samples and how were they collected?

(105) was dropped on already prepared and dried agar plates in duplicates (nutrient, MacConkey and salmonella/shigella). These were spread evenly on agar media with aid of sterile glass rod (sterilized by dipping in absolute alcohol and flaming in bunsen flame).

The inoculated plates were allowed to dry and incubated at 37° C for 18 - 24 hours before examining for growth.

## **2.4 Test for Bacterial Load in stored Kunu**

A set of freshly prepared kunu were kept at room temperature and another in the refrigerator at about 4° C after the initial determination of the bacteria counts in CFU/mL. The counts from the preserved kunu at room and refrigeration temperature were determined on the second and third day respectively.

## **2.5 Identification of Isolated Bacteria**

The bacteria isolated were identified using morphology, cultural, Gram's stain reaction, chemical and biochemical reactions such as citrate, VP, Methyl red, indole, catalase, coagulase and carbohydrate fermentation etc.

## **2.6 Detection of Enterotoxin Producing *E. coli* from Kunu**

PROTM 0157 KIT detects enterotoxin producing *E. coli*. The Hardy Diagnostics PROTM 0157 KIT provides a rapid latex agglutination method to detect *E. coli* serogroup 0157 antigen from colonies isolated in the laboratory. These were *E. coli* producing verotoxin (VT-producing pathogen). Hardy diagnostic *E. coli* PROTM 0157 Kit contains latex particles coated with an antiserum against *E. coli* 0157 antigen. When the coated latex particle is mixed with fresh colonies of *E. coli* serotype 0157, the bacteria will bind to the antiserum, causing the latex particles to visibly agglutinate, which is indicative of positive result.

### **2.6.1 Procedure**

The reagents were allowed to attain room temperature for about 20 minutes prior to use. Then a drop of sterile saline (Cat. no.K59) was dropped in the circle on the test card and overnight cultures of *E. coli* were emulsified by mixing it with the saline on the test card. The Latex Reagents were mixed by inverting the tubes several times, prior to use. One (1) drop of *E. coli* PRO™ 0157 Latex Reagent were dispensed onto the test circle on the test card. The Latex Reagent and the organism suspension were then mixed with the wooden applicator provided, using the complete area of the circle. A new stick was used for each reagent. Then the entire card was gently hand-rocked, allowing the mixture to flow slowly over the ring area for up to 2 minutes. Under normal lighting conditions, agglutination (strong clumping) of the latex particles were examined. All organisms yielding a positive agglutination reaction were retested with the Negative Control Latex Reagent.

## **2.7 Enterotoxigenicity Testing For *S. aureus***

The Prolex™ Staph Latex Kit provides a rapid platform for the identification of Staphylococcal isolates particularly *S. aureus* that produce enterotoxin. The Prolex™ Staph Latex Kit utilizes blue polystyrene latex particles that have been sensitized with fibrinogen and IgG

### **2.7.1 Procedure**

The test kit was removed from the refrigerator 20 minutes prior to use and the latex reagents were allowed to attain room temperature. The latex reagent was re-suspended by inverting the dropper bottle several times. This was followed by dispensing 1 drop of Staph Test Latex Reagent into a circle on the test card. A sterile loop was used to transfer two colonies of the test isolate into the circle. The reagent and colonies were mixed and spread to cover the entire area of the circle and this was rocked gently on the card allowing the mixture to flow slowly over the entire test ring area. Agglutination was observed for 20 seconds. Negative Control Latex Reagent was included as quality control. Strong agglutination within 20 seconds with the Staph Test Latex Reagent indicates positive result.

### 3. RESULTS

#### 3.1

A total of 150 samples of kunu were examined for the presence of bacteria. The result obtained showed that from Yenagooa *S. aureus* was 50 (27.8%), *E. coli* 50 (27.8%), *Bacillus* sp. 50 (27.8%), and *Streptococcus* sp. 30 (16.7%) respectively. From kunu bought from Sagbama, *S. aureus* were 50 (27.0%), *E. coli* 50 (28.6%), *Bacillus* sp. 50 (27.0%) and *Streptococcus* sp. 35 (18.9%) respectively. Kunu purchased from Ogbia town had *S. aureus* 50 (28.6%), *E. coli* 50 (28.6%), *Bacillus* sp. 50 (28.6%) and *Streptococcus* sp. 25 (14.3%) respectively. The overall percentage occurrences of isolated bacteria were *S. aureus* 150 (27.8%), *E. coli* 150 (27.8%), *Bacillus* sp. 150 (27.8%) and *Streptococcus* sp. 90 (16.9%) respectively shown of Table 1.

**Table 1.** Percentage Occurrence of Bacteria Isolated from Kunu Drink

Location	<i>S. aureus</i>	<i>E. coli</i>	<i>Bacillus</i> sp.	<i>Streptococcus</i> sp.	Total
Yenagooa	50 (27.8)	50 (27.8)	50 (27.8)	30 (16.7)	185 (33.3)
Sagbama	50 (27.8)	50 (27.8)	50 (27.8)	35 (27.8)	185 (34.6)
Ogbia	50 (27.8)	50 (27.8)	50 (27.8)	25 (14.3)	175 (32.4)
<b>Total</b>	150 (27.8)	150 (27.8)	150 (27.8)	90 (16.7)	540

**Comment [WU4]:** Table 1 A legend is needed to indicate the table abbreviations (and information presented in the brackets).

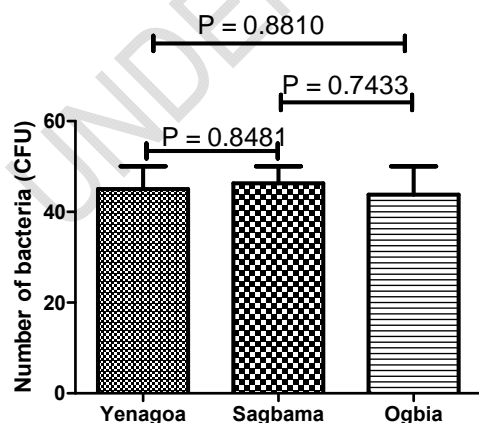


Fig Comparison of the bacteria isolated from different locations

Comment [WU5]: The figure from the line 160-161 has no number. Every figure and table should be referred in the text.

## 4.2 Enterotoxin Producing *S. aureus* and *E. coli* from Kunu

A total of 50 *S. aureus* were isolated from kunu in Yenegoa out of which 7 (14%) were enterotoxin producing strains and 50 *S. aureus* were isolated from Sagbama, 10 (20%) produced enterotoxin, while in Ogbia, Town 50 *S. aureus* were isolated, 8 (16%) were positive for enterotoxin production respectively. Overall total of *S. aureus* that produced enterotoxin were 25 (16.6%) as shown in Table 2.

**Table 2.** Percentage Occurrence of Enterotoxin Producing *S. aureus* and *E. coli* from Kunu

Location	<i>S. aureus</i>	Number positive	<i>E. coli</i>	Number positive
Yenegoa	50	7 (14)	50	7 (14)
Sagbama	50	10 (20)	50	5 (10)
Ogbia	50	8 (16)	50	7 (14)
<b>Total</b>	<b>150</b>	<b>25 (16.6)</b>	<b>150</b>	<b>19 (13)</b>

Numbers in parentheses = percentages

Numbers in parentheses = percentages

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**Table3.** Bacterial Counts from Preserved Refrigerated and Non-Refrigerated Kunu (CFU/mL)

Temperature	0 Hr	24 Hrs	48 Hrs
Refrigeration	$7.48 \times 10^5$	$8.40 \times 10^5$	$8.72 \times 10^5$
Room	$7.48 \times 10^5$	$11.96 \times 10^5$	$13 \times 10^5$

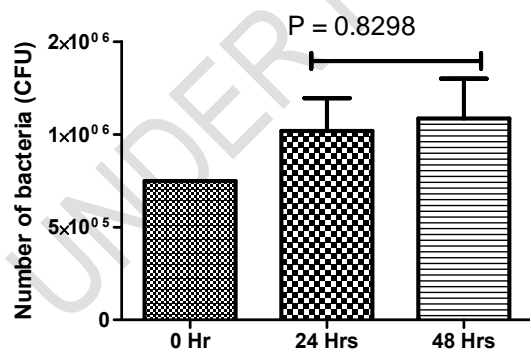


Fig. Comparison of bacterial counts from 0 Hr, 24Hrs and 48Hrs.

187  
188

S/No	Colour	Surface	Edge	Translucency	Texture	Gram Rxn	Size	Shape	Motility	Methyl Red	Voges Proskauer	Oxidase	H <sub>2</sub> S Production	Indole	Coagulase	Catalase	Citrate	Urease	Starch Hydrolysis	Glucose	Lactose	Sucrose	Maltose	Galactose	Mannitol	Arabinose	Oxidative	Fementative	Bac
1	M	R	E	C	D	+	Md	Rd	+	-	-	+	-	-	-	+	-	-	+	A	A	A	A	A	±	-	+	+	<i>Bacillus sp.</i>
2	Cr	S	E	C	Mt	-	Md	Rd	+	-	-	-	-	+	-	+	-	-	-	AG	AG	AG	AG	AG	AG	AG	+	AG	<i>E coli</i>
3	Cr	S	E	C	Nt		Md	Co	-	-	-	-	-	-	-	-	N	N	N	+	+	-	+	-	-	-	+	+	<i>Streptococcus sp.</i>
4	Cr	S	E	C	Mt	+	Md	Co	-	+	+	-	-	-	-	+	N	N	-	A	A	A	-	A	A	-	+	+	<i>S. aureus</i>

Comment [WU7]: Table from the lines 188-189 has no number and no matching in text.

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#### 4. DISCUSSION

The bacteria isolated from kunu drinks in this study were *Staphylococcus* sp, *E. coli*, *Bacillus* sp. and *Streptococcus* sp. The contamination after the boiling process (post CCP contamination) might be responsible for the presence bacteria in kunu. Similar bacteria were isolated from kunu by other researchers in other parts of Nigeria such as *Bacillus* sp. *Salmonella* sp. *Micrococcus*, *Staphylococcus aureus* and *Streptococcus* sp.[13], *E. coli*, *S. aureus*, *Salmonella* sp and *Shigella* sp in Maiduguri [18], In Kaduna, *Lactobacillus*, *Bacillus* sp. and *E. coli* [19], while some reported *E. coli*, *S. aureus*, *Streptococcus pyogenes* [20]. Other researchers had *S. aureus* 4(10%), *Lactobacillus* 9(22.5%), *Proteus* sp. 3(7.5%), *Streptococcus* sp. 4(10%), *Pseudomonas* sp. 3(7.5%), *Bacillus* sp. 6(15%) and *E. coli* 6(15%) respectively [13]. Most researchers had comparable bacteria in Port Harcourt [7], Oyo and Lagos [1,2], Kano [21], Maiduguri [18], Jalingo [3] in Calabar cross River State [5]. The poor microbial quality of kunu produced locally were because of poor hygiene and poor environmental conditions under which kunu were produced. Health hazard may be associated with consumer of locally produced kunu.

Among the bacteria isolated, Staphylococci were the most prevalent organism. *Staphylococcus* is normal inhabitant of the human body which can be found on the skin, mouth, nostril, hands, various surfaces etc. these were possible sources from where *Staphylococcus* sp. can contaminate kunu during processing and packaging. Bacteria might be present in storage containers, sieve used to filter the finished product and contamination from handlers. The percentage of *S. aureus* was 21.7% [11], while in this study *S. aureus* were 26.7%. The prevalence of *E. coli* was 27.8% from this study but others *E. coli* as 33.3% [11] and *E. coli* as 5.0% [5]. *E. coli* is the most prevalent aerobic bacteria in human and mammal faeces. Contamination by *E. coli* might be by faecal contamination, contaminated water, handlers, processing and packing. *Bacillus* sp. were 27.8% occurrence. *Bacillus* sp. are geophilic and the spores are found in the soil, dust etc. The contamination of grains and spices by *Bacillus* sp. and their spores from soil and dust are likely. The percentage of *Bacillus* sp. isolated other workers were 23.3% [5], and 7.6% [12]. Spores may survive during boiling at about 100°C (the only Critical Control Point (CCP) in the processes of kunu production) and germinate to re-contaminate kunu. Kunu should be preserved under refrigeration temperature at 4° C and or pasteurized to reduce the microbial load and to increase the shelf life [21,5].

It was noted that local drinks such as kunu may act as vehicle for the transmission of zoonotic and bacterial infections such as staphylococcosis, salmonellosis, shigellosis, tuberculosis, listeriosis etc. (Umaru et al., 2014)[3]. Kunu as a beverage sold in public places such as markets, schools, offices etc. is patronized because of the cheap price compared to other soft drinks and is served in occasions such as weddings, naming ceremonies, birthday celebrations for economic reasons and public acceptability. The consumption of contaminated kunu drink may result in outbreak of food borne illness. Preparation of kunu in environment with poor sanitary conditions predisposes the preparation and packaging processes to contamination and exposes the consumers to health risk.

The isolation of 13% of *E. coli* and 17% of *S. aureus* capable of producing enterotoxin indicates that the consumption of kunu is of public health concern. The production of enterotoxin in bacteria is mostly associated with gastrointestinal disturbances and/ or food borne illnesses.

**Conclusions:** The production kunu as non alcoholic beverage for public consumption should be regulated by appropriate regulatory agencies to reduce the risk of consumer infection. Producers should be made to have fair knowledge of food preservation and food sanitation. The isolation of 13% of *E. coli* and 17% of *S. aureus* capable of producing

enterotoxin indicates that the consumption of locally produced kunu is a public health concern.

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## DEFINITIONS, ACRONYMS, ABBREVIATIONS

Here is the Definitions section. This is an optional section.

**Term:** Definition for the term

UNDER PEER REVIEW

