



# Determination of Microbial Pathogens and its Abundance in Fresh Kunun-Zaki Drinks: Huge Threat to Public Health

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## Authors' contributions

This work was carried out in collaboration among all authors. Author SEO designed the study and wrote the first draft of the manuscript. Authors SEO, DAN, GNE, JII and OWO executed the study, analysed data, read, revised and approved the final manuscript. All authors read and approved the final manuscript.

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## ABSTRACT

This study investigated the presence and abundance of microbial pathogens in fresh locally produced, packaged and distributed kunun-zaki drinks. A total of 20 samples were randomly purchased from 20 vendors and hawkers. Each of the samples obtained was tenfold serially diluted using sterile peptone water. From the appropriate dilutions, 0.1ml were removed and spread plated on *Salmonella-Shigella* agar, Mannitol salt agar, and Eosin methylene blue agar plates. The inoculated plates were then incubated at 37°C for 24 hours. This study found that of the 20 kunun-zaki samples investigated, 70% were contaminated by *Staphylococcus aureus* which ranged from  $1.0 \times 10^4$  to  $1.21 \times 10^5$  (CFU/ml). *Salmonella* spp ranged from  $1.2 \times 10^4$  to  $4.1 \times 10^4$  (CFU/ml), and contaminated 60% of the samples. *Escherichia coli* ranged from  $0.2 \times 10^4$  to  $1.1 \times 10^4$  (CFU/ml) and

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was found in 45% of the samples. The result showed that the locally produced, packaged and distributed kunun-zaki drinks were highly contaminated by foodborne microbial pathogens and it makes it unsafe for human consumption. It further showed that public health is put at risk when unsafe locally produced kunun-zaki beverage is consumed; hence, local producers and Agencies saddled with the responsibility to monitor, supervise and certify food safety must strictly ensure the protection of consumers and public health by insisting that traditional methods of production, packaging and distribution of kunun-zaki drinks follow, maintain and sustain standards that guarantee quality, safety and accessibility. Consumers must resist the urge to purchase kunun-zaki drinks from unverified sources; producers, vendors and hawkers.

**Keywords:** *Escherichia coli*; *Foodborne microbial pathogens*; *Hawkers*; *Salmonella spp*; *Staphylococcus aureus*; *Vendors*.

## 1. INTRODUCTION

The contamination of food by microbial pathogens is a lingering and critical global health problem and in Nigeria, it is an important and one of the major causes of consistent and persistent foodborne disease out-breaks. In 2018, the World Bank report stated that the overall productivity loss associated with foodborne diseases in developing countries may cost a huge sum of \$95.2 billion annually while the treatment of foodborne illnesses may cost about \$15 billion per year [1]. This worrisome report indicates that foodborne illnesses pose a huge and critical socio-economic danger to developing countries in mostly Asia and Africa. Nigeria being a developing country in sub-Saharan Africa is currently battling foodborne disease problem [1].

Kunun-zaki is a fermented, non-alcoholic and non-carbonated beverage widely consumed in all the regions of Nigeria particularly due to its reported nutritional, medicinal, refreshing and thirst-quenching characteristics including unique taste [3-7]. Kunun-zaki is a cereal-based beverage; highly nutritious, moderately cheap and often used for refreshment. Millet is commonly used as substrate for commercial production of kunun-zaki drinks; though, other cereal substrates such as rice, sorghum and maize can be used [8-9].

In Nigeria, kunun-zaki is mostly produced traditionally according to cultures and preferred tastes. Traditional methods of production and packaging are mostly not supervised and monitored by Government and or certified Agencies. Hence, the environment of production may not be properly sanitized and producers may demonstrate poor personal hygiene practices. To facilitate and promote public health awareness and safety, prevent foodborne diseases and intoxication capable of undermining

socio-economic growth, the current research was carried out to determine the microbial quality and safety of the locally produced, packaged and vended kunun-zaki drinks at Eha-Amufu, Enugu State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This study was conducted at Eha-Amufu, Isi-Uzo Local Government Area of Enugu State, South-East Nigeria. The town has a tropical climatic condition with coordinates 6°39 N, 7°46 E. The seven (7) autonomous communities that make up Eha-Amufu are Amede, Umuhu, Mgbuji, Abor, Agu-Amede, Isu and ihenyi. Both indigenes and non-indigenes engage in trading and agriculture. The town has a tertiary institution; Federal College of Education, Eha-Amufu.

### 2.2 Collection of Samples

A total of 20 fresh kunun-zaki drinks which were all locally produced, packaged and distributed were randomly purchased in batches from 20 vendors and hawkers. The obtained kunun-zaki samples were then properly labelled, arranged in an icebox and moved to the Microbiology Laboratory for bacteriological investigations.

### 2.3 Media Used

We ensured that all culture media used for this current study were strictly and skillfully prepared according to the Manufacturers' instructions. The culture media used include *Salmonella-Shigella* agar (SSA), for isolation of *Salmonella* spp, Mannitol salt agar (MSA), for isolation of *Staphylococcus aureus*, Eosin methylene blue agar (EMB), for isolation of *Escherichia coli* and Nutrient agar (NA), for the storage and preservation of pure isolates for subsequent screenings.

## 2.4 Bacteriological Analyses

All the samples obtained were tenfold serially diluted using sterile peptone water. From the appropriate dilutions, 0.1ml were removed and spread plated on *Salmonella-Shigella* agar, Mannitol salt agar and Eosin methylene blue agar plates. The inoculated plates were then incubated at 37°C for 24 hours. Before inoculation, the culture plates were properly labelled and numbered. After about 24 hours of incubation, black-centred colonies on *Salmonella-Shigella* agar plates were carefully counted and expressed as *Salmonella* spp colony forming units per millilitre (CFU/ml) of each kunun-zaki sample. Also, yellow colonies and a surrounding yellow medium on Mannitol salt agar were counted and expressed as *Staphylococcus aureus* colony forming units per millilitre (CFU/ml) of each kunun-zaki sample investigated. Finally, colonies with a characteristic green metallic sheen on Eosin methylene blue agar were counted and expressed as *Escherichia coli* colony forming units per millilitre (CFU/ml) of each kunun-zaki sample.

## 2.5 Purification, Characterization and Identification of Isolates

To obtain pure isolates, subcultures were done on *Salmonella-Shigella* agar, Mannitol salt agar and Eosin methylene blue agar using representative colonies on culture plates. The pure isolates were then aseptically transferred into prepared Nutrient agar slants and stored for further investigations including biochemical screening.

The biochemical tests conducted after Gram staining include coagulase, citrate utilization, methyl red, catalase, oxidase, indole, and Voges Proskauer [10].

## 3. RESULTS AND DISCUSSION

The prevalence and abundance of microbial pathogens in the kunun-zaki samples is a critical public health problem that demands immediate and sustained intervention. Of the 20 samples screened, Table 2 showed that 70% were contaminated by *Staphylococcus aureus*, 60% contaminated by *Salmonella* and 45% contaminated by *Escherichia coli*. Table 1 indicates that all the samples were contaminated by one or more bacterial pathogens except

sample number K16. The absence of the pathogenic bacteria sought in sample K16 could be attributed to high level of quality control taken by the producer during production, packaging and distribution of the kunun-zaki drinks. The presence of one or more foodborne pathogens in the other samples indicate lack of quality control during production, packaging and distribution.

Table 1 showed that *S. aureus* ranged from  $1.0 \times 10^4$  to  $1.21 \times 10^5$  (CFU/ml). *Salmonella* spp ranged from  $1.2 \times 10^4$  to  $4.1 \times 10^4$  (CFU/ml) while *Escherichia coli* ranged from  $0.2 \times 10^4$  to  $1.1 \times 10^4$  (CFU/ml). The result showed high level of microbial contamination; hence, it poses huge threat to consumers and public health. *S. aureus* is an important foodborne pathogen associated with foodborne intoxication; it causes toxin-mediated gastroenteritis of which the symptoms include cramps, severe nausea, vomiting and in some cases, diarrhoea may occur [11]. Existing studies by Edward et al. [12] and Imoukhuede et al. [13], isolated *S. aureus* from kunun-zaki drinks sold in Umuahia, Abia State and Osun State, Nigeria.

The presence of *Salmonella* spp and *E. coli* in the samples suggest the water used for the kunun-zaki production may have been contaminated or the producers, hawkers or vendors were human carriers and hence, source of contamination. *Salmonella* spp are vital causative agents of reactive arthritis, septicaemia, colitis, osteomyelitis, pancreatitis, meningitis and rheumatoid syndrome [11]. The detection of *Salmonella* spp in kunun-zaki drinks by the current study calls for prompt intervention. Related studies by Anumudu and Anumudu [14.] and Etang et al. [15], isolated *Salmonella* spp from kunun-zaki and kunu drinks. Enterohaemorrhagic *E. coli* infections can lead to life-threatening complications such as haemolytic uraemic syndrome often characterized by acute renal failure, thrombocytopenia and haemolytic anaemia [11,16]. Food contaminated by *E. coli* is considered unsafe for human consumption. This finding also confirms previous studies by Mbachu et al. [17], and Aboh and Oladosu [18] which isolated *E. coli* from kunu drinks sold in Calabar, Cross River State and Abuja Municipal Area Council, Abuja, Nigeria. The result of current study calls for prompt and sustainable intervention and protection of individuals' rights to access safe food to ensure satisfaction, improved health, increased productivity and general well-being.

**Table 1. *Salmonella* spp count (CFU/ml), *Staphylococcus aureus* count (CFU/ml) and *Escherichia coli* count (CFU/ml)**

Kunun-zaki samples	<i>Salmonella</i> spp count (CFU/ml)	<i>S. aureus</i> count (CFU/ml)	<i>E. coli</i> count (CFU/ml)
K1	2.4x10 <sup>4</sup>	3.1x10 <sup>4</sup>	Not Detected
K2	Not Detected	2.3x10 <sup>4</sup>	0.3x10 <sup>4</sup>
K3	1.7x10 <sup>4</sup>	1.2x10 <sup>4</sup>	Not Detected
K4	3.2x10 <sup>4</sup>	Not Detected	0.6x10 <sup>4</sup>
K5	Not Detected	2.7x10 <sup>4</sup>	Not Detected
K6	Not Detected	1.0x10 <sup>4</sup>	Not Detected
K7	2.6x10 <sup>4</sup>	Not Detected	Not Detected
K8	3.9x10 <sup>4</sup>	4.2x10 <sup>4</sup>	0.2x10 <sup>4</sup>
K9	1.6x10 <sup>4</sup>	2.2x10 <sup>4</sup>	0.5x10 <sup>4</sup>
K10	3.0x10 <sup>4</sup>	Not Detected	Not Detected
K11	Not Detected	Not Detected	0.3x10 <sup>4</sup>
K12	1.9x10 <sup>4</sup>	1.5x10 <sup>4</sup>	Not Detected
K13	3.3x10 <sup>4</sup>	6.7x10 <sup>4</sup>	1.1x10 <sup>4</sup>
K14	4.1x10 <sup>4</sup>	Not Detected	0.2x10 <sup>4</sup>
K15	Not Detected	2.1x10 <sup>4</sup>	Not Detected
K16	Not Detected	Not Detected	Not Detected
K17	Not Detected	3.5x10 <sup>4</sup>	0.9x10 <sup>4</sup>
K18	2.8x10 <sup>4</sup>	2.0x10 <sup>4</sup>	0.4x10 <sup>4</sup>
K19	1.2x10 <sup>4</sup>	1.21x10 <sup>5</sup>	Not Detected
K20	Not Detected	1.4x10 <sup>4</sup>	Not Detected

K= Kunun-zaki Drinks (samples)

**Table 2. Percentage occurrence of isolates from the samples**

Isolates	Frequency from samples	Percentage (%) occurrence	Positive	Negative
<i>Salmonella</i> spp	12	60%	40%	
<i>S. aureus</i>	14	70%	30%	
<i>E. coli</i>	9	45%	55%	

Note: N=20

#### 4. CONCLUSION

*Staphylococcus aureus*, *Salmonella* spp., and *Escherichia coli* are important microbial pathogens known to cause food intoxication and varieties of foodborne diseases. The occurrence of these foodborne pathogens in the kunun-zaki drinks investigated by this study indicate gross contamination and predicts huge danger to consumers and complicated risk to public health. The source of contamination could be linked to poor or lack of personal hygiene, inadequate environmental sanitation, use of contaminated water and containers during production and packaging. Additionally, ingredients used to improve taste and nutritional value can be potential sources of transmission and contamination. Producers, hawkers or vendors who are human carriers of any of the pathogens can transmit it and contaminate food. It is paramount to adopt quality control strategies which involves Hazard Analysis Critical Control Point (HACCP), to mitigate or prevent microbial contamination of kunun-zaki drinks during production, packaging and distribution. Government at all levels and Agencies saddled

with the responsibility of supervising, monitoring and certifying food safety must intervene and ensure that kunun-zaki producers, hawkers and vendors adopt sustainable standards and safer traditional methods of production, packaging and distribution in order to guarantee consumers' safety and protect their rights to access to safe food.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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