
# Antibiotic Resistance Profile of *Escherichia coli* From Commercially-Marketed Kunun-zaki in Lafia Metropolis, Nasarawa State, Nigeria

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**Article Info**

**ISSN (online):** 2582-8940
**Volume:** 04
**Issue:** 01
**January-March 2023**
**Received:** 17-02-2023;
**Accepted:** 05-03-2023
**Page No:** 31-34

**Abstract**
Microbial contamination of locally-made beverages is a significant challenge as these beverages can serve as sources for the transmission of potential pathogens. Hence, this study was carried out to determine the prevalence and antibiotic resistance profile of *Escherichia coli* in commercially-marketed kunun-zaki in Lafia, Nasarawa State, Nigeria. Forty (40) kunun-zaki samples were collected from ten (10) different locations and cultured aerobically using standard microbiological techniques. Antibiotic sensitivity testing was carried out using the modified Kirby-Bauer disc diffusion method while the iodometric method was used for the detection of βlactamase production. A total of 13 (32.5%) *E. coli* isolates were recovered from the cultured kunun-zaki. Although all the isolates were susceptible to augmentin, the isolates were resistant to trimethoprim-sulfamethoxazole (23.08%), chloramphenicol, amoxicillin, ofloxacin, and streptomycin (15.38% each), and sparfloxacin, ciprofloxacin, gentamicin, and pefloxacin (7.69% each). Analysis of the resistance profile of *E. coli* isolates showed that 5 (38.46%) were sensitive to all antibiotics, 5 (38.46%) were resistant to one antibiotic, 2 (15.38%) were resistant to 3 antibiotics, and 1(7.70%) was resistant to four antibiotics. Also, only 2 (15.38%) isolates were positive for β lactamase production. High prevalence of *Escherichia coli* in commercially-marketed kunun-zaki is a significant public health concern as this drink is directly ingested after purchase. Hence, producers of this beverage should be properly sensitized on hygienic ways to prevent microbial contamination during the production of kunun-zaki.

**Keywords:** Microbial contamination; *Escherichia coli*; kunun-zaki; beverages; Antibiotic resistance

## Introduction
Food is an essential ingredient for the sustenance of life, be it of plant or animal origin. Hence, its demand can therefore not be overemphasized [1]. In Countries like Nigeria, people depend mostly on indigenous technologies for food preparations, especially foods of plant origin. Some of the foods that originate from plants include beverages such as Zobo and Kunun drinks [2]. The ubiquitous nature of microorganisms guarantees them the opportunity to be found in these locally made beverage drinks and possibly in the water used for its preparation, during storage and other processes involved in its preparation [3]. Unhealthy

processing conditions and poor handling techniques by producers may render these local drinks as vehicles for zoonotic and food-borne diseases or pathogens such as Staphylococcosis, Salmonellosis, Brucellosis, Tuberculosis, Shigellosis, Listeriosis, *Escherichia coli* infection, etc [4].

Kunun-zaki drink is a locally prepared indigenous non-alcoholic beverage which is widely produced and consumed in large quantity in Nigeria, especially the northern part of the country [5]. Though consumed throughout the year, it isextensively consumed during the dry season. 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. Packaging materials for this drink after production are also cheap and readily available. Kunun-zakiis 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 is required [4]. The drink is produced from fermented millet, sorghum, guinea-corn and maize in decreasing order of preference [6]. The methods involved in the production of this local beverage include steeping the grains in some containers such as buckets and some other household utensils followed by grinding the steeped grains into a mash and then mixed up with some spices of choice such as pepper and ginger before it is then divided into two in equal proportions. One of the proportions is then mixed up with hot or boiled water and the other with some ingredients such as malted rice and sweet potato paste [5]. The two proportions are then mixed together at a temperature of about 75-80°C as the mixture is left to undergo fermentation at room temperature within 20-24 hours, after which it is sieved before being considered ready for consumption [6].

The involvement of several people including unemployed school leavers in the production of kunun-zaki for commercial purposes makes the drink prone to microbial contamination [2]. A large number of lactic acid bacteria, coliforms, moulds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content for fermentation processes undesirably. In developing nation like Nigeria, it has not been possible to have control over the processing of hawked foods, because most of the vendors lack the adequateknowledge of food processing and handling practices. As a result of this, there is likely to be high risk of chemical and microbial contamination [7]. The pH of Kunun-zaki is usually too low to allow the growth of pathogenic microorganisms, but the presence of *E. coli*, *S. aureus* and *Streptococcus* spp. could be a matter of serious concern [6]. *E. coli* is an important member of the coliform group which is part of the normal flora of the intestine of human and vertebrates. Some strains of *E. coli* can cause gastroenteritis, diarrhea and urinary tract infections [5]. The presence of these pathogens even in small numbers could render a beverage unsuitable for human consumption.

The production and consumption rate of kunun-zaki in Lafia, Nasarawa state, Nigeria is high, hence the need for adequate hygiene knowledge in processing and handling this local drink. To achieve this, proper investigation must be carried out on the presence and antibiotics susceptibility of *Escherichia coli* isolates being one of the most implicative species fromkunun-zaki drinks sold in Lafia, Nasarawa state, Nigeria. Since little or no work has been done in this area, this research seeks to address the subject.

## 2. Material and methods

### 2.1. Study Area

The research was carried out in Lafia, a town in North Central Nigeria, Nasarawa State, Nigeria. It is bounded by the coordinates 8º30'N, 8º27'31E'.For the purpose of this study, ten (10) locations in this area were explored, viz: GRA, BukanSidi, Millionaires Quarters, Shinge Road, Project Quarters, Almakura Street, Nasara Estate, Tudun Kauri, Ombi one, and SabonPegi.

### 2.2 Sample Collection

A total of forty (40) different samples of kunun-zaki were collected from ten (10) different locations, four samples from each location. The samples were purchased with the packaged bottles and were properly labeled with their respective locations. They were then transported tothe microbiology laboratory of Innovative Biotechnology Limited, Keffi, Nasarawa State for analysis.

### 2.3 Isolation and Identification of *E. coli*

1 mL of individual sample was cultured in 9 mL nutrient broth contained in a bijoux bottle and incubated for 18 – 24 hours at $37^0$C. After incubation, a loopful from each broth culture was cultured on freshly prepared MacConkey agar and Eosin methylene blue (EMB) agar and incubated aerobically at $37^0$C for 18 – 24 hours. *Escherichia coli* was presumptively identified by metallic sheen colonies on EMB agar, and dry, slightly elevated, spherical pink-coloured colonies on MacConkey agar. Presumptively identified *E. coli* isolates were then identified using Gram's differential staining technique and conventional biochemical tests such as indole test, citrate test, and methyl red/Voges Proskauer test. *Escherichia coli* was identified as indole-positive, citrate-negative, methyl red-positive, and Voges-Proskauer negative Gram-negative bacilli.

### 2.6 Antibiotic Sensitivity Testing

The antibiotic sensitivity pattern of *E. coli* isolates was determined using the modified Kirby-Bauer disc diffusion method [8]. Distinct colonies from overnight culture of the test *E. coli* isolates were suspended in normal saline till the turbidity of the inoculum was equivalent to0.5 McFarland standard. Each standardized inoculum was then inoculated on freshly prepared Mueller-Hinton agar plates to create a bacterial lawn. Then, antibiotic-impregnated discswere aseptically placed on the surface of the bacterial lawn and allowed to stand at room temperature for pre-diffusion before incubating for 18 – 24 hours at $37^0$C. Individual *E. coli* isolates were tested against Amoxacillin (30 µg), Augmentin (30 µg), Trimethoprim-Sulfamethoxazole (30 µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Gentamicin (10 µg), Pefloxacin (10 µg), Ofloxacin (10 µg) and Streptomycin (30 µg). The sensitivity pattern of individual *E. coli* isolate was determined by comparing the measured inhibition zone diameter (IZD) around the antibiotic disc with standard break-point values reported by the clinical and laboratory standards institute [9].

### 2.7 Detection of Beta-lactamase Production

Amoxicillin-resistant strains of *E. coli* were screened for the production of beta-lactamases using the iodometric method [4, 10]. Colonies of amoxicillin-resistant *E. coli*strains were

streaked on freshly prepared nutrient agar plates supplemented with 2% w/v soluble starch and incubated at $37^0$C for 18 – 24hours.After incubation, the culture plate was flooded with freshly prepared 10, 000 unit/mL of Penicillin G (0.06mg/ml in 0.1M phosphate buffer, and 7.0 pH) and incubated for 60 minutes at room temperature. Thereafter, one drop of iodine solution was added. Isolates whose colonies turned blue-black with colorless halos were considered as beta-lactamase producing isolates.

## 3. Results

From the study, a total of 13 (32.5%) *Escherichia coli* isolates were recovered from commercially marketed kunun-zaki in Lafia, Nasarawa State. Of the ten (10) locations studied, GRA, Project Quarters, and Sabon Pegi had *E. coli* recovery rate of 2 (15.38%) each while the remaining locations had *E. coli* recovery rate of 1 (7.69%) each (Table 1). Table 2 shows the antibiotic resistance profile of *E. coli* isolates. The isolates showed resistance to trimethoprim-sulfamethoxazole (23.08%), chloramphenicol, amoxicillin, ofloxacin, and streptomycin (15.38% each), and spafloxacin, ciprofloxacin, gentamicin, and pefloxacin (7.69%). All the isolates were however, sensitive to augmentin. Table 3 shows the multiple antibiotic resistance index of *E. coli* isolates as 5 (38.46%) were sensitive to all tested antibiotics, 5 (38.46%) were resistant to one antibiotic, 2 (15.38%) were resistant to three antibiotics, and 1 (7.69%) was resistant to four antibiotics. Table 4 shows the prevalence of β lactamase production in isolates of *E. coli*. Two (15.38%) isolates were positive for β lactamase production and they are also resistant to amoxicillin.

**Table 1:** Prevalence of *E. coli*in commercially-marketed kunun-zaki in Lafia, Nasarawa State

| S/N | Locations | No. of Samples | Frequency (%) |
|---|---|---|---|
| 1 | GRA | 4 | 2 (15.38) |
| 2 | BukanSidi | 4 | 1 (7.69) |
| 3 | Millionaires Quarters | 4 | 1(7.69) |
| 4 | Shinge Road | 4 | 1(7.69) |
| 5 | Project Quarters | 4 | 2(15.38) |
| 6 | Almakura Street | 4 | 1(7.69) |
| 7 | Nasara Estate | 4 | 1(7.69) |
| 8 | Tudun Kauri | 4 | 1(7.69) |
| 9 | Ombi one | 4 | 1(7.69) |
| 10 | SabonPegi | 4 | 2(15.38) |
| | Total | 40 | 13 (32.50) |

**Table 2:** Antibiotic resistance profile of *E. coli*from commercially-marketed kunun-zaki in Lafia, Nasarawa State

| S/N | Antibiotics | Frequency (%) |
|---|---|---|
| 1 | Trimethoprim-Sulfamethoxazole | 3 (23.08) |
| 2 | Chloramphenicol | 2 (15.38) |
| 3 | Sparfloxacin | 1 (7.69) |
| 4 | Ciprofloxacin | 1 (7.69) |
| 5 | Amoxicillin | 2 (15.38) |
| 6 | Augmentin | 0 (0.00) |
| 7 | Gentamicin | 1 (7.69) |
| 8 | Pefloxacin | 1 (7.69) |
| 9 | Ofloxacin | 2 (15.38) |
| 10 | Streptomycin | 2 (15.38) |

**Table 3:** Multiple antibiotic resistance index of *E. coli*isolated from commercially-marketed kunun-zaki in Lafia, Nasarawa State

| S/N | MAR Index | Frequency (%) |
|---|---|---|
| 1 | 0.00 | 5 (38.46) |
| 2 | 0.10 | 5(38.46) |
| 3 | 0.20 | - |
| 4 | 0.30 | 2 (15.38) |
| 5 | 0.40 | 1 (7.70) |
| | Total | 13 (100.00) |

**KEY:** MAR: Multiple Antibiotic Resistance

**Table 4:** β lactamase production among *E. coli* isolated from commercially-marketed kunun-zakiin Lafia, Nasarawa State

| S/N | Isolates | Amoxicillin sensitivity | βlactamase |
|---|---|---|---|
| 1 | GRA | S | - |
| 2 | BukanSidi | S | - |
| 3 | Millionaires Quarters | S | - |
| 4 | Shinge Road | R | + |
| 5 | Project Quarters | R | + |
| 6 | Almakura Street | S | - |
| 7 | Nasara Estate | S | - |
| 8 | Tudun Kauri | S | - |
| 9 | Ombi one | S | - |
| 10 | Sabon Pegi | S | - |

**KEY:** S: Susceptible; R: Resistant; + = Positive; - = Negative

## 4. Discussion

Non-alcoholic beverages are a mainstay in the African culture and are frequently consumed by individuals to different extent. However, the production process of the majority of these beverages predisposes them to microbial contaminations emanating from varying sources. *Escherichia coli* is a significant coliform bacterium, whose presence is an indication of fecal contamination.

Kunun-zaki drink is known to be widely produced and consumed in large quantity in Lafia, Nasawara state, Nigeria [5]. Though consumed throughout the year, it isextensively consumed during the dry season. The involvement of several people including unemployed school leavers in the production of kunun-zaki for commercial purposes makes the drink prone to microbial contamination [3]. Results of this study revealed thepresence of *E. coli*isolates in some of the kunun-zaki samples collected from the study locations. This result is consistent with the findings of [3] who also found *E. coli* isolates present in kunun samples collected from different states in Nigeria. The presence of *E. coli* isolates in some of the kunun-zaki samples collected from the study locations in this research is an indication of high level of contamination of the kunun-zaki samples which to a large extent can be attributed to lack of effective precautions in hygiene practice in handling procedures during processing of the beverage. The practice of addition of some quality of water to kunun-zaki after fermentation may also be a source of microbial contaminants which might have come from the water itself or the utensils used for such purposes [5]. According to, the presence of *E. coli* in water indicates faecal contamination and most of the coliforms found associated with hawked kunun-zaki are known to be causative agents of food borne gastroenteritis and bacterial diarrhea diseases.

The results obtained from this study revealed that a total of thirteen (13) *E. coli*isolates were obtained from different study locations. The highest number of *E. coli* isolates was

obtained from kunun-zakisamples collected from GRA, Project Quarters and Sabonpegiwhich were high with two *E. coli* isolates each. This is indicative of the fact that the level of contamination of kunun-zaki samples produced in these locations is high compared to other locations which can be attributed to lack of effective precautions in hygiene practice in handling procedures during processing of the beverage.

Table 2 shows the resistant *E. coli*isolates profile from kunun-zakisamples sold at different locations in Lafia, Nasarawa State, Nigeria. The*E. coli* isolates showed least resistance [0.0%] to Augmentin and highest resistance [23.08%] to Septrin. Three (3) *E. coli* isolates exhibited multiple antibiotic resistance while the rest were resistant to only one antibiotic as shown in table 3. One isolate had an MAR index of 0.3 while two isolates had an MAR index of 0.4. Multiple antibiotic resistances exhibited by the isolates are indicative of possible abuse or misuse of antibiotics and this has serious health implications.

Beta-lactam antibiotics have been used over the years to successfully treat infections caused by pathogenic *E.coli* [10]. However, currently, the utility of Beta-lactams is being challenged severely by a large number of hydrolytic enzymes-the Beta-lactamases expressed by the bacteria [11]. These enzymes are produced by many bacteria that inactivate Beta-lactam antibiotics by opening the beta-lactam ring [12]. From the results presented in table 4, out of the thirteen (13) *E. coli* isolates obtained from kunun-zakisamples sold at different locations in Lafia, Nasarawa State, Nigeria, two Amoxacillin resistant isolates tested positive for beta-lactamase production.This result agrees with the findings of [12] who also obtained *E. coli* isolates resistant to Amoxacillin in kunun samples obtained in different states of Nigeria.

## Conclusion

This study was carried out to determine the prevalence and antibiotic resistance profile of *Escherichia coli* from commercially-marketed kunun-zaki in Lafia metropolis, Nasarawa State, Nigeria. The recovery rate of *E. coli* in this study is 32.50%. Although all the recovered isolates were susceptible to augmentin, they were most resistant to trimethoprim-sulfamethoxazole (23.08%), and chloramphenicol, ofloxacin, amoxicillin, and streptomycin (15.38% each). However, the larger percentage of the isolates were either susceptible to all antibiotics or resistant to only one antibiotic (38.46% each). High prevalence of *E. coli* in commercially-marketed kunun-zaki is of public health significance as it indicates potential fecal contamination of the beverage. Hence, producers of kunun-zaki should be properly educated and sensitized on hygienic practices that can prevent microbial contamination during the production of the beverage.

The presence of resistant strains of *E. coli*in kunun-zaki sold in Lafia, Nasawara state, Nigeria, suggests that the consumption of this locally made beverage has potential health hazard to its consumers. Resistance of this pathogenic organism to some antibiotics may most probably have resulted from abuse or misuse of these antibiotics or non-adherence to dosage prescriptions. The production of beta-lactamase enzymes by some strains of *E. coli*can confer resistance on these organisms and this can lead to failure in antibiotic therapy.

## Acknowledgments

We want to express our thanks to all those who contributed directly or indirectly to this study.

## Disclosure of conflict of interest

The authors state that there was no conflict of interest.

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