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# Microbial Quality of Traditional Alcoholic Beverages Consumed in Dar es Salaam, Tanzania

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**Three traditional alcoholic beverages available locally in Dar es Salaam namely 'mbege', 'mnazi' and 'komoni' were subjected to microbiological assessments in order to determine their microbial quality and possible resistance to antibiotics among the isolated microorganisms. Twenty-seven samples were randomly collected from local drinking clubs in all the three districts included in the study. The microorganisms were isolated and identified by the conventional methods. Microbial counts were determined for the beverages. The total viable yeast counts in 'mbege', 'mnazi' and 'komoni' were 2.37 x $10^{16}$, 3.9 x $10^{16}$ and 7.0 x $10^{10}$ cfu/ml respectively, while the total viable counts for bacteria in the same beverages were 9.50 x $10^{9}$, 2.22 x $10^{10}$ and 1.88 x $10^{10}$ cfu/ml respectively. Antimicrobial sensitivity tests on the microorganisms showed that all bacteria were susceptible to a majority of the assayed antibacterials, whilst yeasts were resistant to all the antifungal agents tested except clotrimazole. The results indicate that these beverages pose a danger to public health due to the observed high 'bio-burden' and call for better and safer means for the preparation, processing and handling of the products.**

**Key words:** Traditional beverages, microorganisms, antibacterials, antifungal agents.

## INTRODUCTION

Traditional alcoholic beverages in Tanzania play an important role in the daily social, economic, nutritional and cultural life of the people. Some of the traditional Tanzanian beers include 'mbege' and 'komoni' made from fermented bananas and maize respectively, while traditional wines include 'mnanasi', 'wanzuki', 'mofru' and 'mnazi' also known as 'tembo' [1]. The last beverage is palm wine and is largely confined to the coast of Tanzania. Evaluation of some homemade but commercially available alcoholic beverages in Dar es Salaam revealed that the ethanol concentrations of the brewed samples ranged from 2.2 to 8.5 % w/v while the distilled beverages, 'gongo', contained 24.2 to 29.3 % w/v ethanol. Aflatoxin B1 was also found in 9 out of 15 beverages suggesting the use of contaminated grains or fruits for their production. Moreover, these beverages may also contain a number of bacteria and fungi which can cause water and food borne diseases like cholera (*Vibrio cholerae*), typhoid (*Salmonella typhi*), paratyphoid fever (*S. paratyphi*), food poisoning (*Salmonella spp*) and bacterial dysentery (*Shigella spp*, *Campylobacter spp* and *E. coli*) [2].

Most of the bacteria found in traditional alcoholic beverages belong to the *Staphylococcus* species that are normal flora and are widely distributed in the environment. With the exception of *Staphylococcus aureus* which is the most pathogenic species within the group, the incidence of other pathogenic microorganisms such as *S. epidermidis* which is associated with nosocomial infections is low. On the other hand, *Pseudomonas* spp, particularly *P. aeruginosa, P. pseudomallei* and *P. maltophilia* are found in a wide range of environments. *P. aeruginosa* is mostly found in hospital environments while *P. pseudomallei* is found in the mud of riverbanks and stagnant water [2-3].

There is extensive brewing and drinking of homemade or informal-sector drinks, estimated to

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be 2.4 billion liters a year or more, which accounts for 90 % of all the alcohol consumed in Tanzania [4]. Bottled beer is about six times more expensive when compared to maize beer. Most of the outbreaks of food-borne diseases in Dar es Salaam city have mainly been associated with the poor hygiene of foods and locally prepared beverages sold within the community. Therefore, continuous daily drinking of these beverages is certain to increase health risks [5-6].

Additionally, owing to the HIV/AIDS crisis, infected individuals are more prone to a myriad of opportunistic infections as compared to uninfected individuals. Hence, if the microbial load in these traditional alcoholic beverages is beyond the tolerable limits, it may result in their deterioration. For that reason, this study was aimed at assessing the levels of microbial contamination and hence the quality of traditional alcoholic beverages available in the Dar es Salaam informal sector drinking clubs/bars, and consequently at creating awareness among the authorities and the public at large.

## MATERIALS AND METHODS

The study involved three districts in Dar es Salaam city namely Temeke, Kinondoni and Ilala. Three clubs/bars were randomly selected from each district and from each club/bar three different brew samples were collected in sterilized universal bottles three times at different time intervals and analyzed in duplicate for statistical reasons. The collected samples were 'mbege' and 'komoni' as the traditional beers and 'mnazi', consumed as traditional wine.

All growth media and the necessary apparatus were sterilized by autoclaving. The test samples were directly inoculated into differential and non-selective growth media and incubated for 24-48 h at 37 $^{o}$C. Subsequent subcultures were made onto solid culture media namely Sabouraud's dextrose agar, nutrient agar, MacConkey agar and blood agar, which were similarly incubated at 37 $^{o}$C for 24-48 h. About 15-20 ml each of sterile thioglycolate fluid medium, yeast extract fluid and soy-casein digest fluid medium was inoculated in universal bottles containing 1 ml of each test sample beverage. The mixture was incubated overnight at 37 $^{o}$C. Distilled water was used as a control and was also incubated under the same conditions. All the procedures outlined above were aseptically conducted.

Yeast cells isolated from Sabouraud's dextrose agar medium were subjected to the germ tube test [2]. A positive germ tube indicated the presence of *Candida albicans* while a negative test implied the presence of other species of *Candida* or other yeasts. The same colony subjected to the germ tube test was also tested for sugar metabolism in order to identify different *Candida* species, based on their capacity to metabolize carbohydrates (sugar) to produce lactic acid, with production of gas serving as an additional proof [2, 7].

Colony counting of viable bacteria and yeast cells was done by inoculating the diluted samples onto nutrient agar and Sabouraud's dextrose agar media respectively. Serial dilution was performed by mixing 1 ml of sample with 99 ml of sterile water to make a total of 100 ml. Further decimal dilutions ($10^{-1}$ to $10^{-6}$) were prepared and pour-plated using differential media and incubated at 37 °C for 24-48 h. The resultant colonies were counted and recorded as colony forming units per millilitre (cfu/ml). This procedure was repeated three times and the average cfu/ml were calculated. Standard microbial limits were set at less than $10^3$ to$10^4$ cfu/ml for an acceptable final product, $10^5$ to $10^6$ cfu/ml for medium contamination and more than $10^6$ cfu/ml for heavy contamination [2]. Identification of the isolated microorganisms was carried out as previously described [2, 7-8].

The isolated bacteria and yeasts were also tested for susceptibility to antibacterial and antifungal agents commonly used for the treatment of infections using the agar disk diffusion technique. Disks of blotting paper of known size were impregnated with known volumes and appropriate concentrations of antimicrobial agents. Upon vacuum drying, the disks were placed on ISO-sensitest and Sabouraud's dextrose agars inoculated with bacterial and fungal cells respectively. The plates were then incubated overnight at 37 °C and the diameter, in

millimeters, of each inhibition zone was subsequently determined.

**Table 1. Different species of *Candida* that metabolize different sugars and results of sugar fermentation by these species.**

| *Candida* spp | Sugar Fermentation | | | | Results | |
|---|---|---|---|---|---|---|
| | Glucose | Maltose | Sucrose | Lactose | Gas produced | Fermentation |
| *C. albicans* | Ag | Ag | A | _ | _ | _ |
| *C. tropicalis* | Ag | Ag | Ag | _ | _ | _ |
| *C. cruzei* | Ag | _ | _ | _ | + | + |
| *C. gucillemondi* | Ag | _ | Ag | _ | _ | _ |

Ag = acid and gas production, A = acid produced

The antifungal agents used for disk sensitivity testing were ketoconazole, nystatin, griseofulvin, fluconazole, terbinafine, and clotrimazole. Doxycycline, chloramphenicol, cefuroxime, cotrimoxazole, ampicillin, gentamicin and ceftriaxone were used to assay *Pseudomonas* spp sensitivity. For both *Staphylococcus* spp and *Streptococcus* spp ampicillin, gentamicin, penicillin, chloramphenicol, cotrimoxazole, doxycycline, cefuroxime and ceftriaxone were used. The control microorganisms were *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Candida albicans*.

Statistical analyses of mean differences among the groups were determined by the Paired Samples t-test (SPSS software) and the significance level was set at $p < 0.001$.

## RESULTS

All the 27 brews collected (100 %) were found to be contaminated with various microorganisms. The Gram staining technique revealed the presence of Gram positive cocci, oval shaped Gram positives (yeast cells) and Gram negative bacilli. The Gram negative, small, dark red colonies isolated from MacConkey agar were catalase negative suggesting that they were *Streptococci* while the whitish colonies of Gram positive cocci isolated from the same medium were catalase positive which suggested that they were *Staphylococci* (figures not included). The catalase positive cocci were also coagulase positive indicating the presence of *Staphylococcus aureus*. The other possible bacteria would be *S. epidermidis* and *S. saprophyticus* that are coagulase negative.

The Gram negative bacilli isolated from the solid media were oxidase positive suggesting the presence of *Pseudomonas* spp, *Alcaligenes*, *Vibrio* or *Aeromonas*. The presence of *Pseudomonas* was confirmed by its positive reaction in the indole and citrate tests as well as its ability to ferment glucose. The yeast cells isolated from Sabouraud's dextrose agar plate could not produce the germ tubes, suggesting the absence of *Candida albicans*, and therefore the presence of other *Candida* species or yeasts. The same yeast cells were subjected to sugar fermentation test and yielded a positive test on glucose and gas production confirming the presence of *Candida cruzei* (Table 1). The total viable yeast and bacterial counts from the solid growth media expressed as colony forming units per milliliter (cfu/ml) found in the assayed beverages are depicted in Table 2.

**Table 2. Total viable microbial counts**

| Assayed beverage | Bacterial mean cfu/ml | Fungal mean cfu/ml |
|---|---|---|
| *Mbege* (n= 9) | $9.50 \times 10^{9}$ | $2.37 \times 10^{16}$ |
| *Mnazi* (n= 9) | $1.88 \times 10^{10}$ | $7.0 \times 10^{10}$ |
| *Komoni* (n= 9) | $2.22 \times 10^{10}$ | $3.9 \times 10^{16}$ |

The results for the antimicrobial susceptibility tests on the isolated microbes are summarized in Tables 4, 5 and 6 and are based on measuring the inhibition zone radii and comparing them with those of standard drugs and microorganisms as described under Materials and Method (figures not included). The results were interpreted as sensitive (S) for a zone radius not smaller than that of the control by more than 3 mm,

intermediate (I) for a zone radius more than 3 mm smaller than that of the control but not less than 3 mm in actual size, resistant (R) for no zone of inhibition or when the zone radius measured 2 mm or less and dead disk (Dd) when there was no zone of inhibition on both the control and test sample [2]. The findings revealed that *Pseudomonas spp* were resistant to chloramphenicol and ampicillin, *Streptococcus spp* were resistant to ampicillin alone while *Staphylococcus spp* were sensitive to all the assayed drugs. *Candida cruzei* was resistant to all the assayed antifungal agents except clotrimazole.

## DISCUSSION

This study revealed that the microorganisms present in the traditional alcoholic beverages assayed were *Staphylococcus spp*, *Pseudomonas spp*, *Streptococcus spp*, and *Candida cruzei*. With the exception of *Pseudomonas spp,* the other identified microorganisms are part of the normal flora of the human body and are widely distributed in nature. This implies that microorganisms from human and water sources contaminated these beverages during the preparation process.

However, the yeasts that yielded negative results in all the biochemical tests conducted for *Candida* could be other types of fungi used in the fermentation of the assayed beverages for example *Saccharomyces cerevisiae* as previously reported [4-5].

Our study further revealed high microbial counts in these beverages (Table 2), which could be due to poor hygienic conditions during preparation and the use of water of poor quality. The absence of coliform and enteric bacteria that are mostly found in water might be due to the acid produced during the fermentation process as well as the alcohol content of these beverages [5]. The persistence of *Staphylococcus* spp*, Streptococcus* spp*, Pseudomanas* spp and *Candida cruzei* could indicate that these microorganisms are resistant to the acidic and alcoholic environment within the beverages. There were statistically significant differences ($p<0.001$) with regard to the total viable bacteria count among the assayed beverages despite the fact that all the assayed samples were heavily contaminated as compared to the control ($10^3$ to $10^4$ cfu/ml). The most contaminated sample was 'komoni', though there was no significant difference with respect to 'mnazi' ($p = 0.022$). The level of contamination is of great concern since one million cells of *Staphylococcus aureus* per milliliter can produce enough enterotoxin to cause illness [7].

On the other hand, viable microbial counts obtained from Sabouraud's dextrose agar show that 'mnazi' contains a significantly ($p <0.001$) lower yeast cell count as compared to 'mbege' and 'komoni', though it is still higher compared to that of the control. This could be interpreted to imply that 'mbege' and 'komoni' pose a greater danger to immunocompromised individuals, taking into account the resistance manifested by *Candida cruzei* to most of the assayed antifungal agents. The bacteria isolated were sensitive to most available antibacterial agents. In addition, our findings concur with one previous study which reported some trace elements as well as microbial contaminants in traditional alcoholic beverages [6].

In conclusion, this study revealed that the unregulated production and use of traditional brews poses a health risk since the brews are contaminated with bacteria and fungi. While most of the isolated and identified microorganisms are normal flora, they may cause serious health problems in individuals who are immuno-compromised. Among the bacteria isolates found, *Streptococcus* spp*.,* which forms part of the normal flora of the gastrointestinal system, causes infection if ingested. Special attention should be paid to *Candida cruzei* as it causes disease and is resistant to most antifungal agents. Further studies should be carried out on some yeast species which were isolated but could not be identified owing to lack of facilities.

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**Table 3. Results of susceptibility test on *Pseudomonas spp* isolates to different antibiotics**

| Drug | Conc. (µg/µl) | Zone of inhibition (mm) | | Inference |
|---|---|---|---|---|
| | | Test sample | Control | |
| Doxycycline | 20 | 14 | 14 | S |
| Gentamicin | 10 | 10 | 8 | S |
| Cotrimoxazole | 25 | - | - | Dd |
| Cefuroxime | 30 | 10 | 14 | I |
| Chloramphenicol | 30 | 2 | 6 | R |
| Ceftriaxone | 30 | 9 | 14 | I |
| Ampicillin | 10 | - | 2 | R |

S = Sensitive; R = Resistant; I = Intermediate and Dd = dead disk (no zone of inhibition on control and test sample).

**Table 4. Results of susceptibility test on *Staphylococcus spp* isolates**

| Drug | Conc. (µg/µl) | Zone of inhibition (mm) | | Inference |
|---|---|---|---|---|
| | | Test sample | Control | |
| Gentamicin | 10 | 9 | 9 | S |
| Cefuroxime | 30 | 15 | 16 | S |
| Ceftriaxone | 30 | 17 | 16 | S |
| Ampicillin | 10 | 12 | 11 | S |
| Doxycycline | 20 | 15 | 14 | S |
| Chloramphenicol | 30 | - | - | Dd |
| Cotrimoxazole | 25 | 14 | 13 | S |

**Table 5. Results of susceptibility test on *Streptococcus spp* isolates**

| Drug | Conc. (µg/µl) | Zone of inhibition (mm) | | Inference |
|---|---|---|---|---|
| | | Test sample | Control | |
| Doxycycline | 20 | 5 | 8 | I |
| Cefuroxime | 20 | 5 | 9 | I |
| Ampicillin | 10 | 2 | 12 | R |
| Gentamicin | 10 | 10 | 10 | S |
| Cotrimoxazole | 30 | - | - | Dd |
| Chloramphenicol | 30 | - | - | Dd |
| Ceftriaxone | 30 | 9 | 10 | S |

**Table 6. Results of susceptibility test on Candida cruzei and other yeast isolates**

| Drug | Conc. (µg/µl) | Zone of inhibition (mm) | | Inference |
|---|---|---|---|---|
| | | Test sample | Control | |
| Nystatin | 12 | - | 4 | R |
| Griseofulvin | 10 | - | - | Dd |
| Terbinafine | 12 | - | 6 | R |
| Fluconazole | 15 | - | 5 | R |
| Ketoconazole | 15 | 2 | 12 | R |
| Clotrimazole | 15 | 5 | 11 | I |

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