Isolation of Some Pathogens in Burukutu, a Local Drink, Sold in Sengere Village, Girie Local Government, Adamawa State

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Research Article

Isolation of Some Pathogens in Burukutu, a Local Drink, Sold in Sengere Village, Girie Local Government, Adamawa State

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ABSTRACT

A total of sixteen (16) samples of burukutu were collected from different houses in Sangere village, Girei LGA for determination of prevalence of some pathogens in these samples. The pH of the samples was in the range of 3.24-3.82. Gram staining and biochemical tests revealed that the samples contain some pathogens namely Staphylococcus aureus and species of Shigella and Salmonella. The values of total bacteria count of the samples were in the range of $1.11 \times 10^4$-4.00x$10^4$ in nutrient agar medium and 0.50x10$^4$-2.20x10$^4$ in salmonella shigella agar medium.

Keywords: Burukutu, Salmonella, Shigella, Staphylococcus.

INTRODUCTION

The earliest reported traditional African fermented product is that of production of beer from cereal extracts invented in Egypt some 5000 to 6000 BC. In the famous Benin and Ghana empires, various alcoholic beverages were used for spiritual and ceremonial occasions. For centuries the production of these beverages has gradually evolved as an art of craft which is passed on from one generation to another, without proper understanding of scientific basis of the art (Scott and Bloomfield, 1981). The traditionally fermented alcoholic beverages from cereal grains, such as guinea corn ($S. \text{ vulgaris}, S. \text{ bicolor}$), and the sap of various palm trees like the raphia palm ($R. \text{ hooke}, R. \text{ sudanica}$ and $R. \text{ vinifera}$) and coconut palm ($Cocos \text{ nicifera}$) were reported to be consumed by many Africans. In some African beer, however, sediment from previous brews is added to boost the spontaneous fermentation process.

The production of burukutu is by uncontrolled fermentation. It is one of the indigenous alcoholic beverages; it is produced mainly from grains of guinea corn ($S. \text{ vulgaris}$ and $S. \text{ bicolor}$). It is a thick, creamy, sour and alcoholic beverage (Achinewhu, 1983). Burukutu is an alcoholic beverage with 3.3-1.0% alcoholic content (Charalambus, 1984). It is found in bars areas dispensing alcoholic beverages, either in their own right or accompany meal. Burukutu is important to most rural Nigerian population who could not afford the price of a beer and other beverages. Most people take these beverages to add variety to their diet and for stimulating effects. Since this beverage is obtained from guinea corn, it is good source of essential minerals e.g. calcium, iron, zinc and copper. It has high content of suspended solids.

The major problems associated with the traditional processing of burukutu include, an availability of potable processing 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 micro-organisms. 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) favours acetic fermentation and oxidative spoilage of burukutu, leading to the production of harsh and vinegary products, low temperature fermentation and storage is not practiced.
Several types of diseases may be associated with the consumption of contaminated burukutu. The most common food poisoning is caused by the ingestion of enterotoxin produced by *Staphylococcus aureus*, *Bacillus cereus* that can cause disease. The high cost of larger beer and poor financial status of most people in the urban and rural area has resulted in increased demand and consumption of local brews such as pito, burukutu and palm wine. These beverages especially burukutu with its high nutrient content are potential media for growth of toxigenic and indicator micro-organisms. Contamination of brewing materials leads to high viable counts (Bryan et al., 1986). A number of food processed locally have been shown to be highly contaminated with *Staphylococcus sp*, *B. cereus* and other bacterial (Antai, 1988). Food poisoning outbreaks are often recognized by the sudden onset of illness within a short period of time among many individuals who have drunk contaminated burukutu.

Sangere is located near the University campus; students and staff are the regular customers of different houses in this village. The environmental conditions surrounding these houses are very unhygienic and the most of the sellers are poor and uneducated people and for these reason they may lack appreciation for safe handling and processing of burukutu. This research is aimed at assessment of the microbial quality of this beverage produced in different houses of this village and possibly highlights the risk involved for the consuming staff, students and local public.

**MATERIALS AND METHODS**

**Collection of samples**

Four samples of freshly prepared burukutu were collected at different times from each of different burukutu houses between the months of August-September, 2007 in Sangere, Girei local Government of Adamawa State, Nigeria. The samples were collected in 500ml sterile plastic bottles and immediately transferred to the microbiological laboratory of the Federal University of Technology, Yola for isolation and enumeration of the bacterial isolates. The sixteen samples collected were labelled as A1-A4, B1-B4, C1-C4, and D1-D4.

**Characteristics of samples**

The consistency and color of the samples were observed and noted. The pH of each sample was determined using pH meter (Corning 35).

**Isolation and enumeration of bacteria in Burukutu samples**

This was carried out using the pour plate method. The samples were serially diluted up to 10⁻⁴ and 1ml of each dilution was introduced onto dry agar medium. Nutrient agar, MacConkey agar and Salmonella Shigella agar were used for this purpose. A sterile glass spreader was used to spread the suspension onto the surface of the agar medium. The plates were then incubated at 37°C for 18-48 hrs and the colonies were counted using Gallenkamp colony counter. The total bacterial count was then expressed as cfu/ml (Lateef et al., 2004).

For isolation purpose, four media namely Nutrient agar (NA), McConkey agar (MAC), Salmonella-Shigella agar (SSA) and Mannitol salt agar (MSA) were used for isolation purpose using pour plate method. The plates were incubated at 37°C for 18-48 hrs and the discrete colonies were selected and were then re-inoculated onto appropriate medium. All the isolates were kept at 4°C in the refrigerator for identification purpose.

**Identification of organisms**

The bacterial isolates were then identified following standard microbiological procedure as described by Cheesbrough (2002). The colonial morphology of the isolates on different media was observed and noted. The procedures for identification of isolates were described below:

**Gram Staining:** Gram staining was done according to method as described in Cheesbrough (2002).

**Biochemical tests:** The Biochemical tests were performed according to the methods as described in Cheesbrough (2002).
Catalase Test

A drop of 3% hydrogen peroxide was placed on a glass slide. A bit of growth of each isolate was collected from the medium using a wire loop and the growth was emulsified in the drop. A positive test was indicated by bubbling and frothing, negative test did not show bubbling or frothing.

Coagulase Test

The slide method test was used for this study. A drop of saline on two separate spots was placed on the same grease free slide, speck of growth of the test organism was picked and emulsified in both spots, to one spot a drop of plasma was added and to the other a drop of saline was added, both mixtures were mixed thoroughly by rocking. A positive test indicates coagulation in the emulsion in the spot to which plasma was added.

The presence of clotting indicates positive test for Staphylococcus aureus.

Indole Test

The test organism was grown in peptone water and incubated at 37°C for 24 hours to give optimum accumulation of indole. A positive result of this test was indicated when a red coloration was observed in the uppermost layer of the tube, after adding 0.5ml of kovac's reagent to 5ml of peptone water culture.

Kligler Iron Test (KIA)

In this method each isolate was grown in a medium containing (KIA) 0.1% glucose and 0.1% lactose. KIA tubes were inoculated with a wire loop full of pure colony. The wire loop was stabbed into the deep (butt), the bottom of the tube while the slant surface was streaked with a back- and- forth motion. Inoculated tubes were placed into an incubator at 35°C for 18 to 24 hours.

Motility

A single colony of each of the organisms was inoculated into labelled test tubes containing peptone water (5mls) and the tubes incubated at 37°C over night. A drop of the well-mixed organism in peptone water incubated over night was placed on a cover slip and the edges surrounded with oil immersion. A microscope slide was then placed over the cover slip taking care that the slide those not touch the drop on the cover slip but suspended by the oil immersion. The slide was then turned quickly but gently. This preparation was then observed under the microscope for motile bacteria under x 100 objectives.

Triple sugar iron test

Using a straight wire a speck of each of the isolates was inoculated into each of TLS agar slants in tubes by stabbing the butt twice and smearing the slope. Each of the tubes was capped loosely and then incubated at 37°C for 18-25 hrs. After incubation, a red colour slant with blackening of the butt and the appearance of bubbles with cracks or complete pushing of the butt indicates no fermentation of lactose and sucrose, and is indicated as positive reaction.

RESULTS AND DISCUSSION

The color and consistency of the samples were brown and semi solid. The pH values of the samples of burukutu collected from different houses were in the range of 3.24-3.82. It is in agreement with Achi (2005) who reported that during the 48 hrs fermentation periods in burukutu production, the pH falls from 6.4-3.7. This level of acidity of burukutu has been described by several researcher including Efuvwevwere and Akoma (1995) and Akoma et al. (2006) who attributed these to certain spp of lactic acid bacteria during the fermentation process. Similar local drinks with acidic pH values have been reported for pito and bili-bili (kolawale et al., 2007). The acidity tends to increase with increase in fermentation period resulting into spoilage.

The values of total bacterial count of all the collected samples were shown in figure 1a and 1b. The values were in the range of 1.1X10⁴- 4.0X10⁴ in NA and 0.5X10⁴-2.2X10⁴ in SSA. These high colony counts may be due to poor hygiene condition of environment and may also be due to unhygienic storage process. The utensils used like calabash and storage pots may also be responsible for this type of contaminated burukutu sold in these houses.
Fourteen isolates were obtained from the collected samples. The isolates were labelled as X1-X5, Y1-Y4 and Z1-Z5. Result of gram staining is shown in Table 1. The cultural and morphological characteristics of isolates are listed in Table 1. The biochemical characteristics of isolates are listed in Table 2.

The presence of *S. aureus* in the samples may be attributed to handling during production. *S. aureus* is a normal flora of the skin and mucus membrane and a common etiological agent of septic arthritis. The presence of *Salmonella* spp in the alcoholic beverages could be attributed to the contaminated water used for germination sprout and poor environmental condition surrounding the burukutu house. Also the presence of *Shigella* spp can be traced to contaminated water and symptomatic carrier working in the brews house (Prescott *et al.*, 2002). The presence of some pathogens in burukutu may be as a result of transferred of these organisms by flies. Poor presentation and storage of this local alcoholic beverage attracts flies that pitch on it and sometimes falls into the beverages (Nester *et al.*, 1998). Sediments which are added to aid in the fermentation process are agricultural commodities, which may contain a high level of microbial impurities (Adyem and Umar, 1994). These can be source of spoilage and pathogenic micro organisms (Bibek, 2001).

This study reveals that burukutu samples collected in Sangere village were contaminated with pathogens like *S. aureus*, *Salmonella* and *Shigella* spp. These pathogens are associated with some diseases like typhoid fever, urinary tract infection and food poisoning.

**Fig. 1a:** TBC values of different burukutu samples on MA

- **Horizontal axis = TBC X10,000**
- **Vertical axix = Samples of burukutu**
Samples

Fig. 1b: TBC values of different burukutu samples on SSA
Horizontal axis = TBC X 10,000
Vertical axis = TBC X 10,000

Table 1: Cultural and morphological characteristics of isolates

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Media</th>
<th>G.S</th>
<th>Possible organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA and MSA</td>
<td>MAC</td>
<td>SSA</td>
</tr>
<tr>
<td>X₁ – X₅</td>
<td>Yellow cream</td>
<td>Cream</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-2mm colonies</td>
<td>0.1 – 0.5mm colonies</td>
<td></td>
</tr>
<tr>
<td>Y₁ – Y₄</td>
<td>-</td>
<td>Pale colour 1-2mm</td>
<td>Red – pink colonies 2- 4mm</td>
</tr>
<tr>
<td>Z₁ – Z₄</td>
<td>-</td>
<td>Grey – white 2 – 3mm colonies</td>
<td>Pale colonies with blackening in the medium</td>
</tr>
</tbody>
</table>

Key: - No growth

GS – Gram staining GPC – Gram positive cocci
GNR – Gram negative rod NA - Nutrient Agar
MAC – MacConkey Agar SSA – Salmonella Shigella Agar

Table 2: Biochemical characteristics of isolates

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Indole</th>
<th>TST</th>
<th>Motility</th>
<th>Catalase</th>
<th>Coagulase</th>
<th>KIA</th>
<th>Mannitol</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁ – X₅</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>+</td>
<td>+</td>
<td>NC</td>
<td>+</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Y₁ – Y₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>NC</td>
<td>Shigella spp.</td>
</tr>
<tr>
<td>Z₁ – Z₅</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>NC</td>
<td>Salmonella spp.</td>
</tr>
</tbody>
</table>

Key
+ : Positive Reaction
- : Negative Reaction
TST : Triple sugar test.
KIA : Klingler Iron sugar
NC : Not carried out
REFERENCES


