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Bacteriological evaluation of Kunu beverages sold in university communities of Bokkos, Plateau State, Nigeria

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ABSTRACT: Kunu is a widely consumed indigenous non-alcoholic beverage in northern Nigeria, valued for its nutritional benefits, cultural significance, and economic importance. This study assessed the bacteriological quality of kunu, a traditional non-alcoholic fermented beverage, sold within the university communities of Bokkos Local Government Area, Plateau State, Nigeria. A total of 30 kunu samples were randomly collected and subjected to standard microbiological procedures to isolate, characterize, and identify bacterial contaminants. Colonial morphology, Gram staining, and biochemical tests were employed for bacterial identification. The total viable bacterial counts were also determined using the pour plate method. Four bacterial species were consistently isolated: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, and *Enterobacter aerogenes*. The most frequently detected organism was *S. aureus* (35.71%), followed by *E. coli* (28.57%). Morphological and biochemical characteristics of the isolates corresponded with standard descriptions, validating their identification. Bacterial loads ranged from 0.0 to 5.5 × 10^5 CFU/mL, with most samples exceeding the maximum acceptable limit of 10^4 CFU/mL for ready-to-drink beverages, as recommended by the International Commission on Microbiological Specifications for Foods. Statistical analysis revealed no significant difference ($p = 0.4142$) in the distribution of bacterial species across the samples. The presence of enteric and pathogenic bacteria in most samples suggests poor hygienic practices during preparation, handling, and storage. The study underscores the need for public health education, improved sanitary practices among vendors, and regulatory monitoring to ensure the safety of traditional beverages like kunu consumed by the public.

Keywords: Bacteriological quality, Bokkos, Kunu, Plateau State.

INTRODUCTION

Kunu is a popular indigenous non-alcoholic beverage widely consumed across northern Nigeria for its nutritional, cultural, and economic value (Ndukwe *et al*., 2023). Typically prepared from cereals such as millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), or maize (*Zea mays*), *kunu* is often enriched with additives such as sweeteners, ginger, and spices to enhance its flavour and palatability (Akubuenyi and Sylvanus, 2022). Its affordability, availability, and refreshing qualities have made it a preferred drink among urban and rural populations, including students and residents of university communities.

The traditional production of kunu generally involves cleaning and steeping the grains, wet milling, sieving the slurry, partial fermentation, boiling part of the filtrate into a pap, and then mixing it back with the unboiled portion to achieve the desired consistency and taste (Sadiq and Atteh, 2023). After cooling, sweeteners and spices are added before packaging for immediate sale. While this method enhances the beverage's sensory appeal, each step, from grain washing to mixing and packaging, poses potential points for microbial contamination, particularly when hygienic standards are inadequate. Beneficial microorganisms such as lactic acid bacteria (*Lactobacillus*

spp.) and yeasts typically dominate the natural fermentation, contributing to the drink's sour-sweet flavour and mild preservation effect, while opportunistic and pathogenic bacteria (*E. coli*, *Salmonella* spp., *Shigella* spp., *S. aureus*) can contaminate the product under poor sanitary conditions (Sowemimo *et al.,* 2021).

Despite its popularity, *kunu* production in Nigeria remains largely informal and home-based, with minimal adherence to sanitary and hygienic standards. According to Ndukwe *et al.* (2023), vendors often prepare and sell the beverage under unhygienic conditions, exposing it to potential microbial contamination from raw materials, water sources, utensils, handling practices, and storage environments. Several studies (Sowemimo *et al.,* 2021; Ogunremi *et al.,* 2022; Sadiq and Atteh, 2023; Olawoye *et al.,* 2024) have reported the presence of pathogenic and indicator bacteria such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* in locally made beverages, raising public health concerns. Typically, kunu has an acidic pH range of 3.5–4.5, which limits many spoilage microbes but still allows survival of acid-tolerant pathogens if handling is poor (Akubuenyi and Sylvanus, 2022). The consumption of such contaminated drinks may result in foodborne illnesses, particularly among vulnerable groups such as children, the elderly, and immunocompromised individuals.

In the context of Bokkos Local Government Area, where Plateau State University is situated, kunu remains a staple drink among students and local residents. However, there is a dearth of empirical data on the bacteriological quality of the kunu sold in these communities. Given the increasing reliance on street-vended foods and drinks, especially in university settings, assessing the microbial safety of such beverages is critical. This is particularly important in resource-limited settings where regulatory oversight is weak or absent and where outbreaks of foodborne diseases could have far-reaching implications for public health.

This study, therefore, seeks to evaluate the bacteriological quality of different varieties of kunu sold within the university communities of Bokkos LGA, Plateau State. By identifying the bacterial load and types of microbial contaminants present, the study aims to provide evidence-based recommendations for improved hygiene practices and public health interventions. The findings will also contribute to the body of knowledge on food safety and support policy efforts aimed at regulating the production and sale of local beverages in Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted in Bokkos Local Government Area of Plateau State, North Central Nigeria. The area is home to Plateau State University and comprises several university host communities where a wide range of street-vended foods and beverages, including kunu, are readily available. The region experiences a tropical climate with a distinct wet and dry season, which may influence the microbial profile of consumables due to variations in temperature and sanitation practices.

Study design and sample collection

This study employed a cross-sectional design conducted between April and June, 2024. Researchers collected kunu beverage samples from randomly selected vendors across three major points of sale within the Plateau State University community in Bokkos, Plateau State. A total of 30 kunu samples were aseptically collected using pre-sterilized 250 mL glass bottles. To maintain sample integrity and prevent contamination, aseptic techniques were rigorously observed throughout the collection process, including the use of sterile gloves and immediate sealing of containers after sample acquisition. All samples were promptly stored in an ice-packed cooler and transported within two hours to the Microbiology Laboratory of Plateau State University for bacteriological analysis.

Determination of bacterial load

Bacteriological analysis followed standard microbiological procedures as described by Ogunremi *et al.* (2024). Each *kunu* sample was first homogenized, and a tenfold serial dilution was prepared using sterile normal saline up to a dilution factor of 10^{-5}. From each appropriate dilution, 1 mL aliquots were aseptically inoculated onto sterile Petri dishes using the pour plate technique. Nutrient Agar was used for estimating total viable bacterial count, while selective and differential media, including MacConkey Agar (MCA), Eosin Methylene Blue Agar (EMBA), Mannitol Salt Agar (MSA), and Salmonella-Shigella Agar (SSA), were employed for the isolation and differentiation of specific bacterial species. All plates were incubated aerobically at 37°C for 24 hours for Nutrient Agar and EMBA, while MSA and MCA plates were incubated at 37°C for 24–48 hours. SSA plates were incubated at 37°C for 24 hours to selectively isolate enteric pathogens. Bacterial loads were subsequently recorded and expressed as colony-forming units per millilitre (CFU/mL).

Isolation and identification of bacteria

For the isolation and identification of specific bacterial pathogens, separate aliquots were plated onto selective and differential media: MacConkey Agar for Gram-negative enteric bacteria, Eosin Methylene Blue (EMB) Agar for *Escherichia coli*, Salmonella-Shigella (SS) Agar

for *Salmonella* and *Shigella* spp., and Mannitol Salt Agar for *Staphylococcus aureus* (Ogunremi *et al.*, 2022). After incubation, distinct colonies were sub-cultured onto fresh Nutrient Agar plates and incubated at 37°C for 24 hours. This sub-culturing process was repeated for at least two successive passages to ensure pure cultures were obtained. The purified isolates were then subjected to Gram staining and a series of biochemical tests, including catalase, coagulase, indole, citrate, urease, and triple sugar iron (TSI) tests, to facilitate accurate identification.

Data analysis

Data analysis was conducted using R Console Version 4.4.1. Data were presented in tabular form for clarity and ease of interpretation. Bacterial count for each kunu sample expressed in colony-forming units per millilitre (CFU/mL). To determine the prevalence and distribution of bacterial isolates and as well as determine the bacteria count, the Chi-square test was employed. Differences were considered statistically significant at $p<0.05$.

RESULTS

Frequency distribution of bacterial isolates from *Kunu* sample

A total of four bacterial species were isolated from the 30 kunu samples collected across the various university communities in Bokkos, Plateau State. The identified microorganisms included *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, and *Enterobacter aerogenes*. Among these, *Staphylococcus aureus* exhibited the highest prevalence, accounting for 35.7% of the total isolates. This was followed by *Escherichia coli* (28.6%), *Streptococcus pyogenes* (21.4%), and *Enterobacter aerogenes* (14.3%) (Table 1). However, the analysis showed no statistically significant difference ($\chi^2 = 2.8571$, df = 3, $p = 0.4142$) in the prevalence of bacteria isolated from the kunu samples.

Morphological features of the isolates on different media

Table 2 presents the colonial morphology of bacterial isolates on various culture media. The growth patterns and pigmentations observed provided preliminary differentiation of the isolates. *Staphylococcus aureus* and *Streptococcus pyogenes* were distinguishable by their hemolytic reactions on Blood Agar, while *E. coli* and *Enterobacter aerogenes* exhibited characteristic lactose fermentation and colony appearances on MacConkey and EMB agars. Selective inhibition on certain media, such as SSA and MSA, further aided in confirming isolate identity. These observations formed the basis for subsequent confirmatory biochemical testing.

Table 1. Prevalence of bacteria isolates from *Kunu* sample.

Organisms	Number isolated (%)
Streptococcus pyogenes	6(21.43)
Escherichia coli	8(28.57)
Enterobacter aerogenes	4(14.29)
Staphylococcus aureus	10(35.71)
Total	28(100.00)

($\chi^2 = 2.8571$, df = 3, P = 0.4142).

Biochemical characteristics of the bacterial isolate

Table 3 presents the biochemical profiles of the bacterial isolates recovered from *kunu* samples. The Gram staining reactions, enzyme activities, and carbohydrate fermentation patterns confirmed the identities of *Streptococcus pyogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterobacter aerogenes*. Notably, *S. aureus* exhibited positive catalase and coagulase activity typical of the species, while *E. coli* showed strong indole production and lactose fermentation. *S. pyogenes* was catalase-negative and oxidase-positive, consistent with streptococcal characteristics. Although *E. aerogenes* showed variable results, its lactose fermentation and indole positivity supported its classification. These biochemical characteristics aligned with the colonial morphology findings and supported accurate identification of the isolates.

Bacterial counts of the *Kunu* samples (CFU/ml)

Table 4 shows the total viable bacterial counts expressed in colony-forming units per millilitre (CFU/mL) for 30 kunu samples labelled S1 to S30. The bacterial counts ranged from 0.0 CFU/mL (samples S6 and S15) to as high as 5.5×10^5 CFU/mL (sample S9). Several samples, including S5, S10, S20, S25, and S26, also showed high bacterial loads exceeding 4.0×10^5 CFU/mL. In contrast, some samples such as S1, S4, S7, S16, and S30 exhibited relatively lower counts, ranging between 1.2×10^5 and 2.4×10^5 CFU/mL. Therefore, there was a very high significant difference ($\chi^2 = 1781037$, df = 29, $P < 0.001$) in the bacterial count of the kunu samples examined.

DISCUSSION

This study demonstrated the presence of multiple bacterial species in kunu beverages sold within university communities in Bokkos, Plateau State. The isolates, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, and *Enterobacter aerogenes*, are commonly associated with food contamination and pose considerable health risks. The prevalence of these organisms aligns

Table 2. Morphological features of the isolates on different media.

Isolates	B A	MCA	SSA	EMBA	MSA
Staphylococcus aureus	Greenish discolouration, α-hemolytic	Pale yellow colonies	Inhibited	Inhibited	Yellow colonies
Streptococcus pyogenes	Whitish colonies with β-hemolytic	Inhibited	Inhibited	Inhibited	Poor growth
Escherichia coli	No Haemolysis	red/pink non mucoid colonies	Small pink to red colonies	Blue-black colonies with green methalic sheen	Inhibited
Enterobacter aerogenes	No Haemolysis	Pink mucoid colonies	Mucoid, pale opague cream to pink colonies	Pink dull colonies	Inhibited

Key: BA: Blood Agar; MCA: MacConkey Agar; SSA: Salmonella-Shigella Agar; EMBA: Eosin Methylene Blue Agar; MSA: Mannitol Salt Agar.

Table 3. Biochemical characteristics of the bacterial isolate.

Biochemical tests	*Streptococcus pyogenes*	*Escherichia coli*	*Staphylococcus aureus*	*Enterobacter* aerogenes
Gram reaction	+ve	-ve	+ve	-ve
Catalase	-	+	+	-
Oxidase	+	-	-	-
Coagulase	-	-	+	+
Citrate	-	-	-	-
Urease	-	-	+	-
Indole	-	+	-	+
Starch hydrolysis	-	-	-	-
Motility	-	-	-	-
Methyl red	-	+	-	+
VP-Voges Proskauer	-	-	+	-
Glucose	+	+	+	-
Sucrose	+	+	+	-
Fructose	-	-	+	-
Galactose	-	-	-	-
NO_3	-	+	+	-
Lactose	-	+	+	+

Key: +ve: Positive; -ve: Negative; - : Nill.

with findings from similar studies across Nigeria and other regions. For instance, Oyedum and Agbala (2022) reported comparable bacterial contaminants in locally prepared cereal-based drinks in Niger State, Nigeria, while Akubuenyi and Sylvanus (2022) observed similar microbial profiles in fermented beverages in Cross River State, Nigeria. Globally, studies such as that of Cárdenas *et al.* (2024) in Ecuador and Zulfakar *et al.* (2021) in Pakistan also identified these pathogenic bacteria in street-vended beverages, underscoring the universal challenge of ensuring microbial safety in informal food sectors. Although this study did not find a statistically significant difference in the prevalence of isolates across the different kunu varieties, the consistent detection of potentially pathogenic bacteria across samples indicates widespread contamination. Such contamination can originate from multiple sources, including poor personal hygiene of vendors, contaminated water, use of unclean utensils, and lack of standardised processing techniques, as documented by Amala *et al.* (2021) and Echeonwu and Eruteya (2024) in Nigerian street foods. The presence of pathogens such as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* poses serious public health concerns, as their ingestion can lead to foodborne illnesses ranging from mild gastroenteritis to severe diarrheal diseases and systemic infections. In immunocompromised individuals, children, and the elderly, these infections can escalate rapidly, resulting in significant morbidity and potential mortality. Frequent exposure to contaminated *kunu* may also contribute to the endemicity of diarrheal diseases in affected communities, undermining public health efforts to reduce the burden of

Table 4. Bacterial counts of the Kunu samples (CFU/ml).

Samples	Colony Forming Unit per 1 Millilitre (CFU/ml)
S1	$2.3x10^5$
S2	$3.4 x10^5$
S3	$4.2 x10^5$
S4	$2.4 x10^5$
S5	$4.6 x10^5$
S6	0.0
S7	$2.1 x10^5$
S8	$3.5 x10^5$
S9	$5.5 x10^5$
S10	$4.3 x10^5$
S11	$3.1 x10^5$
S12	$2.8 x10^5$
S13	$3.9 x10^5$
S14	$4.4 x10^5$
S15	0.0
S16	$1.2 x10^5$
S17	$3.0 x10^5$
S18	$4.1 x10^5$
S19	$2.6 x10^5$
S20	$4.6 x10^5$
S21	$3.2 x10^5$
S22	$1.8 x10^5$
S23	$2.4 x10^5$
S24	$1.9 x10^5$
S25	$4.7 x10^5$
S26	$5.2 x10^5$
S27	$3.3 x10^5$
S28	$2.9 x10^5$
S29	$3.6 x10^5$
S30	$1.6 x10^5$

($\chi^2 = 1781037$, df = 29, $P < 0.001$).

preventable water- and food-borne infections.

The colonial morphology of the isolates on selective and differential media reinforced their identification and reflected expected patterns consistent with standard microbiological profiles. For example, *S. aureus* exhibited typical yellow colonies on Mannitol Salt Agar and α-hemolysis on Blood Agar, indicative of mannitol fermentation and hemolytic activity (Cheebrough, 2010). Similarly, *E. coli* produced characteristic blue-black colonies with a green metallic sheen on Eosin Methylene Blue Agar, signalling vigorous lactose fermentation, a hallmark trait of this species (Mustafa *et al.,* 2024). The inhibition of certain bacteria on selective media, such as Salmonella-Shigella Agar, further aided in differentiating closely related species. These morphological observations are consistent with previous research by Ogunremi *et al.* (2022) in East and Western Nigeria, who reported similar colonial features in isolates from traditional beverages. Such culture-dependent methods remain foundational in microbiology for preliminary bacterial identification before biochemical confirmation.

The biochemical profiles presented align well with classical descriptions of the isolates. *Staphylococcus aureus* displayed catalase and coagulase positivity, confirming its identity as a coagulase-positive staphylococcus, a feature consistently reported in studies by Sule *et al.* (2022) in Kwara State and Susan *et al.* (2024) in Plateau State. The negative catalase reaction for *Streptococcus pyogenes* distinguished it from staphylococci, corroborating findings by Shakir *et al.* (2021). The positive indole and methyl red tests for *Escherichia coli* indicated mixed acid fermentation and tryptophan metabolism, matching observations from earlier studies (Li *et al.,* 2020; Reeds *et al.,* 2024). In this study, *Enterobacter aerogenes* was identified primarily through its distinct morphological and biochemical characteristics, consistent with prior descriptions (N'Tcha *et al.,* 2023). Although coagulase testing is not typically applicable to *Enterobacter* species, which are inherently coagulase-negative, this underscores the practical challenges of relying solely on biochemical tests in resource-limited settings. Carbohydrate fermentation patterns further supported accurate identification: *E. coli* and *S. aureus* fermented multiple sugars, including glucose and lactose, while *S. pyogenes* exhibited limited fermentation, aligning with its known metabolic profile (Cheebrough, 2010). These classical biochemical traits remain vital for preliminary differentiation, where molecular diagnostics are not routinely available, but confirmatory molecular assays are strongly recommended to resolve ambiguous or atypical results.

The bacterial loads measured in the *kunu* samples ranged from undetectable levels to 5.5×10^5 CFU/mL, highlighting significant microbial contamination in most samples. This bacterial density exceeds the acceptable limits for ready-to-drink beverages set by various food safety authorities, such as the World Health Organisation and Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC), which recommend total viable counts below 10^4 CFU/mL in beverages (Udu-Ibiam *et al.,* 2025). Similar high microbial loads have been reported in traditional fermented beverages in Nigeria and other African countries. For example, Opeyemi and Obuneme (2020) found comparable bacterial counts in kunu samples from Keffi, Nasarawa State Nigeria, while Echeonwu and Eruteya (2024) observed elevated counts in fermented cereals sold in Portharcourt, Nigeria. The presence of high bacterial loads increases the risk of foodborne infections, especially where pathogenic species are present. The samples with zero bacterial counts likely reflect either freshly prepared or properly handled beverages or possible sampling/analytical variation. Nonetheless, the general trend underscores the urgent need for improved hygiene, vendor training, and regulatory enforcement to reduce microbial contamination in street-vended beverages.

The high significant difference observed in the bacterial counts among the kunu samples examined in this study indicates considerable variability in the microbial load across different samples, reflecting inconsistencies in the hygienic quality of the beverages. Such significant variation may be attributed to differences in preparation methods, water quality, handling practices, storage conditions, and environmental exposure during processing and vending. The finding underscores the non-uniform nature of microbial contamination in traditionally produced kunu, and it highlights the urgent need for standardized hygiene protocols to ensure product safety and protect public health. This result aligns with those of other related studies, both within Nigeria and across other parts of Africa. For instance, Akubuenyi and Sylvanus (2022) reported wide variations in microbial loads of kunu sold in Calabar, attributing the disparities to differences in water sources, hygiene practices, and environmental exposure during production and vending. Similarly, Ezemba *et al.* (2021) found significant differences in microbial contamination levels in street-vended beverages in Awka, Nigeria, linking them to unregulated preparation methods and lack of sanitary oversight. Beyond Nigeria, Wafula *et al.* (2022) in Kenya observed significantly varied bacterial counts in fermented cereals, highlighting that informal food processing practices often lead to unpredictable microbial profiles. In Ghana, Aboagye *et al.* (2020) reported high variability in the microbial content of traditional drinks sold in urban markets, reinforcing the influence of local hygiene and processing conditions.

Conclusion

This study demonstrates that kunu beverages sold within the university communities of Bokkos Local Government Area contain diverse bacterial contaminants, with microbial loads that exceed acceptable limits for ready-to-consume drinks and pose potential health risks to consumers. These findings corroborate global and regional evidence highlighting the vulnerability of traditional, informally prepared beverages to microbial contamination. If left unaddressed, such contamination can contribute to foodborne illnesses and undermine community health, especially among students and other high-consumption groups.

To mitigate these risks, it is recommended that targeted public health education be intensified for local vendors and consumers to promote hygienic practices during preparation, storage, and vending of kunu. Health authorities should develop and enforce clear sanitation guidelines and regular inspection protocols for street-vended beverages. Furthermore, empowering vendors through training and access to safe water and sanitary production facilities can help reduce contamination risks. Future research should also explore cost-effective preservation methods and conduct routine monitoring to ensure the microbiological safety of traditional drinks like kunu.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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