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

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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 from4.0x102 to8.6x102cfu/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

Index terms—

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Different workers have reported that Kunuis rich in vitamins, minerals, carbohydrates and proteins **??**Adebayo et

1 Materials and Methods

2 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.

3 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.

4 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?.

5 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.

6 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.

7 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).

8 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. 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).

9 III.

10 Results

IV.

11 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% 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.

12 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.

[1]

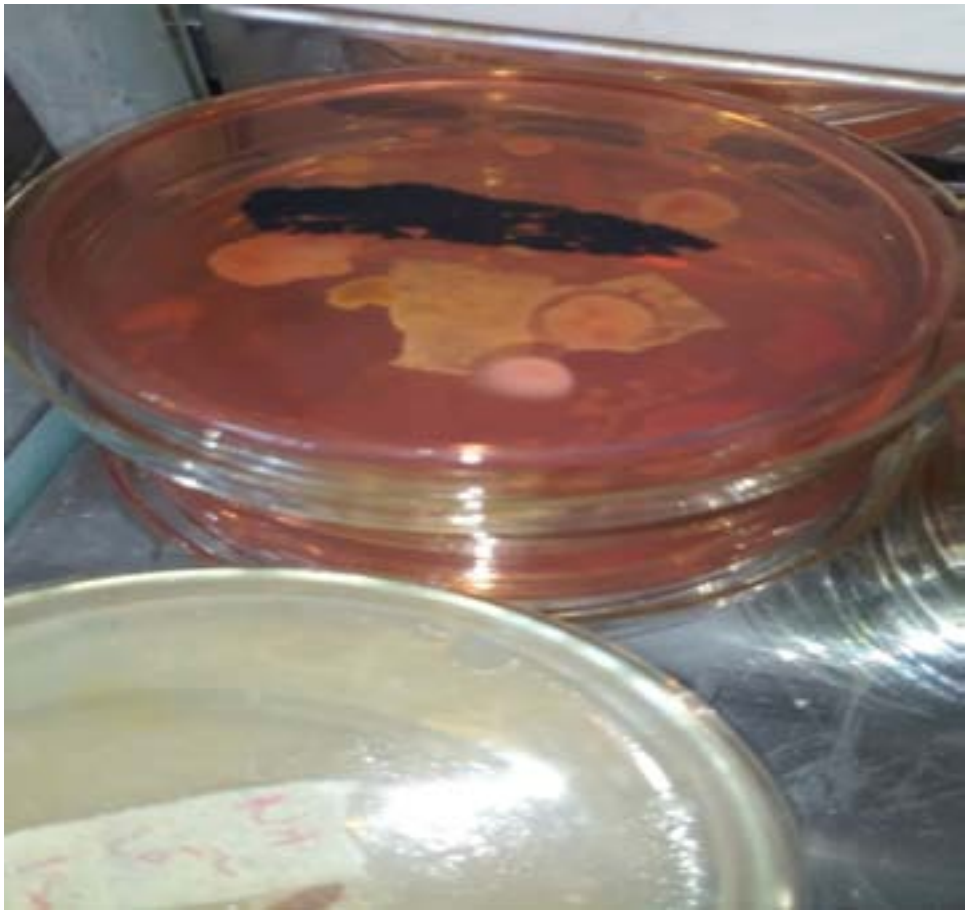

Figure 1:

Figure 2: Plate 1 :

Figure 3:

1

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.

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	

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

Figure 4: Table 1 :

2

Locations						
Bacteria Isolates						
Escherichia coli	+	+	+	+	+	+
Staphylococcus aureus	+	_	_	+	_	+
Streptococcus spp	_	_	_	_	+	+
Enterobacteria aerogenes	+	+	+	_	+	+

Figure 5: Table 2 :

3

Figure 6: Table 3 :

the antibiotics tested. However, E. coli had very high
sensitivity to Pefloxacin (100%), followed by Gentamicin
(88%), Augmentin (75%), tarivid (68.7%) and Percentage Resistance of Isolates Streptomycin (68.7%). Strep
Antibiotics Conc. (µg) susceptible isolates which had very high sensitivity Escherichia coli Enterobacter Aer

Septrin30 Chloramphenicol, Chloramphenicol 30 Amoxicillin, Perfloxacin, respectively. Staphylococcusaureu

Sparfloxacin 5 Amoxicillin,	68.7	35.7
Ciprofloxacin 5	50.0	7.1
Amoxicillin 30	12.0	35.7
Augmentin30	25.0	50.0
Gentamicin10	12.0	14.3
Pefloxacin10	0.0	42.9
Tarvid 30	31.3	28.8
Streptomycin 10	31.3	14.3

Figure 7:

[Adebayo] , G B Adebayo .

[Eur. J. Food Res. Rev] , *Eur. J. Food Res. Rev* 1 p. .

[Omeke et al. ()] 'Antibacterial activity of leaf extract of Chromolaena odorata and the effect of its combination with some conventional antibiotics on Pseudomonas aeruginosa isolated form wounds'. P O Omeke , J O Obi , N A Orabueze , A Ike . *Journal of Applied Biology Biotechnology* 2019. 7 (03) p. .

[Mcgeer et al. ()] *Antimicrobial resistance in Ontario: Are we making progress? Laboratory Proficiency Testing Program Newsletter*, A Mcgeer , C A Fleming , K Gree , D E Low . 2010. 293 p. .

[Chesebrough ()] *District laboratory practice in tropical countries: Part 2*, M Chesebrough . 2006. New York: Cambridge University Press.

[Adedokun and Onyeneke ()] 'Effect of Aframomum danellians black pepper crude extracts on physio-chemical and sensory properties of Kunun-zaki during storage'. I I Adedokun , E Onyeneke . *J. Food Techn* 2012. 10 p. .

[Maji et al. ()] *Effect of chemical treatment and pasteurization on the shelf life of Kunun zaki (sorghum and maize gruel)*, A A Maji , J & O E Omale , Chigozie . 2011.

[Folasade and Oyenike ()] 'Effect of sesame seed addition on the chemical and sensory qualities of sorghum based Kunun-zaki drink'. M & O Folasade , Oyenike . *Afr. J. Food Sci. Techn* 2012. 3 p. .

[Essien et al. ()] 'Evaluation of the nutritional and microbiological quality ofKunu (A Cereal Based Non-alcoholic Beverage) in Rivers State'. E Essien , C Monago , E Edor . *Nigeria. The Internet Journal of Nutrition and Wellness* 2009. 10 p. .

[Lawal ()] 'Microbial quality of Kunun-zaki beverage sold in Ile-Ife, Osun State'. O A Lawal . *J.Food Techn* 2012. 10 p. .

[Amusa and R Aswaye ()] *Microbiological and Nutritional Quality of Hawked Kunun (A Sorghum Based Non-Alcoholic Beverage) Widely Consumed in Nigeria*, O Amusa , B R & Aswaye . 2009.

[Oluwajoba and Adedeji ()] 'Nutritional composition of a non-alcoholic beverage spiced with Zingiber officinale extract produced from Sorghum bicolor stem sheath'. I B T O Oluwajoba , Adedeji . *Int. J. Food Sci. Nutr. Eng* 2013. 3 p. .

[Bukar et al. ()] 'Occurrence of some entropathogenic bacteria in some minimally and fully processed ready-to-eat foods in Kano metropolis'. A Bukar , A & T I Uba , Oyeyi . *Nigeria. Afr. J. Food Sci* 2010. 4 p. .

[Csli ()] 'Performance standards for antimicrobial susceptibility testing'. Csli . *Seventeenth Informational Supplements* 2007. p. . Cinical and Laboratory Standard Institute

[Otunola and Ajao ()] 'Physiochemical, microbiological and sensory characteristics of Kunu prepared from millet, maize and guinea corn and stored at selected temperatures'. G Otunola , T A Ajao . *Adv. J. Food Sci. Techn* 2010. 2 p. .

[Falagas et al. ()] 'Resistance to polymyxins: Mechanisms, frequency and treatment options'. M E Falagas , P I Rafailidis , D K Matthaiou . *Drug Resist. Update* 2010. 13 p. .