

Microbial Quality Assessment of *Kunun Zaki* Local Beverages Sold in Wukari Metropolis, Taraba State, Nigeria

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Abstract

Kunun-zaki is a traditional indigenous beverage widely consumed in Northern Nigeria, typically prepared from millet, guinea corn, or maize. Milky in appearance, kunun-zaki is rich in carbohydrates, vitamins, and minerals, but low in protein content, and is often consumed within a few hours of production. This study aimed to assess the microbial quality of kunun-zaki sold in Wukari metropolis to evaluate its safety for consumer intake. A total of 40 kunun-zaki samples were collected from vendors across four different clusters, with ten samples obtained from each zone. The samples were analyzed for microbial load using standard microbiological procedures. Results indicated total aerobic plate counts ranging from 9.8×10^5 to 1.0×10^5 cfu/ml in Zone A; 9.7×10^5 to 1.21×10^5 cfu/ml in Zone B; 9.7×10^5 to 1.0×10^5 cfu/ml in Zone C; and 4.96×10^5 to 1.12×10^5 cfu/ml in Zone D. Bacterial isolates identified across all zones included *Staphylococcus* spp. (24.6%), *Escherichia coli* (20.9%), *Micrococcus* (19.7%), *Pseudomonas* (9.2%), *Salmonella* spp. (7.4%), *Bacillus* spp. (6.17%), *Klebsiella* spp. (4.3%), *Vibrio* spp. (3.08%), and *Enterobacter* spp., which appeared in Zones A (2.4%) and D (4.87%). The high bacterial load and presence of pathogenic species indicate significant contamination, posing potential health risks to consumers. The study concludes with a call for

stringent hygienic practices among producers and vendors during the preparation and storage of kunun-zaki to safeguard public health.

Keywords: Kunun Zaki; Microbial Quality; Food Vendors; Health Hazard; Hygienic Measures

INTRODUCTION

Kunun-zaki is an indigenous fermented non-alcoholic beverage that is widely consumed for its thirst quenching properties. Though consumed throughout the year, it is extensively consumed during the dry season. The drink is produced from fermented millet, sorghum, guinea-corn and maize in decreasing order of preference. In some cultures, the grains are used in a composite from especially millet, guinea-corn and sorghum in a ratio of 1:2 w/w [1]. It is sweetened with honey and sugar together with small quantities of sweet potatoes and spices (ginger, black pepper or clove). Akoma *et al.*, [2] worked on the production and consumption pattern of kunun-zaki and concluded that kunun-zaki was identified to be the most preferred (67%) among the nine types of Kunu produced in Nigeria. Housewives were observed to be actively involved (26%) in Kunu production. The lesser involvement of tertiary school graduates (17%) and the greater participation of un-educated individuals (30%) in Kunun production may be there as on why its production is still on a small-scale level; there is therefore, the need for the development of a scientific process for its production in order to encourage large-scale production.

The method of production is crude, not standardized with levels of ingredients not quantified and largely a family art. The procedure involves steeping the cereals in local household utensils such as buckets, calabashes and earthenware vessels. This is then followed by grinding of the steeped grains into a mush which is then mixed with spices (clove, red or black pepper and ginger). Significant variations exist in the procedures depending on taste and cultural habits leading to differences in quality and stability.

While some cultures prefer kunun-zaki with much pepper or sweet taste, others prefer it with no pepper or sugar [3] It is usually packaged and sold in 1litre and 500 ml plastic bottles or even tied in small polyethene bags. Kunun-zaki must be consumed within 18 - 36 h of production due to its poor keeping quality.

The dominance of *Lactobacillus leimannii* and *L. fermentum* in a study samples led Akoma *et al.* [2] concluded that kunun-zaki is a lactic acid bacteria fermented beverage. The acidity of Kunun drinks may be due to the presence of some bacteria such as *Lactobacillus*, *Acidophillus*, *Candida* species and *Saccharomyces cerevisiae* which help in acid fermentation of the Kunun products and essential to human beings [2,4,5].

The drink is very cheap because the cereals and additives used in its production are locally sourced as they are grown throughout the savannah belt of West Africa and the packaging materials are also cheap and easily available.

Agarry *et al.*, [6] have reported that kunun-zaki is rich in carbohydrates, vitamins and minerals but low in proteins. Furthermore, the methods of production are simple and cheap as no elaborate equipment and expertise required [6].

Mbachu, *et.al.*, [7] worked on the microbiological assessment of kunun-zaki marketed in Abuja Municipal Area Council (AMAC) in the Federal Capital Territory (FCT), Nigeria and concluded that the presence of the isolated organisms in kunun-zaki samples analyzed could serve as indicator so there is need to promote awareness about the possible health hazards that could arise due to handling and processing of the beverage. The range of microorganisms isolated pose serious threat to food safety and hence the need to ensure microbial safety during the production and distribution of this drink that is widely consumed in most part of Northern Nigeria while Iruolaje *et al.* [8] also worked on the microbial evaluation of kunun-zaki prepared and sold in the Bauchi metropolis. From the result obtained, it was reported that the organisms associated with kunun-zaki were *Escherichia coli*, *Staphylococcus species*, *Streptococcus species*, *Lactobacillus species*, *Candida species* and *Aspergillus species* and the action or presence of these organisms in Kunun-zaki rendered it unfit for human consumption.

The high water content coupled with crude methods of production and packaging under improper sanitary conditions predisposes *kunun-zaki* to microbial contamination. The production of Kunu in environment with poor sanitary condition could predispose consumers of the drink to pathogens of medical or public health importance. Furthermore the drink has short shelf-life and is prone to microbial spoilage if not adequately stored and could act as important medium for the transmission of pathogenic microorganisms. Therefore, the aim of this study is to assess the microbial quality of kunun-zaki sold in Wukari metropolis in order to ascertain its quality and safety to the consumers.

MATERIALS AND METHODS

Materials

Different samples of Kunun-zaki were purchased from four (4) different zones in Wukari metropolis of Taraba state, Nigeria.

Samples Collection

Forty (40) samples of Kunun-Zaki were collected from the sellers in four different zones (clusters), (Fig 1.1), and ten (10) samples from each Cluster. The zones comprises of Wapan nghaku, Federal university Wukari, mission quarters , old Market and Old BB, (Zone A), Tatum Junction, New site, Shagari Low-cost, New Market and Urban Planning Quarters area (Zone B) Abuja area, Yam Market, Holy spirit Agidiku and East (Zone C)and General Hospital, GSS, Prison, St. Mary, Angwan Kabari (Zone D).

The samples were labelled appropriately, placed in ice packed in cooler in separate plastic bags and conveyed to Microbiology laboratory of the Federal University Wukari for analyses. The samples were stored at refrigeration temperature until when needed for analyses.

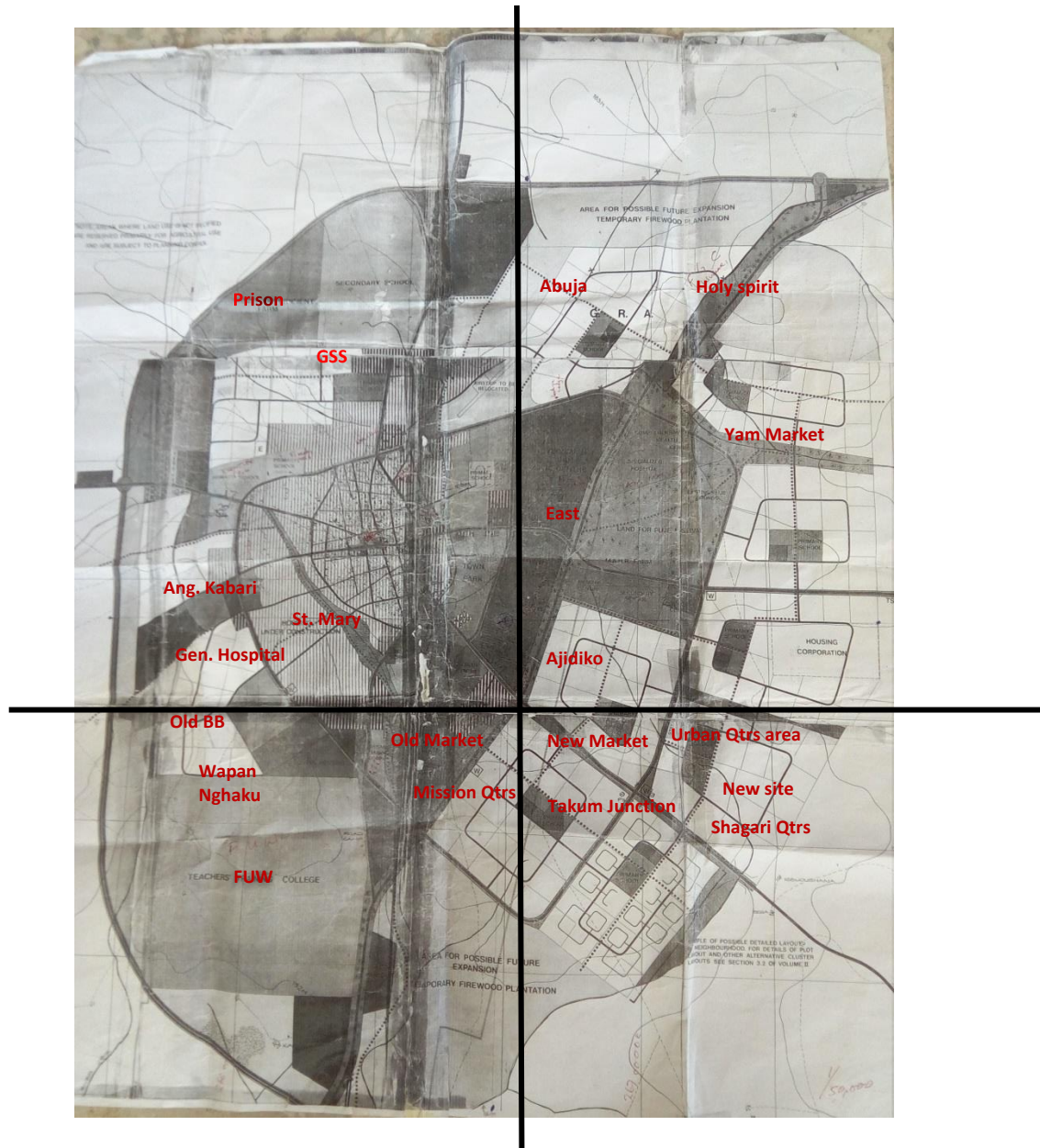


Figure 1. Map of Wukari Metropolis

Microbiological Analysis

Media preparation

The media used Nutrient and MacConkey agar were weighed and dissolved in distilled water and sterilized by autoclaving at 121° for 15 minutes at 15psi according to the manufacturer's instruction.

Enumeration of Microorganisms

Total bacterial count was determined using the method of Edem and Elijah [9]. Ten fold serial dilution(1:10, 1: 100, 1:1000...10,0000). was used. Aliquots (0.1 ml) of appropriate dilutions(10^2 and 10^4) of kunun samples were poured on Nutrient and MacConkey agar plate and incubated at 37°C for 24 - 48 hours for total aerobic bacterial and coliform count respectively. Distinct colonies were counted after incubation. All enumeration was expressed as colony forming unit per milliliter (cfu/ml).

Purification and Maintenance of Microbial Isolates

Bacteria isolates was transferred onto fresh agar medium of isolation and incubated at 37 °C for 24 hours. Pure colonies of bacteria were maintained on slant and stored at 4 °C until needed.

Characterization and Identification of the Isolates

Standard inoculum was prepared from the preserved stock culture by taking a loopful of the isolates and aseptically inoculating onto sterile nutrient agar plates. The plates was incubated at 37°C for 24 h. Morphological and biochemical tests such catalase, coagulase, oxidase, citrate utilization were carried out using standard methods [10], while characterization and identification were done for confirmation of isolate using Microgen kit (microgen Tm GNA+B-ID system)((Microgen Bio Products) Surrey, United kingdom.

Microgen GNA-ID Identification

The biochemical reactions of the bacterial species were carried out on the isolates from Kunun-zaki as described by Amna et.al 2015, using an identification kit Microgen Tm GNA+B-ID system (Microgen Bio Products) Surrey, United kingdom for the confirmation of the identity of the suspected organisms isolated from the various samples of the beverage. The microgen identification system comprises of 60A tests micro well strip containing 12 standardized biochemical substrate for identification of the family of Enterobacteriaceae and commonly encountered non-fastidious oxidase positive and negative gram bacilli; these include, lysine, ornithine, hydrogen sulphide, glucose manitol, xylose, ortho-nitrophenol(ONPG), nitrate, indole, urease, vogues proskauer, citrate, tryptophan deaminase reagent (T.D.A) and nitrate. The dehydrated substrate in each well was reconstituted culture to be identified.

Procedure

A single colony of the bacterial cells was emulsified from 18-24 hours culture in 3ml sterile 0.85% normal saline inoculated into the GNA micro well test strips. 3-4 drops using a sterile Pasteur pipette (100microlitre) of the bacterial suspension were added to each of the micro well test strips. After inoculation, 3-4 drops of the mineral oil reagent were added into the micro well of numbers, 1, 2, 3 and 9. The top of the micro well tape and incubated at 35-37⁰c for 18-24 hours. At the end of incubation the adhesive tape removed and all the positive reactions with aid of the colour chart was recorded. Appropriate reagents were added to the following wells 7, 8, 10 and 12 and the results were recorded accordingly. 2 drops of kovac's reagent was added to well 8 and the results recorded after 60 seconds, formation of a red colour indicates a positive result. One drop of vogues proskayer (vp 1 and vp 2) reagent were dropped in well 10 and read after 15-30 minutes. Formation of a deep pink/red colour indicates a positive result. One drop of Tryptophan Deaminase Reagent (TRA) reagent was added to the well 12 and after 60 seconds formation of a cherry red colour indicates a positive result.

Statistical Analysis

Analysis of variance (ANOVA) was used to test levels of significant values ($p \leq 0.05$). Mean values were separated using Duncan multiple range test.

RESULTS AND DISCUSSION

Table 1 shows the total aerobic bacterial plate counts of Kunun-zaki sold at the different locations in (Zone A, B, C and D) Wukari metropolis. The counts ranged from 9.8×10^{-5} to 1×10^{-5} cfu/mL in zone A, zone B 9.7×10^{-5} to 1.21×10^{-5} cfu/mL, zone C 9.7×10^{-5} to 1.01×10^{-5} cfu/mL and zone D 4.96×10^{-5} to 1.12×10^{-5} cfu/mL respectively.

The relative microbial counts recorded were indicative of high level of microbial contamination.

There was no significant difference in the microbial counts of the Kunun-zaki sold at the different locations of the metropolis which is indicative that the beverage sold at the different locations most likely had similar microbial quality. This may be due to the fact that similar handling procedures are employed during processing and marketing of the beverage. The high microbial counts may be attributed to lack of effective precautions on

hygiene practice in handling procedures during processing of the beverage. The practice of addition of some quantity of water to Kunun-zaki after fermentation may also be a source of introducing microbial contaminants which may have come from the water itself or from the utensils used for such purposes. Amusa and Ashaye [11] and Echeonwu and Eruteya [12] had earlier reported that the presence of coliforms such as *Escherichia coli* in hawked Kunun-zaki was as a result of contaminated water, containers, as well as dirty environment where the Kunun-zaki were being processed and hawked.

The occurrence of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp and *Enterobacter Pseudomonas* spp, *Klebsiella*, *Salmonella*, *Pseudomonas*, *Bacillus* *Vibrio* spp and *Micrococcus* in the samples analyzed is a pointer to the fact that the Kunun-zaki drink sold in the different locations in Wukari metropolis is contaminated with potentially pathogenic bacteria and this may have come from the water used for domestic purposes, or the human handlers during processing and sales of the product, respectively. This result obtained in the study is in agreement with Amusa and Ashaye [11] and Umaru *et al.*[14], who had reported also that water used for production coupled with the crude method of production and packaging under improper sanitary conditions predisposes Kunun-zaki drink to microbial contamination by an array of both gram negative and gram positive bacteria. There is therefore need for surveillance by Public Health officials to ensure safety of the Kunun-zaki being sold in Wukari for public consumption. There is need to also ensure that the water used for production especially post-heating processing of the Kunun-zaki is safe and free from microbial contaminants. The source of contamination may also have come from the spices used additives [15,16].

Thus, bacterial species such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp and *Enterobacter Pseudomonas* spp, *Klebsiella*, *Vibrio* spp and *Micrococcus* was recorded in all the zones in Wukari metropolis.

Table 1. Total aerobic plate count of bacteria cells (cfu/ml) isolated from kunun-zaki

S/No	Zone A		Zone B		Zone C		Zone D	
	Sample Code		Sample Code		Sample Code		Sample Code	
1	KZ1	5.2x	KZ11	5.2 x	KZ21	1.01 x	KZ31	4.96 x
2		10 ⁻⁵		10 ⁻⁵		10 ⁻⁵		10 ⁻⁵
3	KZ2	1.07 x	KZ12	4.5	KZ22	7.6 x	KZ32	3.01 x
		10 ⁻⁵		x10 ⁻⁵		10 ⁻⁵		10 ⁻⁵

S/No	Zone A		Zone B		Zone C		Zone D	
	Sample Code		Sample Code		Sample Code		Sample Code	
4	KZ3	4.7 x 10 ⁻⁵	KZ13	6.5 x 10 ⁻⁵	KZ23	4.2 x 10 ⁻⁵	KZ33	4.21 x 10 ⁻⁵
5	KZ4	2.12 x 10 ⁻⁵	KZ14	1.21 x 10 ⁻⁵	KZ24	7.7 x 10 ⁻⁵	KZ34	4.51 x 10 ⁻⁵
6	KZ5	1.91 x 10 ⁻⁵	KZ15	5.7 x 10 ⁻⁵	KZ25	5.1 x 10 ⁻⁵	KZ35	4.09 x 10 ⁻⁵
7	KZ6	2.09 x 10 ⁻⁵	KZ16	1.01 x 10 ⁻⁵	KZ26	1.49 x 10 ⁻⁵	KZ36	3.08 x 10 ⁻⁵
8	KZ7	1.79 x 10 ⁻⁵	KZ17	2.8 x 10 ⁻⁵	KZ27	1.21 x 10 ⁻⁵	KZ37	4.08 x 10 ⁻⁵
9	KZ8	1 x 10 ⁻⁵	KZ18	1.62 x 10 ⁻⁵	KZ28	9.7 x 10 ⁻⁵	KZ38	4.35 x 10 ⁻⁵
10	KZ9	7.8 x 10 ⁻⁵	KZ19	7.9 x 10 ⁻⁵	KZ29	2.09 x 10 ⁻⁵	KZ39	1.12 x 10 ⁻⁵
	KZ10	9.8 x 10 ⁻⁵	KZ20	1.21 x 10 ⁻⁵	KZ30	3.02 x 10 ⁻⁵	KZ40	3.91 x 10 ⁻⁵

Characterization and Identification of micro flora isolated from kunun-zaki

The result in Fig 4.1, revealed the presence of species (spp) of *Staphylococcus spp*, *Bacillus spp*, *Pseudomonas spp*, *Micrococcus spp*, *Escherichia spp*, *salmonella spp* and *vibrio spp* in fresh kunu. The Prevalence of bacterial strains isolated from Kunun-zaki showed *Staphylococcus* occurring highest (24.39%), followed by *E.coli* (19.51%), *Micrococcus* (14.63%), *Bacillus* (12.19%), *Pseudomonas* (9.75%), *Salmonella* (7.31%), *Enterobacter* (4.87%) and *Vibrio spp*, *Klebsiella* and *shiegella* occurring least (2.43%) sequentially (fig.4.1).

Based on the characterization and identification of the strains, *Staphylococcus spp* has the highest percentage in all the zones (A, B, C, D), (23.80%) for zone A; zone B (25.8%), zone C (25%) and zone D (24.3%).

Micrococcus occurs highest in samples from zone B (23.0%) followed by zone A (21.4%), zone C (17%) and least occurrence in zone D (14.62%) respectively. *E.coli* occurrence was highest in zone B (23%) followed by zone A (21%), zone C (20%) and zone D (19.5%) respectively. *Pseudomonas* occurs highest in zone A (14.28%), zone D (9.75%), zone C (7.5%) and zone B(5.12%), *Bacillus* occurs highest in zone D (12.19%), zone B(5.12%), zone A(5.20%) and zone A(2.38). *Salmonella* occurs highest in zone C (10%), zone B (7.6%), zone D(7.31)% and zone A (4.76%). *Enterobacter* occurs only in two of the zones

(A and D) with zone D having the highest occurrence (4.87%) and zone A (2.38), *Shiegella* occurs within the same range in the four zones; zone C (2.5%), zone B (2.56%), zone D (2.43) and zone A (2.38) in that order.

The presence of these pathogens (*E.coli*, *S.aureus*) among others could be a matter of serious health concern comparing these result with work done by [17], this contamination could be from water used and crude methods of production and packaging under improper sanitary condition was also reported to predispose Kunun-zaki to heavy microbial contamination which was the case with Kunun-zaki sold in Wukari Metropolis.

Relating the above result with work done by Echeonwu and Eruteya [12], it shows that consumption of Kunun-zaki produced in Wukari Metropolis has potential health hazard to the consumers.

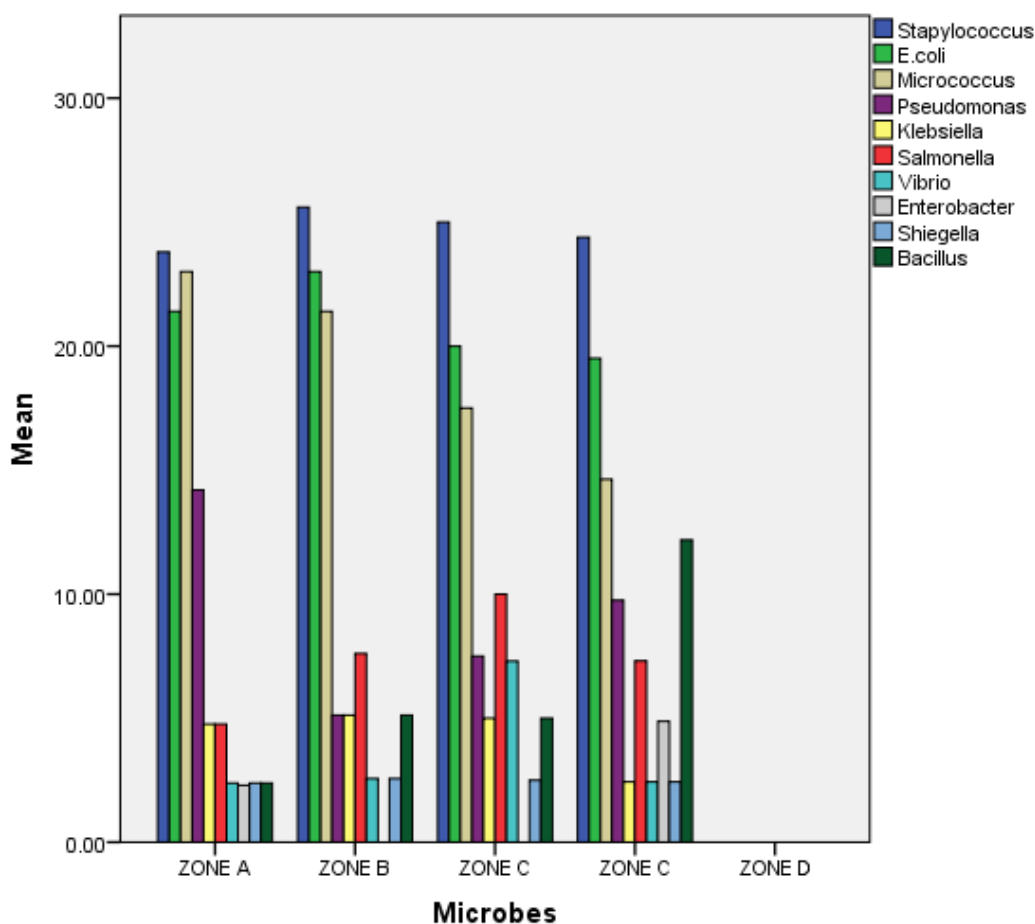


Fig 1. Prevalence of bacterial strains isolated from Kunun-zaki

CONCLUSION

This study has identified the presence of *Escherichia coli*, *Staphylococcus* species, *Streptococcus* species, *Lactobacillus* species, *Candida* species, and *Aspergillus* species in samples of Kunun-zaki, indicating significant microbial contamination. The presence of these organisms renders the beverage unfit for human consumption and poses a potential risk to public health.

The findings contribute to the growing body of evidence on food safety concerns in traditionally prepared beverages, emphasizing the critical need for improved hygiene practices and regulatory oversight. This study underscores the importance of ensuring microbiological safety in local food production processes to prevent health hazards associated with contaminated consumables.

To mitigate these risks, food safety agencies should implement routine monitoring of local production practices and conduct public sensitization campaigns on hygiene and sanitation during the preparation and processing of Kunun-zaki and similar beverages. Additionally, the use of clean, potable water in production must be enforced to reduce microbial contamination and enhance product safety and quality. Further research may explore intervention strategies and microbial risk assessments to support the development of standardized safety protocols in traditional beverage production.

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