Assessment of Pathogenic and Antibiotic Resistant Bacteria in Kunu Sold in Major Markets in Ibadan

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Abstract

Kunu, a traditional non-alcoholic beverage widely consumed in Nigeria, particularly in the southwestern region, is produced from a blend of cereals such as millet, maize, guinea corn, and sometimes rice. Its popularity stems from its affordability, rich taste, and perceived nutritional benefits. However, the methods used in its preparation and sale raise significant public health concerns, especially regarding microbial contamination. This study was conducted to evaluate the presence of pathogenic bacteria in Kunu sold across major markets in Ibadan and to assess the antibiotic susceptibility profiles of these organisms. Samples were obtained from six major markets (Mokola Market, Ogunpa Market, Dugbe Market, Orita Market, Toll gate market and Sabo market) in Ibadan, Nigeria. Microbiological analyses, biochemical analysis and antibiotic susceptibility testing was conducted using standard methods. The findings revealed the presence of common foodborne pathogens such as *Staphylococcus aureus, Escherichia coli, and Streptococcus spp.* in significant quantities. The consistent observation of hemolysis among the isolates is an important indicator of their pathogenic potential. Hemolytic activity reflects the ability of these bacteria to produce extracellular enzymes and toxins that facilitate tissue destruction, nutrient acquisition, and evasion of host immune defenses. Many of the isolates demonstrated varying degrees of sensitivity to widely used antibiotics. The abundance of microbial contaminants found, underscore the urgent need for public health action, including vendor education, improved hygiene practices, and stricter regulatory oversight of locally produced beverages. The study highlights the risk posed to consumers and the broader public health implications of unchecked microbial contamination which can lead to community-acquired infections.

Keywords: kunu, pathogenic bacteria food safety, street vended beverages.
Word Count: 254

Introduction

Foodborne illnesses remain a significant public health challenge globally, with developing countries experiencing a higher burden due to inadequate sanitation, limited access to clean water, and poor food safety practices. In Nigeria, the informal food sector plays a crucial role in food supply, with street-vended foods and drinks forming a major part of daily nutrition for many urban dwellers. Among these popular street beverages is Kunu, a cereal based, non-alcoholic drink that is enjoyed across different regions of the country (Obasi et al, 2023).

Kunu is traditionally made from fermented cereals such as millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), maize (*Zea mays*), and occasionally rice (*Oryza sativa*), often blended with sweeteners and spices such as ginger, pepper, and cloves. (Ndukwe et al 2023). The production process typically involves steeping, grinding, sieving, partial farmentation, and sometimes heating. These steps, while effective in enhancing the drink's flavor and appeal, are often carried out in unregulated and unsanitary environments. Vendors often prepare and store Kunu in home settings or makeshift stalls where hygiene standards are suboptimal, thus increasing the risk of microbial contamination. (Roy et al, 2022).

Studies have shown that food and drinks prepared under such conditions are highly susceptible to contamination by a variety of pathogens, including *Staphylococcus aureus*, *Escherichia coli, Salmonella spp.,* and *Streptococcus spp*. These organisms, if ingested in large quantities, can lead to severe gastrointestinal disturbances, urinary tract infections, and systemic infections, particularly among children, the elderly, and immunocompromised individuals. Compounding the risk is the increasing prevalence of antimicrobial resistance (AMR), which renders many first-line antibiotics ineffective in treating common bacterial infections. (Nwaiwu et al, 2020).

Literature Review

Kunu is a popular traditional beverage widely consumed in Nigeria and across West Africa. Kunu production is predominantly carried out at the household or micro-enterprise level. The processes involved in its preparation, including soaking, wet milling, sieving, fermentation, and partial heating, are largely manual and informal. (Effiong et al, 2023).

These practices, while traditional and culturally significant, are often carried out without adherence to food safety standards. Because of the low-cost, non-industrialized nature of its production, Kunu is sold in open markets, on roadsides, or by mobile vendors, often in reused plastic containers. These factors contribute significantly to the drink's exposure to microbial contaminants.

Numerous studies have documented the microbial hazards associated with street-vended beverages in sub-Saharan Africa. The lack of regulatory oversight and inadequate vendor training often lead to contamination by a wide range of bacteria, including both spoilage organisms and pathogen (Odo et al, 2021). *E. coli*, for instance, is frequently used as an indicator organism for fecal contamination, suggesting poor water quality or unclean utensils used during preparation. Staphylococcus aureus, another common isolate, is typically associated with human skin and nasal mucosa and often enters the food during handling.

Reports indicates that beverages like kunu are vulnerable to contamination through multiple routes: poor hygiene, contaminated raw materials, inadequate storage, and lack of temperature control. (Nwaiwu et al 2020). In another study, various bacterial pathogens where isolated from traditional beverages sold in southeastern Nigeria, including *Salmonella* and *Klebsiella species*. (Nwiyi et al, 2022). This aligns with findings from another study, where it was noted high microbial loads in local drinks produced under unregulated conditions. The high counts are often above the permissible limits set by food safety agencies (Akinsemolu et al, 2024).

The emergence and spread of antibiotic-resistant bacteria have become a global concern. Food products, particularly those consumed without further cooking, serve as potential vehicles for the transmission of resistant organisms. Resistance in foodborne pathogens can develop through the misuse or overuse of antibiotics in humans and animals. (Kumar et al 2020). These resistant bacteria can then be transferred to humans via the food chain, increasing the difficulty of treating infections.

Antibiotic resistance mechanisms such as efflux pumps, enzyme production (example; lactamases), and gene transfer via plasmids have been well documented. In Nigeria, empirical use of antibiotics without prescription is widespread, contributing to an environment where resistant organisms flourish (Hrustemović et al, 2021). When these

resistant bacteria are present in food items like Kunu, they not only pose a direct health risk but also serve as reservoirs of resistance genes that may be transferred to more virulent organisms.

A recent found that over 60% of *E. coli* isolates from local beverages exhibited resistance to ampicillin and tetracycline, two commonly used antibiotics (Obasi et al. 2023). Similar resistance patterns were noted among *S. aureus* and Streptococcus spp., further emphasizing the need for continuous monitoring and interventions in the informal food sector.

Health Implications of Consuming Contaminated Kunu

The consumption of beverages contaminated with pathogenic and antibiotic-resistant bacteria has far-reaching consequences. Individuals may suffer from mild to severe food poisoning, manifesting as diarrhea, vomiting, abdominal cramps, or even systemic infections in immunocompromised hosts. In cases where resistant organisms are involved, treatment may require expensive or last-line antibiotics, thereby increasing healthcare costs and burdening the already strained Nigerian health system (Akinsemolu et al, 2024)

Children, the elderly, and pregnant women are particularly vulnerable to these infections. Prolonged or untreated gastrointestinal illnesses can lead to dehydration, malnutrition, and, in extreme cases, mortality. The World Health Organization (WHO) estimates that unsafe food causes 600 million cases of foodborne illnesses and 420,000 deaths globally every year, with a large proportion occurring in developing countries.

Materials and Methods

Study Design

The research employed a cross-sectional descriptive design, aimed at assessing the presence of pathogenic and antibiotic-resistant bacteria in Kunu samples obtained from selected markets in Ibadan. This approach was appropriate for establishing both the prevalence of microbial contamination and the antibiotic susceptibility patterns of the isolates at a single point in time.

Collection of Sample Material

The material that was used for this study was kunu drink. Samples were obtained from six major markets (Mokola Market, Ogunpa Market, Dugbe Market, Orita Market, Toll gate market and Sabo market) in Ibadan, Nigeria. It was ensured that the samples were collected under sterile condition. All Samples were labeled accordingly. The samples were transported to the Microbiology Laboratory of Lead City University, Ibadan.

Nutrient agar, MacConkey Agar (MA) and De Man, Rogosa and Sharpe Agar (MRS) were used for isolation bacteria used for the study. **Total Bacterial Count**

The samples were agitated for a while to ensure uniformity and the samples were subjected to 10 fold serial dilution. Using standard microbiological techniques, 10µ was inoculated from dilution factors of 10^7,10^5 and 10^3 in triplicates on agar plates of Nutrient Agar (NA) for bacteria count, MacConkey Agar (MCA) for coliform count and De Man, Rogosa and Sharpe Agar (MRS) for Lactic acid bacteria. Using their morphological characteristics such as shape, appearance, and colony counts, sub culturing was carried out on the isolates until pure cure was obtained following standard microbiological procedures. Plates were incubated at 37°C for 24–48 hours, after which colonies were observed for morphological characteristics.

Suspected isolates were further purified and subjected to biochemical testing, including Gram staining, catalase, coagulase, indole, and oxidase tests, in accordance with Cheesbrough (2006). Identified organisms were compared with known standards using the Bergey's Manual of Determinative Bacteriology.

Antibiotic Susceptibility Testing

The antibiotic susceptibility testing of isolates was determined by the disc diffusion method on Nutrient agar (CM0337 oxoid, England) and interpreted in line. The antibiotic disks (Mast Diagnostica Germany) and their concentrations (in µg) used include Septrin (30µg), Chloraphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (30µg), Amoxacillin (30µg), Augumentin (10µg), Gentamycin (30µg), Pefloxacin (30µg), Tarivid (10µg), Streptomycin (30µg). The antibiotic disks were firmly placed on sterile Nutrient agar (NA) plates previously seeded with a 24hr. old culture of the isolate (106 CFU/ml of 0.5 McFarland Standard). The plates were incubated at 37°C for 24 h and diameters of zones of inhibition

were compared. Multiple antibiotic resistant (MAR) isolates were defined as resistance to greater than or equal to three (≥ 3) classes of the antibiotics tested. Plates were incubated at 37°C and the diameter of zone of growth inhibition was measured to the nearest millilitre and interpreted. Molecular identification was also carried out to identify the bacteria isolated.

Results and Discussion

Bacterial contamination of kunu locally produced and sold in six (6) major markets in Ibadan, Oyo state were assessed. The colony count observed on nutrient agar showed Highest colony count of samples obtained at Mokola was 18×10^7cfu/ml while lowest was 8×10^7cfu/ml , highest for samples from tollgate was 2×10^7cfu/ml 3and lowest was 3×10^7cfu/ml , the highest colony count obtained from samples from Sabo was 18 while $t\times10^7$cfu/ml he lowest was 4×10^7cfu/ml, also highest from Ogunpa was 8×10^7cfu/ml while lowest was 3×10^7cfu/ml, highest colony count from Dugbe was 14×10^7cfu/ml while lowest was 3×10^7cfu/ml,the highest from Orita was 39×10^7cfu/ml while the lowest was 8×10^7cfu/ml.

Total coliform count on MacConkey Agar. the highest and lowest colony counts were recorded as follows; samples from Mokola the highest was 24×10^7cfu/ml with the lowest at 6×10^7cfu/ml,samples from tollgate, the highest was 27×10^7cfu/ml with lowest at 4×10^7cfu/ml, samples gotten from Sabo had 28×10^7cfu/ml as highest while 1×10^7cfu/ml was the lowest. From samples obtained Ogunpa, the highest was 12×10^7cfu/ml while the lowest was 5×10^7cfu/ml and the highest from Dugbe was 12×10^7cfu/ml while the lowest was 4×10^7cfu/ml.

The colony count on De Man Rogosa, Sharpe agar,with the highest group from Mokola at 20×10^7cfu/ml, lowest 1×10^7cfu/ml, Ogunpa with highest at 28×10^7cfu/ml and lowest at 1×10^7cfu/ml, Sabo with highest at 24×10^7cfu/ml and lowest at 4×10^7cfu/ml, Orita with highest at 17×10^7cfu/ml and lowest 4×10^7cfu/ml, and Dugbe with highest at 15×10^7cfu/ml while lowest is 4×10^7cfu/ml.

Table1.0 shows the chemical and cultural characteristics of isolates and probable organism while Table 2.0 shows the percentage distribution of bacterial isolates. Table 3.0

shows the characteristic and properties of partial 16Sribosomal sequence from 3 bacterial isolates obtained from kunu using the nBLAST on GenBank.

Table 4.0 0 shows the antibiotic susceptibility pattern of pathogenic bacteria isolated from kunu samples.

S/NO	probable Identified Organisms	Gram Staining Reaction of the Isolates	Cultural Characteristic on Culture Media					Catalase	Motility	Methyl Red	Acid and Gas Production from Sugar	Haemolysis on Blood Agar
			MRS	M.Z	MacConkey Agar	SSA	M.S.A					
1.	*Bacillus subtilis*	+ve rod	___	Creamy	__	__	yellow	+	+	__	+	Non
2.	*Leuconostoc spp*	+ve cocci	Creamy	Creamy	__	__	__	+	+	__	__	Non
3.	*Micrococcus spp*	+ve cocci	__	Yellowish	__	__	Red	__	__	__	__	Non
4.	*Salmonella spp*	-ve slender rod	__	Creamy	__	Dark Centre	__	+	+	__	__	B
5.	*Lactobacillus spp*	+ve rod	Whitish	Creamy	Cream White	__	__	+	__	__	+	Non
6.	*Staph aureus*	+ve rod	Creamy	Creamy	__	__	yellow	+	__	+	+	Non
7.	*Escherichia coli*	-ve rod	Creamy	Yellow	___	Pinkish	__	+	__	+	__	α
8.	*Pseudomonas*	-ve thick rod	__	Creamy	Pinkish	__	__	__	+	+	__	B -

Table 1: Biochemical and Cultural Characteristics of the Isolated Organisms

Table 2.0 Percentage Distribution of Bacterial Isolates

O	Probable Isolated Microorganisms	DG	TG	OT	OG	/MK	SB	Total	%
1.	*Micrococcus spp*	13	18	24	13	31	23	**122**	**10**
2.	*Leuconostoc spp*	29	30	19	45	_	_	**123**	**11**
3.	*Staph aureus*	30	62	23	14	68	75	**272**	**24**
4.	*Salmonella spp*	2	_	3	_	3	_	**8**	**1**
5.	*Lactobacillus fermentum*	50	50	16	57	19	62	**254**	**22**
6.	*Bacillus spp*	26	5	18	48	23	33	**153**	**13**
7.	*Escherichia Coli*	9	6	11	3	13	21	**63**	**6**
8.	*Pseudomonas*	42	47	23	_	16	17	**145**	**13**
	Total No Occurrence	**201**	**218**	**137**	**180**	**173**	**231**	**1,140**	
	% Occurrence	**18**	**19**	**12**	**16**	**15**	**20**		

Table 3.0: Characteristics and properties of partial 16S ribosomal RNA sequences from three bacteria samples obtained from kunu in Ibadan, Oyo State using nBLAST on GenBank

Sample ID	Accession Number	Properties	Identified species	Details	E-value	Alignment score	Highest query coverage (%)
NG-LCU-MCB-naGP1	OQ344330	1,461 bp	*Escherichia coli*	99.93% similarity using BLAST 2.10.0N+	0.0	>200	100
NG-LCU-MCB-naGP1	OQ344331	1,477 bp	*Pseudomonas aureginosa*	99.80% similarity using BLAST 2.10.0N+	0.0	>200	100
NG-LCU-MCBmrsMKL1	OQ344332	1,457 bp	*Escherichia coli*	99.93% similarity using BLAST 2.10.0N+	0.0	>200	100

Table 4 : Antibiotic Susceptibility Pattern of Pathogenic Bacteria Isolated from Kunu Samples

	Antibiotics Disc Used	**Code**	**Concentration**	**Isolated Pathogenic Bacteria**		
				Pseudomonas	***E. coli***	***E. coli***
1.	Perfloxacin	PEF	30ug	23	20	21
2.	Gentamicin	CN	30ug	20	19	21
3.	Amoxacillin	AM	30ug	22	21	21
4.	Ciprofloxacln	CPX	30ug	22	22	23
5.	Streptomycin	S	30ug	23	20	20
6.	Septrin	SXT	30ug	21	23	22
7.	Augmentin	AU	10ug	23	21	21
8.	Tarivid	OFX	10ug	22	19	21
9.	Chloramphenicol	CH	30ug	22	21	21
10.	Sparfloxacin	SP	10ug	23	20	22

Discussion of Findings

The bacteriological analysis of kunu samples collected from six major markets in Ibadan revealed that all samples were contaminated to varying degrees.

Bacterial species isolated from the samples include *Staphylococcus aureus*, *Lactobacillus fermentum*, *Escherichia coli*, *Pseudomonas species*, *Salmonella*, *Bacillus subtilis Leuconostoc species* and *Micrococcus species*. *Staphylococcus aureus* had the highest occurrence of 24% followed by *Lactobacillus fermentum* 22% and *Bacillus subtilis* with percentage occurrence of 14%. *Salmonella spp.* had the least occurrence of 1% .The presence of E. coli in Kunu samples is particularly concerning because it is a classical indicator of fecal contamination. (Chepkoskei et al, 2025). This suggests the use of contaminated water during production or unhygienic handling practices by vendors. Similarly, *S. aureus* is a commensal organism of the skin and nasal passages and is typically introduced into food through direct human contact.

Antibiogram of the pathogenic bacterial isolates showed zone of inhibition to the antibiotics tested against. The tested bacteria showed susceptibility to all antibiotics in varying degrees compared with reports by (Yadav et al, 2019) which showed increase sensitivity to Gentamicin, Imipenem, Amoxicillin and Ofloxacin against the test bacterial isolates. This indicates that the antibiotics may be administered when combating the test organisms in treatment for effective results. Compared to findings by (Nwaiwu et al, 2020) which showed evidence that antimicrobial resistance is on the rise in Nigeria.

The findings from the hemolytic analyses of the kunu samples revealed that the beverage was contaminated with potentially pathogenic bacterial species, including *Staphylococcus aureus, Escherichia coli,* and *Streptococcus spp*. The presence of these organisms signifies lapses in hygiene during the preparation, handling, or storage of Kunu, reflecting poor sanitary conditions and the use of contaminated materials or water sources in production. (Amala & Daw 2019). The β-hemolytic pattern seen in culture provides a clear indication of the organism's pathogenic potential and explains why *S. aureus* infections can range from mild skin lesions to severe systemic diseases such as septicemia and pneumonia. The presence of hemolytic activity in these isolates, particularly β-hemolysis, suggests the production of streptolysins toxins that contribute to the organism's virulence by lysing red blood cells and damaging host tissues β-hemolytic *Streptococcus species*, such as *S. pyogenes*, are well-known for causing pharyngitis, scarlet fever, and invasive soft tissue infections.(Pontieri., 2018).

The hemolytic activity exhibited by some *E. coli* isolates is indicative of the production of hemolysin (HlyA), a toxin that damages cell membranes and contributes to intestinal and extraintestinal infections. (Pontieri 2018). The presence of such hemolytic strains in Kunu is of great concern because it suggests that the beverage could serve as a vehicle for transmitting virulent and antibiotic-resistant strains to consumers. (Gugu et al 2025)

Although the isolation of multidrug-resistant (MDR) strains from street-vended beverages such as Kunu is alarming, as these organisms are not only harmful to the direct consumers of the product but also contribute to the broader issue of antimicrobial resistance within the community. Regular ingestion of sub-lethal doses of these bacteria may lead to colonization of the human gut, turning healthy carriers into silent disseminators of resistance genes. (Ibironke et al, 2023).

Conclusion

The findings of this study clearly demonstrate that Kunu, though a widely enjoyed traditional beverage, poses a significant risk to public health due to microbial contamination. The presence of hemolytic and pathogenic bacterial isolates in Kunu sold in Ibadan markets indicates that the beverage poses a substantial health risk if consumed without appropriate precautions. The bacteria identified in the samples *Staphylococcus aureus*, *Lactobacillus fermentum*, *Escherichia coli*, *Pseudomonas species*, *Salmonella*, *Bacillus subtilis Leuconostoc species* and *Micrococcus species* are known to cause a range of infections, some of which may be life-threatening, especially in individuals with weakened immune.

Although this current study did not isolate MDR bacteria, other findings have. In a country like Nigeria, where access to effective and affordable healthcare is already limited, the probability of presence of drug-resistant organisms in everyday food and beverages presents a substantial threat.

Immediate and sustained public health interventions are required. These should include:

- Rigorous monitoring of street-vended foods and beverages by health authorities.
- Public awareness campaigns targeting both vendors and consumers
- Introduction of basic food safety training for Kunu vendors
- Enforcement of minimum hygiene standards and licensing for local food and drink processors

If left unaddressed, the current situation could evolve into a larger epidemic of foodborne illnesses and antibiotic-resistant infections.

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