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East and West African milk products are reservoirs for human and livestock-associated *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus frequently isolated from milk products in sub-Saharan Africa (SSA) is a major pathogen responsible for food intoxication, human and animal diseases. SSA hospital-derived strains are well studied but data on the population structure of foodborne *S. aureus* required to identify possible staphylococcal food poisoning sources is lacking. Therefore, the aim was to assess the population genetic structure, virulence and antibiotic resistance genes associated with milk-derived *S. aureus* isolates from Côte d'Ivoire, Kenya and Somalia through *spa*-typing, MLST, and DNA microarray analysis. Seventy milk *S. aureus* isolates from the three countries were assigned to 27 *spa* (7 new) and 23 (12 new) MLST sequence types. Milk-associated *S. aureus* of the three countries is genetically diverse comprising human and livestock-associated clonal complexes (CCs) predominated by the CC5 (n = 10) and CC30 (n = 9) isolates. Panton-Valentine leukocidin, toxic shock syndrome toxin and enterotoxin encoding genes were predominantly observed among human-associated CCs. Penicillin, fosfomycin and tetracycline, but not methicillin resistance genes were frequently detected. Our findings indicate that milk-associated *S. aureus* in SSA originates from human and animal sources alike highlighting the need for an overarching One Health approach to reduce *S. aureus* disease burdens through improving production processes, animal care and hygienic measures.

1. Introduction

Staphylococcus aureus can asymptomatically colonize the skin and nasal cavities of humans and animals. Roughly 20% of humans are persistent carriers while another 30% are considered to be intermittent carriers of *S. aureus* in the nasal vestibules (van Belkum et al., 2009). However, *S. aureus* is also responsible for a wide range of diseases in humans and animals, including skin and soft tissue infections, mastitis, severe invasive infections, Staphylococcal Food Poisoning, and Toxic Shock Syndrome (Tong et al., 2015). Pathogenicity, disease propagation and severity are largely influenced by a large variety of well characterized virulence factors such as the staphylococcal enterotoxins, toxic shock syndrome toxin (TSST-1), Panton-Valentine leukocidin (PVL), adhesins and immune evasion factors, capsule types, accessory gene regulator (*agr*) groups, proteases, DNases and lyases (Argudín et al., 2010; Krakauer and Stiles, 2013; Powers and Bubeck Wardenburg, 2014). However, while *S. aureus* is well characterized in the industrialized world, *S. aureus* is considered to be a neglected agent for disease in tropical and developing countries (Herrmann et al., 2013). This is despite the impact of *S. aureus* diseases on the general population, healthcare, food and livestock production systems of many communities in sub-Saharan Africa that in addition share a lifestyle of close daily livestock interactions (Akindolire et al., 2015; Egyir et al., 2014a, 2014b; Gitau et al., 2014; Maina et al., 2016;

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Abbreviations

ABR	Antibiotic resistance
CMT	California mastitis test
DLV	Double locus variant
CC	Clonal complex
MDR	Multi drug resistant
MLST	Multi locus sequence typing
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin susceptible *Staphylococcus aureus*
*n*LV	n locus variant (n = 4, 5, or 6)
PVL	Panton-Valentine leukocidin
SLV	Single locus variant
ST	Sequence type
TLV	Triple locus variant

Njage et al., 2013). Livestock products of cow, camel or goat origin contribute to the daily food supply in sub-Saharan Africa. Especially milk products including fermented milk products for increased shelf life are traditional staple food items in sub-Saharan Africa, providing important nutrients to pastoral and sedentarized communities (Elhadi et al., 2015; Iannotti and Lesorogol, 2014). High prevalence of zoonotic diseases, poor hygiene and production processes as well as long transportation routes without cooling render these milk products highly susceptible to the outgrowth of foodborne pathogens including *S. aureus* (Jans et al., 2012; Kouamé-Sina et al., 2012; Njage et al., 2013; Noor et al., 2013). This is exemplified by a high presence of clinical and subclinical mastitis and intramammary infections caused by *S. aureus* among dairy animals including camels in sub-Saharan Africa (Gitau et al., 2014; Issa et al., 2016; Regassa et al., 2013; Wanjohi et al., 2013). Furthermore, diarrhoea and vomiting in humans was especially associated with raw milk consumption (Kaindi et al., 2012) and possibly linked to the high prevalence of *S. aureus* in these milk products (Njage et al., 2013). Therefore, increased attention to foodborne *S. aureus* is required especially in terms of understanding the population structure, locally important lineages and their virulence factors to formulate appropriate interventions.

Population structure analysis by multi locus sequence typing (MLST) provides important epidemiological clues on emerging highly virulent lineages such as methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA) as well as associated clonal complexes (CCs) (Enright et al., 2000; Tong et al., 2015). There is some data available on the population structure of *S. aureus* for human and healthcare-associated settings, revealing a predominance of CC5, CC30, CC152 and typically CC88-MRSA-IV in sub-Saharan Africa (Abdulgader et al., 2015; Schaumburg et al., 2014). The emergence of MRSA in Africa is visible, but still significantly lower than in developed countries whereas PVL is highly prevalent, especially among MSSA isolates (Abdulgader et al., 2015; Schaumburg et al., 2014, 2015). While the presence of *S. aureus* and antibiotic resistances (ABR) including multi drug resistance (MDR) is well known in humans, animals and food products in sub-Saharan Africa, knowledge on population structure and virulence factors of *S. aureus* is still mostly limited to human and healthcare associated settings.

Therefore, the aim of this study is to provide a first insight into the population structure of *S. aureus* from raw and fermented milk products as major vehicles of zoonotic infections in relation to human and livestock-associated CCs. This was combined with a detailed investigation of virulence factor and ABR gene carriage in order to understand *S. aureus* epidemiology and public health risk potential within the tightly connected system of animals, food and humans in order to contribute towards the implementation of a One Health approach in *S. aureus* and ABR control strategies.

2. Material and methods

2.1. Milk sample origin

A total of 78 raw and fermented milk samples assigned into subsets 1–3 were incorporated into the current study to provide first insights into milk-associated *S. aureus* of geographically different regions of Africa, in particular *S. aureus* isolated from milk products from Kenya, Somalia and Côte d'Ivoire. The milk samples were collected during three independent studies designed to investigate the microbiota of dairy products in these three East (Kenya and Somalia) and West (Côte d'Ivoire) African countries. General characterization of subsets 1 and 2 milk samples including sample type, location, physicochemical properties and microbiota description were described in previous studies (Jans et al., 2012, 2013; Njage et al., 2013). Subset 1 (n = 28, Kenya and Somalia) and 2 (n = 15, Côte d'Ivoire) comprised 22 raw and 21 fermented milk samples collected in 2007, 2008 and 2010 (Supplementary Table S1) that originated from camel (n = 27), cow (n = 15) and goat (n = 1) milk. Subset 3 was collected and analysed independent of subsets 1 and 2 and the samples have not been previously characterized. It was comprised of raw milk samples collected from randomly selected camels from herds in Isiolo (two herds; n = 10) and Marsabit (one herd; n = 11), as well as milk sampled from public markets (n = 14) in Garissa in Kenya during 2008. Sampling at each location was done on separate days. Samples from camel herds were comprised of pooled udder milk derived from all four quarters. Udders were disinfected using 70% (v/v) ethanol and 3–4 strokes of foremilk were discarded prior to sample collection into sterile 50-mL Falcon tubes. Market raw milk samples (n = 14) were collected in sterile 50-mL Falcon tubes and processed as described previously (Jans et al., 2012). pH measurements and California Mastitis Test (CMT) were performed on all samples of subset 3.

2.2. Staphylococcus spp. source, origin, isolation and enumeration in milk samples

Previously isolated *S. aureus* stored in glycerol stock at −80 °C (n = 52; subset 1; Njage et al., 2013) or isolated during this study from milk samples previously not screened for *S. aureus* (subset 2 and subset 3; Jans et al., 2013) were examined. Subset 2 milk samples could only be qualitatively analysed for this study since long term storage of milk samples at −20 °C would have had an impact on bacterial survival to allow accurate quantitative analysis. The EN ISO 6888-2 protocol for the isolation of coagulase-positive staphylococci (ISO, 1999) was applied. Presumptive *S. aureus* isolates obtained based on colony morphology and presence of an opaque fibrin halo on rabbit plasma fibrinogen agar (Oxoid, Basel, Switzerland) were transferred on to 5% sheep blood agar plates and incubated at 37 °C for 24 h. Post incubation, *S aureus* isolates were confirmed using the Staphaurex latex agglutination test (Oxoid, Basel, Switzerland). For analysis of milk samples in subset 3 an identical isolation approach as previously used for subset 1 was applied during field studies in Kenya using Baird Parker agar medium supplemented with Egg yolk tellurite (BP, Biolife, Milan, Italy) and incubation under aerobic conditions at 37 °C for 2 days (Njage et al., 2013). Colonies of desired presumptive *S. aureus* morphology were picked and streak purified on BP agar. Enumeration of presumptive *S. aureus* performed on the Kenyan subset 3 was performed using serial dilutions followed by plating onto BP agar. Colonies of typical *S. aureus* morphologies on BP agar were further