



Determination of antibiotic resistance and enterotoxigenic potential of *Staphylococcus aureus* strains isolated from foods sold by street vendors in Gaborone, Botswana

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Abstract: *Staphylococcus aureus* is one of the causes of foodborne diseases worldwide. Staphylococcal food poisoning ensues after ingestion of contaminated food and results in symptoms of gastroenteritis such as vomiting, abdominal cramps and diarrhea. The present paper aims to isolate *Staphylococcus aureus* from foods sold by street vendors in Gaborone, Botswana, and to determine its enterotoxigenic potential and antibiotic resistance profile. One hundred eight food samples comprising starch, meat, salads and vegetables portions were collected from these vendors and tested for the presence of *S. aureus*. Identification of *Staphylococcus aureus* to the species level was performed using the Vitek 2 automated identification and susceptibility testing system (BioMerieux, Marcy-l'Étoile, France). Enterotoxins were detected by the Reversed Passive Latex Agglutination method (SET-RPLA). Results showed that 49 (45%) of the samples tested positive for *Staphylococcus aureus*. The organism was isolated at higher frequencies in vegetables and starchy foods (34.7%) than in meats (30.6%). These differences in isolation rates however, were not statistically significant ($p > 0.05$). *Staphylococcus aureus* isolates were found to be resistant to penicillin G (52.4%), tetracycline (38.1%), methicillin (26.2%) and vancomycin (11.9%). Four Staphylococcal enterotoxin types A-D, were detected among the isolates. Staphylococcal enterotoxin D was the most prevalent (52.9%), while enterotoxin C was produced by the least number of isolates (5.9%). Of note, five isolates simultaneously expressed two or more enterotoxin types in varying combinations. The present study underscores a potential risk of staphylococcal food poisoning and transmission of methicillin resistant *S. aureus* strains for consumers of street vended food products in Gaborone, Botswana especially in the absence of a quality assurance regulatory framework. As a mitigating factor, sensitization of street food vendors on the importance of food and personal hygiene is strongly recommended.

Key words: *Staphylococcus aureus*; antibiotic resistance; enterotoxins; street foods; Botswana.

Introduction

In developing countries, street-vended foods are mainly consumed by people in the low-income bracket of society [32]. Although these foods are convenient to people working nearby because of low cost and high nutritional quality, these were reported to cause food-borne diseases [17]. Poor hygiene of street vendors, dusty preparation areas and higher ambient holding temperatures especially in the tropics were ascribed to the multiplication and subsequent contamination of street food by microorganisms [25].

In Botswana, street food vending is an important micro-economic activity especially amongst women and is actively encouraged by the government as a citizen empowerment initiative [7]. However, unlike established food service outlets, this sector remains largely unregulated in terms of quality assurance. This therefore, places the consuming populace at high risk of food-borne illnesses.

Staphylococcus aureus, an important food-borne pathogen, produces heat and pH-stable enterotoxins which when ingested result in food poisoning leading to characteristic manifestations of gastroenteritis such as abdominal pains, vomiting and diarrhea [18]. Most epidemics are due to 'classical' staphylococcal enterotoxins A-E (SEA-SEE) [12], however, other enterotoxins have been described (SEG, SEH, SEI, SEJ, SEK, SEM, SEN, SER, and SEU) [8,12] but their role in food poisoning remains unclear [2]. The presence of enterotoxigenic *S. aureus* in foods poses a notable public health threat, and these strains were thought to be transmitted to the food by food handlers [5, 26].

S. aureus has also garnered substantial public health attention due to its multi-drug resistance which accounted for increased mortalities in health facilities [31]. Methicillin resistant *Staphylococcus aureus* (MRSA) had especially been central to nosocomial and community acquired infections globally [10, 27]. *Staphylococcus aureus* was previously isolated from street

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foods [21], food handlers [22] as well as in the healthcare setting [30, 33] in Botswana but a comprehensive study on the prevalence, enterotoxigenic potential and antibiotic resistance in street foods has not been carried out. Therefore, this aimed to investigate the occurrence of enterotoxigenic and antibiotic resistant *Staphylococcus aureus* in street vended foods collected in Gaborone, Botswana.

Materials and Methods

Sampling

Sample size (n) was determined using SPSS software (version 16.0, SPSS, Chicago), with assumptions that $p > 0.05$ and $r^2 > 0.80$. A total of 108 samples were collected from October 2013 to July 2014 from randomly selected street food vendors in three geographical areas of Gaborone, Botswana namely; University of Botswana food stalls (Latitude: -24.6610233 | Longitude 25.936174800000003); Bus Rank Mall food market, Station Rd, (Latitude: -24.663513 | Longitude: 25.906507); Botswana Building Society Mall, food market, Segoditshane Way (Latitude: -24.627747 | Longitude: 25.934311).

From each food vendor, one dish of food containing starch, meat and salad or vegetable portion was sampled. The samples were placed in separate labeled sterile specimen bags (Lab-Loc® Specimen) and transported to the Microbiology Laboratory, University of Botswana in a cooler box containing ice packs, for further analysis. Strict aseptic techniques were followed to avoid contamination during transport of the samples from the vending sites.

Isolation and presumptive identification of *Staphylococcus aureus* strains

25 g of each food sample was transferred into 225 ml of sterile Buffered Peptone Water (Oxoid, Basingstoke, UK) and then homogenized with the Stomacher (Seward 400, Tekmar, and Cincinnati Ohio, USA) set at medium speed. 0.1 ml of the homogenate was then spread-plated on the surface of sterile Baird Parker Agar (Oxoid) supplemented with 5% egg yolk tellurite enrichment suspension (Oxoid) and then incubated at 37°C for 24h. Typical staphylococcal colonies (gray-black, surrounded by a dull halo) were sub-cultured on agar slants of Brain Heart Infusion (Oxoid). After 24h incubation, the slants were maintained at 4°C for use in biochemical profiling. Presumptive staphylococci were subjected to Gram stain, catalase, mannitol fermentation, DNase activity, and coagulase tests. Identification of *Staphylococcus aureus* to the species level was confirmed using the Vitek 2 automated identification and susceptibility testing system (BioMerieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. Subsequently, confirmed *S. aureus* isolates were preserved in a solution containing 80% Tryptose Soy Broth (Oxoid, UK) and 20% glycerol and stored at -80°C, till further use.

Antibiotic susceptibility testing

Antibiotic Susceptibility Testing (AST) was determined using the disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) [9]. The names and respective disc contents of nine antibiotics tested were as follows: penicillin G 10 IU, streptomycin 300µg, vancomycin 30µg, tetracycline 30µg, erythromycin 15µg, chloramphenicol 25µg, fusidic acid 10µg, methicillin 10µg, and novobiocin 5µg. Cartridges with commercially prepared paper discs containing the appropriate antibiotic dosage were purchased from Mast Diagnostics, Merseyside (UK). Disk diffusion assays were performed on Mueller-Hinton Agar (Oxoid, UK). Each of the confirmed *S. aureus* isolates were incubated at 37°C on a Gallenkamp shaker (200 rpm) for 24 h. The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain turbidity optically comparable to that of the 0.5 McFarland standards. One milliliter of the cell suspension was then spotted on the surface of Mueller-Hinton agar (Oxoid, UK) and then plated evenly. *Staphylococcus aureus* (ATCC 25923) strain was used as quality control.

Detection of enterotoxin production

To determine the enterotoxigenicity of isolated strains, a staphylococcal enterotoxin test kit for the detection of staphylococcal enterotoxins by reversed passive latex agglutination, the SET-RPLA (Oxoid, UK) was used following the manufacturer's instructions. The isolated *Staphylococcus aureus* strains from food sources were cultured in tryptone soy broth (Oxoid, UK) and then incubated at 37°C for 24h, with shaking. The cells were then harvested by centrifugation (Z 233 M-2, Hermle Labortechnik GmbH, and Germany) at 900 X g for 20 min at 4°C. The supernatant thus obtained was used to detect staphylococcal enterotoxin A - D in the culture fluid of each *S. aureus* isolate. The agglutination patterns developed were compared to known standards. *Staphylococcus aureus* ATCC 9144 was used as a control to ascertain enterotoxin production.

Statistical Analyses

Measurements of inhibition zones obtained from the disc diffusion method were analysed individually using the Statistical Package for Social Science (version 16.0, SPSS, Chicago). All triplicate data were presented as mean Standard \pm deviation (SD) manner. Student-Newman-Keuls was employed for comparing two or more than two variable groups respectively. The level of significant difference between mean values was set at p -value < 0.05 .

Ethical Clearance

This research was conducted after ethical clearance from the Institutional Review Board, Office of Research and Development, University of Botswana, and written consent was obtained from all street food vendors who provided the food samples.

Results

Distribution of *Staphylococcus aureus* isolated from street vended food

Data presented in Table 1 shows that only 49 (45%) out of 108 food samples from 3 designated zones around Gaborone, Botswana tested positive for the presence of *Staphylococcus aureus*. The distribution of *S. aureus* in foods from the various zones was 26.5% for University of Botswana food stalls and 36.7% each for both the Bus Rank Mall Food Market and Botswana Building Society Mall. The detection of *S. aureus* in various food sources tested was 30.6% in meat, and 34.68% each in both vegetables and starchy food. It was observed that these differences in the detection rates of *S. aureus* in food types were not statistically significant ($p > 0.05$).

Table 1: Percentage and number of samples tested positive for *Staphylococcus aureus* from various food classes in three vending sites in Gaborone, Botswana.

Vending sites	Food sources			Total n= 108 % Prevalence and number of isolates
	Meat	Vegetables/ Salads	Starch	
UB ¹	8.16 (4)*	10.20 (5)	8.16 (4)	26.5 (13)
BBS ²	10.20 (5)	12.24 (6)	14.28 (7)	36.7 (18)
BR ³	12.24 (6)	12.24 (6)	12.24 (6)	36.7 (18)
Total	30.6 (15)	34.68 (17)	34.68 (17)	45.37 (49)

*The data are not significantly different at 95% ($p < 0.05$).

*Number of samples are indicated in parentheses

¹UB-University of Botswana food stalls

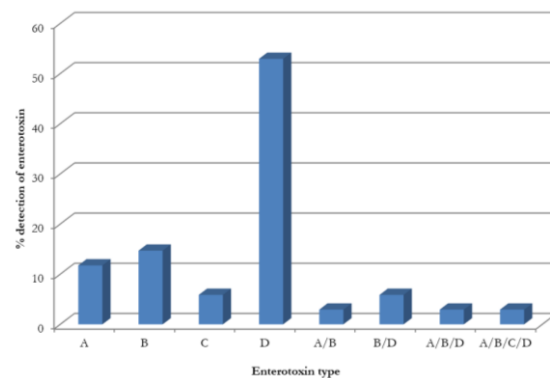
²BBS- Botswana Building Society food market

³BR – Bus Rank mall food market

Detection of *S. aureus* enterotoxin serological types

Only eight enterotoxin serological types were detected from a total of 34 strains of *S. aureus*. These ranged from 2.94% for SEA/B, SEA/B/D and SEA/B/C/D; 5.88% for SEC and SEB/D; 11.76% for SEA; 14.70 for SEB; to 52.94% for SED (Fig. 1). Five of the enterotoxin types were detected in *S. aureus* isolates from meat sources, where the percentage detection rate varied from 8.30% for SEA/B/C/D, SEB and SEC; 25% for SEA to 50.00% for SED. In the vegetable food items, six of the eight enterotoxin types were detected from *S. aureus* isolates. The detection rate varied from 7.69% for SEA/B, SEA/B/D, SEB/D and SEC; 23.07 % for SEB to 46.15% for SED. Four enterotoxins types were recorded from the starchy food sources. The detection rates were 11.11% each for SEA, SEB and SEB/D; and 66.66% for SED. The latter (SED) was the most predominantly detected enterotoxin while SEA/B, SEA/B/D, SEB/D and SEC remained the least detected from *S. aureus* isolates from the various food types sampled. SEA/B and SEA/B/D were detected only in *S. aureus* isolates from vegetables, while SEA/B/C/D was detected only in *S. aureus* isolates from meat (Fig. 1). Pearson correlation analysis indicated no differences in mean and ANOVA indicated that there was no statistically significant difference amongst the means of SE detection for the various food types from various sampling zones ($p = 0.435$; $F = 0.868$).

Fig. 1: Percentage detection of *Staphylococcus aureus* enterotoxin types.



A=SEA,B=SEB,C=SEC,D=SED,A/B=SEA/SEB,B/D=SEB/SED, A/B/D=SEA/SEB/SED,A/B/C/D=SEA/SEB/SEC/SED

Antibiotic susceptibility of *S. aureus* isolated from street vended foods

S. aureus isolates from food survey were subjected to antibiotic sensitivity assays for nine different antibiotics. *S. aureus* was found to be most sensitive to vancomycin (88.1%), followed by erythromycin (81.0%) and least sensitive to tetracycline (50.0%) and penicillin G (47.6%). *S. aureus* had an intermediate profile to four different antibiotics (Table 2).

Table 2: Percentage of antibiotic susceptibility of *S. aureus* isolated from street foods by the disk agar diffusion assay.

Antibiotic	Isolates that were;		
	Sensitive	Intermediate	Resistant
Erythromycin	81.0 ^b	4.8	14.3
Fusidic acid	71.4	0.0	30.0
Methicillin	73.8	NA ^c	26.2
Novobiocin	69.0	4.8	26.2
Penicillin G	47.6	NA ^a	52.4
Streptomycin	66.7	2.4	26.2
Chloramphenicol	80.0	0.0	20.0
Tetracycline	50.0	7.1	38.1
Vancomycin	88.1	0.0	11.9

^bThe data are not significantly different at 95% ($p < 0.05$).

NA^c, no CSLI-approved criteria were available.

Discussion

One of the most frequent foodborne microbial diseases is staphylococcal food poisoning (SFP) which is caused by *S. aureus* metabolites [16]. In our study, an overall prevalence of *S. aureus* in the analysed food samples was 45% with a higher prevalence in vegetables/salads and starch (34.7%), as compared to meat samples (30.6%). The presence of *S. aureus* in foods usually designates contamination that may be introduced into the food by workers who have skin lesions containing *S. aureus*, or by sneezing or coughing [13]. Other contamination sources of *S. aureus* are soil, dust and air [14]. The incidence of *S. aureus* (45%) in the present study was much higher than the rate of between 6 and 8% reported by studies in Turkey [4, 6, 13]. However, much

higher rates were reported in Italy [3] and Brazil [1], with rates of 83% and 66.7% respectively.

Of the many extracellular toxins which are thought to contribute to the pathogenicity, staphylococcal enterotoxins (SEs) pose the greatest risk to consumer health [16]. In the present study, 31.5% of isolated *S. aureus* strains produced enterotoxins. Our findings are in agreement with those reported in other studies [15, 22] and this suggests that human contamination of foods is the primary source of SFP. Most isolated strains produced SED (52.94%), followed by SEB (14.70%), SEA (11.76%) and lastly SEC (5.88%). Three (8.82%) of the enterotoxigenic strains synthesized two SEs, while one strain (2.94%) produced three SEs (SEA, SEB and SED) and one strain (2.94%) also produced four SEs (SEA, SEB, SEC and SED). This may be indicative of the fact that street food vendors in the areas surveyed lacked the necessary food handling and food safety skills and training. Our results highlight the potential high risk for consumers of meat, starch and vegetable products especially in the absence of strict hygienic and preventive measures to avoid SEs production in foods. Several authors have asserted to the fact that presence of *S. aureus* in food commodities may be associated with contamination by food handlers during processing (such as during cutting, chopping or mixing) [15, 29]. Staphylococcal enterotoxin A (SEA) and SED, followed by SEB were the most common toxins implicated in cases of *S. aureus* food poisoning [2]. Staphylococcal enterotoxin A (SEA) was reported to be highly resistant to proteolytic enzymes [19]. Staphylococcal enterotoxin C (SEC) was linked to several staphylococcal food poisoning outbreaks; it was reported in an outbreak in Taiwan during 2001-2003 [8] and also in an outbreak in Japan [24] in 2009. SEA was recovered from 77.8% of all SFP outbreaks in the United States followed by SED (37.5%) and SEB (10%) [20].

The highest resistance rate was observed for penicillin G (52.4%) and tetracycline (38.1%), followed by streptomycin (26.2%) and methicillin (26.2%). Pu *et al.*, [24] demonstrated that different *S. aureus* food isolates had uniform resistance patterns to penicillin, tetracycline, and erythromycin. In contrast, however our isolates were found to be more susceptible to erythromycin (81.0%). Can and Celik [4] observed a comparatively lower resistance of *S. aureus* from food sources to tetracycline (25%) and methicillin (16.6%) than those observed in the current study.

The high rate (26.2%) of resistance to methicillin observed in this study is especially worrisome because methicillin resistant *Staphylococcus aureus* (MRSA) strains are very important in nosocomial and community acquired infections. Food may therefore, represent an avenue of the spread of MRSA and notably, MRSA was reported in street vended foods in Benin [28] albeit at a lower rate (15.18%) than the present study. Resistance to methicillin is mediated by the *mecA* gene located on a transmissible mobile chromosome cassette called SCCmec [23], and therefore, the isolates in the present

study may serve as reservoirs of resistant strains. It should be noted that infections with MRSA are routinely treated with glycopeptides such as vancomycin and oxazolidinones such as linezolid [11]. However, 11.9% of isolates in the present study were resistant to one of the last lines of defence against MRSA (vancomycin). The high proportion of vancomycin resistant isolates in a non-clinical setting is of significant concern, especially in light of the high prevalence rates of HIV/AIDS in Botswana. To date no plausible explanation exists for these observed high rates of MRSA in this particular environment. However, the use of antibiotics is the single most important factor leading to the development of antibiotic resistance.

Conclusion

The results of our study suggest that street vended food could be an important vehicle for food-borne *S. aureus* infections. Our study also highlights the potential high risk of *S. aureus* food poisoning for consumers of street vended food products especially in the absence of strict hygienic and preventive measures. The high incidence of antibiotic resistance of most isolates, especially to methicillin and vancomycin accentuates a development that could burden the healthcare system of developing countries such as Botswana. It is imperative that the relevant authorities be more proactive to ensure the safety of the street food vending trade in Botswana.

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