

*Full Length Research Paper*

## **Staphylococci and other selected microbiota associated with indigenous traditional beer**

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The nature and origin of indigenous traditional beer, makes it is prone to spoilage by a variety of microbiota in particular post-fermentation. In this study, samples of commercially and homebrewed indigenous traditional beer were collected using sterile sampling Whirl-pak<sup>®</sup> bags from local informal brewers in typical marginal urban settlements of South Africa. Both commercially and homebrewed traditional beer recorded the mean counts for total coliforms and *Staphylococcus* spp. *circa* 10<sup>5</sup> cfu.ml<sup>-1</sup> whereas the mean TVC and total fungi counts amounted to 10<sup>6</sup> and 10<sup>7</sup> cfu.ml<sup>-1</sup>, respectively. The counts from homebrewed indigenous traditional beer were about one log-phase higher than its commercial counterpart. Further characterisation of staphylococci identified *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylosus*, *Staphylococcus hominis* and *Staphylococcus saprophyticus*. *S. aureus* was the dominant species in both traditional beers and *S. saprophyticus* and *S. hominis* were the least identified. The implementation of sanitation guidelines, licensing of informal brewers, training programmes in aspects such as good manufacturing practices, five keys to safer food is a prerequisite in the study area and the rest of South Africa.

**Key words:** Indigenous traditional beer, food safety, microbiota, staphylococci.

### **INTRODUCTION**

Indigenous fermented foods and beverages play a major role in the diet of indigenous people, especially in developing countries where they are used for a variety of purposes (Mwesigye and Okurut, 1995; Bvochora and Zvauya, 2001; Jespersen, 2003; Naumova et al., 2003; Almeida et al., 2007; Aloys and Angeline, 2009). These include alcoholic and non-alcoholic beverages, which are mainly cereal-based. Alcoholic beverages, maiza (commercially produced beer) and somfana (home brewed beer) are amongst some indigenous beers consumed in South Africa. Their production by fermentation is considered as an effective method of producing and preserving a beverage safe for human consumption (Ross et al., 2002). In addition, studies have demonstrated that a decrease in pH during fermentation reduces the presence of food-borne bacterial pathogens

(Cho et al., 2011). Although the preservative properties of fermentation are known, the production of good-quality, wholesome indigenous traditional beer still require a high standard of personal and general hygiene by all persons involved in the brewing process. These requirements are needed as this product provides a favourable environment for the multiplication of microbiota post fermentation (Lyumugabe, 2010). In addition, keeping the typical manufacturers and consumer base in mind, these individuals are often ignorant with regard to the quality of the product they produce and/or consume (Ikalafeng, 2008).

Studies have shown that lack of education on the part of food handlers has resulted in negligent practices, especially relating to sanitation and hygiene, during and post- production of indigenous traditional products (Roy et al., 2007; Lyumugabe, 2010). The practice of not washing hands has been shown to result in up to 10<sup>6</sup> cfu.ml<sup>-1</sup> growth of pathogenic organisms under the fingernails of food handlers (Abdussalam and Kaferstein, 1993; Mensah et al., 2002). Martinez-Tome et al. (2000) and Ikalafeng (2008) highlighted the education of food

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handlers as a crucial line of defence in preventing most types of food-borne illnesses. Microbiota associated with food-borne illnesses relating to poor sanitation and improper food handling; are particularly organisms such as coliforms and various species of the *Staphylococcus* genus (Lawrie, 1998; RSA, 1999).

The genus *Staphylococcus* comprises of bacterial species predominantly associated with food-borne illnesses. Within the genus, *Staphylococcus aureus* has been highlighted as the leading cause of gastroenteritis resulting from the consumption of contaminated food. Some *S. aureus* strains are capable of producing staphylococcal enterotoxins (SEs) which are the causative agents of staphylococcal food poisonings. Staphylococcal food poisoning is caused by the absorption of staphylococcal enterotoxins preformed in the food. The presence of *S. aureus* in beer or any food product suggests poor hygiene of handlers as humans are primary sources of *Staphylococcus* spp. (Lawrie, 1998; RSA, 1999).

Although there has been some research done on indigenous foods and beverages in some African countries, the rate of interest in South Africa has only began in the past decade (Mwesigye and Okurut, 1995; Vvochora and Zvauya, 2001; Blandino et al., 2003; Jespersen, 2003; Naumova et al., 2003; Shackelton, 2003; Ikalafeng, 2008; Aloys and Angeline, 2009). Additionally, the microbiological characteristics of *scomfana* and *maiza* are not known. The aim of this study was therefore to determine the prevalence of *Staphylococci* species and other selected micro-organisms in the two types of indigenous traditional beer produced in typical marginal urban settlements area in South Africa.

## MATERIALS AND METHODS

### Preparation of *scomfana* and *maiza*

The two types of indigenous traditional beer investigated in this study were firstly commercial traditional beer known as 'maiza' and secondly traditional beer mixed and brewed entirely at home known as 'scomfana' or 'zamalek'. The basic recipe for *scomfana* as reported by Ikalafeng (2008); includes malt, commercially-produced maize ("Mthombo Mmela"), brown bread, compressed yeast and brown sugar all mixed together with lukewarm water. The mixture is allowed to ferment overnight or longer depending on the desired alcohol level by the consumers (Ikalafeng, 2008). During the mixing process, the informal brewers use their hands to mash the bread, and after fermentation, the solid particles are removed by means of a sieve or a straining cloth. The end product varies from a pinkish to a brownish colour due to large quantities of suspended particles and yeasts (Odumfa, 1985; Shackelton, 2003; Ikalafeng, 2008; Nyabenda, 2008).

Maiza production uses the same recipe as *scomfana* with the only difference being that it is brewed and sold on a commercial basis. The commercial brewing of *maiza* and other known commercially produced traditional beers involves malting, souring, boiling, mashing, straining, and alcoholic fermentation (Haggblade and Holzappel, 1989; Blandino et al., 2003); however, human intervention is limited as a result of the mechanical action inherent to commercial processing.

### Study area and sampling protocol

To secure a representative sample, the stratified method was used to select thirty informal brewers of indigenous traditional beer from the seven peri-urban settlements (Galeshewe, Phutanang, Vergenoeg, Number Two, Phomolong, Two Thousand and Retswelele) surrounding the city of Kimberly in South Africa. Samples of both *scomfana* (homebrewed) and *maiza* (commercially brewed) were collected from these settlements over a period of one month during the summer season. This season was chosen as it is characterised by windy periods and the environmental conditions are favourable because the produced traditional beer is stored at room temperature (Ikalafeng, 2008). The samples were aseptically collected between 09:00 and 14:00, in sterile sampling bags (Whirlpak®, NASCO, SA), kept on ice to restrict microbial proliferation and transported to the laboratory for microbial analysis. Serial dilutions were prepared in buffered peptone water (Biolab, SA) and 0.1 ml of each dilution was plated on various selective media using the spread-plate method (Herbert, 1990). All laboratory analysis was done in triplicate for statistical purposes and relevant controls were used for all media used in this study.

### Microbiological analysis

#### Total viable counts (TVC)

Enumeration of total viable counts was done on plate count agar (PCA) (Merck, SA) after incubation at 25°C for 72 h (Houghtby et al., 1993; Vorster et al., 1994).

#### Total coliforms

Enumeration of total coliforms was done on Chromocult coliform agar (Merck, SA) after incubation at 37°C for 48 h. Colonies were dark-red to purple in appearance.

#### *Staphylococcus* spp.

Baird Parker agar (Biolab, SA) was used for the isolation of *Staphylococcus* spp. and the plates were incubated at 35°C for 48 h. Grey-black colonies with a clear zone around the colony were regarded as *S. aureus*. Identification was confirmed with the rapid latex agglutination test (Slidex Staph Plus test kit, Bio Merieux, France) (Personne et al., 1997; Van Griethuysen et al., 2001). *Staphylococcus* colonies isolated from Baird Parker agar, which were not classified as *S. aureus*, were plated on blood agar and incubated for 24 h at 35°C. These strains were identified using the API-Staph system (Nagase et al., 2002) and APILAB software in accordance with the manufacturer's specifications (Bio Merieux, France).

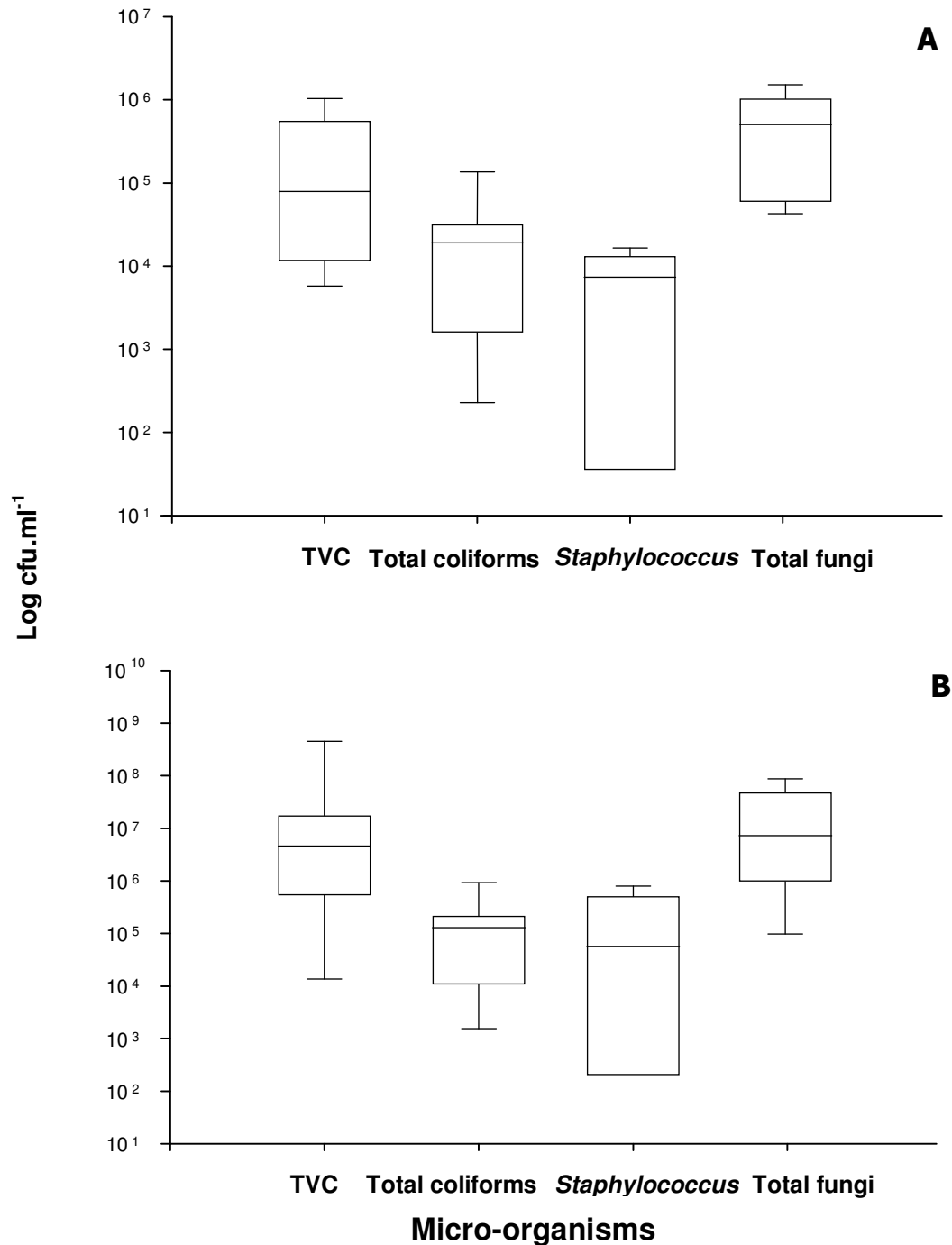
#### Total fungi (yeasts and moulds)

Detection and enumeration of total fungi were done on potato dextrose agar (PDA) acidified to pH 3.5 with tartaric acid (Merck, SA) (Christen et al., 1993; Frank et al., 1993). Plates were incubated for 72 h at 25°C.

## RESULTS AND DISCUSSIONS

### Total viable counts (TVC)

Measuring total viable counts (TVC) is a convenient tool



**Figure 1.** Microbial distribution in commercially produced and homebrewed traditional beers (A) Commercially produced traditional beer 'maiza' and (B) Homebrewed traditional beer 'scomfana'.

in assessing the general microbial contamination of foodstuffs. The mean TVC for maiza samples were  $10^5$  cfu.ml<sup>-1</sup> (Figure 1A) and for scomfana up to  $10^7$  cfu.ml<sup>-1</sup> (Figure 1B). These high counts could have emanated from the brewer's hands post-fermentation since high counts were detected in both beer samples. Brewers handle both beers post-fermentation when it is being

served to their consumers. In assessing the TVC, however, a basal medium and not a selective medium was used; thus the contribution of the yeast and mould population to this count should be kept in mind. The 2-log-phase lower TVC counts in commercially produced traditional beer are likely to be due to the fact the hands are less involved during the process and mechanical

action mostly dominates.

### Total coliforms

Coliforms are widely distributed in the intestines of human and warm-blooded mammals and are also the predominant facultative anaerobes in the bowel (Collins et al., 1995; Hayes et al., 2001). In addition, a number of species in this group are known pathogens and causative agents of food-related illnesses. They are also regarded as indicators of faecal contamination. In traditional beer, these organisms thus provide an estimate of faecal contamination in either the raw materials or during production or consumption (Eisel et al., 1997; Strech and Southgate, 1991).

Total coliforms were isolated from 80% of the commercially produced traditional beer samples with counts in the region of  $10^4$  cfu.ml<sup>-1</sup> (Figure 1A) and 100% isolated from homebrewed traditional beer samples with counts ranging from  $10^5$  to  $10^6$  cfu.ml<sup>-1</sup> (Figure 1B). Roy et al. (2007) reported coliform counts in 92% of samples taken from traditional fermented foods in India. Regarding the fact that the micro-organism *Escherichia coli* dominate the total coliform population, it is likely that the legislative guideline of 10 cfu.ml<sup>-1</sup> would be exceeded in both products investigated in the present study. Moreover, the infective dose level of  $10^5$  cfu.ml<sup>-1</sup> stipulated by the South African Department of Health (RSA: DoH, 2000) for foodstuffs sold to the general public may also be exceeded in the studied products. The samples could have been contaminated by poor handling by the brewers as reported by Ikalafeng (2008) and Lyumugabe (2010).

### *Staphylococcus* spp.

*Staphylococcus* spp. is commonly found on the hands, under the fingernails and in the nose, throat and mouth of healthy people. When present in these areas of the body, the bacteria do not cause any harm and people may be unaware that these bacteria can cause illness due to the toxins that they produce (Trickett, 1998). The organism may contribute to spoilage and produce off-flavours, but food poisoning is normally associated with the production of heat-labile enterotoxins (Anderson et al., 1995; Eley, 1992; Hittu and Punj, 1999; Reed, 1994).

Staphylococci in commercially produced traditional beer (maiza) were *circa*  $10^4$  cfu.ml<sup>-1</sup> and for scomfana  $10^5$  cfu.ml<sup>-1</sup> (Figure 1A and B). These levels exceeded the maximum limit stipulated in legislation ( $10^2$  cfu. ml<sup>-1</sup>) and it is a concern to note that samples of scomfana exceeded the infective dose levels for foodstuffs ( $10^5$  cfu. ml<sup>-1</sup>) (RSA: DoH, 2000). Although high numbers of the aforementioned organisms were expected in scomfana, the fact that they also occurred in the commercial product was notable. Since samples were collected from domestic environments, the counts in these settings are likely to be the result of poor handling (improper storage,

**Table 1.** *Staphylococcus* species in indigenous traditional beer.

Organism	'Scomfana' (%)	'Maiza' (%)
<i>S. aureus</i>	38	56
<i>S. epidermidis</i>	18	30
<i>S. xylosus</i>	1.2	0
<i>S. hominis</i>	0.8	0.4
<i>S. capitis</i>	22	16
<i>S. saprophyticus</i>	0.2	0.4

unclean containers and/or communal sharing of the drink) rather than any processing activities (Ikalafeng, 2008).

### Classification and aetiology of *Staphylococcus* spp.

Maiza samples were contaminated with *Staphylococcus aureus* (38%), *Staphylococcus epidermidis* (18%), *Staphylococcus xylosis* (1.2%), *Staphylococcus hominis* (0.8%), *Staphylococcus capitis* (22%) and *Staphylococcus saprophyticus* (0.2%). *Staphylococcus* species identified in scomfana were *S. aureus* (56%), *S. epidermidis* (30%), *S. hominis* (0.4%), *S. capitis* (16%) and *S. saprophyticus* (0.4%) (Table 1). In terms of *S. aureus*, these levels were to be expected, since human skin has been reported to be the most common source of *S. aureus*, from which point the organism finds its way into the air and onto clothing from where it may further cross-contaminate foods. The relative prevalence of this species in scomfana was notable; however, a number of other staphylococci also produce staphylococcal enterotoxins (SEs) and are thus capable of causing food-borne illnesses.

Kumar et al. (2008) has reported *S. epidermidis* to be a common pus-forming microbe that is responsible for the development of various forms of acne vulgaris. *S. xylosus*, which was isolated from both homebrewed and commercially produced traditional beer, is a common inhabitant on the skin of domestic and wild animals and is predominantly associated with nasal dermatitis or "sore nose" (Nagase et al., 2002). *S. xylosus* has also been isolated from the teat skin of cows, as well as from the nares of humans (Nagase et al., 2002). As is the case with the majority of other *Staphylococcus* species, *S. capitis* (present in 22% of maiza isolates and 16% of scomfana isolates), is commonly associated with the skin of humans and the hides of warm-blooded animals (Euzéby, 2003). The presence of *S. saprophyticus* has in particular been linked to poor sanitary hygiene, as it is frequently associated with acute urinary tract infections.

### Total fungi

Yeasts and moulds play both a positive and a negative

role in fermented products. The positive role of yeasts lies in their contribution to the fermentation process in especially bread, alcoholic beverages and other products. However, on the negative side, they may also act as spoilage organisms. The high yeast counts ( $10^6$  to  $10^7$  cfu.ml<sup>-1</sup>) for both homebrewed and commercially produced beer (Figure 1) were to be expected, as yeasts were included in the ingredients. However, since the scomfana-brewing process makes use of an “open” fermentation process, it was to be expected that numerous wild fungi would contaminate and multiply in the product as this commodity provides favourable conditions for their growth. Due to the predominance of the culture yeasts, however, it is unlikely that wild yeasts and moulds will reach such high numbers in the product after a 24 to 48 h fermentation period as to have a notable effect in terms of sensory quality and safety. Contamination of the product by yeasts other than as part of the ingredients used for fermentation has, however, been shown to produce off-flavours.

## CONCLUSION AND RECOMMENDATIONS

In this study, the majority of samples of both commercially based and homebrewed beer suggests contamination close to or above the infective doses, raising concerns about the risks this would impose on the consumers' health. The higher microbial counts found in homebrewed traditional beer in comparison with commercially produced traditional beer led to the conclusion that homebrewed beer may present a marginally higher risk. The presence of total coliforms, as well as particular species of staphylococci, suggests a degree of ignorance amongst home-based brewers in respect of the fundamental aspects of proper hygiene and safe housekeeping in addition to a lack of basic infrastructure.

It is imperative to increase research on indigenous traditional foods and beverages in Southern Africa to cover aspects such as the biochemical components and microbial characteristics of traditional foods and beverages (Aloys and Angeline, 2009; Greenhill et al., 2009; Stringini et al., 2009). It is advisable that protocols regarding brewing post-fermentation handling of beer be established and brought into effect by local authorities, concomitant to coherent strategies for the licensing of informal breweries. Such strategies should serve as regulatory measures for controlling hygiene and quality.

In order to assist local brewers in brewing beer of sound quality, the following recommendations are made: (1) Brewers should be educated regarding quality control and possible microbial hazards that might be present in the beer; (2) Local municipalities, via environmental health practitioners, should regularly monitor the brewers in terms of proper hygiene, sanitation, management of waste, and refrigeration; (3) The negative health effects of alcohol should be highlighted and the consumer's right to enquire about the ingredients used for brewing should

be stipulated; (4) The benefits of a healthy diet should be emphasised; (5) Entrepreneurship through proper business management should be encouraged; (6) Licensing of brewers should be implemented and properly monitored; and (7) National standards and guidelines should be drafted for microbial chemical parameters in particularly homebrewed beer. Most of all Five Keys to safer food principles should also be used to curb possible contamination during the preparation of indigenous traditional beer (WHO, 2009).

The changes in composition and number of the microbial population present post-fermentation are amongst things that have to be monitored in future. In addition, the use of PCR (Polymerase Chain Reaction) based methods is encouraged as this method is more sensitive than the conventional plating technique and will thus enhance identification of microbes.

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