Version of Record: https://www.sciencedirect.com/science/article/pii/S0956713519301525
Manuscript_c36b5182ab69f3b5c27da6fae33f6294

1 **_Staphylococcus aureus_ Enterotoxin Genes Detected in Milk from various Livestock**

2 **Species in Northern Pastoral Region of Kenya**

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31 **ABSTRACT**

32 *Staphylococcus aureus* (SA) food poisoning results from consumption of preformed *S. aureus*
33 enterotoxins in food. The enterotoxins are one of the most important virulence factors of the
34 bacterium. The risk posed by contamination of milk intended for human consumption by
35 pathogenic *S. aureus* in pastoral areas in Kenya is still generally not well documented yet this
36 information is critical for ensuring safety to consumers who sometimes may take unpasteurized
37 milk. This study, therefore determined the prevalence of *S. aureus* enterotoxin genes in raw
38 milk from cattle, goats, sheep and camels intended for human consumption in northern Kenya.
39 A total of 603 milk samples from 57 zebu cattle, 346 galla goats, 8 red Maasai and dorper
40 sheep, 4 one-humped camel (*Camelus dromedaries)* and 188 pooled from all animals were
41 collected from Isiolo and Marsabit counties of Kenya. *S. aureus* isolates were cultured from
42 milk samples using a selective media, mannitol salt agar (MSA). Suspect colonies of SA were
43 further analyzed using biochemical tests. Polymerase chain reaction and sequencing techniques
44 were used to confirm SA and detect *sea, seb, sec, sed* and *see* enterotoxin genes.

45 Overall, potentially pathogenic *S. aureus* harboring enterotoxic genes were detected in 85
46 (14.09 %, 95 % CI: 11.55-17.1 %) of the total milk samples. Genes encoding enterotoxins were
47 detected in the *S. aureus* bacteria isolated from the milk samples. At least one type of *S. aureus*
48 enterotoxin gene (*SE*) was detected in 74.11% (95 % CI: 63.91-82.24 %) of the 85 isolates.
49 The most frequently encountered gene in the two counties was *see* (51; 60%, 95 % CI: 49.73-
50 69.76 %) followed by *sea* (22; 25.88 %, 95 % CI: 17.76 -36.09 %) and *sec* (19; 22.35 %, 95 %
51 CI: 14.8-32.29 %). None of the isolates tested positive for *sed*. Overall, 21 of the 85 (24.7%,
52 95 % CI: 16.76-34.83 %) strains harbored more than one enterotoxin gene. More than half of
53 the *S. aureus* isolates harbored at least one of the enterotoxin coding genes, indicating milk
54 samples contaminated by *S. aureus* could have a high chance of causing staphylococcal food
55 intoxication.

56 Consumption of raw and sour milk in the region could increase the risk of *staphylococcal* food
57 poisoning and pastoral communities in the region are therefore advised to consume pasteurized
58 milk.

59 Key words: ***Staphylococcus aureus*: milk: enterotoxins: genes: Kenya**

60

61 ## 1. Introduction

62 *Staphylococcus aureus (S. aureus)* is known to widely cause foodborne illnesses in humans
63 with most of the outbreaks resulting from consumption of many food products including milk
64 obtained from different parts of the world (Silva *et al.,* 2000; Silva *et al.*, 2003; Adman *et al.*,
65 2005; Aragon-Alegro *et al*, 2007; Chiang *et al*, 2008; Zouharova and Rysanek, 2008). *S. aureus*
66 can contaminate several food products including pork, beef, mutton, poultry, eggs and milk
67 during farming, and value addition process. The bacterium is normally found in the skin and
68 nasal cavities of humans and animals. About 30 % of humans are thought to be intermittent
69 carriers while the other 20% are known to be permanent carriers (Van Belkum, *et al*, 2009).
70 Food handlers carrying *S. aureus* on their bodies may contaminate food (Crago *et al.,* 2012)
71 indicating that the presence of *S. aureus* in milk can be due to contaminations from skin of food
72 handlers. The contamination of milk with the bacterium can also be from milk of animals with
73 intra-mammary infections (Kümmel *et al.,* 2016), and from the environment as a result of poor
74 hygienic conditions during milking (Dittmann *el al*., 2017).

75 Staphylococcal food intoxication is dependent on a single type of virulence factor that is
76 responsible for the production of heat stable staphylococcal enterotoxins (SEs) by certain
77 strains of *S. aureus* (Le Loir et al., 2003). There is a strong association between the ability of
78 *S. aureus* strains to produce one or more of the SEs and the occurrence of staphylococcal food
79 poisoning (Bennett, 2005). Currently, about 23 SEs have been identified and out of these, five
80 enterotoxins namely *SEA, SEB, SEC, SED*, and *SEE* are considered to be the classical
81 enterotoxins (da Silva Sdos *et al*., 2015). The five *SEs* are encoded by specific enterotoxin
82 genes such as *sea, seb, sec, sed*, and *see* respectively. Some of these enterotoxins are produced
83 by *S. aureus* when the bacterium grows in unpasteurized raw milk. Pasteurizing raw milk,
84 which involves heat-treatment, normally eliminates *S. aureus* from raw milk. However, once
85 the *S. aureus*-enterotoxins have been produced, they can withstand high temperatures of

86 pasteurization (Asao *et al.*, 2003). For example, SEs such as *SEA* is known to be highly resistant
87 to heat treatment and retain their biological activity after exposure to a high temperature of
88 121°C for 28 minutes (Seyoum *et al.*, 2016). Therefore, if raw and unpasteurized milk
89 contaminated with the SEs is consumed they can cause food poisoning sometimes resulting in
90 deaths.

91 Of late, there has been increasing incidences of staphylococcal food poisoning worldwide
92 resulting in serious food safety concerns (Pu *et al.,* 2011). For example, in 2012 enterotoxins-
93 producing *S. aureus* was reported in European Union where it caused 346 foodborne disease
94 outbreaks (FBOs) representing 6.4 % of all the outbreaks documented (Macori *et al.*, 2016). In
95 the United States, staphylococcal food poisoning has been reported to account for
96 approximately 241,000 illnesses annually. Some of these cases required hospitalization and
97 were part of the 56,000 foodborne illnesses in the United States (Scallan *et al.*, 2011; Byrd-
98 Bredbenner *et al.,* 2013). In China, approximately 20–25% of reported bacterial foodborne
99 illnesses have been reported to be caused by *S. aureus* isolated from retail food outlets in
100 Shaanxi (Wang *et al.*, 2014).

101 In sub-Saharan Africa, especially in communities that interact closely with livestock like
102 pastoralist communities, *S. aureus* can cause serious illnesses in general population, healthcare
103 units, as well as in food and livestock production systems (Njage *et al.,* 2013: Gitau *et al.,* 2014
104 ;Egyir *et al.*, 2014a, 2014b; Akindolire *et al.*, 2015; Maina *et al.*, 2016). Furthermore, under-
105 reporting, inadequate investigation of the outbreaks and inadequate diagnostic facilities has
106 led to unreliable data on staphylococcal food poisoning in these regions. In Kenya, *S. aureus*
107 is responsible for up to 38 % of reported foodborne disease outbreaks (Ombui *et al* 2001).
108 Moreover, another study by Ombui *et al.* (1992) also reported a prevalence of 74.2 % for
109 enterotoxins-producing *S. aureus* in raw milk sampled from Nairobi and its environs. Out of
110 these isolates, three (4.17%) were found to produce *SEA* on screening using latex agglutination

111 test. Mathenge *et al* (2015) also identified enterotoxigenic *S. aureus* strains in meat and dairy
112 products in Nairobi County and its surroundings. However, in spite of these reports, the actual
113 risk of staphylococcal food poisoning in the pastoral areas of northern Kenya generally remains
114 unknown yet this information is crucial for the management of foodborne illnesses. We believe
115 that unpasteurized raw milk regularly consumed by the pastoral communities in northern
116 Kenya contain potentially pathogenic *S. aureus,* which harbour enterotoxin genes responsible
117 for the production of heat stable SEs.
118 Therefore, this study has determined the risk of contamination of raw milk of cows, goats,
119 sheep and camels with potentially pathogenic *S. aureus.* We have also established whether the
120 potentially pathogenic isolates*,* harbour enterotoxin genes, which encode the heat-stable SEs
121 responsible for the foodborne illness. The information provided here could assist pastoralist in
122 northern Kenya to mitigate outbreaks associated with fatal Staphylococcal food poisonings.
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129 ## 2. Materials and methods

130 ### 2.1 Study area and livestock population

131 This study was done in Isiolo and Marsabit counties in northern part of Kenya in June 2016
132 and February 2017 (Figure 1). Both counties are part of the arid and semi-arid land (ASAL)
133 areas of the country and are inhabited by pastoralists whose livelihoods are mainly dependent
134 on livestock.

135 ### 2.2 Study design

136 A cross-sectional study design with a household being a unit of analysis was used. Four wards
137 (Burat, Kinna, Merti and Oldonyiro) in Isiolo county and six wards in Marsabit (Karare, Korr,
138 Laisamis, Moyale, Sololo and Turbi) county were conveniently selected as the study sites
139 whereas households within these areas were randomly identified using systematic sampling
140 method along transects defined by feeder roads. In this process, every firth household that kept
141 animals (cattle, sheep, goats and camels) of interest in this study were recruited making a total
142 of 188 households (Figure 2). From each household one pooled milk sample consisting of milk
143 from multiple lactating animals and randomly selected individual lactating animals were
144 collected from animals kept in those households. A total of 603 milk samples from 57 zebu
145 cattle, 346 galla goats, 8 red maasai and dorper sheep, 4 one-humped camel (*Camelus*
146 *dromedaries)* and 188 pooled were collected from Isiolo and Marsabit counties

147 ### 2.3 Sample collection

148 After selected households consented to participate in the study, a total 603 milk samples were
149 collected of which 305 were from Isiolo and 299 from Marsabit. About 10 ml of milk was
150 aseptically collected into a sterile 15 ml falcon after disinfection of the udder using cotton
151 swabs moistened with 70% ethyl alcohol. Individual milk samples were collected mid-stream
152 from all the teats of the lactating cattle, sheep, goats and camels. Additionally, a pooled milk
153 sample from each household was collected into a 50 ml sterile falcon tube identified using

154 unique barcode identifiers. Aliquotes of milk samples were placed into cryovials and stored at
155 -20^{0}C pending laboratory analysis.
156 A questionnaire was administered to capture animal and households data. Information on milk
157 processing before consumption was collected and households geo-referenced using Garmin
158 ETrex hand held GPS units.
159
160 **2.4 Isolation and identification of *Staphylococcus* Species**
161 Ten microliters of milk samples from each collection site was inoculated to selective medium
162 mannitol salt agar (MSA) and incubated at 37°C for 24- 48 h. Growth of yellow colonies on
163 this medium surrounded by yellow zones was considered a presumptive positive for
164 *Staphylococcus aureus* (Kateete *et al.* 2010)
165 The presumptive *Staphylococcus aureus* colonies were sub cultured onto 5 % sheep blood agar
166 and incubated at 37°C for 24 h to get a pure culture. Identification of *S. aureus* and other
167 staphylococci was performed using the following tests: Morphology following Gram staining,
168 catalase activity, mannitol fermentation, coagulase activity and β-hemolysis. The isolates were
169 confirmed by amplification of *S. aureus* specific staphylococcal terminase gene (satm).
170 **2.5 Extraction of *S. aureus* DNA**
171 Genomic DNA extraction was performed using Invitrogen DNeasy DNA extraction protocol
172 for bacterial cultures. Bacterial DNA was extracted according to the protocol provided by the
173 manufacturer. Colonies were harvested and suspended in 180 µL lysozyme digestion buffer
174 and incubated at 37^{0}C for 30 minutes. Twenty microlitres of Proteinase K was added followed
175 by 200 µL of PureLink™ genomic lysis /binding buffer. After incubation at 55^{0}C for 30
176 minutes and addition 200 µl of 96-100% ethanol, DNA was bound to silica-gel-membrane in a
177 brief centrifugation step. The inhibitors of PCR such as the proteins and divalent cations were

178 completely removed in two washing steps, leaving pure nucleic acid behind, which was eluted
179 in the elution buffer. The DNA was stored at 4°C pending subsequent analysis. The DNA
180 quantity and purity was assessed spectrophotometrically at 260-280 nm, with NanoDrop ND-
181 1000 full spectrum UV-Vis spectrophotometer**.**

182 **2.6 Detection of *S. aureus* by PCR**

183 Primers that target the staphylococcal terminase gene (MH678720) were designed using the
184 Primer Blast tool (www.ncbi.nlm. nih.gov/tools/primer-blast/) for detection of *S. aureus*.
185 Oligonucleotide forward primer was 5'-TAACCCCTCATCACCTCCGT-3'and the reverse
186 primer was 5'-ACTGCAAAGCAAGCACGTTT-3'. The annealing temperature was
187 determined using a gradient PCR on the Veriti 96-well thermal cycler (Applied Biosystems,
188 Foster city, CA, USA). A 25µl reaction volume contained 12.5 µl of 1X dreamTaq mastermix
189 (Fermentas, Thermo Scientific, USA) 10000nM of each forward and reverse primers, 1 µl of
190 DNA template and 9.5 µl nuclease free water. Optimized PCR conditions were: 95°C for 3
191 min; 35 cycles of 95°C for 30 sec; 57°C for 60 sec; and 72°C for 1 min with a final extension
192 at 72°C for 10 min. Amplified products were detected using gel electrophoresis. DNA extracted
193 from *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923^{TM}) was used as the positive control
194 and nuclease-free water as the negative control in all the analyses done.
195 Some PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN GmbH
196 Hilden, Germany) and taken for sequencing at Macrogen Inc (Macrogen Europe Meibergdreef,
197 Amsterdam, The Netherlands). Sequences obtained were edited and analysed using the
198 BLASTn tool to confirm identity of the isolates.

199 **2.7 Amplification of staphylococcal enterotoxin genes**

200 The isolates confirmed to be potentially pathogenic *S. aureus* were further evaluated to
201 determine whether they harbor enterotoxin genes responsible for milk-borne food poisoning.
202 Oligonucleotides primers used in a previous study (Table 1) were used for the amplification of

203 the enterotoxin encoding genes (Mehrotra, *et al.,* 2000). Synthesis of oligonucleotides was
204 done by Macrogen Inc (Macrogen Europe Meibergdreef, Amsterdam, The Netherlands). The
205 primers were used for the amplification of *sea, seb, sec, sed* and *see* genes. The amplicons were
206 further purified and sequenced as described above. The sequenced genes were then subjected
207 to Blastx analysis to confirm whether the amplified genes encoded enterotoxin proteins.

208 **2.8 Data management and analysis**

209 Animal and household data were cleaned and merged with the aliquoting databases for both
210 counties as well as the results databases in access files on R software. The merged document
211 was imported into STATA version 13 for analysis. This involved descriptive statistics of the
212 prevalence data with both sample type and sampling area. Chi ($\chi 2$) square tests were used to
213 calculate the prevalence as well as odds ratios.

214

215 **3. RESULTS**

216 **3.1 Identification of SA phenotypes from raw milk**

217 A total of 603 milk samples comprising on 415 individual and 188 pooled samples were
218 collected in this study; 304 samples were from Isiolo and the other 299 were from Marsabit.
219 Two hundred and fifty presumptive *S. aureus* were isolated on culture with 223 (89.2 %, 95 %
220 CI: 84.74-92.47 %) isolates being catalase positive**.** On further biochemical tests, 151 (60.4 %,
221 95 % CI: 54.22-66.26 %) isolates were β-hemolytic while 129 (51.6 %, 95 % CI: 45.43-57.72
222 %) isolates were coagulase positive from which 85 were confirmed to be *S. aureus* through
223 amplification of *satm* gene. Among the 85 isolates, 43 isolates (14.38 %, 95 % CI: 10.86 % -
224 18.81 %) were from Marsabit and 42 isolates (13.82 %, 95 % CI: 10.39 % - 18.15 %) were
225 from Isiolo counties.

226 Overall, the proportion of *S. aureus* isolated from pooled milk samples was significantly (p = 227 0.0001) higher (23.94 %, 95 % CI: 18.40-30.52 %) as compared to individual lactating animals 228 (9.64 %, 95 % CI: 7.12-12.86 %). In Marsabit county, there was a significantly higher (p= 229 0.0001) proportion of isolates from pooled milk (25.44 %, 95 % CI: 18.34-34.14 %) as 230 compared to individual lactating animals (7.37%, 95% CI: 4.44- 11.99%). The trend was 231 similar in Isiolo county where proportion of isolates from pooled milk samples (21.62 %, 95% 232 CI; 13.77-32.27 %) was higher as compared to milk from individual lactating animal (11.56 %, 233 95 % CI: 8.01-16.39 %) (p =0.03).

234 **3.2 Confirmation of *S. aureus* by PCR and sequencing**

235 The primer targeting the terminase gene amplified the fragment yielding a specific band 236 corresponding to approximately 510bp (Figure 2). The sequenced PCR products were 237 confirmed to be *S. aureus* gene by Blastn analysis using Genbank of the NCBI database. The 238 blastn results revealed that the genes were homologous to *S. aureus* DNA Pathogenicity Island, 239 one of the genetic determinants responsible for pathogenicity of the bacterium. The nucleotide 240 identities of the sequenced isolates as compared with the homologues above were between 95 241 % and 98 %. Subsequently, this result confirmed that the 85 (14 %) isolates obtained from the 242 milk samples were actually potentially pathogenic *S. aureus*. The accession numbers of the 243 sequenced terminase gene of *S. aureus* are available in the Genbank under the accession 244 numbers MH678717-MH678720. Blastx analysis revealed that the translated amino acid 245 sequences were homologous to the *S. aureus* terminase small sub unit protein revealing 246 sequence identity of 100 %.

247 **3.3 Enterotoxin genes detected by genetic analysis**

248 Overall, 63 (74.11%, 95% CI: 63.91-82.24 %) of the 85 *S. aureus* were found to harbor at least 249 one gene of the enterotoxin genes. A total of 100 enterotoxin genes were detected in this study. 250 Among the genes that code classic enterotoxins, *see* gene was the most frequent, carried by 51

251 (60%) isolates, followed by *sea* 22 (25%). The s*eb* gene was detected in 9 (10.6%) isolates
252 only while *sed* gene was not detected in all the *S. aureus* evaluated (Table 4). Of the *S. aureus*
253 isolates (85), 21 (25%) strains harbored more than one enterotoxin gene and more than half of
254 the isolates harbored at least one of the enterotoxin coding genes. There was no significant
255 difference (p= 0.47) in the proportion of enterotoxins detected in *S. aureus* from individual
256 milk samples (17.8%, 95% CI: 14.45- 21.8%) as compared with *S. aureus* pooled milk samples
257 and (15.43%, 95% CI: 10.96-21.28%)
258 Sequencing and blastx analysis revealed that these enterotoxin genes were homologues to the
259 enterotoxin sequences of *S. aureus* revealing high amino acid identities of 76 % and 91 % for
260 enterotoxin A and E respectively. The presence of enterotoxins C and B was also confirmed.

261 **3.4. Enterotoxin genes from milk of various species**

262 High levels of enterotoxin genes were detected in *S. aureus* isolates from goat milk (44 %)
263 followed by cattle (25 %) and sheep (2 %) in that order (Table 3).
264

265 **4. Discussion**

266 This study determined the presence of potentially pathogenic *S. aureus* in raw milk intended
267 for human consumption in northern Kenya. *Staphylococcus aureus β*-hemolysin is one of the
268 cytotoxic molecules responsible for its pathogenicity (Dinges *et al*., 2000). These exotoxins
269 together with coagulase are known to be some of the virulence factors responsible for its ability
270 to cause infections (Hennekinne *et al.,* 2012). In this study, a number of *S. aureus* isolated
271 from milk were *β*-hemolytic and coagulase positive indicating that these bacterial isolates could
272 be potentially pathogenic. Terminase gene was used in this study to identify potentially
273 pathogenic *S. aureus* from the milk samples because it forms one of the core genes in the
274 staphylococcal pathogenicity islands (SaPIs), which is a mobile genetic element responsible
275 for the bacterial virulence (Malachowa and DeLeo, 2010). This study found that the *S. aureus*

276 sequences were homologous to *S. aureus* DNA Pathogenicity Island with the corresponding
277 translated amino acids sequences being homologous to the *S. aureus* terminase small sub unit
278 protein suggesting that these isolates were pathogenic.
279 In this study, the overall occurrence of *S. aureus* in the analyzed samples was 14 % for milk
280 samples in Marsabit and Isiolo Counties. This prevalence was lower than that of Asiimwe *et al*
281 (2017) who reported a prevalence of 20.3 % for *S. aureus* detected in bulk can-milk and 12.1
282 % in sour milk consumed in pastoral areas of Uganda. The prevalence was also lower than that
283 reported by Mathenge *et al* (2015) in which an overall prevalence of 36 % in meat and milk
284 products was found in Nairobi county and its surroundings. Higher prevalence rates in milk
285 were also reported in various studies done in other countries including Turkey (Kiymet *et al*
286 2010), USA (Lubna *et al* 2015), Zimbabwe (Gran *et al* 2003) and in Malaysia (Chye *et al* 2004).
287 Overall, the proportion of *S. aureus* isolated from pooled milk samples was significantly (p =
288 0.0001) higher than those from individual lactating animals. During pooling of milk, there is
289 risk of contamination from human skin carrying *S. aureus* thereby resulting in higher
290 contamination level as seen above. Alternatively, pooling of milk under poor hygienic
291 conditions involving use of contaminated utensils could have been response for the relatively
292 high contamination. For individual milk samples, contamination by *S. aureus* isolates may have
293 been due to clinical and subclinical mastitis resulting from intramammary infections caused by
294 the bacterium. Usually the bacterium is known to enter the udder through the teat canal from
295 the surrounding environment (Smith *et al.,* 2005) and this can act as a source of infection and
296 milk contamination. Nevertheless, other modes of contamination cannot be ruled out and
297 further studies are needed to establish the other possible sources of contamination of milk with
298 *S. aureus*.
299 Increased awareness of pastoralists is necessary in order to minimize contamination of milk
300 through improved hygiene practices as well as through diagnosis and treatment of infected

301 animals. Pastoralists in northern Kenya live in geographically different ecosystem and practice
302 livestock production system different from other systems in other regions of the country. It is
303 possible that this difference in geographical ecosystem and production systems could be
304 responsible for the disparities in the contamination levels seen with the other previous studies.
305 Nevertheless, this claim needs to be confirmed by performing further studies because other
306 factors could have been responsible for the high level of Staphylococcal-contamination. The
307 high level of *S. aureus* detected in raw milk may also be as a result of contamination by milk-
308 handlers potentially harboring the bacterium. Indeed, it has been reported that 50% of the
309 human population may be carriers of *S. aureus* as commensal microorganisms (Mathenge *et*
310 *al*., 2015).
311 In this study, we detected the genetic determinants that are responsible for the production of
312 enterotoxins from the *S. aureus* isolates. At least one type of *S. aureus* enterotoxin gene *(SE)*
313 was detected in 74.11 % of the isolates, similar to a previous study done in Kenya by Mathenge
314 *et al* (2015). Furthermore, other similar studies have detected high levels of *S. aureus* harboring
315 enterotoxin genes in milk and dairy products, one in Italy (Morandi *et al.* 2007), and two in
316 Japan (Omoe *et al*.,2002; Katsuda *et al.,* 2005) thereby corroborating our findings. Another
317 previous study done in Switzerland reported a high prevalence (65.2 %) of *S. aureus* isolates
318 carrying one or more enterotoxin gene (Scherrer *et al*., 2003); a finding closer to that observed
319 in the current study. Therefore, from these reports, it appears that enterotoxin- producing *S.*
320 *aureus* is gaining global significance and may not just be a problem of pastoral communities
321 alone. We therefore recommend creation of awareness on good hygienic practices during milk
322 handling. Pasteurization of raw milk is also encouraged in the region to avoid the risk.
323 The *see* gene exhibited the highest prevalence (60 %) in this study. It is carried by a prophage
324 (Cao *et al*., 2012) and can be easily disseminated among *Staphylococcus* Spp. strains. In
325 another study, Normanno *et al,* (2005) suggested that the classical *sea* is the most frequently

326 observed enterotoxin gene in enterotoxigenic strains of *S. aureus*; however in our study it was
327 the second highest (22 %) of the strains. This is probably due to the fact that different strains
328 from different foods carry different enterotoxins, as observed elsewhere (Asiimwe *et al.*2017).
329 A previous study in Nairobi, Kenya revealed that 4.17 % of *S. aureus* isolated from raw milk
330 produced SEA on latex agglutination test (Ombui *et al*. 1992). Staphylococcal enterotoxin A
331 is frequently associated with food poisoning since it is toxic at low concentrations (Morandi *et*
332 *al*, 2007). Enterotoxin A is produced at the beginning of the exponential phase and its
333 expression is not regulated by the accessory gene regulator (*agr*), different from enterotoxins
334 B, C, and D, which depend on the *agr* system for maximum expression (Balaban and Rasooly,
335 2000: J.-A. Hennekinne *et al.,* 2011).
336 The *sec* gene is located on pathogenicity islands and can be divided into three subtypes (sec1,
337 sec2, and sec3) based on antigenic differences and on the animal host associated with it. Some
338 studies suggest that the heterogeneity of enterotoxin C is related to selection for modified *sec*
339 sequences that facilitate the survival of *S. aureus* in their respective hosts (Smyth *et al*, 2005).
340 In the present study, *sec* was the third most common classical enterotoxin after *see* and *sec.*

341 The *SED* gene was not detected in any of the strains studied. The *SED* gene is located on
342 plasmid pIB485 (Hennekinne *et al.,* 2011) and enterotoxin D is the second most common toxin
343 associated with food poisoning (Balaban and Rasooly, 2000). A small amount of this
344 enterotoxin is able to cause illness, mainly in children and the elderly (Aydin *et al.,* 2011).
345 Nonetheless, the absence of *sed* in the strains studied here suggests that it is scarcely related
346 with *Staphylococcus* Spp. isolates from raw milk in Northern Kenya and consequently low risk
347 of causing food poisoning. In this study, 21 isolates of the 85 (25 %) had more than one
348 enterotoxin gene. Based on various studies, *se* genes can be located on plasmids (*sed* and *sej*,
349 phages (*sea* and *see*), pathogenicity islands (*seb* and *sec* and chromosomes (*seg*, *sew,* and *sej*);
350 therefore, several *se* genes can be harbored by enterotoxigenic *S. aureus* strains (Asiimwe *et*

351 *al.*2017). This study reports the first case of staphylococcal enterotoxin genes in milk from the
352 pastoral region of northern Kenya.

353 The detection of *S. aureus* enterotoxin genes isolated from milk of goats is important since
354 information from this region of the country is scarce. Enterotoxigenic *S. aureus* was high in
355 milk samples from goats (45 %) followed by Cattle (27 %) and sheep (2 %) (Table3). The
356 presence of the SEs was probably due to improper hygiene and poor management practices
357 among the pastoralists. High prevalence of *sea* and *see* genes in goat milk in this study may be
358 difficult to understand, however, improving the hygienic conditions of the milking environment
359 and/or utensils may reduce the prevalence of *S. aureus* in milk and prevent its transmission to
360 humans. (Abo- Shama, 2014)

361 Raw milk is one of the leading foods contaminated with *S. aureus* (Aydin *et al*., 2011). The
362 risk of infections and staphylococcal food poisoning increases when food contaminated with
363 *S. aureus* is not cooked properly or when the bacterium contaminate some ready-to-eat food
364 by cross contamination. It is reported that about 95% of staphylococcal food poisoning (SFP)
365 are associated with the classical SEs namely *SEA, SEB, SEC, SED*, and *SEE* (Aydin *et al.,*
366 2011). Indeed, some of the genes encoding the classical SEs were detected in many *S. aureus*
367 isolates identified in this study indicating that these SEs may be produced by the bacterium
368 thereby accumulating in raw milk and cause food poisoning especially if such a product is
369 stored at inappropriate temperature.

370 **4. Conclusion**

371 *Staphylococcus aureus* strains harbouring genes responsible for production of enterotoxins that
372 cause food poisoning were detected for the first time in raw milk from livestock in northern
373 Kenya. This has a big implication on the safety of milk and milk products consumed in the
374 region. The risk of staphylococcal food poisoning could be increased by consumption of raw

375 and sour milk by the pastoral communities. Extensive studies aimed at prevention and control
376 of milk contamination by enterotoxin producing *S. aureus* should be done in the pastoralist
377 regions. Regular surveillance on prevalence of *S. aureus* in raw milk and enterotoxin gene
378 carriage is recommended in order to monitor the risk of food poisoning with *S. aureus*.
379
380 **Funding Information**
381 This study was part of a project entitled Accelerated Value Chains Development – Livestock
382 Value Chains that was jointly funded by the United States Agency for International
383 Development (USAID) and the CGIAR Research Program Agriculture for Nutrition and Health
384 (A4NH).
385
386 **Conflict of interest statement**
387 The authors declare no conflict of interest. The findings and conclusions in this paper are those
388 of the authors and do not necessarily represent the official position of the participating
389 institutions or the funding organization.

390 **Acknowledgements**
391 We thank all participants for agreeing to be part of this study. We also thank the staff of the
392 Accelerated Value Chains Development project for mobilization of farmers and coordination
393 of field work activities in Isiolo and Marsabit counties respectively.
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578 **Figure Legends**

579 **Figure 1.** A map of Kenya showing the counties where the samples were collected and

580 questions administered; B) shows specific maps of Isiolo and Marsabit counties

581 **Figure 2.** PCR amplification of S. aureus-terminase gene from representative isolates obtained

582 from milk samples. The amplification of the gene is seen by presence of a specific band

583 corresponding to approximately 500bp.

584 **Figure 3.** Gel showing PCR amplified products of *S. aureus*-enterotoxin gene from

585 representative isolates obtained from milk samples.

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Figure 1. Omwenga *et al.,* 2018

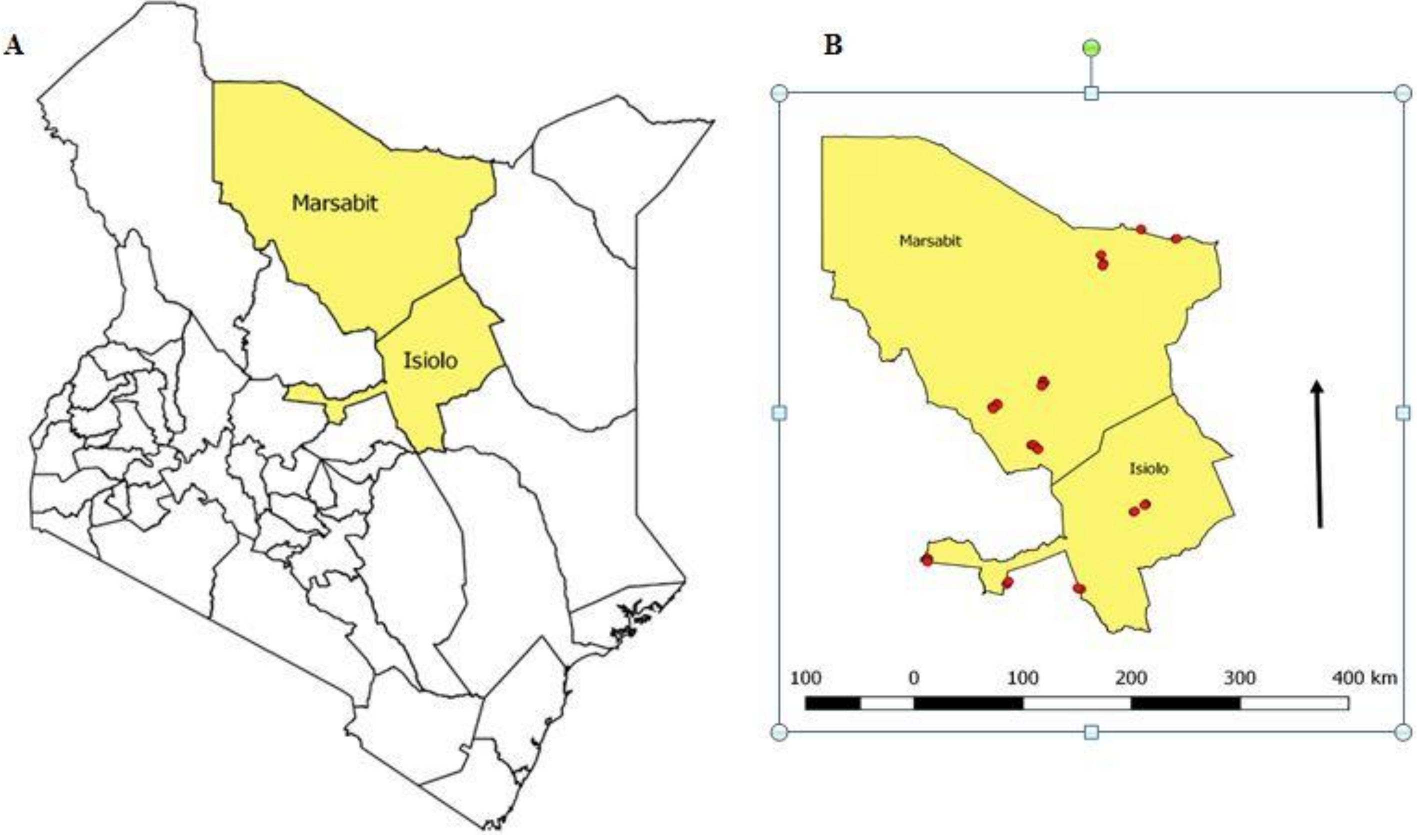


Figure 2. Omwenga *et al*., 2019

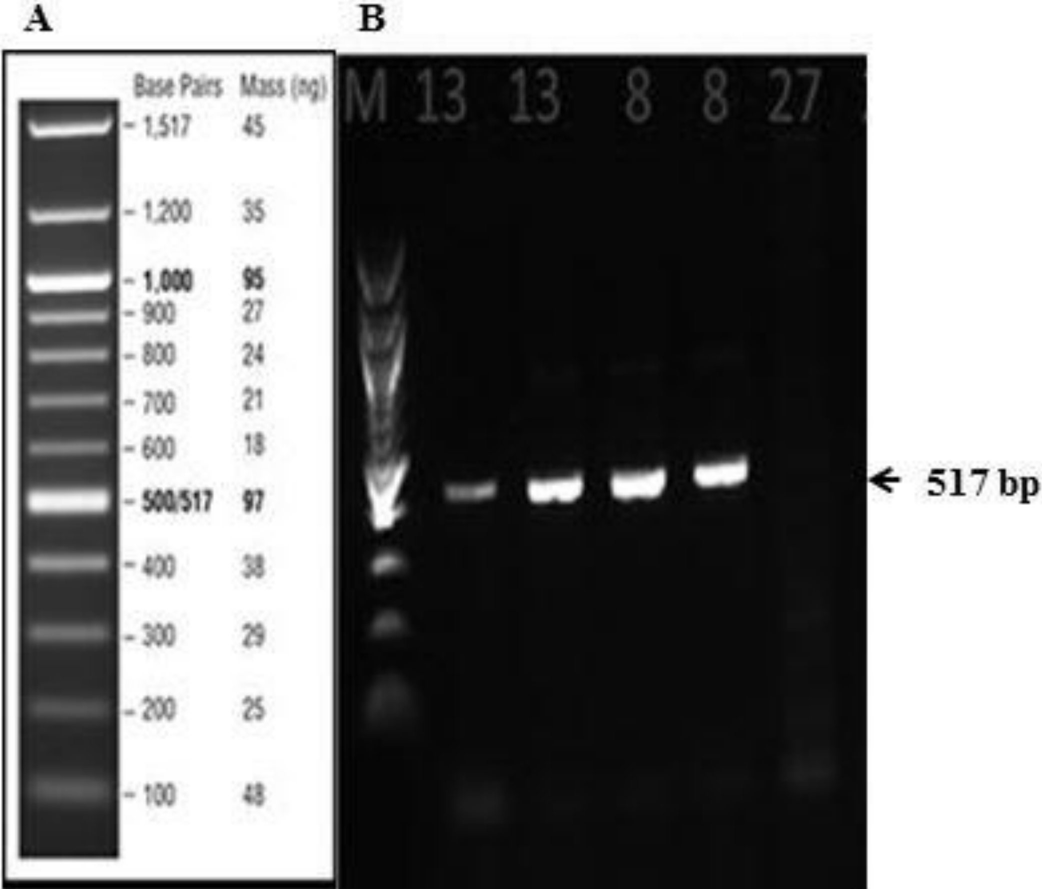
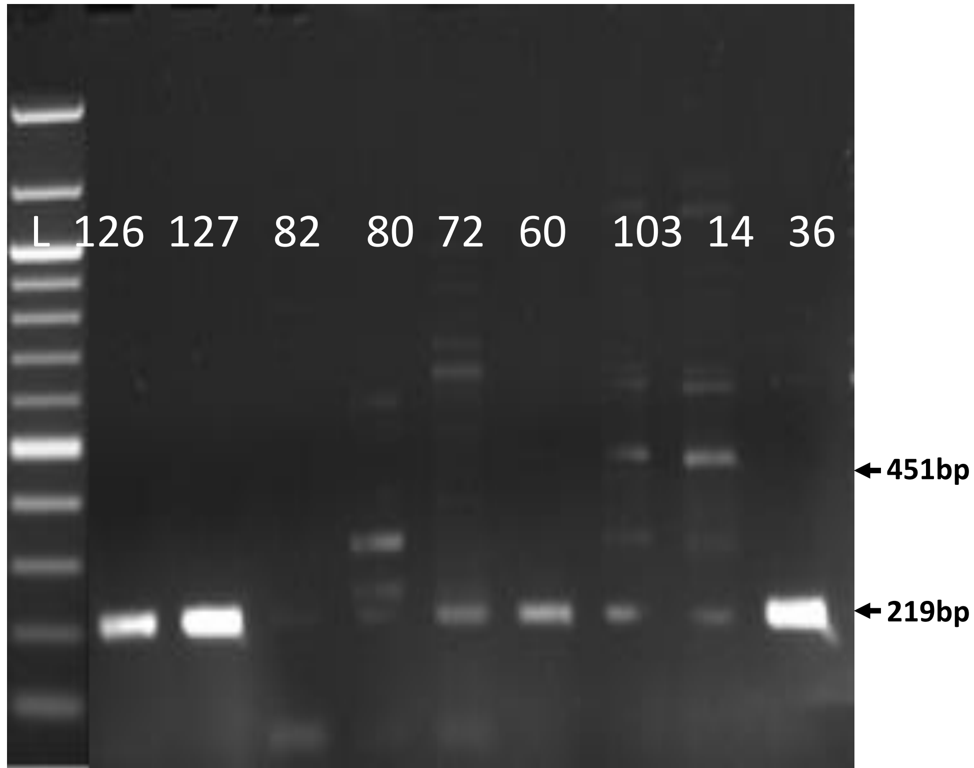


Figure 3. Omwenga *et al*. 2018



List of tables

Table 1. Primers used for the PCR amplification of *S. aureus* enterotoxin genes

Gene	Primer name	Oligonucleotide sequence (5'- 3')	Amplicon size (bp)
sea	GSEAR1[1]	5'- GGTTATCAATGTGCGGGTGG-3'	102
	GSEAR2[1]	5'- CGGCACTTTTTTCTCTTCGG – 3'	
seb	GSEBR1[1]	5'- GTATGGTGGTGTAACTGAGC – 3'	164
	GSEBR2[1]	5'- CCAAATAGTGACGAGTTAGG- 3'	
sec	GSECR1[1]	5'- AGATGAAGTAGTTGATGTGTATGG-3'	451
	GSECR2[1]	5'- CACACTTTTAGAATCAACCG-3'	
sed	GSEDR1[1]	5'- CCAATAATAGGAGAAAATAAAAG-3'	278
	GSEDR2[1]	5'- ATTGGTATTTTTTTTCGTTC-3'	
see	GSEER1[1]	5'- AGGTTTTTTCACAGGTCATCC-3'	209
	GSEER2[1]	5'- CTTTTTTTTCTTCGGTCAATC-3'	

Primers adopted from Mehrotra, et al., (2000)

Table 2. Enterotoxin genes of *S. aureus* isolates detected by PCR and sequencing

Enterotoxin gene	Overall (%, n=85)	Marsabit (%, n=43)	Isiolo (%, n=42)
1. *see*	51(60)	25(58.1)	26(61.9)
2. *sea*	22(25.9)	13(30.2)	9(21.4)
3. *sec*	18(21.2)	11(25.6)	7(16.7)
4. *seb*	9 (10.6)	9(20.9)	0(0)
5. *sec/see*	11(12.9)	7(16.3)	4(9.5)
6. *seb/see*	4(4.7)	4(9.3)	0(0)
7. *sea/sec*	8(9.4)	6(14)	2(4.8)
8. *sea/sec/see*	5(5.9)	3 (7)	2 (4.8)
9. *sea/seb/sec*	1(1.2)	1(2.3)	0(0)

Table 3: Distribution of *S. aureus* enterotoxin genes in milk from various species of animals in Isiolo and Marsabit counties

	Overall	**Marsabit**					**Isiolo**				
	Enterotoxin gene *(%)*	*sea*	*seb*	*sec*	*sed*	*see*	*sea*	*seb*	*sec*	*sed*	*see*
Cattle	27(27)	0	5	2	0	9	3	0	2	0	6
Goats	45(45)	10	0	5	0	16	4	0	3	0	7
Sheep	2(2)	0	0	0	0	0	1	0	0	0	1
Camels	0	0	0	0	0	0	0	0	0	0	0
Pooled milk	26(26)	3	6	4	0	0	1	0	2	0	12