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Microbiological Quality of Juice Beverages Available in Dar es Salaam and Resistance Profiles of Microbial Contaminants

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Eight most widely available juice beverages each constituted by four extemporaneous and four industrially made juices were bought from different localities in Dar es Salaam. Extemporaneous juices were randomly collected using sterile containers. Each sample was microbiologically analyzed using conventional methods. The identified microbes were subjected to antimicrobial resistance assays against 15 commonly used antibiotics using the Kirby-Bauer disk diffusion method. Reference strains of *Escherichia coli* (ATCC25922), *Candida albicans* (ATCC90028) and *Staphylococcus aureus* (ATCC25923) served as control microorganisms. Four microbial contaminants were isolated, namely *Streptococcus* spp, *Escherichia coli*, *Staphylcoccus aureus*, and *Klebsiella* spp. High content of contamination (4.1 × 10^5 cfu/ml of bacteria and 6.4 × 10^6 cfu/ml of fungi) were found in all extemporaneous juices. About 5.4 × 10^4 cfu/ml of bacteria and 6.8 × 10^4 cfu/ml of fungi were isolated from industrially produced juices. Microbial contaminants in juices exceeded acceptable limits by 100-fold. The *Streptococcus* spp and *Escherichia coli* isolates were resistant to ampicillin, amoxicillin, ceftriaxone and doxycycline.

Key words: Antimicrobial resistance, microbial contamination, extemporaneously and industrially made juices

INTRODUCTION

Juice beverages are among food products which are highly consumed in Tanzania, particularly in Dar es Salaam City. The city has a very humid climate and relatively high temperatures with temperatures rising to 30 °C. The high temperature and humidity make residents to experience discomfort which obliges them to ingest a lot of water and juice products [1]. Moreover, consumption of juice beverages is strongly recommended by nutritionists because of their high vitamin content [2-5]. However, high water content coupled with crude methods of production and packaging under improper sanitary conditions predisposes the juices to microbial contamination [6-8]. Juices that are prepared extemporaneously at points of sale are at even higher risk of microbial contamination because they do not undergo quality control process prior to their consumption as compared to industrially produced ones.

Microbial contamination of juices and soft drinks are of two major types namely microbial growth and deterioration of the products leading to spoilage and microbial contamination of the products by pathogenic microorganisms [9,10]. Nevertheless, previous studies had raised concerns regarding the microbiological quality of juice beverages indicating the presence of bacteria and yeasts [10,11]. But these studies did not include antimicrobial resistance profiling of the contaminating microorganisms.

Dysentery, typhoid fever, cholera and other food-borne diseases are caused by micro-organisms which are accidentally introduced into foodstuffs and beverages during their preparation, particularly under poor sanitary conditions [12,13]. Further, sick persons are advised to take nutritive food stuffs including

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juices. Individuals whose immune systems are compromised become even more prone to several opportunistic infections [6,14,15]. Hence, high presence in these juices of potentially pathogenic microorganisms beyond the tolerable limits can lead to enhanced susceptibility to infections. Therefore the quality and safety of these beverages are of crucial importance to consumers.

The outbreaks of food-borne diseases experienced in Dar es Salaam City have been mainly attributed to poor sanitary conditions as a result of malfunctioning drainage and wastes disposal systems as well as unhygienically prepared foodstuffs and/or traditional beverages that are consumed by majority of low-income citizens [16,17]. The present study was designed to highlight potential sources of food-borne infections in Dar es Salaam through isolating, identifying and antimicrobial resistance profiling of the microbial contaminants.

MATERIALS AND METHODS

Collection of samples and storage

Juice samples were collected from different localities of Dar es Salaam City. Three different samples of extemporaneously made juices of each of the following components, passion, mango, tamarind and sugar cane were collected using sterile containers. While samples of industrially produced juices designated as A, C, S and P samples were purchased in their original containers. All collected samples were stored at 4 °C in their original containers until use (time lapse not exceeding 6 h). Only unexpired industrially made juice samples were used this purpose.

Microbiological analysis of juice beverage samples

Each juice sample was aseptically opened by swabbing the container with 70% ethanol. Each sample was serially diluted in sterile distilled water and 1 ml aliquot was separately subjected to spread-plate count test. Various selective and differential media were used: Nutrient agar (NA) for total viable count, Eosine methylene blue agar (EMB) for coliforms; Thiosulfate citrate bile sucrose agar (TCBS) for *Vibrio*; Salmonella-Shigella agar (SSA) for Salmonella; Sabouraud's dextrose agar (SDA) for fungal molds and Yeast extract-dextrose peptone agar (YEDP) for yeasts [18,19]. All plates were incubated at 37 °C for 24-48 h. At the end of the incubation period, microbial growths were counted and recorded as cfu/ml. Further physiological and biochemical tests were performed for confirmation of the identity of each isolated microorganism. Fecal and non-fecal coliforms were determined by indole, methyl red, Voges-Proskauer and citrate (IMiVC) test as described in literature [18].

Antibiotic resistance profiling

The isolated microbial contaminants were subjected to antibiotic resistance assays against commonly used antibiotics, using the Kirby-Bauer disc diffusion method on RPMI-glucose agar and Mueller-Hinton agar for fungal and bacterial contaminants respectively [18-20]. A 24-h broth sub-culture of each microorganism was used for this purpose. This was preceeded by comparison of microbial suspension against that of 0.5 MacFarland turbidity standards prior to the antibiotic resistance testing. The reference strains of microorganisms employed were: *Escherichia coli* (ATCC25922), *Candida albicans* (ATCC90028) and *Staphylococcus aureus* (ATCC25923). The antibiotic discs used were: ciprofloxacin 5 µg, doxycycline 30 µg, cefuroxime 30 µg, clavum 30 µg, and ampicillin 10 µg (Pharmathene-SA, Athens, Greece); amoxicillin 25 µg, gentamicin 10 µg, and ceftriaxone 30 µg (Remedica, Limassol, Cyprus); nystatin 12 µg and terbinafine 12 µg (Pharmathene-SA, Athens, Greece); and fluconazole 15 µg, ketoconazole 15 µg, and clotrimazole 15 µg (Himedia Lab., Mumbai, India). The selected antimicrobial agents represented the most commonly available and used antibiotics in Tanzania as per the National Standard Treatment Guidelines [21].

Data analysis

All assays were conducted in triplicate and performed twice for statistical purposes and consistency of results. Statistical analysis of numeric data was performed by independent samples T-test using the Statistical packages for social science (SPSS version 17, Chicago-USA). Differences in inhibition zones between the reference strains of microorganisms and isolated microbial contaminants were considered significant at $p < 0.05$.

RESULTS

Three samples of each of the four extemporaneously produced juice samples (mango, passion, tamarind and sugar cane) and four industrially manufactured juices designated as S, P, C and A were microbiologically analyzed. The industrially produced juice samples A, S and C were registered by the Tanzania Food and Drug Authority (TFDA) in 2006, 2009 and 2008 respectively, while sample P could not be found in the TFDA register [22].

The analyses revealed presence of microbial contaminations of varying degrees in both extemporaneously and industrially made juice beverages (Tables 1 and 2). Tamarind juices were the most microbiologically contaminated with both bacterial and fungal contaminants. However, all extemporaneous juices harboured more fungal than bacterial contaminants (Table 1). Industrially manufactured juice P had the lowest number of bacterial contaminants (5.0×10^3 cfu/ml), while juice A had the least fungal contaminants (1.2×10^2 cfu/ml) as indicated in Table 2. In both cases, the fungal contaminants were due to *C. albicans*. The detected microbial contamination from the extemporaneous juices exceeds the acceptable limits by 100-fold while that of industrially juices exceed by 5-6 times [8].

A variability of antimicrobial resistance profiles was observed among the isolated bacterial contaminants as illustrated in Figures 1-2.

Table 1: Total viable counts in extemporaneous juices

Type of juice	Isolated microorganisms (cfu/ml)	
	Fungal	**Bacterial**
Mango	5.2×10^5	5.6×10^4
Tamarind	6.2×10^6	4.1×10^5
Sugar cane	6.21×10^5	4.0×10^5
Passion	6.0×10^6	5.25×10^4

Table 2: Total viable counts in industrial juices

Type of juice	Isolated microorganisms (cfu/ml)	
	Fungal	**Bacterial**
C	5.2×10^3	1.0×10^4
A	1.2×10^2	5.4×10^4
S	6.8×10^4	2.6×10^4
P	2.2×10^3	5.0×10^3

Isolates of *E. coli* exhibited variability with regard to sensitivity to the antibiotic tested. This microorganism was resistant to ceftriaxone and doxycycline and was of intermediate susceptibility to cefuroxime, ampicillin and amoxicillin (Figure 1). The isolated *Klebsiella* contaminants were susceptible to all tested antibiotics except one isolate that yielded less than 20 mm-diameter inhibition zone when tested against ceftriaxone as shown in Figure 1.

Isolates of *S. aureus* were coagulase-positive and were found to be sensitive to all the tested antibiotics (Figure 2). Two isolates of *Streptococcus* spp were resistant to amoxicillin and ampicillin but of intermediate susceptibility to gentamicin and ceftriaxone as indicated in Figure 2. All candida isolates were relatively sensitive to the antifungal agents used as compared to the control strain (*C. albicans*-ATCC90028) except terbinafine to which the fungal contaminants were moderately susceptible (Figure 3).

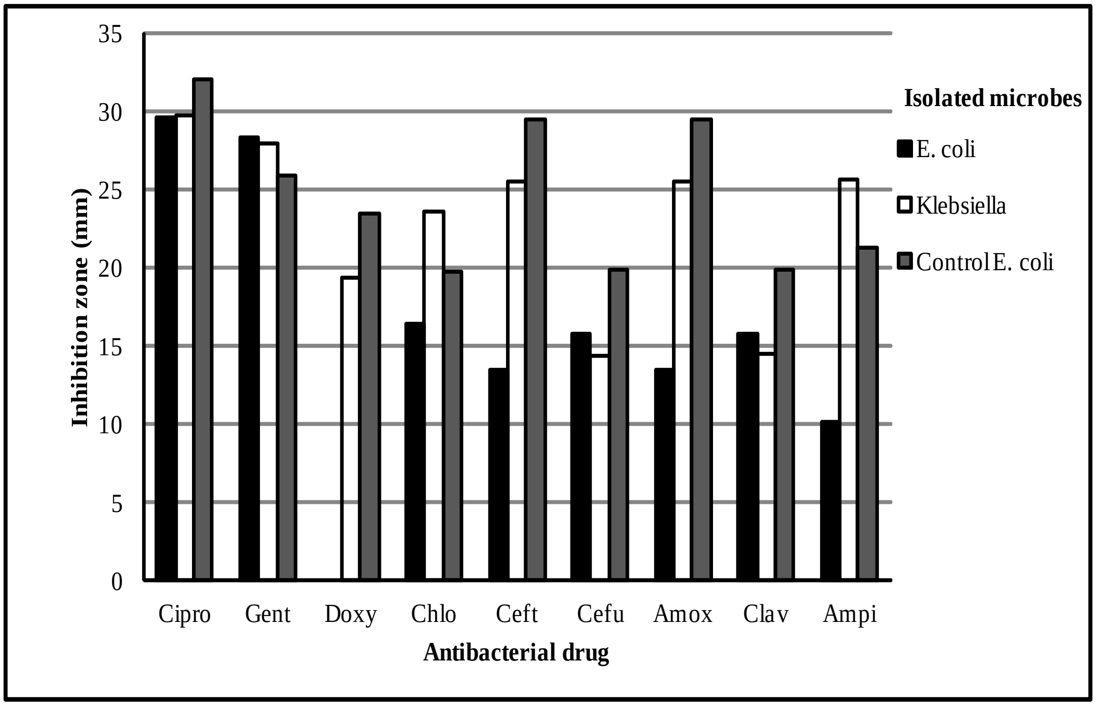

Figure 1. Resistance profiles of Gram-negative bacterial contaminants (*E. coli* and *Klebsiella* spp). Cipro = ciprofloxacin; Gent = gentamicin; Doxy = doxycycline; Chlo = chloramphenicol; Ceft = ceftriaxone; Cefu = cefuroxime; Amox = amoxicillin; Clav = clavum; Ampi = ampicillin.

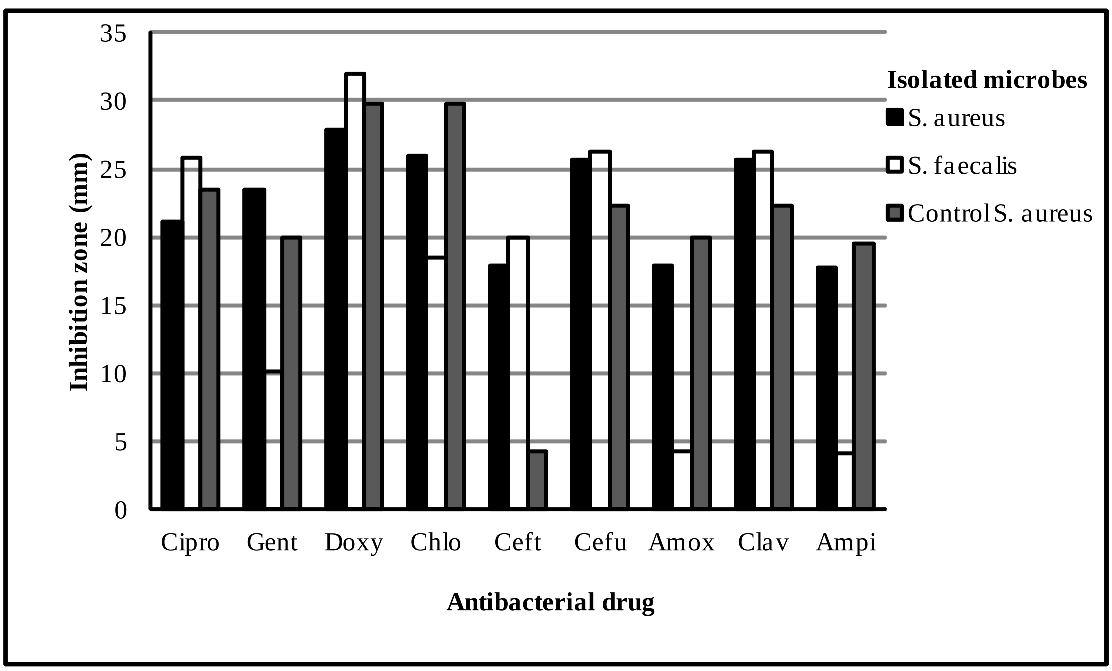

Figure 2. Resistance profiles of Gram-positive bacterial contaminants (*S. aureus* and *Strep. faecalis*).

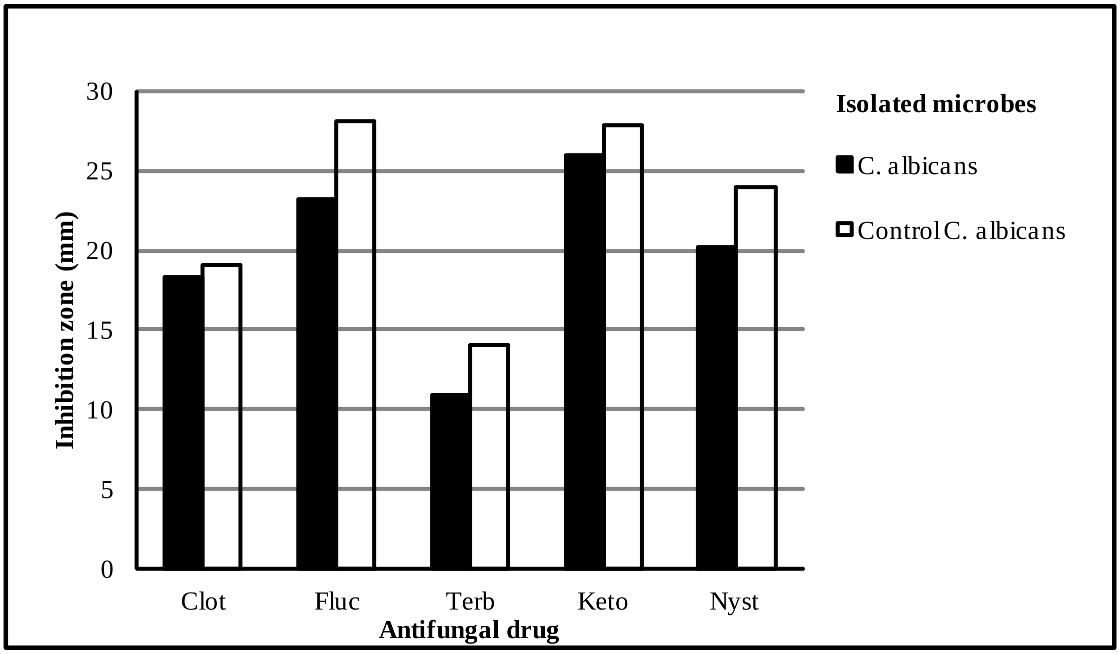

Figure 3. Resistance profile of *C. albicans*. Clot = clotrimazole; Fluc = fluconazole; Terb = terbinafine; Keto = ketoconazole; Nyst = nystatin.

DISCUSSION

The presence of different microorganisms in commercially available juices is of major health concern [23-25]. Microbiological contamination of juices may occur when the microorganisms enter the processing plant or found on the surface of the fruits/raw materials having originated from the fields. The degree of contamination of such products varies depending on how the raw materials are handled from field and in the processing plant. Therefore proper handling, washing and sanitizing of fruits can contribute significantly to the product good quality. It has been documented that an increase of microbial contamination of fresh juices is attributable to pathogenic microorganisms that are becoming more resistant to preservation techniques. The microorganisms also rapidly multiply spread fast. This is especially important for immmunocompromised patients who are quite sensitive to low levels of pathogenic microorganisms [14,15,26].

The low pH of fruit juices greatly limits the number and the type of microorganisms that can survive in them but some acidophilic microorganisms thrive well under low pH conditions [27]. In addition, presence of some microorganisms may alter the pH making it favorable for pathogenic ones [23]. The use of unsafe water for preparation of juices, prolonged preservation without refrigeration, unhygienic surroundings and air bone dust can also act as sources of microbial contamination to both raw materials and ready-to-use juices [27].

This study has revealed heavy microbial contamination for both extemporaneous and industrially made juices. The main microbial contaminants were *Staphylococcus* spp, *Streptococcus* spp, *E. coli*, *Klebsiella* spp and *Candida albicans.* The findings collaborate with a previous study conducted by Elmahmood *et al.*, who isolated S*treptococcus* spp, *Staphylococcus aureus*, *E. coli* and yeasts from non-alcoholic beverages though the bioburden was relatively lower [9]. In addition to *E. coli*, the results of the present study indicate the presence of *Klebsiella* spp both of which are enteric bacteria that are commonly found in water [12,16]. Moreover, since all the isolated microorganisms are normal flora of human, it inferred that they originated from humans.

Our results underscore a possible health hazard as a consequence of consuming extemporaneous juices not only due to the observed heavy microbial contamination, but also because of the fact that the microbial counts could increase further when the juices are taken home and stored at room temperature which favours microbial proliferation.

Although, the isolated microorganisms are not naturally pathogenic to human, they may cause serious health problems in individuals who are immunocompromised especially HIV/AIDS patients or those on immunosuppressive drugs [13,15]. The observed microbial counts were significantly higher ($p < 0.05$) compared to the acceptable limits for *E. coli* and other enteric bacteria [8].

The presence of coagulase-positive *S. aureus* in these juices could pose great danger to the consumers. In the human host, the action of coagulase produces clotting of the plasma in the immediate vicinity of the bacterium. The resulting increased effective diameter of the bacterium makes it difficult for the defense reactions of the host to handle the microorganism [15].

The antibiotic resistance to amoxicillin, ampicillin, doxycycline and ceftriaxone exhibited by some of the isolated microbial contaminants cannot be overlooked, since they are widely used for treatment of prevalent microbial infections in the community [29,31].

Although the prevalence of drug resistance in fungi is below that observed in bacteria, many mycologists consider that selective pressure will, over time, lead to more widespread resistance [32,33]. However, in the present study, fungal contaminants were susceptible to all the tested antifungal agents with the exception of terbinafine to which they were moderately sensitive.

In order to protect its consumers from any detrimental effects, the food industry has to put in place the Hazard Analysis Critical Control Point system (HACCP) that ensures food safety including juices. When properly applied, the system controls hazardous elements in the food such as contaminants, pathogenic microorganisms, physical objects (glass and metal), toxins, chemicals (pesticide residues) and raw materials. Usually any deviation in the HACCP system needs to be corrected [34,35]. All these measures are hardly carried out by majority of local juice producers.

CONCLUSION

The microbial contaminants in the analyzed juices were relatively high exceeding the tolerable limits by 100-fold. Higher microbial counts were revealed in extemporaneous juices than the industrially manufactured ones. *Staphylococcus* spp, *Streptococcus* spp, *E. coli*, *Klebsiella* spp and *Candida* spp were the main microbial contaminants. Isolates of S*treptococcus* spp and *E. coli* were resistant to ceftriaxone, amoxicillin, ampicillin and doxycycline.

The microbiological safety and quality of juices is a major health concern since may serve as vehicle for microbial infections. The TFDA and other competent authorities need to enforce more rigorous measures to ensure that juices are prepared and sold in good sanitary and hygienic conditions. Most importantly, the general public should be informed of risks involved in consumption of microbiologically contaminated products. Issuance of licenses for large scale production of juices should be subjected to review with respect to compliance with the good manufacturing practices (GMP).

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