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Prevalence of the Staphylococcal Enterotoxins Genes in Raw and Milk Products along the Value Chain in Mbeya and Mbozi Districts, Tanzania

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Abstract

The study determined the prevalence of genes coding for Staphylococcal enterotoxins (SEs) from *Staphylococcus aureus* isolates in milk produced and sold in Mbeya and Mbozi districts in Tanzania. Samples of raw milk (n=300), boiled hot (ready-to-consume) milk (n=72) and sour milk (n=72) were randomly collected from smallholder dairy farmers, milk collection points (MCP) and milk shops. Laboratory analysis showed that 59.7% of the milk samples contained *Staphylococcus species*. Biochemical tests showed that 12.4% of the isolates were positive for *S. aureus*, of which 5.6, 2.5 and 4.3% were from samples collected from farmer's herd milk, MCP and milk shops, respectively. Furthermore, multiplex Polymerase Chain Reaction (mPCR) results showed that 36.4% of the total *S. aureus* isolates (n=55) had SEs genes. Frequently observed gene was *Sea* (32.6%) while *Sej* was not detected in any of the isolates. The distribution of the SEs genes along the milk market channel showed 35, 15 and 50% of the genes came from isolates samples collected at farm level, MCP and milk shops, respectively. Moreover, no statistical difference were observed for SE coding gene between the districts and seasons, though higher (65%) prevalence of *S. aureus* isolates carrying SEs genes were observed in dry than wet season (35%). The prevalence of SE coding gene in raw, boiled hot and sour milk were 4.3, 5.6 and 4.2%, respectively. The results obtained show that milk produced and marketed in the two districts contained *S. aureus* isolates expressing gene for enterotoxins production which pose a potential public health risk. Hence, the results indicate the need to institute proper hygienic measures by all milk stakeholders in order to avoid contamination of milk with *S. aureus*. Further studies on the diversity and distribution of enterotoxins producing *S. aureus* in the Southern highlands and other areas in the country are recommended.

Keywords: Boiled hot milk, sour milk, enterotoxins, Staphylococcus aureus, Multiplex PCR

1. Introduction

Staphylococcus aureus (S.aureus) is recognized worldwide as a major pathogen that causes food poisoning and various infections in animals and humans (Peles *et al.* 2007). The primary habitat of *S. aureus* is the nasal passage of humans and on the skin and hair of warm-blooded animals (Schmitt *et al*. 1990). It is estimated that 20 – 30% of healthy human population harbours *S. aureus* in their body (van Belkum *et al.* 2009). *Staphylococcus aureus* is able to grow in a wide range of foods at temperatures between 7°C to 48.5°C (optimum of 30 to 37°C), pH 4.2 to 9.3 (optimum of 7 to 7.5) and Sodium chloride concentrations of up to 15% (Schmitt *et al*.1990).

Milk and milk products are common vehicles for staphylococcal food poisoning and *S. aureus* was reported to be the most frequent pathogen isolates of raw milk (Jørgensen *et al*. 2005). The lack of proper hygienic measures during milk handling and processing increases the probability of contamination with *S. aureus,* especially in manually prepared products (Asao *et al*. 2003). The risk to public health is particularly associated with the ability of 50% of isolates to produce thermo stable enterotoxins associated with food poisoning (Le Loir *et al.* 2003). Although pasteurization kills *S. aureus*, thermo stable Staphylococcal enterotoxins genes (SEs) retain their biological activity (Kadariya *et al*. 2014). To date, over 20 SEs have been identified and most of *S. aureus* strains can synthesize more than one type of toxins (Loncarevic *et al.* 2004). More than 95% of staphylococcal food poisoning outbreaks are caused by staphylococcal enterotoxins (SEs) types SEA to SEE defined as a classical enterotoxins. The remaining 5% of outbreaks are associated with newly described SE's (SEG, SH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER and SEU) (Kerouanton *et al.* 2007).

A study on foodborne disease outbreaks conducted in Europe showed that *S. aureus* was one of the most common causative pathogens associated with milk-related outbreaks (Huong *et al*. 2010). According to the U.S. Food and Drug Administration (FDA) (2012), the concentration of $>10^5$ CFU/g *S. aureus* is capable of causing Staphylococcal intoxication. It is considered that when a *S. aureus* strain has an enterotoxigenic gene, it can potentially produce food toxins; hence detection of SEs genes in milk is important in order to know their

prevalence along the milk marketing channels. The production of toxins and detection of SEs genes has been reported in dairy products by previous workers in Kenya (Mathenge *et al*. 2015) and Egypt (Elbargisy *et al.* 2016). Since more than 85% of milk produced in Tanzania is sold to consumers in its raw form (Kurwijila 2006), there is a considerable potential threat to the health of humans who consume inadequately heated raw milk and its derived products.

Although several studies on SEs have been reported in other parts of the world, there is limited information regarding SEs in Tanzania. The aim of this study was therefore to determine the occurrence of genes in *S. aureus* from dairy cows in the Mbeya region in the Southern highlands of Tanzania that codes for SEs. This will provide baseline information on the prevalence of Staphylococcal enterotoxins genes in the Southern Highlands Zones as a basis for development of strategies to reduce contamination of milk and milk products with pathogenic microorganisms.

2. Materials and methods

2.1 Study area

The study was conducted in two districts namely Mbozi and Mbeya rural districts in Mbeya region. Mbozi district is located in the south western corner of Mbeya region (Fig 1). The district is located between latitudes 8° and 9° 12’ S and longitudes 32° 7’30’’ and 33° 2 0’East. The rainfall pattern in Mbozi district is unimodal which starts from November to May, while the dry seasons start from June to October. Annual rainfall ranges from 800 mm to 1100 mm. Annual temperature ranges between 16^oC and 23^oc (URT1997). Mbeya rural district lies between latitude 8° 38’ and 9° 20’ S and longitude 33° and 33° 52’E. The rainfall pattern in Mbeya district is also unimodal, and ranges from 800 mm to 1800 mm annually. Annual temperature ranges between 16^oC and 20^oC (URT 1997).

Figure 1. A map of Mbeya region showing the study districts

2.2 Study design and sampling procedure

A cross-sectional study was conducted to determine the presence of *S. aureus* in milk from dairy farmers' herds, milk collection points and milk shops in the two districts of Mbeya region. The sample size for households keeping dairy cattle and milk samples for microbiological analysis were calculated by the formula described by Fox *et al.* (2007). The household sample size was based on proportion of dairy farmers (4%) in the two districts (NBS 2013). The sample size for milk was based on prevalence of 20.4 % of *S. aureus* reported in Tanzania (Mdegela *et al.* 2004). Sample size was determined at 95% confidence interval and 5% margin for random error where $N = P\ (100\% - P)/\ (SE)^2$, $SE = MRE/1.96$.

Where; N = Sample size, P = Prevalence of subclinical mastitis, SE = Standard error, MRE = Margin for random error (5%) and 1.96 = tabular value for 95% confidence interval.

From the above formula, sample size was as follows; 96 herd milk samples, 18 samples from milk collection points and 36 samples from milk shops. Furthermore, two more samples (boiled and sour milk) were collected from each milk shop for microbial analysis. The criterion for household inclusion in the sampling frame was any household that had at least one lactating dairy cow during the study period.

2.3 Sampling procedure

Twelve villages were randomly selected by using a table of random numbers, from a sampling frame comprised of a list of all villages in the study area, which were obtained from the District Livestock office. Proportional sampling was adopted to obtain the number of villages from each of the two districts. Within each village eight households, two collection points and three milk shops were randomly selected for milk sampling. In this study, milk collection points were selected based on the presence of cooling facility, container for receiving and storing milk, quality control and members capable of collecting not less than 150 litres of milk per day. Two of these had milk cooling tanks while 16 used refrigerator and cold water for cooling milk and six collection points did not meet the condition and were removed from the study.

Samples were collected between 6.15 am and 7.30 am, and 4.15 pm and 6.30 pm (local time) from farmers' herds, milk collection centre and milk shops. Samples were collected in sterile universal bottles of 50 ml with a screw lid and kept in a cool box at less than $4^{o}C$ and then transported to the Tanzania Livestock Research Institute (TALIRI), Uyole, and stored at $-20^{o}C$. The next day after morning collection, samples were transported to Sokoine University of Agriculture (SUA) and the microbiological analysis was performed within 36 hrs.

2.4 Isolation of S. aureus from milk samples

In the laboratory, enumerations of Total Staphylococcus Counts were done by using standard procedures (ISO 1999, 6888-2). Briefly, tenfold dilution of each milk sample was prepared using sterile peptone water. The dilution of 10^{-2} to 10^{-4} was used and 0.1 ml of each dilution was transferred using sterile pipette and spread onto sterile plates containing Manitol Salt Agar (MSA) (Oxoid) using a sterile glass spreader for each sample. The plates were then kept in an incubator at $37^{o}C$ for 24-48 hrs. The colonies that were yellow in colour from each MSA plate were further purified by sub-culturing into MSA plates and incubated aerobically at 37°C for 24 h – 48 hrs. Then, pure colonies were retained for further bacterial identification.

2.4.1 Bacterial identification

Gram staining was performed according to Cruikshank *et al.* (1975) and Gram-positive cocci, which occurred in clusters under the microscope, were selected and subjected to preliminary biochemical tests such as catalase and oxidase tests. Furthermore, haemolysis and coagulase tests were performed for confirmation of *S. aureus.* The haemolysis test was conducted by using blood agar base (Oxoid). After sterilization, 10% of horse blood was added into the solution and poured in the plate and incubated for 24 h at $37^{o}C$. After 24 hrs of incubation, the pure colony was streaked onto plate containing blood agar and incubated for 24 hrs at $37^{o}C$ and haemolysis observed. The coagulase test was performed according to the tube agglutination test method using rabbit plasma in a 1:10 dilution in a nutrient broth (Oxoid) and performed according to the manufacturer's instructions. The tubes were monitored for clot formation at 30 minutes intervals for the first 6 hours and then after 24 hours incubation. Isolates that produced clots were recorded as *S. aureus* and maintained at 4°C in 30% glycerol for further characterization.

2.5 DNA Extraction and Multiplex PCR amplification

Extraction of DNA was conducted by boiling method. Two to four colonies of pure culture of *S. aureus* were dispensed into eppendorf tubes containing 100 µl of sterile distilled water. The suspension was heated for 10 min at $95^{o}C$ and then centrifuged for 10 min at 13 000x g for 10 minutes. The supernatant was collected and stored at $-80^{o}C$ and later used in the PCR reaction as template DNA (Martin *et al.* 2003).

2.6 PCR amplification

DNA extracts were allowed to thaw for 5-10 minutes while preparing the Master Mix. The master mix contained 0.2 ml reaction tubes in a final reaction volume of 25µl. The PCR mixture consisted of 5 mM $MgCl_2$, 200 µM dNTPs, buffer, 2 U of Taq polymerase, and 5 µl of DNA. Nine pairs of primers (Table 1) for detection of staphylococcal enterotoxins genes namely; *sea, seb, sec, sed, see, seg, sei, seh* and *sej* were used. DNA amplification was performed in a Takara thermal cycler (MJ Research, Inc. Tokyo Japan) using the following conditions: initial denaturation for 5 min at 94°C followed by 40 cycles of denaturation (94°C for 30 sec), annealing (90 sec at 57°C), initial extension for 72°C at 60 sec. A final extension step (72°C for 10 min) was performed after the completion of the cycles. The amplified PCR products were visualized by standard gel electrophoresis in a 2% agarose gel stained by Gel red (5 µg/mL). The gel electrophoresis was run for 60 minute at 110 V in order to achieve a visible separation of bands. The gels were photographed under ultraviolet light using the Gel-Doc 2000 system (Bio-Rad, USA).

Table 1. Oligonucleotide primers for amplification of genes encoding staphylococcal enterotoxins

Gene	Primer	Primer sequence 5' to 3'	Amplification size	Reference
sea	GSEAR-1	GGT TAT CAA TGT GCG GGT GG	102	Mehrotra *et al*.(2000)
	GSEAR-2	CGG CAC TTT TTT CTC TTC GG		
seb	GSEBR-1	GTA TGG TGG TGT AAC TGA GC	164	Mehrotra *et al*.(2000)
	GSEBR-2	CCA AAT AGT GAC GAG TTA GG		
sec	GSECR-1	AGA TGA AGT AGT TGA TGT GTA	451	Mehrotra *et al*.(2000)
	GSECR-2	TGGCAC ACT TTT AGA ATC AAC CG		
sed	GSEDR-1	CCA ATA ATA GGA GAA AAT AAA	278	Mehrotra *et al*.(2000)
	GSEDR-2	AGATT GGT ATT TTT TTT CGT TC		
see	GSEER-1	AGG TTT TTT CAC AGG TCA TCC	209	Mehrotra *et al*.(2000)
	GSEER-2	CTT TTT TTT CTT CGG TCA ATC		
seg	SEG-1 SEG-2	TGC TAT CGA CAC ACT ACA ACC	704	Mehrotra *et al*.(2000)
		CCA GAT TCA AAT GCA GAA CC		
seh	SEH-1 SEH-2	CGA AAG CAG AAG ATT TAC ACG	495	Mehrotra *et al*.(2000)
		GAC CTT TAC TTA TTT CGC TGT C		
sei	SEI-1	GAC AAC AAA ACT GTC GAA ACT G	630	Mehrotra *et al*.(2000)
	SEI-2	CCA TAT TCT TTG CCT TTA CCA G		
sej	SEJ-1	CAT CAG AAC TGT TGT TCC GCT AG	142	Mehrotra *et al*.(2000)
	SEJ-2	CTG AAT TTT ACC ATC AAA GGT AC		

2.7 Statistical analysis

The data were analyzed using SPSS program, (2010). Simple descriptive statistics and frequency distribution were used to explore the variability of the studied parameter involved in the evaluation of prevalence of SEs in the milk samples. Chi square was used to compare the differences between the district, source and season and differences were considered significant at values of $p < 0.05$.

3. Results

3.1 Prevalence of Staphylococcus species

Out of 444 milk samples, 265 (59.7%) samples were positive for *Staphylococcus species* (Table 2). The prevalence of *Staphylococcus species* in the farms, milk collection points (MCP) and milk shops were 19.1, 17.6 and 23%, respectively. Out of 265 samples that were positive for *Staphylococcus species,* 55 (12.4%) samples contained *Staphylococcus aureus* of which 3.2, 4.0 and 5.2% were from farms, MCP and milk shops, respectively. The prevalence of *S. aureus* in Mbeya and Mbozi were 5.9 and 6.5%, respectively and the difference was insignificant ($P > 0.05$). The concentration of this bacterium in milk sample ranged from 10^3 to 10^6cfu/ml in both districts. Generally, dry season had significant ($p<0.052$) higher prevalence of *S.aureus* compared to wet season.

Table 2. Prevalence of *Staphylococcus species and S. aureus* detected from milk sold in Mbeya and Mbozi districts.

	Total		Mbeya		Mbozi	
Characteristics	N	%	Frequency	%	Frequency	%
Staphylococcal species tested	444	100	222	50	222	50
Sample not yield Staphylococcal species	179	40.3	82	18.4	97	21.8
Staphylococcal species detected	265	59.7	129	29.1	136	30.6
Staphylococcus aureus detected	55	12.4	26	95.9	29	6.5

3.2 Prevalence of SE coding gene in raw, boiled hot and sour milk

The prevalence of SEs coding genes is shown in Table 3. The results showed that overall prevalence of SEs coding gene in raw, boiled hot and sour milk was 4.3, 5.6 and 4.2%, respectively.

Table 3 Prevalence of SEs coding gene in raw, boiled hot and sour milk sold in Mbeya and Mbozi districts.

	Total		Mbeya		Mbozi	
Type of milk	N	%	Frequency	%	Frequency	%
Raw	300	100	150	50	150	50
Absent	286	95.3	140	48.9	146	51.1
Present	13	4.3	7	2.3	6	2.0
Boiled hot	72	100	36	50	36	50
Absent	68	94.4	33	45.8	35	48.6
Present	4	5.6	1	1.4	3	4.2
Sour milk	72	100	36	50	36	50
Absent	70	97.2	35	48.6	35	48.6
Present	3	4.2	1	1.4	2	2.8

3.3 Detection of SEs genes by multiplex PCR and their prevalence

Multiplex PCR results showed that 20 (36.4%) out of 55 isolates were positive for one or more SEs genes (Table 4). The distributions of the SE genes were 16.4% and 20% for Mbeya and Mbozi districts, respectively. The genes that code for classic enterotoxins (Staphylococcal enterotoxins coding gene type A to type E (*sea-see*), *sea* was the most frequently observed at 32.6% followed by *sed* (20.9%), *seb* (18.6%), *sec* (9.3%) and *see* (4.7%) among *S. aureus* isolates in the study area. Their corresponding frequency in Mbeya and Mbozi districts are shown in Table 4. Newly characterized enterotoxins genes detected in this study were *sei*, *seg* and *seh.* The *sei* was frequently detected (7%) followed by *seh* (4.7%) and lastly *seg* (2.3%).

Table 4: Frequency of genes coding for SEs from *S. aureus* isolates from milk samples collected in Mbeya and Mbozi district, Tanzania

			Mbeya		Mbozi	
Parameters	N	Total	Frequency	%	Frequency	%
S. aureus isolates eliciting enterotoxins gene	55	100	26	47.3	29	52.7
Absent	35	63.6.	16	29.1	19	34.5
Present	20	36.4	9	16.4	11	20
Gene detected of which(n=43)						
Sea	14	32.6	6	14.0	8	18.6
Sed	9	20.9	5	11.6	4	9.3
Seb	8	18.6	5	11.6	3	7.0
Sec	4	9.3	2	4.7	2	4.7
See	2	4.7	1	2.3	1	2.3
Sei	3	7.0	1	2.3	2	4.7
Seg	1	2.3	0	0.0	1	2.3
Seh	2	4.7	0	0.0	1	2.3

Sea– Seh Staphylococcus enterotoxins gene type a-h

The combination of Staphylococcus enterotoxins genes are shown in Table 5. Isolates coded by one SEs gene were observed in 50% of the isolates tested in the study area. Although there was no statistical difference, the isolates coded by more than one gene were higher in Mbozi than Mbeya district (30% vs. 20%). Furthermore, the result showed that the highest combination observed was five genes detected in one isolate from Mbozi district (Figure 2).

Table 5. Combination (genotype) of Staphylococcal enterotoxins genes detected from *S. aureus* isolates (n=20)

			Mbeya		Mbozi	
Genotype	**N=20**	Overall	**Frequency**	**%**	**Frequency**	**%**
Sea	8	40	4	20	4	20
Seb	1	5	0	0	1	5
Sed	1	5	1	5	0	0
Sea/Seb	1	5	1	5	0	0
Seb/Sed	1	5	0	0	1	5
Sed/Sec	1	5	1	5	0	0
Sea/Sed/Sec	1	5	0	0	1	5
See/Sed/Seb	1	5	1	5	0	0
See/Sed/Seg	1	5	0	0	1	5
Sea/Seb/Seh	1	5	0	0	1	5
Sea/Seb/Sed/Sei	1	5	0	0	1	5
Sea/Sed/Sec/Sei	1	5	1	5	0	0
Sea/Sed/Sec/Seh/Sei	1	5	0	0	1	5

Sea – Sei Staphylococcus enterotoxins gene type a-i

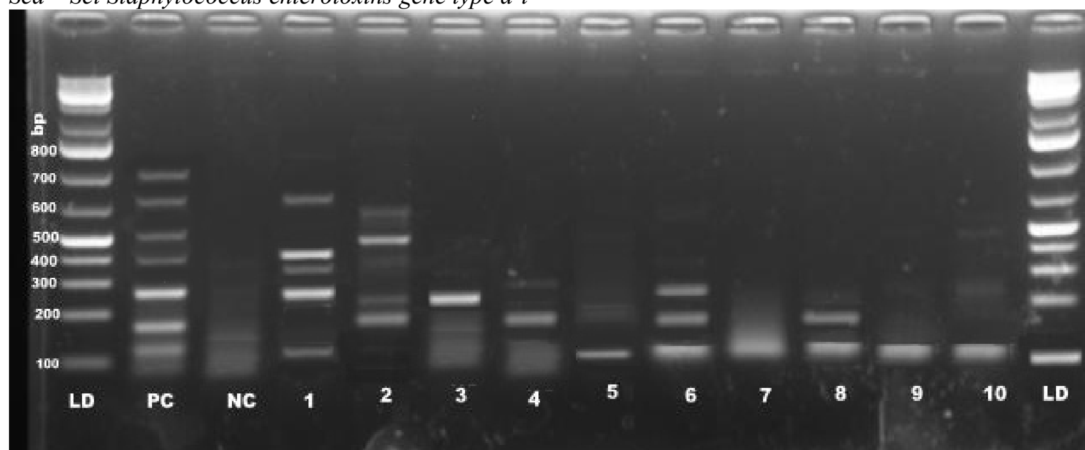

Figure 2. Agarose gel electrophoresis showing the results of multiplex PCR for detection of enterotoxins genes among the *S. aureus* isolates of milk samples. Lane LD: 100 bp ladder DNA molecular weight marker, Lane PC: Positive control for *sea, seb, sed, sec see, seh, and sei* genes; NC: Negative control. Lane 1-10 showed positive targeted genes *sea seb, sed, sec, see, seh* and *sei* of *S. aureus* isolates from milk sample.

3.4 Gene distribution along the milk value chain

Gene distribution along the milk value chain in the study area is shown in Figure 3. Results showed that 15%, 5% and 35% of SEs genes were detected in milk collected at farm level, milk collection points and milk shops, respectively in Mbozi district. The corresponding values for SEs genes in Mbeya district were 20%, 10% and 15%. Furthermore, the level of SEs genes detected in milk (raw, boiled and cultured) from the milk shop was similar in Mbeya district. They were however, different from samples collected from milk shops in Mbozi district. About 65% of SEs coding genes were detected after the milk leaving the farm.

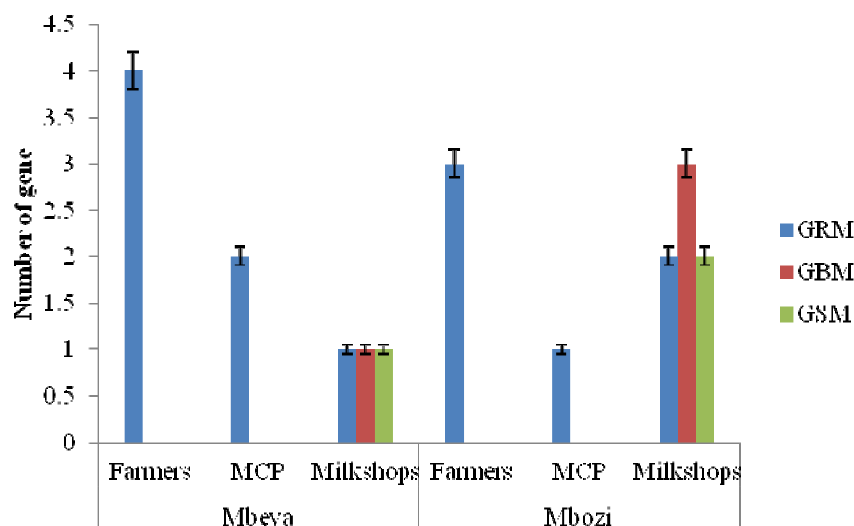

Figure 3. Distribution of SEs gene along milk value chain in Mbeya and Mbozi district (GRM- Gene isolated from raw milk, GBM-gene from boiled milk, GSM- gene from sour milk, MCP- Milk collection Point)

3.5 Seasonal distribution of SEs in the study area

Table 6 shows seasonal distribution of gene coding for Staphylococcus enterotoxins detected in milk produced and marketed in Mbeya and Mbozi districts. The distribution of SEs gene in Mbeya district was 15% and 30% genes for wet and dry seasons, respectively. In Mbozi district 20% SE coding genes were detected in the wet while 35% in the dry season. No isolates carried genes coding for SEs in boiled and cultured milk were observed in the wet season in Mbeya district, while in the dry season raw milk collected from milk shop carried no SE coding gene. In Mbozi district, the distribution of gene in the wet and dry season were 15% and 40%, respectively, of which 5% and 10% of the isolates were detected from boiled hot milk in the wet and dry season, respectively. On the other hand, 10% of the isolates from cultured milk carried SEs gene in the dry season. Statistically no significant ($p > 0.05$) difference was observed between wet and dry season, though numerically dry season had higher values compared to wet season.

Table 6. Seasonal distribution of SEs genes in the production and milk marketing channel in Mbeya and Mbozi district, Tanzania

District	Season	Source	Type of milk	SEs gene (N=20)	%
	Wet	Farmer	Raw	1	5
	Wet	MCC	Raw	1	5
	Wet	Milk shop	Raw	1	5
			Boiled hot	0	0
Mbeya			Cultured	0	0
	Dry	Farmer	Raw	3	15
	Dry	MCC	Raw	1	5
	Dry	Milk shop	Raw	0	0
			Boiled hot	1	5
			Cultured	1	5
	Wet	Farmer	Raw	1	5
	Wet	MCC	Raw	0	0
	Wet	Milk shop	Raw	1	5
			Boiled hot	1	5
Mbozi			Cultured	0	0
	Dry	Farmer	Raw	2	10
	Dry	MCC	Raw	1	5
	Dry	Milk shop	Raw	1	5
			Boiled hot	2	10
			Cultured	2	10

4. Discussion

The prevalence of SEs coding gene found in this study is comparable to the results of other studies conducted

elsewhere around the world. It has been reported that the proportion of isolates expressing or containing enterotoxins genes varied from 12% (Shuiep *et al.* 2009) to 77% (Hwang *et al.* 2010). This could be due to geographical location, initial concentration of *S. aureus* in the milk and method of extraction. The prevalence of SEs genes in the study area for the raw milk was statistically similar ($p > 0.05$). This could be due to the similar management, milking procedures and milk handling.

The concentration of *S. aureus* determined the production of enterotoxins genes that can produce a detectable toxin in the food. In this study the concentration of *S. aureus* in the milk sample ranged from 10^3 to 10^6cfu/ml. Two samples (4%) out of 55 samples had concentration of 10^3 cfu/ml and yielded *S. aureus* able to encode enterotoxins gene. It has been demonstrated that for a detectable SEs production in food samples, *S. aureus* population between 10^5 and 10^7 cfu/g is required (Fujikawa & Morozumi 2006; Huong *et al.* 2010). Concentrations of 10^3cfu/ml of *S. aureus* have been reported in Egypt by Abdi El-fatah & Tahoun, (2013) to encode *S. aureus* enterotoxins genes. Fifty six percent of the samples in the present study had *S. aureus* counts of $\geq 10^5$ which have higher possibilities of producing toxins. Annual reports of food-borne diseases from seven countries showed that milk and milk products were causing 1-5% of the total bacterial outbreaks and *S. aureus* was by far the most frequent pathogen associated with food-borne disease outbreaks (De Buyser *et al.* 2001). The observed contamination level of *S. aureus* in milk and milk products could be originating from milk handlers who harbour the bacteria. It has been reported that 20-30% of the human population carries *S. aureus* as commensals (Le Loir *et al.* 2003). Other contamination sources may originate from animals with clinical or subclinical mastitis, soil, water, dust and air during milking and processing which may vary in different production and environmental condition.

In the present study, *S. aureus* isolates were screened by multiplex PCR in order to detect the Staphylococcal enterotoxins genes *(sea, seb, see, sed see, seg, seh, sei and sej)* from the milk samples. *Staphylococcus aureus* isolates coding for one or more SE genes observed in this study concurs with the results reported by El-Jakee *et al.* (2013) who found enterotoxigenic genes to be 35.7% in milk. However, other studies by Veronica *et al.* (2011) and Rahimi *et al.* (2012) reported respectively lower percentages of 11.7% and 20.8% enterotoxigenic genes. Furthermore, Rall *et al.* (2008) found that 68.4% out of 57 strains isolated from raw or pasteurized bovine milk were positive for the presence of at least one SE gene. The differences in the frequency of gene coding strains from this study might be due to the source of bacteria isolation which could vary between animals (depending on the stage of mastitis), milk handlers, milking environment and concentration of *S. aureus* in the milk.

Regarding gene encoding for classical enterotoxins, it was found that *sea* gene was most frequently detected. Similar findings were reported in studies conducted in Egypt (Abdi El-fatah & Tahoun 2013) and Kenya (Mathenge *et al.* 2015). The dominance of *sea* genes has also been reported by other researchers (Bendahou *et al.* 2009; Fooladi *et al.* 2010) for *S. aureus* recovered from food samples. *Sea* genes have been mentioned as the main cause of food poisoning worldwide and it is most frequently isolated from humans being. This is an indication that most of the contamination of milk and milk products originate from faulty milk handling practices by personnel.

Other SEs genes detected in higher frequencies in this study were *seb* and *sed*. Similar observations have been made by (Morandi *et al.* 2009) who reported that *sed* was the most frequently isolated toxin gene type, after *sea*, in staphylococcal food-poisoning outbreaks involving dairy products. The present study shows that the *sea, seb* and *sed* genes were dominant. The dominance of enterotoxins *sea* and *sed* have shown a wide variation in several studies and from different countries such as France, Japan and Norway (Jørgensen *et al*. 2005; Katsuda *et al.* 2005;Villard *et al.* 2005), where enterotoxins *sec* were frequently isolated from milk and cheeses made from raw milk. Other researchers (Normanno *et al.* 2007) showed that the most frequently isolated strains in Italian dairy products were *sed*, followed by *sea*, *sec*, and *seb*. These variations could be related to geographical location, concentration of bacteria and type of food material. Also the pattern for *S. aureus* intramammary infection which often differ from herd to herd and these patterns could be linked to strain differences (Piccinini *et al.* 2010).

In the present study, *see* gene occurred in combination with other genes and was detected in two *S. aureus* isolates in combination with other genes. A study conducted in Kenya by Mathenge *et al.* (2015) reported this gene to occur in six isolate singly and in combinations in ten isolates with other classical enterotoxins. Several authors (Arcuri *et al.* 2010; Rahimi 2013; Abdi El- Fatah & Tahoun*,* 2013; Ferreira *et al*. 2014) did not detect this gene in their study. Also, other authors (Rall *et al.* 2008; Ostyn *et al.* 2010; Seyoum *et al.* 2016) have detected this gene in their study in varying percentages. The results of this study and other studies indicate that staphylococcal enterotoxic genes have a broad distribution and also, showed that the geographical distributions of these genes are variable.

Newly characterized SEs genes were detected in four samples and in combination with classical enterotoxins. The detected genes were *seh, seg* and *sei. Sei* was found in three isolates in combination with classical enterotoxins. These results were similar with those reported by Rahimi (2013) who found these genes to occur in combination with other enterotoxins genes. These genes are frequently found together with classical

enterotoxins because they are within the same genetic cluster (EGC) (Jarraud *et al.* 2001). In the present study, no *sej* gene was detected in any of the isolates. This finding was in line with Ahmady & Kazemi (2012) who did not detect any *sej* genes out of 25 isolates. On the other hand, contrasting results were reported by Rall *et al*. (2008) who detected three (7.7 %) positive strains, and Zschöck *et al*. (2005) who found 37.7 % positive strains for *sej*. But Jorgensen *et al*. (2005) reported *sej* gene in association with *seg* and *sei* in *S. aureus.*

It was observed that milk along the milk value chain in the study area was contaminated with SEs genes. Milk samples from shops were detected to have more SEs genes, followed by milk from farmer's herds and milk collection points. This could be due to personnel handling and time taken to sell the milk, fluctuation of temperature during milk storage and recontamination. It was observed that most of milk shops owners switch off electricity during the night and switch on their fridges on the following day in order to lower electricity costs. It was indicated that exposure of milk in different temperatures favours the growth of *S. aureus* and SEs production (Peles *et al.* 2007). Farmer's bulk milk had higher number of isolates that carried SEs genes than milk collection point (MCP). This could be due to subclinical mastitis, contamination from milker's hands and utensils used to handle the milk. In the MCP the SEs gene were fewer than any other nodes, the reasons could be due to dilution effects because MCP receives milk from different sources and some source may have milk which is not contaminated with *S. aureus*. Another reason could be the inability of *S. aureus* to compete with other bacteria and making their growth difficult (Kim *et al*. 2010).

Seasonal distribution of *S. aureus* isolates showed that samples collected in the dry season carried more genes coding for SEs. It is well known that higher temperatures favours the growth and multiplication of *S. aureus*, therefore in the dry season there is high possibility for *S. aureus* to grow and reach its potential characteristics than in the wet season. Similar observation was made by Peles *et al*. (2007) who reported that higher temperature is an important requirement for SEs and toxin production. Furthermore, few boiled hot and sour milk (read-to-consume) had isolates carrying genes coding for SEs. The occurrence of these genes in the boiled hot milk could be due to insufficient boiling of milk, recontamination of milk from the utensils and milk handlers. Another plausible reason for the occurrence of genes coding for SEs in the boiled hot milk is that, there is a possibility of the milk being contaminated by SEs toxin before the boiling process. It has being indicated that once the toxins are produced in the milk, normal boiling cannot inactivate the SEs (Asao *et al*., 2003) and the gene detected could be toxic, therefore poses risk to milk consumers. According European Commission for Health and Consumer Protection (ECHCP) (2003), crude SEA in buffer was reduced from 21 µg/ml to < 1µg/ml after heating at 100°C for 130 minutes and purified SEA (0.2 mg/ml) was completely inactivated in buffer after heating at 80°C for 3 min or 100°C for 1 minute.

5. Conclusion and recommendations

This study showed that milk and milk products produced and marketed in Mbeya and Mbozi districts were contaminated with *S. aureus,* encoding SEs gene, which poses a health risk for milk consumers.

A total of 14 *S. aureus* isolates were positive for one or more SE coding genes and *sea* gene was the most prevalent. The frequencies of SEs coding gene in the milk shops samples were higher than in the farms bulk herd milk and milk collection points. This is a public health concern because 35% of ready to consume milk sold in the milk shops contained SEs coding genes.

Personnel hygiene and proper handling should be emphasized so as to avoid recontamination on ready to consume milk.

Further study is required to determine the diversity and toxins production among detected SEs genes in the study area and other parts of the country as well.

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Conflict of interest

The authors declare that they have no conflict of interest and this document is their original research work done in the Southern Highlands Zone of Tanzania and no part of it has been submitted somewhere else for conference presentation or publication.

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