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Research Article
Microbiological Evaluation and Antibiotic Susceptibility Pattern of Bacteria Associated with 'Burukutu', a Non-alcoholic Beverage

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Abstract

Background and Objective: The possible health hazard associated with the traditional production of non-alcoholic beverages calls for regular microbiological quality control check to reduce the potential menace on humans' health. The current study was planned to investigate the microbiological quality and antibiotic susceptibility pattern of bacteria associated with burukutu beverage sold in Akure metropolis with a view to establish its biosafety. **Materials and Methods:** This research was carried out between April and July, 2016. Total aerobic microbial counts were determined using pour plate technique on nutrient agar, MacCankey agar, potato dextrose agar and sabroud dextrose agar. Afterward, the counts were reported as colony forming unit per milliliter (CFU mL^{-1}) for bacteria and spore forming unit per milliliter (SFU mL^{-1}) for fungi. The antibiotic sensitivity test was carried out using agar diffusion method. The susceptibility of the bacterial isolates to commercial antibiotics were determined by measuring the zone of inhibition in millimeter and interpreted according to Clinical and Laboratory Standards Institute (CLSI). **Results:** The highest bacterial and fungal loads of 35.0×10^{9} and 105.0×10^{9} SFU mL^{-1}, respectively was obtained from the samples collected from the Army Barrack (Seller 3), while the samples collected from Road block (Seller 1) had the least values. The identified microbial isolates were: *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *E. coli*, *Lactobacillus fermentum*, *Streptococcus lactis*, *Aspergillus niger*, *Penicillum italicum*, Rhizopus *japonicum*, *Fusarium* spp. and *Saccharomyces cereviseae*. *Streptococcus lactis* and *Lactobacillus fermentum* had the highest percentage occurrence of 60% from samples collected from Road block and Army barrack respectively, while *Aspergillus niger* had the highest percentage occurrence of 62.5% from the sample collected from Road block. Appreciable numbers of the bacterial isolates were sensitive to commercial antibiotics. The isolates *Streptococcus lactis* showed resistant to all the antibiotics except Amoxycillin and Androcephin. *Escherichia coli*, *Bacillus subtilis*, *Streptococcus* and *Staphylococcus aureus* showed resistant to Amoxycillin, Zinacef, Septrin and Streptomycin. **Conclusion:** The presence of antibiotic resistant bacteria and mycotoxin-producing fungi in the samples are of great health concern both to human and animals.

Key words: Microbiological quality, antibiotic susceptibility, burukutu, agar diffusion, health hazard

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cereals constitute major part of our food and as raw materials in our industries for optimum use in the production of high quality products for man use. It is easily accessible, available and the rate of its production in Nigeria could serve as alternative in the formulation of basal diet for animal feeds which is less cost effective in terms of yield and final products[1].

Consumption of fermented cereal products in the last decades has gained its popularities in different socio-cultural mostly in Africa and other parts of the world[2]. In Africa, alcoholic beverages such as burukutu, pito, sekete and many others were consumed for pleasure soon after brewing or tapping[3], these products were rarely traded in the market[4]. Alcoholic and non-alcoholic beverages production and consumption can be purposely used for traditional rituals, festival and other social activities which include marital arrangements, child-naming, settling of quarrels between individuals and between families and communities and also as expression of hospitality to visiting guests[5]. Mostly among the village settings, consumption of locally brewed beverages brings togetherness, traditional obligations and nutritional, economic, daily, social, cultural life of its people and as source of income[6].

Burukutu is a rich non-alcoholic beverage characterized with vinegar-like flavor and a cloudy suspension, it is produced mainly from the grains of either *Sorghum bicolor* or *S. vulgare*. The grains of *Sorghum bicolor* or *S. vulgare* serves as a staple food for the poor and less privileged individuals from the developing countries in which Nigeria is one and it is constituted by energy and protein rich compounds[7]. It is the main source of carbohydrates and protein for millions of people, mostly in the Southern Sahara[8]. It can be preserved for 8 days from the day of production and its short shelf life might be connected with the presence of low lactic acid content, low titratable acidity, low alcohol content, high concentration of vitamins and fermentable sugars[7].

The major problem associated with the traditional production of burukutu includes non-availability of potable water, most often local brewers depends on untreated water supplied by hawkers and such water could be a potential vehicle for the spread and contamination of the brew with pathogenic microorganisms. The processing areas are filthy and in some cases are located near toilet. Utensils, cups and other measuring devices such as calabash are not properly washed after use or before serving customers. Other problems include uncontrolled fermentation processes. This often leads to the production of very poor quality of burukutu due to excessive fermentation periods. The brewing conditions (excessive fermentation period and temperature) favor acetic fermentation and oxidative spoilage of burukutu, leading to the production of harsh and vinegary products, low temperature fermentation and storage is not practiced[9]. This research was therefore carried out to evaluate the microbiological status and antibiotic susceptibility pattern of bacteria associated with burukutu beverage sold in Akure metropolis with a view to establish its biosafety and possible health hazard.

MATERIALS AND METHODS

Collection of samples: A total of five freshly prepared burukutu samples were purchased from various vendors at two different locations in Akure, Ondo State, Nigeria. The samples were collected in 200 mL sterile plastic bottles and immediately transferred to the microbiology laboratory of the Federal University of Technology, Akure, Nigeria in iced parked cooler for microbial analysis.

Visual observation and pH determination of the samples: The consistency and color of the samples were observed and noted before microbial analysis. The pH of each sample was determined using a pH meter (Jenwey Model)[10].

Isolation, numeration and identification of microorganisms: Total microbial load from the samples were determined using pour plate technique. Nutrient agar, MacCankey agar, potato dextrose agar and sabroud dextrose agar were used for the isolation of total aerobic bacteria, coliform and fungi respectively. Total microbial colonies from the afore-mentioned agar media were counted and reported as colony forming unit per milliliter (CFU mL^{-1}) for bacteria and spore forming unit per milliliter (SFU mL^{-1}) for fungi. The pure bacterial and fungal isolates obtained from repeated streak on appropriate bacteriological and mycological media were identified following standard microbiological procedures as described by Buchanan and Gibbons[11] and Cheesbrough[12] for bacteria and taxonomic schemes as described by Ainsworth *et al.*[13] and Mislivec *et al.*[14] for fungi.

Antibiotic susceptibility testing: Antibiotic susceptibility against the bacterial isolates associated with the samples was determined on Mueller Hinton agar using the disc diffusion method according to the prescription of the Clinical and Laboratory Standards Institute CLSI. Inoculum was prepared

by direct colony suspension and growth methods depending on whether the bacterium is Gram-positive or Gram-negative. The standardized inoculum of each isolate with a uniform optical density was spread over Mueller Hinton agar plate using sterile cotton swabs. The standard antibiotics discs were placed at the equidistance on the surface of inoculated agar plates. The susceptibility patterns of the isolates were determined by measuring the zone of inhibition in millimeter and interpreted according to CLSI. Commercial antibiotics were Androcephin (A) 25 µg, Pefloxacin (PEF) 5 µg, Gentamicin (GN) 15 µg, Ampiclox (APX) 5 µg, Zinacef (Z) 10 µg, Amoxicillin (AM) 5µg, Rocephin (R)25 µg, Ciprofloxacin (CPX) 10 µg, Streptomycin (S) 10 µg, Septrin (SXT) 10 µg and E- Erythromycin (E) 5 µg[15].

Arithmetical determination of multiple antibiotic resistance (MAR): Arithmetical method was adopted in the determination of MAR index by analyzing the antibiotic susceptibility/resistance pattern of the bacteria against commercial antibiotics[16]. MAR index was estimated and reported as follows:

$$MAR = \frac{A}{B}$$

where, A is a symbolic representation of the number of antibiotics to which the bacteria is resistant, B represents total number of antibiotics employed.

RESULTS

Visual examination of the samples and pH determination: Table 1 showed the visual observation and pH determination of the samples sold at Army Barrack and Road Block. The pH ranged from 2.6 (Road Block seller 1 and Army Barrack seller 3) to 2.9 (Army Barrack seller 1). The visual observation showed the opaque coloration of the burukutu samples collected from Army Barrack and Road Block, Akure, Nigeria.

Microbial counts: The total aerobic bacterial, coliform and fungal counts were presented in Table 2 and 3. The burukutu purchased at Army Barrack Akure from seller 3 had the highest bacterial counts of 3.5×10^{10} CFU mL^{-1} and the lowest mean value of 1.1×10^{10} CFU mL^{-1} obtained from Road Block seller 1. Total coli form counts on MacCan key agar from Army Barrack seller 2 recorded the highest value of 1.05×10^{11} CFU mL^{-1}, followed by the sample collected from Army Barrack seller 3 with a mean value of 6.5×10^{10} CFU mL^{-1} while the least mean value of 5.0×10^{9} CFU mL^{-1} was recorded for the sample bought from Road Block seller 1.The sample collected from Road Block seller 1 had no coliform growth on EMB while sample collected from Army Barrack seller 3 had highest. The total fungal counts from burukutu sold at Road Block by seller 2 recorded the highest number of fungal population on potato dextrose agar (PDA) followed by the sample collected from Army Barrack seller 3 while the least count was obtained from Army Barrack seller 1. The samples collected at Road Block and Army Barrack from seller 2 and 3, respectively had the highest fungal population of 2.00×10^{6} SFU mL^{-1} on Sabroud dextrose agar (SDA) and the least value of 1.20×10^{6} SFU mL^{-1} was obtained from Army Barrack seller 1.

Percentage occurrence of bacterial and fungal isolates from the samples: The percentage occurrence of bacteria and fungi isolated from burukutu sold at Road Block and Army Barrack were represented in Table 4 and 5. *Streptococcus* spp. and *Lactobacillus* spp. had the highest percentage occurrence from the sample collected from the Road block and Army Barrack, respectively. The highest percentage occurrence of 62.5 and 60% was obtained for *Aspergillus niger* and *Rhizopus* sp. from the samples collected from Road Block and Army Barrack respectively.

Table 1: Visual observation of the samples and pH

Samples	Colour	pH
RBS_1	Opaque	2.6
RBS_2	Opaque	2.7
ABS_1	Opaque	2.9
ABS_2	Opaque	2.8
ABS_3	Opaque	2.6

RBS_1: Road block seller 1, RBS_2: Road block seller 2, ABS_1: Army barrack seller 1, ABS_2: Army barrack seller 2, ABS_3: Army barrack seller

Table 2: Total bacterial and coliform counts from the samples

Samples	Total bacterial count (CFU mL^{-1})	Total coliform count on MAC (CFU mL^{-1})	Total coliform count on EMB (CFU mL^{-1})
RBS_1	1.1×10^{10}	5.00×10^{9}	Nil
RBS_2	3.0×10^{10}	3.50×10^{10}	2.5×10^{10}
ABS_1	2.5×10^{10}	3.00×10^{10}	1.5×10^{10}
ABS_2	2.0×10^{10}	1.05×10^{11}	2.9×10^{10}
ABS_3	3.5×10^{10}	6.50×10^{10}	4.5×10^{10}

RBS_1: Road Block seller 1, RBS_2: Road Block seller 2, ABS_1: Army Barrack seller1, ABS_2: Army Barrack seller 2, ABS_3: Army Barrack seller 3

Antibiotic sensitivity pattern of bacterial isolates to commercial antibiotics: Antibiotic sensitivity pattern of various bacterial isolates was presented in Table 6. *Bacillus subtilis* and *Staph. aureus* were resistant to septrin (SXT) and Zinacef (Z), respectively. *Bacillus cereus* and *Lactobacillus fermentum* were sensitive to the entire antibiotic employed. *Streptococcus lactis* was resistant to almost commercial antibiotic evaluated except for Androcephin (A) and Amoxicillin (AM) where appreciable susceptibility was observed. *Escherichia coli* was susceptible to 90% of the antibiotic evaluated. In Table 7, MAR index showed that the value obtained for *Streptococcus lactis* was higher than 0.2 and this suggested contamination with high risk from a source where antibiotics is frequently used as antimicrobial agents.

Table 3: Total fungal counts from the samples

Samples	Total fungal counts on PDA (SFU mL^{-1})	Total fungal counts on SDA (SFU mL^{-1})
RBS_1	6.80×105	7.20×10^5
RBS_2	2.40×10^6	2.00×10^6
ABS_1	1.00×10^5	1.20×10^6
ABS_2	1.04×10^6	1.48×10^6
ABS_3	1.05×10^6	2.00×10^6

RBS_1: Road block seller 1, RBS_2: Road block seller 2, ABS_1: Army barrack seller 1, ABS_2: Army barrack seller 2, ABS_3: Army barrack seller 3

Table 4: Percentage occurrence of bacterial isolates from burukutu sold at Road Block and Army Barrack

Bacterial isolates	Road block	Army barrack
Escherichia coli	50.0	50.0
Bacillus subtilis	55.6	44.4
Staphylococcus aureus	50.0	50.0
Streptococcus lactis	60.0	40.0
Lactobacillus fermentum	40.0	60.0

Table 5: Percentage occurrence of fungal isolates from burukutu sold at Road Block and Army Barrack

Fungal isolates	Road block	Army barrack
Aspergillus niger	62.5	37.5
Fusarium spp.	55.6	44.4
Penicilium italicum	42.9	57.1
Rhizopus japonicum	40.0	60.0
Saccharomyces cereviseae	50.0	50.0

DISCUSSION

The various industrial and domestic exercise involved human efforts in the transformation of raw materials into edible products will not only improve the nutritive and organoleptic properties of such products but also a vehicle for contaminants with great health implication on human[17]. Thus, the improved level of personal and environmental hygiene with adequate microbiological quality control check of non-alcoholic beverages in the developing countries would render them safe for human consumption.

The pH values of the samples collected from different sources ranged from 2.6-2.9. Amy[18] had reported pH ranged from 3.03-3.18 for freshly prepared burukutu while a range between 2.75 and 3.48 was reported by Fadahunsi *et al.*[19] for refrigerated burukutu. The production of organic acids by lactic acid bacteria during fermentation of cereals for burukutu production might responsible for pH lowering. Organic acids production is desired during fermentation process in that it serves as antimicrobial agents against pathogenic and food spoilage organisms[20]. The pH is an important physical parameter that contributed to the growth and survival of microorganisms. Each organism has an optimum pH or range at which its metabolic activity is permitted. Microbial metabolism is best achieved at optimum pH, at this point, the enzymes required for fermentation has its best catalytic efficiency. At optimum pH, microorganisms strive to maintain the ecological balance of the medium and the surrounding environment.

The burukutu purchased from different sellers in Akure metropolis revealed higher total aerobic bacterial, coliform and fungal counts than what were earlier reported by other investigators. Eze *et al.*[7] reported total aerobic bacterial count ranged from1.6×10^7-7.8×10^7 CFU mL^{-1}, coliform count ranged from 1.9×10^5-6.6×10^5 CFU mL^{-1} and fungal count ranged 1.3×10^2-6.3×10^2 SFU mL^{-1}. Similarly, Alo *et al.*[21] reported total aerobic bacterial, coliform and fungal counts ranged from 2.8×10^7-7.6×10^7 CFU mL^{-1},

Table 6: Antibiotic sensitivity pattern of bacterial isolates to commercial antibiotics (mm)

Isolates/antibiotics	CN	APX	Z	AM	A	CPX	S	SXT	E	PEF
Bacillus subtilis	13.0	15.5	7.5	5.0	13.5	20.0	15.0	R	13.0	16.0
Bacillus cereus	20.5	21.0	19.5	20.0	20.0	22.0	21.5	19.5	23.0	29.5
Lactobacillus fermentum	23.5	23.0	22.0	24.0	21.0	29.0	22.0	22.0	21.0	24.5
Staphylococcus aureus	16.5	16.0	R	14.0	19.5	25.0	23.0	16.5	20.0	28.0
Streptococcus lactis	R	R	R	15.0	19.5	R	R	R	R	R
Escherichia coli	25.0	20.0	15.0	R	23.0	21.0	16.0	26.0	R	23.0

A: Androcephin, PEF: Pefloxacin, GN: Gentamicin, APX: Ampiclox, Z: Zinacef, AM: Amoxicillin, R: Rocephin, CPX: Ciprofloxacin, S: Streptomycin, SXT: Septrin, E: Erythromycin

Table 7: Arithmetical determination of multiple antibiotic resistance

Organisms	MAR
Bacillus subtilis	0.1
Bacillus cereus	0.0
Lactobacillus fermentum	0.0
Staphylococcus aureus	0.1
Streptococcus lactis	0.8
Escherichia coli	0.2

2.8×10^5-8.2×10^5 CFU mL^{-1} and 2.2×10^2-5.8×10^2 SFU mL^{-1}, respectively for burukutu produced from a mixture of sorghum and millet after 24 h of fermentation. The presence of bacteria, fungi and coliform of great significance in food and medical science, ranged from human normal flora to pathogenic organisms. The presence of these microorganisms with varied loads or population might be linked to factors such as water used in the preparation of the samples, human involvement during preparation and prevailing environmental factors[7, 15]. According to Eze *et al.*[7] and Olaniyi[10], microbial contamination comes from human, sewage, handling and storage condition and rodents. The isolation of microorganisms of food and medical importance from alcoholic and non-alcoholic beverages had been reported by Kolawole *et al.*[22] , Yabaya[23] and Anaukwu *et al.*[24]. The possibility of scaling down microbial contamination or load is achievable through improper hygiene to a tolerable level for human consumption, although total elimination of undesirable organisms at different stages is rarely obtained because of the fact that some bacteria and fungi posses inherent tolerant capability to withstand adverse processing techniques[25,26].

The presence of pathogenic and lactic acid bacteria such as *E. coli*, *B. cereus*, *Staph. aureus* and *L. fermentum* from the samples corroborates the reports of Eze *et al.*[7], Alo *et al.*[21] and Olaniyi[10]. Olaniyi[10] isolated and identified same bacteria from selected fruit juices, while Eze *et al.*[7] and Alo *et al.*[21] confirmed their presence in burukutu. The roles of some microorganisms encountered in this study cannot be underestimated in food processing however, certain number indicated food spoilage or potential outbreak of food borne illnesses[7,10]. For instance, *Staph. aureus* which had the highest occurrence in all the samples obtained from the two locations is a normal flora of the skin and mucous membrane and a common etiological agent of septic arthritis in human and several animals[27]. It is an opportunistic pathogen and has been implicated in nosocomial infections[9,24]. This bacterium gets into the food during harvesting, processing or even storage. It is also the only known cause of staphylococcal food poisoning which is characterized by diarrhea and vomiting[28]. The presence of coliform is not only an indication of poor hygiene and handling but also places consumer at high risk of contacting food borne infections. *Escherichia coli*, a prominent member of *Enteriobacteriaceae* family, a component of helpful intestinal flora of humans and vertebrates with many benefits. However, some strains of *E. coli* had been implicated in gastroenteritis and urinary tract infection as well as diarrhea in infants and young children. Its presence in burukutu poses a health threat and care should be taken during its preparation to ensure zero tolerance of *E. coli*[24]. Its presence also indicates contamination of the samples with faeces and poor hygienic conditions of the environment where the product is being made and the personnel involved in the production and sale of the commodity[29]. The presence of notable spore-forming bacterium *B. cereus* indicates potential condition of distress which might result from the ingestion of food contaminated with its toxins. The toxins produced by this bacterium cause illnesses characterized by diarrhea, nausea and vomiting[10,28].

The fungal isolates encountered in this study include *S. cereviseae*, *Fusarium* spp., *A. niger* and *Penicilium italicum*. This is in tandem with the reports of Kolawole *et al.*[22], Chinedu *et al.*[30], Adebayo *et al.*[31], Eze *et al.*[7] and Oyarekua[32]. Yeasts and moulds are known for their organic acids utilization potentials and this might influenced the isolation of these fungi. *S. cerevisiae* is a yeast species responsible for the alcoholic fermentation known to be the dominant yeast in burukutu production[33]. Apart from the fermentation of the cereals by *S. cerevisiae*, its metabolic activity would also generate secondary metabolites such as higher alcohols, esters and precursors of sulphur compound. Some of these secondary metabolites impact desirable flavor and aroma on fermented beverages and serve as natural preservatives against spoilage organisms[34]. The presence of *P. italicum* and *Fusaruim* spp. are of great health concern both to human and animals since they are known for fumonisins and zearalenone production, respectively[35]. Fumonisin B1 is one of the most toxic and abundant metabolites produced by fungi and it have been reported to be a cause of esophageal cancer in humans. *P. italicum* infested wet and partially processed grains with Fumonisin B1 intended to be used for animal feed and beverages and when the residual is consumed, impairment of immune system occurs. The growth and zearalenone secretion on grains before harvest by *Fusaruim* spp. is favored during moist and cold seasons[35].

The present study has also revealed the antibiotic susceptibility profile of microorganisms isolated from burukutu. The antimicrobial susceptibility profile indicated that all the bacterial isolates with the exception of *Streptococcus lactis*, exhibited varied degree of susceptibility

to the antibiotics evaluated. The resistance of bacterial isolates to certain antibiotics might be connected to the acquired resistant genes through the process of transformation in these organisms coupled with loss of target site[33,36].

CONCLUSION

In conclusion, production of burukutu with wholesome and clean utensils will reduce the microbial population that might be of health concern. Microbiological safety of the fermented alcoholic drinks must be checked to ensure human safety on consumption. Therefore, there is need for sensitization on hygienic standard and mass awareness among producers, vendors and consumers of burukutu on the health implication of consuming spoilt or contaminated burukutu. It is advisable that burukutu when produced should be consumed fresh.

SIGNIFICANCE STATEMENT

The implication of the current study revealed potential pathogenic microorganisms in burukutu, non-alcoholic beverage sold in Akure metropolis. Thus, regular microbiological quality control check and sensitization on hygienic standard is required to guide against food borne diseases.

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