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Characterization and Antibiotic Sensitivity Testing of Clinical Bacteria Species Isolated from Kunu Drink Sold in Rumuolumeni, Rivers State

By Okwelle, Austin Achinike
Ignatius Ajuru University of Education

Abstract- The isolation, characterization and antibiotic sensitivity tests of some clinical bacteria species isolated from Kunu drink sold in Rumuolumeni, Rivers State was carried out. Samples of the Kunu drink was bought from vendors indifferent locations and their bacteriological counts enumerated using standard microbiological methods by the pour plate technique. The antibiotic sensitivity pattern of the pure bacteria isolates against some antibiotics was determined using the disc diffusion method. The total bacterial counts of the Kunu in the different locations ranged from$4.0x10^2$ to$8.6x10^2$cfu/ml. Four species of bacteria including *Escherichia coli, Enterohacter aerogenes, Staphylococcus aureus* and *Streptococcus* spp were isolated and identified by grain staining and their biochemical reactions. The most prevalent isolate in terms of occurrence was *Escherichia coli* (50%) followed by *Enierobacter aerogenes* (30%), *Staphylococcus areus* (10%) and *Streptococctts* spp (10%).

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I. Introduction

According to Maji *et al.,*(2011), Kunu drink is a locally prepared indigenous non-alcoholic beverage normally prepared and consumed in large quantity in Nigeria, especially in the northern part of the country (Amusa and Aswaye, 2009). It can be consumed during the wet and dry seasons due to its thirst quenching properties. Umaru *et al.,*(2014) reported that Kunu drink is sold in many public places such as markets, offices, schools, motor parks and as drinks during festival, weddings and naming ceremonies. It is an appetizer and food complement used to quench hunger (Adelekan *et al.,* 2014). Kunu drinks are usually produced using maize, guinea corn, millet or sorghum in varying proportions (Maji *et al*, 2011) to which sweet potato sometimes is added to increase the taste of the Kunu, which is a major factor that attracts consumers to the product. This common drink is usually packaged and sold in 50ml to 1L plastic bottle and at times tied in some disposal polythene bags the drink is mostly consumed within 20-35 hours of production due to its poor keeping quality (Akoma *et al,* 2012). This drink is not expensive because the grains and other ingredients used for production are locally sourced. The packaging materials are also readily available, cheap and affordable within the communities.

Different workers have reported that Kunuis rich in vitamins, minerals, carbohydrates and proteins (Adebayo *et al.,* 2010; Essien *et al*., 2009; Folasade & Oyenike, 2012). Oluwajoba *et al,* 2013).also noted that the nutritional content of Kunu drink include protein (2.31-3.63%), fat (3.55-3.63%), ash (1.66-1.21%) and carbohydrate (82.92-83.55%). The health benefits of kunu drink is that, it lowers blood pressure and promotes good functioning of the heart, improves healthy pregnancy and adequate breast milk flow, boosts sperm count in men, relaxes personal mood and promotes good sleep and reduces menstruation pains for women.

The local kunu drink could act as a vehicle for food borne infections like Brucellosis, Tuberculosis, Shigellosis, Listeriosis and *Staphylococcus* etc. Most of the pathogens found in the drink such as *Staphylococcus* sp, *Bruce1la* sp, *Pseudomonas* sp, *Clostridium*, *Salmonella*, *Vibrio cholerae* and *Escherichia coli* can led to change in the physical and nutritional qualities of kunu. Also, activities of the natural food enzymes could also contribute in the spoilage of the final product. The high water content (about 85%) coupled with crude method of production and packaging under inadequate sanitary conditions can also predispose kunu drink to microbial contamination (Aya *et al.,* 2010).

According to Mbachu *et al.,* (2014), the short life of kunu drink is a major problem faced by the producers and consumers. The introduction of microbes into kunu

Author: Department of Biological Sciences, Faculty of Natural and Applied Scioence, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. e-mail: okwelleaa@yahoo.com

drink results from the processing activities and materials used such as water, handling and presentation techniques. The water content coupled with the crude method of production and packaging under poor sanitary conditions predisposes the drink to sudden microbial contamination (*Akoma et al*, 2012). Again, there is no public health regulatory agency that monitors the production processes in spite of the associated harm that kunu drink causes Bukar *et al.,* (2010). The aim of this study is to characterize and determine the antibiotic sensitivity of some pathogenic bacteria species isolated from Kunu drink sold in Rumuolumeni, Rivers State.

II. Materials and Methods

a) Study Area

The study was conducted in Rumuolumeni, Port Harcourt, Rivers state, Nigeria. Port Harcourt lies between latitude 4°46'38.71"N and longitude 7°00'48.24"E. and located in the tropical rainforest in Nigeria.

b) Collection of Sample

Five bottles of hawked kunu samples were bought from vendors in different locations in Rumuolumeni, properly labelled, placed in a sterile plastic container and transported to the Biology laboratory, Ignatius Ajuru University of Education for microbiological analysis.

c) Processing of Sample

The samples were mixed gently and 10ml of each was added to 90 ml distilled water with a clean pipette. The solution was mixed and diluted serially by transferring 1 ml of the stock sample into sterilized test tubes containing 9ml of peptone water. The procedure was repeated for the third and fourth test tubes to make a dilution of 10^3 and 10^4.

d) Preparation of Media

All the glassware used such as petri-dishes, conical flasks, test tubes and pipettes were washed with detergent, rinsed in water, dried and sterilized in the hot air oven at 60°c for 1 hour. Different culture media such as Nutrient Agar, MacConkey Agar, Salmonella-Shigella Agar (SSA), Trisulphate Citrate Bile Salt Agar (TCBS) and Manitol Salt Agar (MSA) were used for isolation. Each of the media was prepared by weighing out appropriate quantities according to the manufacturers instruction and dissolved completely in the required volume of distilled water. The media were autoclaved at 121°C for 15 minutes and allowed to cool at 45-50°C. The media was dispensed aseptically into the petri-dish plates and left on the table to solidify at room temperature.

e) Isolation and Preservation

Using a sterile loop, discrete colonies were all sub-cultured onto another media to obtain pure colonies. This was done by streaking a loopful of a particular isolate into freshly prepared culture media plates for bacteria. The sub-cultured nutrient agar plates were incubated at 37°c. Bacteria pure cultures were accordingly stored in sterile agar slants for preservation and further analysis at 4°c.

f) Identification of Isolates

The isolates were identified using gram staining and biochemical tests such as: motility test, urease test, citrate utilization test, indole test, oxidase test, coagulase test, catalase test, vogues proskauer reaction and methyl red test. Identification was based on comparison of the characteristics of the isolates described by (Chess brough, 2006).

g) Antibiotic Susceptibility Test

The isolates were screened for antimicrobial sensitivity using the Kirby-Bauer agar disk diffusion method (CLSI, 2009). A suspension of each isolate was prepared in peptone water to match 0.5 McFarland turbidity standards in order to standardize the inoculum. The standardized inoculum of each isolate was inoculated onto the surface of plain Mueller-Hinton agar plates and Septrin (30 μg), Chloramphenicol (30 μg), Sparfloxacin (5 μg), Amoxycillin (30 μg), Ciprofloxacin (5 μg) Augmentin (30 μg), Gentamicin (10 μg), Pefloxacin (10 μg), Tarivid (30 μg) and Streptomycin (10 μg) discs were placed and incubated at 37°C for 24 hours. The zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute.

III. Results

Table 1: Total bacteria count of kunu sold at the different location in Rumuolumeni Port Harcourt metropolis

Location	Kunu
A	5.1×10^2
B	6.2×10^2
C	4.6×10^2
D	7.1×10^2
E	4.1×10^2
F	8.5×10^2

Keys: A= Waterside, B = Big tree market, C = Akar Junction, D = Iwofe school gate, E = St. John's, F = Town Hall

The bacterial counts from the different samples of Kunu ranged from 4.6×10^2cfu/ml (which was the lowest recorded for St. John's) to 8.6×10^2 cfu/ml enumerated in Town Hall.

Table 2: Bacteria isolates identified from Kunu samples in different locations

Bacteria Isolates	Locations					
	A	B	C	D	E	F
Escherichia coli	+	+	+	+	+	+
Staphylococcus aureus	+	–	–	+	–	+
Streptococcus spp	–	–	–	–	+	+
Enterobacteria aerogenes	+	+	+	–	+	+

Keys: A= Waterside, B = Big tree market, C = Akar Junction, D = Iwofe school gate, E = St. John's, F = Town Hall

Table 2 shows the bacterial species isolated from samples of Kunu drinks sold at the different locations in Rumuolumeni. The isolates were *Escherichia coli, Staphylococcus aureus, Streptococcus* spp. and *Enterobacter aerogenes. Escherichia coli* was the most predominant isolate with very high percentage occurrence (100%), followed by *Enterobacter aerogenes* (70%) and *Staphylococcus aureus* had the least occurrence (30%).

Plate 1: Bacterial colonies growing on petri dish plates

Table 3: Antibiotic resistance pattern of bacteria isolates from Kunu drink

Percentage Resistance of Isolates				
Antibiotics Conc. (μg)	Escherichia coli	Enterobacter Aerogenes	Strept. spp	Staphyloccus Aureus
Septrin30	68.7	14.3	0.0	16.7
Chloramphenicol 30	75.0	21.4	0.0	0.0
Sparfloxacin 5	68.7	35.7	33.3	16.7
Ciprofloxacin 5	50.0	7.1	33.3	16.7
Amoxicillin 30	12.0	35.7	0.0	0.0
Augmentin30	25.0	50.0	33.3	0.0
Gentamicin10	12.0	14.3	0.0	33.4
Pefloxacin10	0.0	42.9	0.0	33.4
Tarvid 30	31.3	28.8	38.3	0.0
Streptomycin 10	31.3	14.3	33.3	16.7

Table 3 shows the Antibiotic resistance pattern of the bacterial species isolated from Kunun drinks sold in Rumuolumeni, Port Harcourt metropolis. *E. coli* exhibited very high percentage resistance to chloramphenicol (75.0%) followed by Septrin (68.7%) and Sparfloxacin (68.7%) respectively, whereas there was no resistance to Perfloxacin (0.0%). The highest percentage resistance for *Enterobacter aerogenes* was recorded with Augmentin (50.0%) and the least resistance was recorded with Ciprofloxacin (7.1%). The percentage resistance Streptococcus spp. isolated was relatively low which ranged from 38.3% to 33.3% for the antibiotics to which this species showed resistance (Trivid, Sparfloxacin, Ciprofloxacin, Augmentin and Streptomycin). However, the isolates of the *Streptococcus* spp. showed completely no resistance (0.0%) to Septrin, Chloramphenicol, Amoxicillin, Gentamicin and Perfloxacin. Similarly, the percentage resistance of *Staphylococcus aureus* isolated was relatively low which ranged from 33.4% to 16.7% for the antibiotics to which these isolates showed resistance (Gentamicin, Perfloxacin, Septrin, Sparfloxacin, Ciprofloxacin and Streptomycin).

IV. Discussion

The relative high numbers of microbial counts obtained from the different samples of kunu in the study were indicative of high level of microbial contamination of the prpduct. The Kunu sold at Town Hall had the highest counts of 8.6×10^2 cfu/ml, while the one from St Johns location had the lowest counts of 4.0×10^2 cfu/ml. The high microbial counts experienced 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 Kunu after fermentation may also be a source of microbial contaminants, which may have come from the water itself or from the utensils used for such purposes. In an earlier report, Amusa and Ashwaye (2009) had stated that the presence of coliforms such as *Esherichia coli* in hawked Kunu was as a result of contaminated water, containers, as well as dirty environment where the Kunu were being processed and hawked. The identification of *Escherichia coli, Staphylococcus aureus, Streptococcus* spp and *Enterobacter aerogenes* in the samples analyzed is a positive sign to the fact that the Kunu drink sold in the different locations in the community was 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 is in agreement with Amusa and Ashwaye (2009) and Akoma *et al.,* (2013), who had noted that water used for production coupled with the crude method of production and packaging under improper sanitary conditions predisposes Kunudrink to microbial contamination of both gram negative and gram positive bacteria. The source of contamination may also have come from the spices used additives (Essien *et al.,* 2009, Lawal, 2012). There is therefore need for surveillance by Public Health officials to ensure safety of the Kunu sold for to public. There is need to also ensure that the water used for production especially post-heating processing of the Kunu is safe and free from microbial contaminants.

Antibiogram of the isolates revealed varying levels of resistance to the antibiotics tested. *Escherichia coli* showed high resistance to chloramphenicol (75%), followed by Septrin (68.7%) and Spafloxacin (68.7%), while *Enterobacter aerogenes, Streptococcus* spp and *Staphylococcus* aureus had low rates of resistance to all

the antibiotics tested. However, *E. coli* had very high sensitivity to Pefloxacin (100%), followed by Gentamicin (88%), Augmentin (75%), tarivid (68.7%) and Streptomycin (68.7%). *Streptococcus* spp were the most susceptible isolates which had very high sensitivity (100%) to five of the antibiotics tested, namely, Septrin, Chloramphenicol, Amoxicillin, Gentamicin and Perfloxacin, respectively. *Staphylococcusaureus* was also very sensitive (100%) to Chloramphenicol, Amoxicillin, Augmentin and Tarivid, respectively. The sensitivity of these isolates to the antibiotics used are comparable to earlier reports (Falagas *et al*., 2010, McGeer *et al*., 2010 & Omeke *et al*.., 2019). The prevalence of resistant strains of *E. coli, Enterobacter aerogenes, Streptococcus* spp and *Staphylococcus aureus* in Kunu is a reflection of the use and misuse of the antibiotics in the society. This is not surprising because outside the hospital environment, the general populace have access to various kinds of antibiotics at any drug store even without any prescription from a medical practitioner. The Public Health implication of this study is that antimicrobial resistant strains of pathogenic bacteria may colonize the human population through consumption of contaminated Kunun and this would lead to chemotherapeutic failures among the human consumers of this popular beverage in the Rumuolumeni, Port Harcourt metropolis.

V. Conclusion

The presence of resistant strains of *E. coli, Enterobacter aerogenes, Staphylococcus aureus* and *Streptococcus* spp in Kunun sold in Rumuolumeni suggests that consumption of this beverage has potential health hazard to the consumers in Rumuolumeni, Port Harcourt, Nigeria. The consumers of this popular drink are therefore at health risk, which may culminate into failures of commonly used clinical antibiotics for the treatment of the infections.

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