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Genetic Features of *Staphylococcus aureus* from Tanzania: Strain-Specific Phenotypic Behaviors Typing of *S. aureus* within the African-German StaphNet Consortium

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List of Abbreviations

agr	Accessory Gene Regulator
AIDS	Acquired Immune Deficiency Syndrome
ARGs	Antimicrobial Resistance Genes
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
BDH	Bagamoyo District Hospital
BRTC	Bagamoyo Research and Training Center
BSA	Bovine Serum Albumin
CA-MRSA	Community-Acquired Methicillin-Resistant *S. aureus*
CC	Clonal complex
CRF	Case Report Form
CSFE	Carboxyl Fluorescein Diacetate Succinimidyl Ester
CTC	Care and Treatment Clinic
FDNA	Deoxyribonucleic acid
DFG	German Research Foundation
DNA-MCA	DNA microarray
d-UTP	2'-Deoxyuridin-5'-Triphosphate
EDTA	Ethylene diamine tetra acetic acid
FACS	Fluorescence Activated Cells Sorting
FCS	Foetal Calf Serum
FR	Freiburg
HaCat	Human Epidermal Keratinocytes
HA-MRSA	Healthcare-Acquired Methicillin Resistant *S. aureus*
HIV	Human Immunodeficiency Virus
HS	Homburg/Saar
IEC	Immune Evasion Cluster
IHI	Ifakara Health Institute
IT	Ifakara/Tanzania
LDH	Lactate Dehydrogenase
LG	Lambaréné/Gabon

MALDI-TOF	Matrix Assisted Laser Desorption Ionization - Time of flight
MDR	Multidrug-resistant
MGE	Mobile Genetic Element
MHC	Major Histocompatibility Complex
MLST	Multi-Locus-Sequence-Typing
MM	Manhiça/Mozambique
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSCRAMMs	Microbial Surface Component Recognizing Matrix Molecules
MSSA	Methicillin-Sensitive *Staphylococcus aureus*
MW	Münster/Westfalen
OD	Optical Density
PBS	Phosphate Buffer Saline
PCR	Polymerase-Chain-Reaction
PFGE	Pulsed-field-gel-electrophoresis
PMN	Polymorphonuclear Neutrophil
PVL	Panton-Valentine Leukocidin
RPM	Revolution per minute
S. aureus	*Staphylococcus aureus*
SCC	Staphylococcal cassette chromosome
SN	Single Nucleotide Polymorphism
SSSS	Staphylococcal-Scalded-Skin-Syndrome
ST	Sequence Type
TBE-Buffer	Tris-Borate-EDTA-Buffer
TSA	Trypticase Soy Agar
TSB	Trypticase Soy Broth
TNF	Tumor necrosis factor
TSST-1	Toxic shock-syndrome toxin
UKS	University Hospital of Saarland
VISA	Vancomycin-intermediate resistant *Staphylococcus aureus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WHO	World Health Organization

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SUMMARY

1.1 English version

The Gram-positive bacterium *Staphylococcus aureus* is a common colonizer of mammals and is regularly found in humans, particularly in the nasopharynx, on the skin, and in the intestine. At the same time, this bacterium is a feared cause of a large number of different infectious diseases that occur both in the community and in the hospital environment. The bacterial species exhibits a high degree of genetic heterogeneity and differs in the repertoire of genes that code for virulence factors. On the genetic level, the species *S. aureus* can be divided into different clonal complexes (CCs) or sequence types (STs) using modern typing methods. While the genetic composition of CCs/STs circulating in society or the hospital environment is well studied for the Western world, our knowledge of the genetic composition of CCs/STs circulating in the community/healthcare system is quite fragmentary for many regions of Africa. Similarly, very few phenotypic studies exist on the virulence characteristics of *S. aureus* isolates circulating on this continent. Therefore, one of the primary objectives of this PhD thesis was to collect information on the genetic composition of *S. aureus* strains circulating in Tanzania and their virulence potential. A second aim of this dissertation was to investigate whether there are differences in the composition and virulence potential of *S. aureus* isolates circulating asymptomatically in the community or causing infections in Tanzania, and which antibiotic resistances can be found in these isolates.

For this purpose, I collected *S. aureus* isolates that on the one hand led to community-acquired infections in the Bagamoyo area and on the other hand originated from asymptomatic (nasal) carriers. These isolates were subsequently genotypically characterized using DNA microarray technology, and a representative selection of these isolates were phenotypically examined using various test methods with regard to their virulence potential and antibiotic resistance profile. For the latter investigations, a selection of isolates collected and genotypically characterized by the African-German StaphNet Consortium was added. These investigations revealed that the *S. aureus* strain repertoire circulating in the Bagamoyo area of Tanzania is very heterogeneous on the genotypic level. At the CC level, CCs 152, 121, 8, 15, 88, and 5 were the most commonly found CCs in this region, each accounting for between 13 and 9%. Interestingly, the two most frequently found CCs, CC152 (13%) and CC121 (12%), were preferentially isolated from infection, while isolates of CCs 15 and 8 were mainly obtained from asymptomatic carriers. Concerning the virulence factor repertoire, larger differences could be identified between the individual isolates, but also at the CC level, which were particularly striking with regard to the repertoire of cell-damaging toxins such as hemolysins and leukocidins. For example, the majority of isolates of CCs 152, 121, 88, 80 and 30 harbored the *lukF-PV* and *lukS-PV* genes coding for the Panton-Valentine leukocidin (PVL) subunits F and S, respectively, whose gene products are thought to play an important role in the development of necrotic *S. aureus* infections. Moreover, unlike almost all other isolates of this strain collection, CC152 isolates harbored an intact *hlb* gene, whose gene product, β-hemolysin, plays an important role in *S. aureus* biofilm formation and host tissue damage, while the gene *seb*, which codes for enterotoxin B, was preferentially found in isolates of CC121.

Interestingly, all four virulence genes were detected significantly more frequently in infection-associated isolates than in isolates obtained from nasal swabs.

The resistance gene profiles of *S. aureus* isolates circulating in Bagamoyo, Tanzania, showed a low prevalence for the methicillin-resistance mediating gene *mecA* (2.7%), but a very high prevalence for the penicillinase gene *blaZ* (99.6%) and *fosB* (74%), encoding a metallothiol transferase conferring resistance to fosfomycin. In addition, increased rates of the resistance determinants *ermC* (56%, mediating macrolide/linkosamide/streptogramin resistance) and *tetK* (47%, conveying tetracycline resistance) were detected in CC152 isolates. Phenotypic antimicrobial susceptibility testing confirmed the high penicillin-resistance rate (>90%), and revealed considerable frequencies of resistance to the commonly prescribed antibiotics erythromycin (29%), clindamycin (24%), and tetracycline (19%).

My strain-specific phenotypic behavior typing studies of *S. aureus* isolates circulating in Tanzania and from the African-German StaphNet Consortium revealed that isolates of CC152 had a higher virulence potential than isolates of other CCs in almost all test procedures. Treatment of human erythrocytes with supernatants of CC152 isolates resulted in hemolysis titers that were higher than those induced by isolates of other CCs tested. Similarly, treatment of human keratinocytes with supernatants of CC152 isolates resulted in significantly more cell damage than with other CCs tested. However, when the virulence potentials of the isolates were correlated with their origin, in most of the assays carried out, no clear difference was found with regard to whether the isolates were obtained from infection or isolated from asymptomatic donors.

Taken together, these studies indicate that infections caused by CC152 isolates represent an increased risk for the patient to develop a more severe course of disease. However, the lack of clear differences in virulence potential between isolates recovered from clinical infection and those recovered from asymptomatic donors suggests that commensal isolates have a similar potential to infect humans as infection-related isolates. The very high frequencies of *blaZ* and *fosB* in Tanzanian *S. aureus* isolates indicate that antibiotics such as penicillin and fosfomycin are widely used on the community level in this geographical region.

1.2 German version:

Das Gram-positive Bakterium *Staphylococcus aureus* ist ein häufiger Kolonisierer von Säugetieren und beim Menschen insbesondere im Nasen-Rachenraum, auf der Haut und im Darm vorzufinden. Gleichzeit ist dieses Bakterium ein gefürchteter Verursacher einer Vielzahl von verschiedenen Infektionskrankheiten, die sowohl in der Gesellschaft als auch im Krankenhausumfeld auftreten. Die Bakterienart selbst weißt eine hohe genetische Heterogenität auf und unterscheidet sich insbesondere in Hinblick auf das Repertoire an Genen, die für Virulenzfaktoren codieren. Die Spezies *S. aureus* lässt sich mit modernen Typisierungsverfahren in verschiedene klonale Komplexe (CCs) bzw. Sequenztypen (STs) unterteilen. Während für die westliche Welt die genetische Zusammensetzung der in der Gesellschaft oder im Krankenhausumfeld kursierenden CCs/STs gut untersucht ist, ist unser Wissen um die genetische Zusammensetzung der in vielen Regionen Afrikas in der Gesellschaft und im Gesundheitswesen kursierenden CCs/STs noch sehr fragmentarisch. Ebenso existieren nur sehr wenige phänotypische Untersuchungen zu den Virulenzeigenschaften der auf diesem Kontinent kursierenden *S. aureus* Isolate. Daher war es eines der vorrangigen Ziele dieser Doktorarbeit, Informationen über die genetische Zusammensetzung der in Tansania kursierenden *S. aureus* Stämme und deren Virulenzpotential zu sammeln. Des Weiteren sollte im Rahmen dieser Dissertation untersucht werden, ob es Unterschiede in der Zusammensetzung und im Virulenzpotential der in Tansania in der Gesellschaft asymptomatisch kursierenden bzw. infektionsauslösenden *S. aureus* Isolate gibt und welche Antibiotikaresistenzen in diesen Isolaten vorzufinden sind.

Um diese Ziele zu erreichen, wurden von mir *S. aureus* Isolate gesammelt, die zum einen in der Region Bagamoyo zu aus der Gesellschaft erworbenen Infektionen führten und zum anderen von asymptomatischen Trägern stammten. Diese wurden nachfolgend mithilfe der DNA-Microarray-Technologie genotypisch charakterisiert und eine repräsentative Auswahl dieser Isolate mittels verschiedener Untersuchungsmethoden phänotypisch in Hinblick auf ihr Virulenzpotential und ihr Resistenzprofil hin untersucht. Für letztere Untersuchungen wurde des Weiteren auch auf eine Auswahl von Isolaten zurückgegriffen, die über das Afrikanisch-Deutsche StaphNet-Konsortium gesammelt und genotypisch charakterisiert wurden. Diese Untersuchungen zeigten, dass das in der Region Bagamoyo, Tansania, kursierende *S. aureus* Kollektiv, genotypisch betrachtet, insgesamt sehr heterogen ist. Auf klonaler Ebene waren in dieser Region vor allem die CCs 152, 121, 8, 15, 88 und 5 vorzufinden, deren Anteil jeweils zwischen 13 und 9% betrug. Interessanterweise wurden die beiden am häufigsten vorgefundenen CCs, CC152 (13%) und CC121 (12%), bevorzugt aus dem Infektionsgeschehen heraus isoliert, während Isolate der CCs 15 und 8 mehrheitlich von asymptomatischen Trägern gewonnen wurden. Auch in Hinblick auf das Virulenzfaktor-Repertoire konnte zwischen den einzelnen Isolaten, aber auch auf CC-Ebene, zum Teil große Unterschiede identifiziert werden, die insbesondere in Hinblick auf das Repertoire an zellschädigenden Toxinen wie Hämolysine und Leukozidine auffällig waren. So wiesen Isolate der CCs 152, 121, 88, 80 und 30 mehrheitlich die für die Panton-Valentine Leukozidin Untereinheiten F und S kodierenden Gene *lukF-PV* and *lukS-PV* auf, deren Genprodukte eine wichtige Rolle bei der Entstehung nekrotischer *S. aureus* Infektionen zugeschrieben wird. Isolate des CCs 152 wiesen zudem, anders als nahezu alle anderen Isolate dieses Stammsets, ein intaktes *hlb* Gen auf,

dessen Genprodukt, das β-Hämolysin, wichtige Funktion bei der Biofilmbildung von *S. aureus* und der Schädigung von Wirtsgewebe übernimmt, während das Gen *seb*, welches für das Enterotoxin B kodiert, bevorzugt in Isolaten des CCs 121 vorgefunden wurde. Spannenderweise wurden alle vier Gene deutlich häufiger in Infektions-assoziierten Isolaten detektiert als in Isolaten, die aus Nasenabstrichen gewonnen wurden.

In Hinblick auf die Resistenzgenprofile der in Bagamoyo, Tansania, kursierenden *S. aureus* Isolate zeigte sich eine niedrige Prävalenz für das Methicillinresistenz-vermittelnde Gen *mecA* (2.7%), aber sehr hohe Prävalenzen für das Penicillinase-Gen *blaZ* (99.6%) und für *fosB* (74%), welches für eine Metallothiol-Transferase kodiert, die eine Fosfomycinresistenz vermittelt. In CC152 Isolaten wurden des Weiteren erhöhte Raten der Resistenzdeterminanten *ermC* (56 %, vermittelt eine Resistenz gegenüber Makrolid/Linkosamid/Streptograminantibiotika) und *tetK* (47 %, vermittelt eine Resistenz gegenüber Tetracyclinen) festgestellt.

In meinen phänotypischen Untersuchungen der in Tansania kursierenden *S. aureus* Isolate zeigte sich, dass Isolate des CCs 152 in nahezu allen Testverfahren ein höheres Virulenzpotential aufwiesen als die Isolate anderer CCs. So führte die Behandlung von menschlichen Erythrozyten mit Überständen von CC152 Isolaten zu einem Hämolyse-Titer, der sich mehrheitlich signifikant von den Hämolyse-Titern unterschied, die mit den Isolaten der anderen CCs ermittelt wurden. Des Weiteren führte die Behandlung von humanen Keratinozyten mit Überständen der CC152 Isolaten zu einer deutlich stärkeren Zellschädigung, wenn mit der Zellschädigung anderer CCs verglichen. Wurden hingegen die Virulenzpotentiale der Isolate mit deren Ursprung korreliert, so zeigte sich in der überwiegenden Mehrheit der Untersuchungen keine klaren Unterschiede in Hinblick darauf, ob die Isolate aus dem Infektionsgeschehen heraus isoliert wurden oder von asymptomatischen Spendern gewonnen wurden. Die antimikrobiellen Empfindlichkeitstestungen bestätigten die hohe Penicillinresistenzrate (>90%) und brachten eine erhöhte Häufigkeit von Resistenzen gegenüber den in Tansania häufig im Klinikalltag genutzten Antibiotika Erythromycin (29%), Clindamycin (24%) und Tetracyclin (19%) zum Vorschein.

Zusammengenommen weisen diese Untersuchungen darauf hin, dass Infektionen, die durch CC152 Isolate verursacht werden, ein erhöhtes Risiko für den Patienten darstellen, einen schwereren Krankheitsverlauf zu entwickeln. Das Fehlen eindeutiger Unterschiede im Virulenzpotenzial zwischen aus klinischer Infektion gewonnener Isolate und aus der Nase von asymptomatischen Spendern gewonnener Isolate lässt jedoch darauf schließen, dass kommensale Isolate ein vergleichbares Infektionspotenzial für den Menschen aufweisen wie klinische Isolate. Das häufige Vorkommen von *blaZ* und *fosB* in den von mir untersuchten Stämmen aus der Region Bagamoyo, Tansania, weist darauf hin, dass Antibiotika wie Penicillin und Fosfomycin in dieser geographischen Region im Haushaltsgebrauch sehr häufig Verwendung finden.

2. INTRODUCTION

2.1 Background

Staphylococcus aureus, a Gram-positive round-shaped bacterium, is of significant importance in public health. Belonging to the Staphylococcaceae family, it is a well-known commensal organism frequently residing in the upper respiratory tract, on the skin, and on the mucous membranes of mammals. A striking fact is that approximately 20 - 30% of the global healthy human population persistently carries *S. aureus* in their nasal passages, while most of the population may intermittently serve as carriers (1,2). Colonization with *S. aureus* is associated with an elevated risk of both, endogenous subsequent infections and the transmission of the pathogen to others in close contact (3). The bacterium can cause a wide spectrum of infections in livestock and humans, ranging from superficial to invasive diseases (4–6). In humans, *S. aureus* is a common cause of diseases such as uncomplicated skin and soft tissue infections (SSTIs) like pimples, boils, carbuncles, impetigo, cellulitis, folliculitis, and abscesses (7). However, especially in immunocompromised humans, the pathogen may also cause life-threatening diseases such as bacteremia, sepsis, pneumonia, osteomyelitis, endocarditis, and meningitis (8). Depending on its toxin repertoire, certain *S. aureus* strains may cause toxin-mediated diseases such as staphylococcal food poisoning (SFP), toxic shock syndrome (TSS), staphylococcal scalded skin syndrome, or necrotizing pneumonia (9,10).

The transmission of *S. aureus* infections can occur through contact with asymptomatic carriers or direct contact with actively infected individuals. Inanimate objects used by infected persons, such as clothing, towels, or bedding, can also serve as vehicles for transmission. Despite of the fact that *S. aureus* is not capable of forming permanent states (spores), the pathogen exhibits a remarkable ability to survive in various environments, making it a leading cause of both community and hospital-acquired infections (11). The pathogenicity of *S. aureus* to humans depends largely on the genetic repertoire of virulence factors and regulatory elements and may differ substantially between individual strains. With about only 80% of the genome being conserved between individual *S. aureus* isolates, this bacterium belongs to the more heterogenous species in the phylum Firmicutes (12). From a phylogenetic perspective, most of the isolates circulating in the human and livestock populations can be grouped into different clonal complexes (CCs). Members of a CC are usually closely related to each other and clearly separated from members of other CCs. Another clinically important characteristic of *S. aureus* is its ability to acquire resistances to virtually any antibiotic currently on the market. While antimicrobial therapy remains a crucial strategy to combat *S. aureus* infections, its effectiveness has been compromised by the emergence and dissemination of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). These resistant strains pose significant challenges in the treatment of infections caused by *S. aureus* and have been a cause for concern in the medical community for several decades (13). Despite a century of awareness regarding

antibiotic resistance in *S. aureus*, it continues to be a formidable and pervasive pathogen with an unsettling ability to develop resistance even to last-resort systemic antibiotics like glycopeptides. In 2017, the global health organizations designated *S. aureus*, particularly MRSA and VRSA, as priority antimicrobial-resistant pathogens requiring heightened research efforts for the development of new antimicrobial agents (14) , and this categorization was recently confirmed for MRSA(15). Thus, addressing the issue of antimicrobial resistance in *S. aureus* remains a critical focus in the field of infectious diseases and microbiology.

2.2 History of *S. aureus*

Historically, *Staphylococcus aureus* is one of the oldest bacteria to be documented. Its discovery can be traced back to the year 1881 when Sir Alexander Ogston, a Scottish surgeon, first encountered this bacterium, while examining pus discharges from an infected wound, a seminal moment in the understanding of infectious agents (16). Sir Ogston named this newly discovered bacterium "Staphylococcus," drawing from the Greek words "staphyle" (meaning a bunch of grapes) and "kokkos" (meaning berry) about its grape-like microscopic appearance (16,17).

In 1884, another pioneering scientist, German physician Friedrich Julius Rosenbach, went a step further by giving this bacterium the name we now recognize: "*Staphylococcus aureus*." He made this distinction to differentiate it from its sibling bacterium, *Staphylococcus epidermidis* (formerly known as *Staphylococcus albus*). This distinction was made based on the colour of their colonies, with *S. aureus* forming golden or yellowish colonies and *S. epidermidis* forming colonies of a different hue (8, 18, 19). In the early 1930s, the coagulase test emerged as a more efficient method for differentiation between the coagulase-positive *S. aureus* and other coagulase-negative Staphylococcus species (CoNS, *i.e. S. epidermidis*, *S. haemolyticus*, *S. lugdunensis*, etc)(20). The enzyme coagulase activates the host factor prothrombin to convert fibrinogen to fibrin and is produced within the Genus Stapylococcus almost exclusively by *S. aureus* (21).

Besides coagulase, *S. aureus* possesses several distinctive features that collectively serve as noteworthy signatures for its basic identification in diagnostic microbiology. These characteristics include its Gram-positive reaction, its round-shaped cells that may appear as single cells or in pairs, tetrads, or irregular grape-like clusters. Another distinguishing feature is its classic gold pigmentation (staphyloxanthin) and the ability to induce a complete hemolysis of red blood cells called beta (β)-hemolysis (22,23); though are only weak distinguishing features, as *S. aureus* variants with neither staphyloxanthin nor (β)-hemolysin do exist (24,25). The bacterium is also known for its capacity to secret nucleases, and the fermentation of mannitol, resulting in the production of acid (9,26). These collective characteristics have long been essential in the identification and differentiation of *S. aureus* in the realm of diagnostic microbiology.

2.3 The epidemiology of *S. aureus*

S. aureus stands out as one of the most widespread staphylococcal species, exhibiting a remarkable ability to thrive in diverse habitats and hosts. Its reach extends to humans and animals, and it serves as an environmental reservoir, persisting in various settings, including soil, food, plants, water, and surfaces such as clothing, utensils, and furniture (27,28). Globally, *S. aureus* is recognized as a pathogen of significant concern in public health. Its success as a prominent human pathogen can be primarily attributed to its virulence capabilities and its ubiquitous presence as a commensal organism in the human population (29).

Within the immunocompetent human host, *S. aureus* establishes itself as a commensal organism, commonly colonizing the upper respiratory tract, particularly the nostrils, and external skin surfaces. It is also found in areas such as the gastrointestinal tract (GIT) and inguinal regions (7,30). The prevalence of normal nasal carriage of *S. aureus* can vary among populations. Estimates suggest that approximately 20-30% of healthy individuals may serve as transient or long-term carriers of *S. aureus* (3,31). However, in some populations, the rate of *S. aureus* colonization is notably higher, exceeding 50%. A high colonization rate is also observed in individuals with underlying health conditions or co-morbidities, such as diabetes, those undergoing dialysis, individuals with human immune-deficiency virus (HIV), recipients of surgical procedures, patients with extended hospital stays, and those utilizing medical devices like catheters (32).

Conversely, *S. aureus*, as a significant pathogenic agent, is responsible for a wide spectrum of diseases, broadly categorized into three main groups.{1}Superficial skin and soft tissue infections (SSTIs): These include various skin infections characterized by lesions, abscesses, and localized inflammation.{2} Systemic and life-threatening infections: This category encompasses severe infections affecting vital organs and systems, such as endocarditis, osteomyelitis, pneumonia, meningitis, and bacteremia.{3} Toxinoses: Many *S. aureus* isolates produce toxins that lead to conditions such as food poisoning, scalded skin syndrome, and toxic shock syndrome (TSS)(33). In SSTI, lesions typically manifest as abscesses filled with pus and damaged white blood cells, surrounded by necrotic tissue. The severity of an infection often depends on the individual's immune response, the virulence factor repertoire of the specific *S. aureus* strain, and the size of the bacterial inoculum (34,35).

The source of *S. aureus* infection can be attributed to either endogenous or exogenous factors, acquired from carriers (colonization) or actively infected individuals (36–38). Human-to-human transmission of *S. aureus* occurs primarily through direct contact from one person to another. Secondary transmission may involve contact with pus, dried exudate discharges from *S. aureus*-infected individuals, or the sharing of personal items such as clothing, towels, or bed sheets (9,39). Additionally, transmission can occur via fomite-contaminated surfaces like doorknobs and through the ingestion of contaminated food (40–42).With the emergence of CC398, livestock also constitutes a relevant reservoir for *S. aureus* colonization and infection.

In fact, starting as a comparably rare observation in human clinics at the beginning of this century (43), this CC is nowadays the most prominent variant isolated from bloodstream infections seen in intensive care units in France (44) and Switzerland (45). *S. aureus* exhibits the potential to cause both localized and disseminated infections, affecting nearly all human tissues and anatomical sites (see Table 1 below).

Table 1: Human infections caused by *S. aureus*

Infection site	Clinical syndrome
Skin and soft tissues	Boils, abscesses, impetigo, wound infection, scalded skin syndrome, necrotizing fasciitis, cellulitis
Bone	Osteomyelitis
Joints	Septic arthritis
Blood	Bacteremia, toxic shock syndrome, septic thrombophlebitis
Lung	Pneumonia
Brain	Brain abscess, meningitis
Heart	Endocarditis
Intestine	Food poisoning
Urinary tract	Urinary tract infection

Adapted from Malak et al. (10)

The entry of this bacterium into the human body can occur by various means, such as entering through a cut, during a surgical procedure, or even through a minor lesion. Once inside, it can spread within the bloodstream or from cell to cell, giving rise to invasive infections. Additionally, *S. aureus* may spread as an airborne pathogen by dissemination through sputum aerosols expelled from the lungs of individuals with *S. aureus* bronchopneumonia (46).

In terms of its origin, *S. aureus* infections can be acquired within the community, the healthcare (nosocomial) settings, and from an animal reservoir (*i.e.* livestock, pets), respectively. Consequently, clinical *S. aureus* infections are classified into three distinct categories based on their source: community-acquired, healthcare-associated, and livestock-associated. These categories exhibit different clinical presentations, antimicrobial susceptibility profiles, and genetic characteristics of the infecting *S. aureus* strains. It is worth noting that this bacterium has now been identified as the leading cause of human diseases in both hospital and community settings (47,48). The use of antibiotics, which dates back to the 1940s, has played a pivotal role in the emergence of antibiotic resistance within *S. aureus*. The bacterium has developed resistance to virtually every antimicrobial agent introduced into clinical use, with a particular focus on the rise of resistance to methicillin, the first penicillinase-resistant β-lactam antibiotic brought into market. *S. aureus* developed this

resistance by acquiring a penicillin binding protein 2 variant called PBP2a (encoded by *mecA* and *mecC*, respectively), which is still capable of crosslinking the peptidoglycan chains of the murein sacculus in presence of β-lactam concentrations that inhibit the activity of the native PBPs of this bacterium (49). This resistance poses a significant public health challenge, resulting in substantial healthcare costs and elevated rates of morbidity and mortality when compared to methicillin-susceptible strains (50,51). The threat posed by MRSA has grown significantly over the years, dating back to the 1960s, and it has become a global concern (48). Infections caused by MRSA, which originally confined to healthcare settings, are nowadays being observed within the community and lifestock, respectively (52).

While the epidemiological landscape of *S. aureus* in developed countries is well documented, the situation in developing countries presents a contrasting picture. Albeith of the fact that there is only limited information on the strain composition in developing countries, there is evidence of a high disease burden and elevated mortality rates associated with severe *S. aureus* infections, particularly in developing countries. Among these nations, sub-Saharan African countries bear a substantial load of *S. aureus* diseases (53–56). Notably, also the spectrum of *S. aureus*-related infections seems to differ between African countries and other regions of the world, with higher rates in SSTIs as well as *S. aureus*-related pyomyositis being reported for Africa (57). Unfortunately, information about the epidemiology and prevalence of staphylococcal infections in sub-Saharan Africa (SSA) remains scarce when compared to developed regions (58,59). Essentially, the same holds true for the antimicrobial resistance repertoires of *S. aureus* strains circulating in this geographic region. This scarcity can be attributed to limited resources and a lack of well-established public health laboratories in the region. The shortage of data on the epidemiology, molecular biology, phylogeny, and pathomechanisms of *S. aureus* strains circulating in sub-Saharan Africa and its associated diseases is in stark contrast to the size and population of the region, highlighting the need for stronger connections with other parts of the world (59).

2.3.1 *S. aureus* in Tanzania

In Tanzania, as in other parts of the developing world and sub-Saharan Africa (SSA), the presence of *S. aureus* has been documented in both commensal and infectious contexts. This versatile bacterium has been recovered from various illnesses, both invasive and non-invasive, originating from both the hospital and the community settings (60–62). While a handful of studies in the country have bacteriologically detected *S. aureus*, the data available for Tanzania remains limited when it comes to describing *S. aureus* genotypes, antimicrobial resistance profiles, and patterns of virulence factors (62). Even from the few existing molecular data, it was observed that there is a wide genetic diversity among *S. aureus* lineages that colonize and infect the Tanzanian population (60,63–65). However, there is a notable absence of information on further typing to ascertain strain-specific phenotypic characteristics of the Tanzanian *S. aureus*.

The available data on Tanzanian *S. aureus* largely stem from specific studies conducted in tertiary healthcare facilities. Most of these studies are centered on research institutions or university-affiliated hospitals. Unfortunately, there is a shortage of data from many other healthcare facilities, including regional and district referral hospitals. Furthermore, it is worth noting that microbiology diagnostics, even in the majority of tertiary healthcare facilities, are either not readily available or rarely performed as a routine practice for monitoring and evaluating antibiotic therapy in patients' management (66). Instead, empirical treatment strategies are commonly employed, which can potentially lead to treatment errors and contribute to the development and dissemination of antimicrobial resistance. This highlights the need for improved diagnostics and data collection to better understand the dynamics of *S. aureus* infections in Tanzania and to inform more effective treatment strategies.

2.4 *S. aureus* pathogenesis and associated virulence factors

The wide spectrum of infections attributed to *S. aureus* can be traced back to a multitude of virulence factors, each with the capacity to act either independently or in concert with others (67). The array of virulence factors employed by *S. aureus* is extensive, encompassing more than 40 distinct proteins (28). In the context of pathogenesis, these virulence factors fulfill specific roles, working in concert to enhance attachment to host cells, act as anti-phagocytic agents, subvert host immune defenses, facilitate tissue invasion, induce sepsis, and trigger toxin-mediated syndromes (68). *S. aureus* virulence factors are usually categorized based on their mechanisms of action and their roles in the pathogenic process. A common classification is outlined in Table 2 below, adapted from a review by Gnanamani et al (69).

Table 2: Virulence factors of *S. aureus* and their characteristics

Factors	**Characteristics**
Factors mediating microbial attachment to host tissue(s)	
Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)	Cell wall-anchored surface proteins, which include protein A, fibronectin-binding proteins A and, B, collagen-binding protein & clumping factors A & B. They promote binding to host factors such as collagen, fibronectin, and fibrinogen, thus enabling primary attachment of bacteria to host tissue, an important stage in infection establishment. They are also involved in the evasion of immune responses and biofilm formation (70)
Factors breaking/evading the host immune system	
Polysaccharide microcapsule	Extracellular matrix component reducing phagocytosis & killing by polymorph nuclear phagocyte (71)

Protein A (Spa)	Cell wall-anchored surface protein that interferes with the innate and adaptive immune responses by binding to the Fcγ portion of immunoglobulins and protects *S. aureus* from opsonophagocytic killing (72)
Panton-Valentine leukocidin (PVL)	Two component cytotoxin that forms membrane pores in the cell membrane of host cells, leading to cell death (73)
α-hemolysin (Hla)	Pore forming exotoxin causing cell death of different host cell types (74)
Chemotaxis-The chemotaxis-inhibitory *S. aureus* (CHIPS):	Secreted protein interfering with chemotaxis of neutrophils and monocytes (75)
Tissue invasion supporting factors	
Extracellular adherence protein (Eap)	Exoprotein with multiple functions that supports binding and evasion of host cells, and exhibits diverse immune-modulatory functions (76)
Proteases, lipases, nucleases, hyaluronate lyase, phospholipase C	Extracellular enzymes that cause tissue destruction and, thus, aid in bacterial entrance into host tissues.
Toxinosis inducing factors	
Enterotoxins (SEs)	Heat-stable exotoxins that act as superantigens and cause food poisoning (77).
Toxic shock syndrome toxin (TSST-1)	Superantigen causing toxic shock syndrome especially in menstrual women (68).
Exfoliative toxins A and B (ETs)	Serine proteases recognizing and hydrolyzing desmosomal proteins in the skin (78).

Genetic investigations have played a pivotal role in unraveling the complex relationship between *S. aureus* genotypes, expressed virulence factors, and the associated diseases. These studies have provided comprehensive insights into how the distribution of virulence-associated genes can vary among different *S. aureus* strains. Such variations in expression of virulence elements are regulated by multiple elements that are responsive to environmental cues and host factors during growth of the bacterium (79). Figure 1, which is adapted from a previous study (30), provides a schematic representation of the *S. aureus* virulon, highlighting both structural components and secreted products, and their growth-phase-dependent expression.

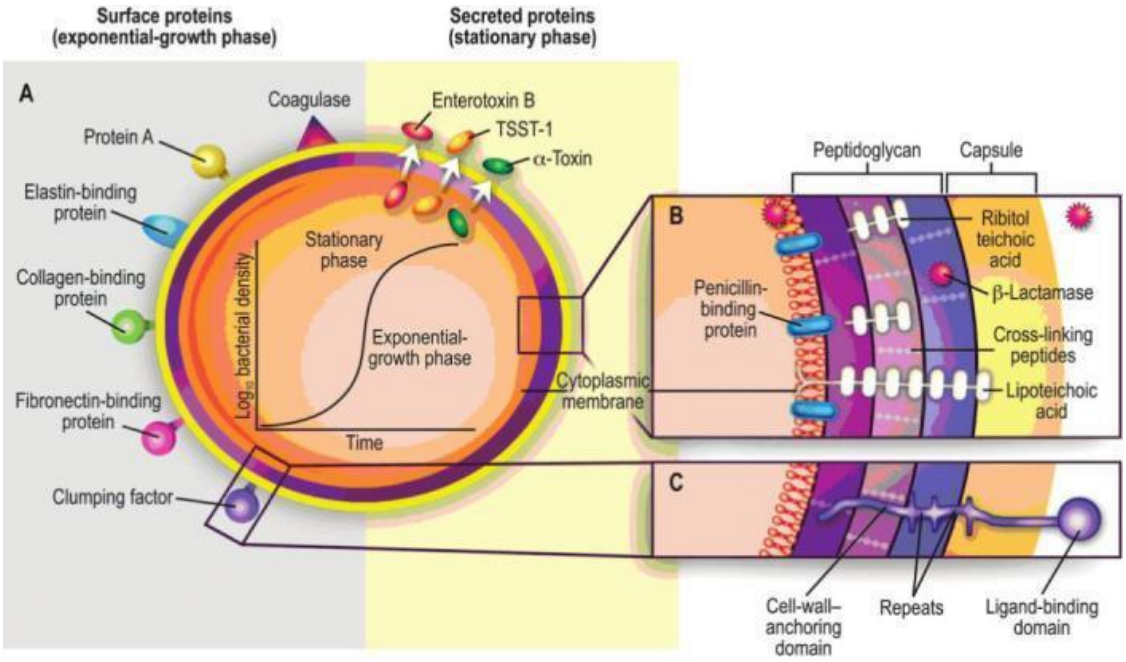

Figure 1: Pathogenic factors of *S. aureus*, with structural and secreted products, both playing roles as virulence factors. A: Surface and secreted proteins. **B, C:** Cross-sections of the cell envelope. TSST-1, toxic shock syndrome toxin. Figure taken from Gordon and Lowy(30).

S. aureus can invade and exert a series of effects on a wide spectrum of human cells. This includes professional phagocytes such as granulocytes, monocytes, and macrophages, as well as non-phagocytes like lymphocytes, erythrocytes, keratinocytes, fibroblasts, osteoblasts, endothelial cells, and epithelial cells (80–84).

Neutrophils are a pivotal component of innate immunity, playing a significant role in defence against *S. aureus* infections (85). Despite the bacterium's efforts to evade the neutrophil attack, such as the expression of a polysaccharide capsule that conceals the bacterial surface and surface-associated proteins to prevent recognition by neutrophils (86), *S. aureus* can further inhibit neutrophil recruitment through the secretion of chemotaxis inhibitory proteins of staphylococci (*chps*) (80,87). *S. aureus* deploys additional protective mechanisms to thwart the natural neutrophil killing through the production of reactive oxygen species. Factors like *S. aureus* carotenoid pigment (staphyloxanthin), recognizable by its characteristic yellow pigment, and superoxide dismutase, safeguard the bacterium against neutrophil attack (8,87). Via the secretion of nucleases and the extracellular adherence protein (*eap*), *S. aureus* also interferes with the capacity of neutrophils to form extracellular nets, thought to capture and kill bacteria (88). Moreover, *S. aureus* produces a range of toxins, including alpha (α)-hemolysin, Panton-Valentine leukocidin (PVL), gamma (γ)-hemolysin, and leukocidin E/D, which have been implicated in lysing leukocytes, thereby enhancing its virulence (48,83). PVL, in particular, is a secreted bi-component leukotoxin frequently associated with *S. aureus* skin infections (89–91).

This toxin exerts cytotoxic effects on human PMNs, creating non-specific pores in the leukocyte plasma membrane that increase permeability and ultimately lead to host cell lysis (92).

While neutrophils that manage to phagocytose *S. aureus* are typically cleared from the host system through efferocytosis by macrophages, *S. aureus*-ingested neutrophils can express a "don't eat me" signal that reduces their uptake by macrophages. This interference with the efferocytosis process leads to blocked lysis, thereby impeding the resolution of tissue inflammation (93). Additionally, *S. aureus* has demonstrated the ability to survive within human monocyte-derived macrophages (94).

Staphylococcal toxins with hemolytic activity, such as hemolysins, contribute to the disruption of red cell membranes and have been identified in various studies to be of importance for the infectivity of *S. aureus* (82,95–97). In particular, α-hemolysin (encoded by *hla*) and β-hemolysin (encoded by *hlb*) have cytotoxic effects on human erythrocytes, lymphocytes, monocytes, macrophages, epithelial cells, and keratinocytes (83). *S. aureus* is known for its capacity to invade epithelial/endothelial cells and to either persist intracellularly or to induce apoptosis, leading to host cell death and dissemination of the bacterium within the infected tissue (98). The wide array of virulence factors possessed by *S. aureus* exerts a profound impact on a wide range of host cells, influencing the bacterium's lifestyle as both a commensal and a formidable pathogen in humans.

2.5 Antimicrobial resistance of *S. aureus* and the emergence of MRSA

Antimicrobial resistance (AMR) is one of the most critical global public health concerns in the 21st century. As already outlined above, *S. aureus* is also with respect to AMR one of the major human pathogens of concern. The global dissemination of resistant *S. aureus* strains, both in healthcare and community settings, has led to substantial challenges in finding effective treatment options. The history of antibiotic resistance in *S. aureus* dates back 83 years ago when resistance to penicillin was first observed (49). Notably, in the 1940s, penicillin resistance emerged soon in *S. aureus* after its introduction as a treatment for staphylococcal infections (99). Penicillin-resistant *S. aureus* produce an enzyme called penicillinase or beta (β)-lactamase, which degrades penicillin's β-lactam ring, rendering it ineffective by altering its structure (100). To combat penicillin resistance, methicillin, a penicillinase-stable semi-synthetic penicillin, was introduced in 1960 (13). Methicillin initially demonstrated effectiveness against penicillin-resistant *S. aureus*, but it also faced resistance, as MRSA strains emerged shortly thereafter. MRSA strains carry a mobile genetic element called SCC*mec* (staphylococcal cassette chromosome *mec*), which harbors the *mecA* or *mecC* gene, respectively, that encodes a modified form of PBP2, leading to reduced affinity against all β-lactam antibiotics (101).

MRSA poses a substantial public health threat, as it is associated with difficult-to-treat conditions and higher mortality rates compared to methicillin-sensitive *S. aureus* (MSSA) (51). In striking contrast to most MSSA, MRSA strains often exhibit multidrug resistance (MDR), rendering them resistant to various antimicrobial agents. MDR is reported among MRSA strains commonly to most available antimicrobial agents

such as tetracycline, sulphonamides, chloramphenicol, lincosamides (clindamycin), aminoglycosides, and quinolones (ciprofloxacin) (19,102). Some MRSA strains have even developed resistance to last-resort antibiotics, such as vancomycin, linezolid, and daptomycin, leading to the emergence of strains that are non-susceptible to vancomycin (vancomycin-intermediate *S. aureus*/vancomycin-resistant *S. aureus* [VISA/VRSA]), daptomycin (DRSA), and linezolid (LRSA), respectively (19,103,104).

The rise of community-acquired MRSA (CA-MRSA) in the 1980s, distinct from healthcare-associated MRSA (HA-MRSA), added to the challenge for the public health (47). CA-MRSA infections tend to affect a younger and healthier population without traditional risk factors associated with healthcare exposure (105). CA-MRSA strains are genetically diverse and characterized by their susceptibility to different antibiotics and the carriage of smaller-sized SCC*mec* elements, typically of types IV or V (106,107). Another source of MRSA are derived from livestock, so-called livestock-associated MRSA (LA-MRSA), which is particularly evident in regions with a high density of pig farms (108).

The classification of MRSA into HA-MRSA and CA-MRSA is nowadays becoming less significant as some strains overlap in both hospital and community settings. Over time, the diversity of MRSA strains and their genetic features have become increasingly important for researchers seeking to address AMR and develop new antibacterial agents. Understanding the characteristics of MSSA and MRSA strains from various regions and sources, including hospitals, communities, and livestock is crucial for overcoming the challenges posed by their genetic diversity and identifying potential targets for combating antibiotic resistance. The World Health Organization (WHO) has recognized MRSA as an antibiotic-resistant microorganism of high concern, underscoring the urgency for further research and the development of effective strategies to combat AMR (15)

2.6 Laboratory diagnosis and typing of *S. aureus*

Microbial diagnosis and typing are a vital part of the clinical microbiology laboratory aiming to support health care interventions; however, they are also potential epidemiological tools in monitoring the spread of different bacterial strains and their resistant ancestors. Owing to the nature of *S. aureus*, being both a commensal (normal inhabitant) and a significant pathogen, and having the propensity to acquire resistance to most available antibiotics, its accurate diagnosis is important for timely intervention in the therapy and prevention strategies.

The primary diagnosis of *S. aureus* is based on phenotypic features; microscopic observation as spherical Gram-positive cocci arranged in irregular grapelike clusters. The bacterium is non-flagellate, non-motile, non-spore forming, with a diameter of 0.5 to 1.0 μm, and able to grow anaerobically and aerobically (22). On a rich medium, *S. aureus* forms medium size, cream to orange-pigmented ("golden") colonies (109). The “golden” pigmentation of colonies is due to the production of the carotenoid staphyloxanthin, which has been documented to be an indication of virulent strains (8). The carotenoid-pigment enhances the bacterium

pathogenicity by inactivating the microbicidal effect of superoxides and other reactive oxygen species (ROS) (110). On blood agar plates, colonies of *S. aureus* often cause β-hemolysis (Figure 2), which is also one of its distinct diagnostic features (16). Staphylococcal bacteria produce catalase, an important virulence factor that degrades the ROS superoxide (H_2O_2) into oxygen ($O_{2)}$ and water (H_2O) (112). The majority of *S. aureus* strains produce coagulase, which is, as already mentioned above, another important diagnostic element, distinguishing *S. aureus* from other staphylococci species (112). Of note, *S. aureus* is fairly salt tolerant with the ability to grow in a medium containing up to 7.5% sodium chloride and it ferments mannitol sugar to lactic acid, giving rise yellowish colonies when grown on mannitol salt agar (69).

In routine laboratory diagnostics, the primary objective is to determine whether the detected *S. aureus* is the causative agent of a specific disease. Simply confirming the presence of *S. aureus* may not be sufficient; it is also crucial to identify its antibiotic resistance status, especially methicillin resistance. Since MRSA presents significant challenges in antibiotic therapy, a systematic diagnostic approach is essential for promptly identifying MRSA and initiating appropriate antibiotic treatment.

Figure 2: Typical colonies of *S. aureus*, grown on Columbia agar with 5% defibrinated sheep blood
Cells were cultured for 24 hours under aerobic atmosphere at 37°C.
(https://www.microbiologyinpictures.com/bacteria%20photos/staphylococcus%20aureus%20photos/STAU16.html)

Species confirmation of *S. aureus* can be achieved through various tests, including slide and tube coagulase tests, latex agglutination tests, and polymerase chain reaction (PCR)-based tests (69,113). To detect antimicrobial resistance, particularly MRSA, methods such as determining the minimum inhibitory concentration (MIC) of oxacillin or cefoxitin by using the broth micro-dilution method, the cefoxitin disk

screen, the oxacillin agar screen, latex agglutination tests for PBP2a, and molecular methods for detecting *mecA* may be used (69). In developed countries, more rapid microbiology testing methods, such as MALDI-TOF mass spectrometry and similar rapid detection technologies, are widely employed for pathogen identification (114).

Bacterial strain typing plays a crucial role in characterizing microbial species and properties. It enables the discrimination of microbial strains at a detailed level, helping to determine whether they originate from a single parental organism within a bacterial species (115). Two main systems of bacterial typing are phenotyping and genotyping. Phenotyping involves describing a bacterium based on its morphology, biochemical tests, serology, and antibiotic susceptibilities; however, it may not provide sufficient discrimination for closely related strains. Genotyping, on the other hand, relies on the genetic content of bacteria, offering high resolution and throughput (116). Genotyping is essential for discriminating between different bacterial isolates within the same species, aiding in source tracing and disease management. In the case of *S. aureus*, which consists of multiple strain types, genotyping is valuable for understanding strain-specific phenotypic behaviors.

Current genotyping methods fall into three main categories: DNA banding pattern, DNA sequencing, and DNA hybridization-based methods. DNA banding pattern-based methods distinguish strains by differences in the size of DNA bands generated through DNA amplification or cleavage using restriction enzymes. DNA sequencing-based methods directly determine the nucleotide sequence, allowing discrimination based on DNA polymorphisms. DNA hybridization-based methods, like DNA microarrays (DNA-MCA), differentiate strains by analyzing DNA hybridization patterns of strains to probes with known sequences (116). In the absence of whole genome sequencing (WGS) capacities, DNA-MCA technology provides a useful tool for an in depth genotypic characterization of *S. aureus* with high discriminatory power and the capability of identifying the strain type, virulence gene composition, and presence/absence of resistance genes in a single experiment (117,118).

Genotyping of *S. aureus* strains is becoming increasingly important in modern microbiology. However, in many developing countries, including Tanzania, there is limited knowledge about the characteristics of *S. aureus* strains, both as commensals and pathogens. Limited laboratory capacity, especially in advanced microbiology and molecular biology techniques, has resulted in a lack of information regarding defined genotypic profiles and strain-specific phenotypic characteristics of *S. aureus* in these regions. Given the challenges posed by diverse *S. aureus* strains, a detailed characterization is crucial for addressing public health concerns effectively.

2.7 Study Objectives

The broad objective of this study was to describe genetic characteristics of the Tanzanian *S. aureus* strain composition from Bagamoyo and to typify strain-specific phenotypic behaviors of genotypically well characterized *S. aureus* strains from Tanzania and within the African-German StaphNet consortium.

2.7.1 Specific objectives

1. To investigate genetic characteristics of *S. aureus* isolates originating from colonization and clinical infections recovered from the Bagamoyo area in Tanzania.
2. To describe strain-specific phenotypic behaviors of *S. aureus* isolates originating from the Bagamoyo area in Tanzania, with a primary focus on the predominant strains associated with colonization and clinical infections.
3. To describe strain-specific phenotypic behaviors of *S. aureus* isolates within "the African-German StaphNet consortium" focusing on the predominant strains in Germany and sub-Saharan Africa, precisely from Tanzania, Gabon, and Mozambique.

3. MATERIALS & METHODS

3.1 Materials

3.1.1 *S. aureus* isolates

S. aureus isolates used in this study were part of the African-German StaphNet multicentre project entitled, "*Infection Biology and Epidemiology of Staphylococci and Staphylococcal Diseases in sub-Saharan Africa"*. The objective of the African-German StaphNet project was to compare staphylococcal isolates from carrier persons and patients both from developing and developed countries. The study aimed to ascertain the role of *S. aureus* in the tropics (particularly in sub-Saharan Africa), with respect to pathogenicity, virulence, antimicrobial resistance, colonization, transmission efficacy, and consequently presentation of clinical disease, and how it differs in its role in temperate regions such as Germany.

3.1.1.1 Tanzanian *S. aureus* isolates

Tanzania, as a participant in the African-German Staphnet consortium, collected 258 *S. aureus* isolates recovered from the Bagamoyo area, in the Pwani region located in the Eastern part of Tanzania. The 258 *S. aureus* isolates included a first set of 200 isolates comprised of 100 isolates from healthy volunteers collected in the vicinity of the Bagamoyo district hospital (BDH) and 100 isolates from various clinical infections collected from outpatients attending BDH and five different health facilities (Fukayosi, Kiwangwa, Makurunge, Mapinga, and Yombo) around Bagamoyo during 2010-2012, respectively. The remaining 58 isolates (24 clinical infections and 34 commensals) were collected from HIV-infected individuals attending

the HIV care and treatment clinic (CTC) at BDH during July 2014-July 2015. A subset of 98 Tanzanian *S. aureus* isolates was selected from the 258 isolates based on DNA-MCA results, the dominant strain types (CCs: clonal complexes), for further characterization to compare their in-vitro-phenotypic behaviours, *i.e.* their haemolytic, cytotoxic, and phagocytosis-preventing activities.

3.1.1.2 Analyzed *S. aureus* isolates from the African-German StaphNet consortium

A comparison set of *S. aureus* strains was selected from the African-German StaphNet consortium for in-vitro-phenotypic behaviours typing. In the prospective cohort study of the African-German-StaphNet on staphylococci and staphylococcal disease (DFG PAK 296), a total of 1200 community-associated isolates were collected from three African countries (Gabon, Mozambique, and Tanzania), and three different German study sites (Homburg, Saarland; Freiburg, Baden-Württemberg; Münster, Nordrhein-Westfalen). Each study site contributed 200 *S. aureus* isolates, i.e. 100 from commensals collected from healthy volunteers (nasal carriage), and 100 clinical isolates collected in accordance to the predefined case-related-forms (CRFs) of the African-Germany StaphNet consortium to exclude hospital acquired *S. aureus* strains (119).

The identity of all 1200 *S. aureus* isolates was phenotypically confirmed by MALDI-TOF mass spectrometry and genotypic characterization by DNA-MCA analyses. Based on the CC distribution depicted in Figure 3, a set of *S. aureus* strains (shown in the Table 3) was selected and tested for their phenotypic behaviors as outlined above.

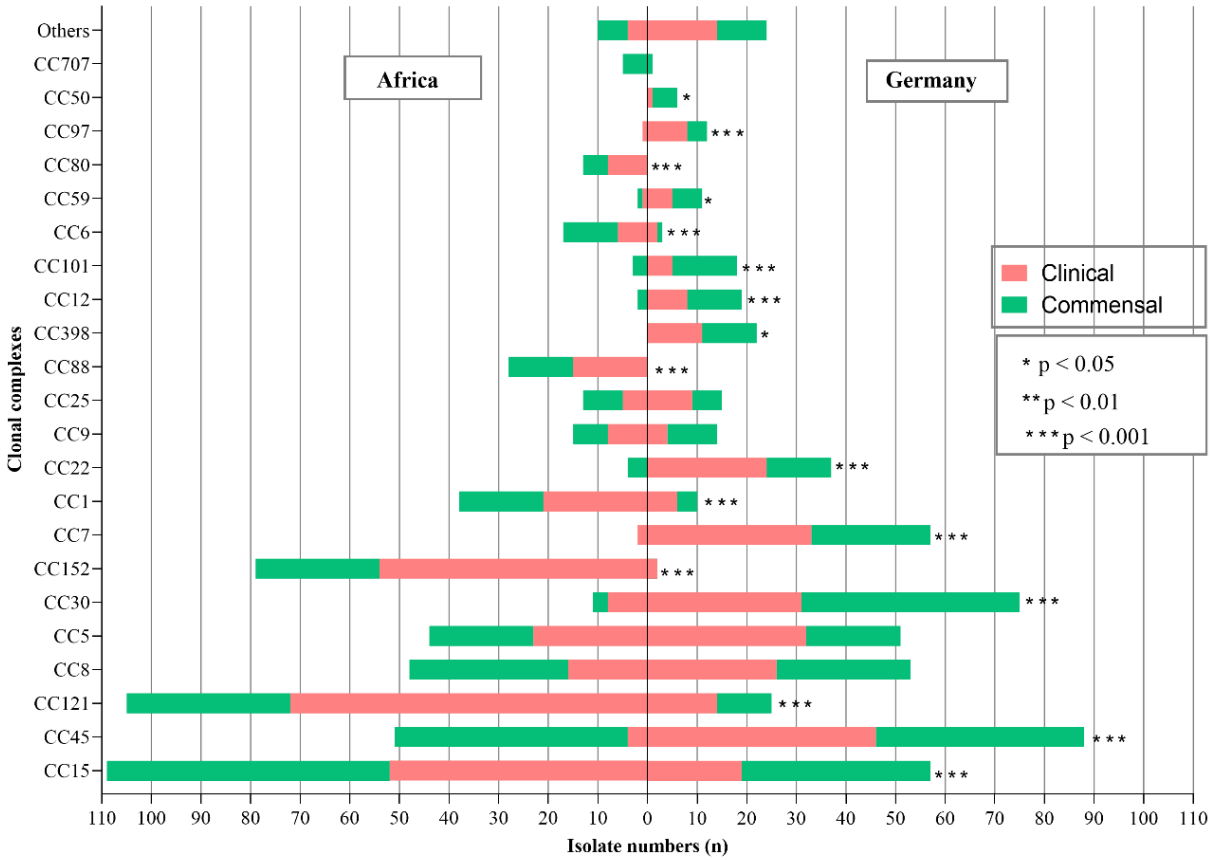

Figure 3: Distribution of clonal complexes (CCs) of *S. aureus* isolates from the African and German Consortium

Distribution of the 22 most prevalent clonal complexes (CCs) in Africa and Germany among isolates from colonization and infection. CCs of low prevalence (*n*<6 isolates) were grouped together (others). The CCs were sorted in ascending order according to the total number of isolates in the respective CC. The proportions of clinical (red) and nasal (green) isolates in the African and German groups are shown. Differences in the distribution of CCs between Africa and Germany were calculated with Fisher's exact test: *, *P*<0.05; ***, *P*<0,001. Image taken from Ruffing et al (119).

The first batch included a set of 150 *S. aureus* isolates selected from 10 different CCs, which were dominant in African and German sites. The second batch included 90 MSSA strains from two CCs, CC121 (n=60) and CC152 (n=30), which were highly dominant in African isolates. This second batch was tested to confirm the differences in the phenotypic behaviors of both CC groups observed with the first batch of isolates.

Table 3: List of *S. aureus* from the African-German StaphNet Consortium for phenotypic activities typing

First Batch: A set of the selected 10 different *S. aureus* CCs			
CC type	**Clinical infection**	**Commensal**	**All**

CC121	10	5	15
CC152	10	5	15
CC15	10	5	15
CC1	10	5	15
CC22	10	5	15
CC30	10	5	15
CC45	10	5	15
CC5	10	5	15
CC8	10	5	15
CC88	10	5	15
All	**100**	**50**	**150**
Second Batch: A set of the selected CC121 and CC152 *S. aureus* strains			
CC type	**Clinical infection**	**Commensal**	**All**
CC121	30	30	60
CC152	15	15	30
All	**45**	**45**	**90**

3.1.2 Chemicals, consumables, instruments, and software:

All chemicals, consumables, instruments, and software used in this study are presented in Table 4**.**

Table 4: List of chemicals, consumables, instruments, and software used in this study

1. Antibiotics		
Antibiotic	**Manufacturer**	**Concentration Used**
Penicillin-Streptomycin	SIGMA- ALDRICH, USA	50 µg/ml in MCDB153
Antimicrobial Susceptibility testing drug discs: Penicillin (10 IU) Trimethoprim/sulfamethoxazole (1.25/23.75µg) Gentamicin (10µg) Erythromycin (15 µg) Clindamycin (2µg) Tetracycline (30µg) Chloramphenicol (10µg) Cefoxitin (30µg)	Oxoid; Basingstoke Hampshire, United Kingdom	

2. Buffer and Solution		
Buffer/Solution	**Manufacturer**	**Recipe/Formula**
FACS-Clean	BD (Heidelberg)	
FAC-Lysing™	BD (Heidelberg)	
FACS Buffer	BD (Heidelberg)	20 ml FCS 5 ml NaN_3 Stock solution (1M) add 1000 ml PBS
FACS-Rinse	BD (Heidelberg)	
Formaldehyde-Solution 16 %		Formaldehyde 16 % add 1000 ml H_2O
NaN_3 Buffer (10 mM)		NaN_3 0.65 g add 1000 ml PBS
NaN3 Stock solution (1M)		NaN_3 65 g add 1000 ml H2O
PBS 10x		40 gm NaCl 2 g/l KCl 14.2 g/l Na_2HPO4 2.7g/l KH_2PO4 Add 1000 ml H_2O, pH 7.4; autoclave
Sodium Chloride (NaCl) solution 0.9 %		NaCl 9 g Add 1000ml H_2O, autoclave

3. Cell culture		
Cell line:	**Relevant features:**	**Reference**
HaCaT	Spontaneously immortalized, human, epidermal keratinocytes	Boukamp *et al.*, (120)

4. Dye/Stain	**Manufacturer**
Carboxy fluorescein succinimidyl ester (CFSE)	Invitrogen (Darmstadt)
Trypan blue dye 0.4% solution	Bio-Rad Laboratories (München)

5. Enzyme and Protein		
Enzyme/Protein	**Name**	**Manufacturer**
Trypsin (TrypLE)	Tryp-LE® Express	Gibco - Life Technologies, Denmark

Amino acid	MEM NEAA (100X)	Gibco-UK
Bovines Serum Albumin		VWR (Darmstadt)

6. Laboratory Machines/Equipment and Systems	
Equipment and Systems	**Manufacturer**
Cell Counter	BioRad (München)
Centrifuge 5417R	Eppendorf (Hamburg)
Centrifuge 5418	Eppendorf (Hamburg)
Centrifuge 5810R for Cell Culture	Eppendorf (Hamburg)
Centrifuge Sigma 4K15	Sigma Laboratory Centrifuge GmbH (Osterode)
Centrifuge Universal 32R	Hettich (Tuttlingen)
Countess™ Automated Cell Counter	Invitrogen (Darmstadt)
Counting slides, a dual chamber for cell count	Bio-Rad – (USA)
DFC420 CCD Kamera	Leica (Wetzlar)
Keutz electrophoresis chamber	Laboratory technology (Reiskirchen)
Eppendorf Research Fix® Pipette set	Eppendorf (Hamburg)
Electrophoresis Power supply	Bio-Rad (München)
FacsCalibur™ Durch Flow cytometer	BD (Heidelberg)
GeneQuant pro -1300 Spectrophotometer	Biochrom (Berlin)
Hera Safe Safety Cabinet Class II	Thermo Fisher Scientific (Karlsruhe)
HeraCell150: CO_2 Incubator	Thermo Fisher Scientific (Karlsruhe)
HeraCell 240: CO_2 Incubator	Thermo Fisher Scientific (Karlsruhe)
Heraeus B5042E	Willi Fischer KG Laboratory supplies (Frankfurt)
Microscope: Carlzeiss Axiovert10	Carl Zeiss Meditec AG (Jena-Germany)
MilliQ™ Synthesis A10	Millipore (Billerica, USA)
Multiple Shaking Incubator	Infors (Bottmingen, Switzerland)

Nanodrop 2000	Thermo Fisher Scientific (Karlsruhe)
PIPETBOY ACU	2 INTEGRA Biosciences AG (Zizers, Switzerland)
PH M220 pH-Meter Radiometer,	Meterlab (Meckenheim)
Thermo-mixer Compact	Eppendorf (Hamburg)
Transfer pipette™ S	Brand Laboratory supplies GmbH + CO KG (Wertheim)
Wallac 1420 Victor^2 Multilabel Counter	Pelkin-Elmer (Waltham, USA)
Vortex mixer VV3	VWR (Darmstadt)
Anticoagulated blood collection (Li-Heparin) tubes: S-Monovette	Sarstedt, Germany
Sterile filter (Filtropur S 0.2)	Sarstedt, Numbrecht Germany
7. Medium for the cultivation of bacteria	
Medium	**Manufacturer/Producer**
Trypticase Soy Agar II (TSA) with 5% sheep blood	BD (Heidelberg)
Trypticase Soy Broth (TSB)	Bacto®, BD (Heidelberg)
8. Medium for the cultivation of cell culture	
Medium	**Manufacturer/Producer**
MCDB153 Basal Medium	Biochrom AG (Berlin)
9. Reagent kit	
Reagent kit	**Manufacturer**
Cytox 96 Non–Radioactive Cytotoxicity assay-Substrate	Promega-Madson, USA
10. Software	
Software	**Manufacturer**
ABI PRISM® 7000 SDS 1.2.3	Thermo Fisher Scientific (Karlsruhe)
CellQuest Pro®	BD (Heidelberg)
Nanodrop 2000 operation software v1.6	Thermo Fisher Scientific (Karlsruhe)

Nanoscope Analysis v1.4	Bruker (Santa Barbara, USA)
NanoScope v8.11	Bruker (Santa Barbara, USA)
Office® 2010/2011	Microsoft (Redmond, USA)
R v3.2	R foundation for statistical computing (Wien, Austria)
SigmaPlot® -SigmaStat® v10/11	Systat Software GmbH (Erkrath)
Wallace 1420 Manager	Pelkin-Elmer (Waltham, USA)

3.2 Methods

3.2.1 Isolation and identification of the Tanzanian *S. aureus*

Primary isolation of *S. aureus* from samples was performed on 5% sheep blood agar and mannitol salt agar (MSA) with incubation at 35°C for 18-24 hours. Preliminary identification of *S. aureus* was based on colony morphology, Gram stain status, and positive reactions for catalase and tube coagulase tests performed at the Microbiology unit of the Ifakara Health Institute (IHI)-Bagamoyo Research and Training Center (BRTC)' laboratory in Bagamoyo, Tanzania. All identified *S. aureus* isolates were stored at -20°C until shipment to the Institute of Medical Microbiology and Hygiene at Saarland University, Homburg, Germany, for *S. aureus* confirmation by Matrix Assisted Laser Desorption Ionization - Time of flight (MALDI-TOF) analysis and further characterisation

3.2.2 Antimicrobial Susceptibility Testing of the Tanzanian *S. aureus* isolates

Antimicrobial susceptibility testing (AST) of the Tanzanian *S. aureus* isolates was performed at BRTC laboratory in Tanzania by the Kirby-Bauer disk diffusion assay on Mueller-Hinton agar (MHA). A panel of eight antibiotic discs were tested: penicillin (10 units), cefoxitin (30µg), tetracycline (30µg), gentamicin (10µg), chloramphenicol (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), erythromycin (30µg) and clindamycin (2µg). Briefly, about 100 µl of a bacterial suspension equivalent to a McFarlands of 0.5 were evenly distributed with a cotton swab on the surface of an MHA plate, and up to six different antibiotic-loaded discs placed onto the surface of the inoculated plate. Plates were incubated at 35°C for 18–24 hours. Inhibition zones were measured and interpreted as recommended by the Clinical and Laboratory Standards Institute guidelines (CLSI 100). For all isolates that were clindamycin sensitive but erythromycin resistant, a D-test was performed to detect an inducible clindamycin resistance (121). Briefly, the erythromycin disk was placed in about 15 mm distance (edge to edge) from the clindamycin disk on the same MHA plate for AST as per CLSI guidelines. The presence of an inducible clindamycin resistance is indicated by the growth of the bacterium in

the area between the two discs, displaying a flattening zone like a D-shape of clindmycin near to the erythromycin disc (resistant) (121,122).

3.2.3 DNA-MCA Genotyping and Data Processing

Genomic DNA was extracted from *S. aureus* colonies which were picked from sheep blood agar plates grown for 19-24 hours at 37°C. About 3-4 colonies were resuspended in phosphate buffer and DNA was isolated with the use of the DNAeasy tissue kit following the procedure described by the manufacturer's instructions. Genotypic characterization using the Identibac® *S. aureus* Genotyping DNA microarray was done following a previously established protocol (123). Subsequent data analysis was performed with the software Iconoclust® (version 3.2.r). As described in the manufacturer's notes, the DNA microarray-StaphyType^{TM} kit enables the detection of 334 oligonucleotide probes, covering various genes specific for multi-locus sequence typing (MLST)-clonal complexes (CCs) and sequence type (ST) designations, as well targets attributing for virulence/persistence and ARGs (124).

3.2.4 Phenotypic Behaviours Characterization

A subset of *S. aureus* strains from both Tanzania and the African-German StaphNet consortium was selected based on DNA-MCA results, and was further characterised to compare in-vitro-phenotypic behaviours, *i.e.* the hemolytic capacity on human red blood cells, cytotoxicity on keratinocytes, and phagocytosis-preventing activities.

3.2.4.1 Hemolytic Activity Method

Determination of the hemolytic potential of the selected study strains was carried out with the use of bacterial broth culture supernatants and human red blood cells. Bacteria were isolated on Trypticase soy agar II with 5% sheep blood (TSAB) at 37 °C overnight. Overnight liquid cultures were prepared by inoculating 2-3 colonies from the TSAB plates into 3 ml Trypticase Soy broth (TSB) and incubating the solution at 37°C and 150 rpm for 16 hours. To prepare bacterial supernatants, overnight cultures were adjusted with fresh PBS to A_{600} of 8.0, and subsequently centrifuged at 12.000g for 1minute. Supernatants were carefully collected, filter-sterilized, and serially diluted in PBS (0:0 to 1:64 dilutions). 100 µl of the dilutions were mixed 1:1 with washed human red blood cells obtained from freshly collected whole blood samples obtained from healthy volunteers, which were resuspended in PBS to a final concentration of 10% (vol/vol). Erythrocyte-bacterial supernatant mixtures were placed into the wells of a round-bottom, clear 96-well plate and incubated for 30 min at 37°C, followed by 16 h at 4°C. Examination of the hemolytic potential on each dilution of the culture supernatant was carried by observing the visible erythrocytes pellet at the bottom of the well and a photo was taken for each plate. The highest dilution giving rise to full lysis of erythrocytes was defined as the hemolytic titer.

3.2.4.2 Cytotoxicity Measurements

Cytotoxicity of *S. aureus* cell culture supernatants was measured in combination with human adult low calcium high temperature keratinocytes (HaCaT), a spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin (120). HaCat cells were cultured in MCDB153 Basal Medium (MCDB) supplemented with 10% fetal calf serum (FCS), 1% non-essential amino acids (NEAA) and 1% antibiotics (10 mg/ml streptomycin and 10,000 units/ml penicillin) at 37°C and 5% CO_2. Confluent cultures were washed with PBS, trypsinated with TrypLE and resuspended in fresh, prewarmed medium to a density of 5 x 10^5 cells/ml. 50 µL aliquots of the cells suspension were placed into each well of a 96 well flat-bottom plate and incubated overnight at 37°C and 5% CO_2 before cells were challenged with the bacterial cell culture supernatants. For the bacterial cell culture supernatants, *S. aureus* isolates were cultured overnight on TSAB plates (Becton-Dickinson) at 37°C. On the following day, 2-3 colonies of the freshly grown plate were inoculated into 3 ml TSB in a sterile glass tube and incubated for 16 h at 37°C and 150 rpm. Bacterial cultures were adjusted with fresh TSB to an A_{600} of 8, centrifuged at 4.500 g for 10 minutes, the bacterial supernatants carefully collected and passed through a 0.2 µm filter. The filter-sterilized and optical density-adjusted bacterial cell culture supernatants were serially diluted with fresh TSB medium, and 50 µL aliquots of the dilutions were added to the overnight HaCaT cell cultures. Bacterial cell culture supernatant-challenged HaCaT cells were subsequently incubated at 37°C and 5% CO_2 for 4 hours. HaCaT cells incubated with TSB and 20% ethanol served as negative and positive controls, respectively.

The cytotoxic potentials of the *S. aureus* isolates were determined by measuring the lactate dehydrogenase (LDH) release of the HaCaT cells in response to the bacterial cell culture supernatant challenge using the CytoTox 96® Non-Radioactive cytotoxicity assay following a procedure described by the manufacturer. Briefly, 50 µl aliquots of the supernatants of the bacterial cell culture supernatant-challenged HaCaT cells were transferred into a fresh 96-well plate, mixed with 50 µl of the CytoTox 96® reagent and incubated for 30 min at room temperature. The colorimetic reaction was stopped by adding 50 µl of stop solution and the absorbance at 490 nm was recorded using a Wallace Victor^2 1420 multi-laber counter within 1 h after addition of the stop solution. The cytotoxic potential of the *S. aureus* isolate was calculated with the formula:

$$\% \text{ cytotoxicity} = 100 \times \frac{Experimental\ A490 - Negative\ control\ A490}{Positive\ control\ A490 - Negative\ control\ 490}$$

3.2.4.3 Phagocytosis Escape Studies

The whole blood phagocytosis assay was carried-out essentially as described by Jung et al., (125). *S. aureus* isolates were cultured in 3 ml TSB in a sterile glass tube for 16 hours as outlined before. One ml aliquots of the bacterial overnight culture were centrifuged at 12.000 g for 1 minute and bacterial cell pellets were washed

twice with PBS. Washed bacterial cells were subsequently stained with 25 µl/ml of carboxyl fluorescein diacetate succinimidyl ester (CSFE: Invitrogen) for 15 min at 37° C and 500 rpm. Unbound dye was removed by washing the stained cells thrice with PBS, and stained bacterial cell cultures were adjusted with PBS to an A_{600} of 1.

Human whole blood samples were collected from voluntary healthy donors (n=15) in lithium heparin-containing tubes (S-Monovette: Sarstedt). The blood samples were collected freshly on the day of the experiment, the PMN content of the blood sample determined, and 1 ml aliquots of the whole blood samples challenged with CSFE-stained bacterial cells at a multiplicity of infection (MoI) of 100 in relation to the PMN content of the blood sample. Bacteria-challenged blood samples were incubated at 37° C and 1000 rpm for up to 1 hour, and 200 µl aliquots were removed at 5, 15, 30, and 60 minutes post infection, respectively. Aliquots were placed in 5 ml round bottom polystyrene tubes (Becton-Dickinson) prefilled with 1ml FACS lysing solution (Becton-Dickinson) to lyse erythrocytes. Residues were removed by centrifugation for 5 min at 450 g. Cell pellets were re-suspended in PBS supplemented with 2% FCS and 0.05% sodium azide, and subsequently analyzed by flow cytometry using a FACSCalibur system (Becton-Dickinson), gating monocytes, lymphocytes, and PMNs according to their morphological properties (size and granularity). In parallel, the fluorescence intensity was analyzed for CFSE-labeled bacterial cargo, allowing for quantitative analysis of the interaction between *S. aureus* and the immune cells of interest in the same run. Data were analysed using the Cell Quest Pro Software system (Becton-Dickinson).

3.2.5 Basic Statistical Methods

The statistical analyses in this study were performed using the GraphPad Prism software version 4.0. The significance of differences between groups was either determined with the Mann-Whitney U test for comparison between two unrelated groups, or the paired sample *t*-test when data points in one group were specifically paired with corresponding data points in another group. In both cases, a significance threshold of $p < 0.05$ was chosen.

3.2.6 Ethical Statement

Studies involving human blood were performed in accordance to the declaration of Helsinki and approved by the Ethics Committee of the Medical Association of Saarland (code number 173/17). Informed written consent was obtained from all blood donors.

4. RESULTS

4.1 Genetic Characteristics of the Tanzanian *S. aureus* based on DNA-MCA Analysis

The DNA-MCA used in this study is designed for the rapid genotyping of *S. aureus*, enables the detection of 333 target sequences (probes) corresponding to 185 distinct genes and their allelic variants (124). Target genes included species-specific markers, accessory gene regulator **(***agr***)** alleles, genes encoding for resistance determinants and virulence factors such as staphylococcal superantigen-like or exotoxin-like genes (*set* or *ssl* genes), and genes encoding adhesion proteins, as well as capsule types.

4.1.1 *S. aureus* species confirmation and strain typing

DNA-MCA analysis confirmed the identity of all 258 tested *S. aureus* isolates by showing hybridization signals (positive results) for the *S. aureus*-specific markers (n=7) *rndD1* (domain 1 of 23S rRNA), glyceraldehyde 3-phosphate dehydrogenase (*gapA*), catalase A (*katA*), coagulase (*coA),* thermostable nuclease (*nuc*), protein A (*spa*) and IgG-binding protein (*sbi).* Subsequent analyses of the strain-specific hybridization patterns allowed to subgroup the 258 *S. aureus* isolates into 24 different clonal complexes (CCs) or sequence types (STs). Figure 4 provides a summary of the distribution of the identified CCs/STs across the clinical infections- and commensal *S. aureus* isolates included into this study.

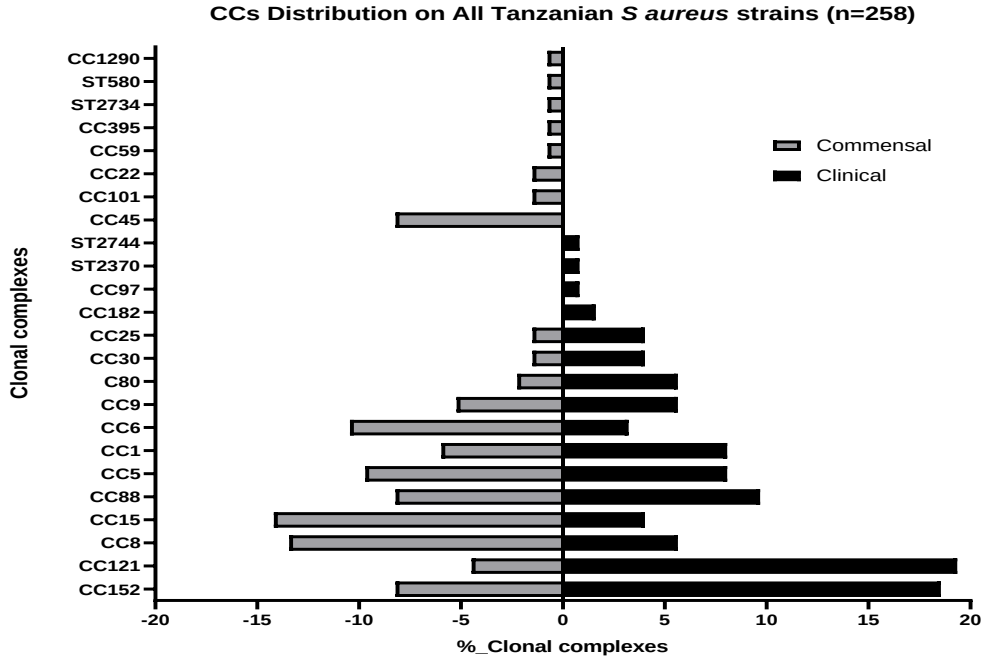

Figure 4: Distribution of clonal complexes (CC)/sequence types (STs) in *S. aureus* isolates collected from Bagamoyo in Tanzania
258 *S. aureus* isolates (134 commensals and 124 infection-related isolates) were tested by DNA-MCA analysis and grouped into different CCs/STs according to their hybridization patterns.

While most of the isolates tested were positive for all 7 markers (96.5%), an unexpected high proportion of isolates lacking a *sbi* signal (20.6%) was observed among the CC152 isolates (Table 5), suggesting a potential polymorphism of this gene in this CC.

Table 5: Profile of identified *S. aureus* clonal complexes (CCs) and the confirmed species markers

CC type	No. of Strains	*rrnD1*	*gapA*	*katA*	*coA*	*nuc1*	*spa*	*sbi*
CC152	34	34	34	34	34	34	33	**27**
CC121	30	30	30	30	30	30	30	30
CC8	25	25	25	25	25	25	25	25
CC15	24	24	23	24	24	24	24	24
CC88	23	23	23	23	23	23	23	23
CC5	23	23	23	23	23	23	23	23
CC1	18	18	18	18	18	18	18	18
CC6	18	18	18	18	18	18	18	18
CC9	14	14	14	14	14	14	14	14
CC45	11	11	11	11	11	11	11	11
CC80	10	10	10	10	10	10	10	10
CC30	7	7	7	7	6	7	6	7
CC25	7	7	7	6	6	7	6	6
CC101	2	2	2	2	2	2	2	2
CC182	2	2	2	2	2	2	2	1
CC22	2	2	2	2	2	2	2	2
CC395	1	1	1	1	1	0	1	1
CC1290	1	1	1	1	1	1	1	1
CC97	1	1	1	1	1	1	1	1
CC59	1	1	1	1	1	1	1	1
ST2744	1	1	1	1	1	1	1	1
ST2734	1	1	1	1	1	1	1	1
ST2370	1	1	1	1	1	1	1	1
ST580	1	1	1	1	1	1	1	1
All (N)	**258**	**258**	**257**	**257**	**256**	**257**	**255**	**249**
All (%)	**100**	**100**	**99.6**	**99.6**	**99.2**	**99.6**	**98.8**	**96.5**

CC152 was also the most abundant CC found in this strain set, followed by CC121, CC8, CC15, CC88 and CC5, which all were present with >20 isolates. The direct comparison between commensal and infection-related isolates identified 12 different CCs that were detected in both commensal and clinical isolates (Figure 4). Notably, while most of the CC121 and CC152 isolates of this strain set originated from infection, the majority of CC6, CC8, and CC15 isolates were obtained from nasal swabs. Eight CCs/STs (CC1290, ST580, ST2734, CC395, CC22, CC101, CC59, and CC45) were found exclusively in the commensal group, while four CCs/STs (CC97, CC182, ST2370, and ST2744) were found in the infection-related isolate group only. However, with the exception of CC45 (11 isolates), all other aforementioned CCs were present in small numbers (<3).

4.1.2 Accessory gene regulator (*agr*) and capsular gene typing

When checking for the *agr* type, 256 out of the 258 Tanzanian *S. aureus* isolates were successfully assigned to one of the four different *agr* groups present on the DNA-MCA (*agr* group *I*, *II*, *III*, and *IV*). Overall, 114 (44.5%) strains from 16 different CCs were associated with *agr* group *I*, led by CC152 (n=34), CC8 (n=25), CC6 (n=18), and CC9 (n=13). Fifty-two strains from four different CCs (CC15, n=24; CC5, n=23; CC1 [ST573/772], n=4; CC9, n=1) were associated with *agr* group II, and 54 strains from five different CCs (CC88, n=23; CC1, n=14; CC80, n=10; CC30, n=6; ST2370, n=1) associated with *agr* group *III*. The remaining 36 strains from CC121 (n=30) and CC45 (n=6) were assigned to *agr* group IV. Notably, 30 strains presented hybridization signals for both, *agr* groups *I* and *IV*, which were mainly from CC152 lineage (n=28).

With respect to the capsule type, nearly all isolates of the Tanzanian strain set grouped into two capsule types, *capsule type 5* (*cap5*) and *capsule type 8 (cap8*). Figure 5 shows the distribution of *agr/cap* types in the Tanzanian strain set, and Table 6 lists representative CCs for each *agr/cap* typing group.

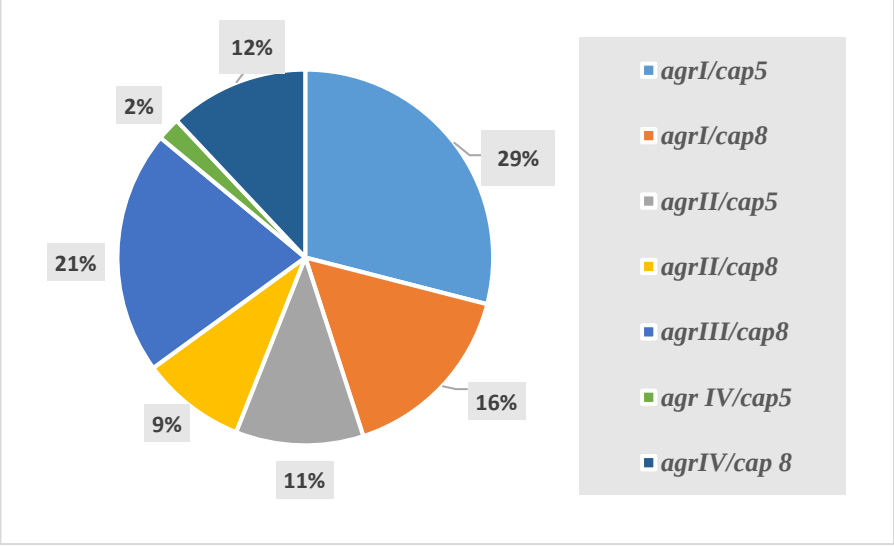

Figure 5: Distribution of seven different *agr/cap* typing groups of *S. aureus* from Tanzania

Table 6: Distribution of *S. aureus* CCs in the seven different *agr/cap* groups

***agr*/*cap* type**	**No. / (%) of Strains**	**CC/Strain type**
agr I/*cap*5	73 (29%)*	Nine different CCs: CC152 (*n*=34), CC8 (*n*=25), CC25 (*n*=6), CC182 (*n*=2), CC22 (*n*=2) and one from each of CC97, ST2744, ST2734, and ST580 strains
agr I/*cap8*	41 (16%)	Seven different CCs: CC6 (*n*=18), CC9[834] (*n*=13), CC45 (*n*=5), CC101(*n*=2) and one from each of ST59-MRSA, CC1290, and CC395 strains
agr II/*cap*5	28 (11%)	Three different CCs: CC1 [ST573/773](*n*=4), CC5 (*n*=23) and CC9 (*n*=1)
agr II/*cap8*	24 (9%)	One CC: CC15 (*n*=24)
agr III/*cap8*	54 (21%)*****	Five different CCs: CC88 (*n*=23), CC1 (*n*=14), CC80 (*n*=10), CC30 (*n*=7), ST2370 (*n*=1)
agr IV/*cap*5	6 (2%)	One CC: CC45 (*n*=6)
agr IV/*cap8*	30 (12%)	One CC: CC121 (*n*=30)
All	256 (99.2%)*****	257 (99.6%)

*****, One CC30-MSSA/*cap8* was not assigned to any *agr* group and one CC25 strain was neither positive for the *agr* nor capsule associated gene probes of the chip. CC45 strains (*n*=11) were assigned into 2 different groups: *agr IV*/*cap*5 group comprised 6 strains, and *agrI*/*cap8* group comprised 5 strains

Only one CC25 isolate showed no hybridization signal to any of the capsule associated genes represented on the DNA-MCA. *Cap8* was the most frequent capsule type detected in 150 isolates (58%), while *cap5* was found in 107 isolates (42%). In sum, seven different groups based on combined *agr/cap* typing were observed: *agrI/cap5* was the most frequently detected group with 73 strains (29%) followed by the *agrIII/cap8* group with 54 strains from five different CCs/STs.

In addition to the *agr*/*cap* gene repertoire, the DNA-MCA also detected genes associated with biofilm formation, *i.e. icaA, icaC, icaD* (encoding the intercellular adhesion protein A, C, and D, respectively), and *bap* (encoding biofilm associated protein). While none of the Tanzanian isolates produced a hybridization signal for *bap*, positive signals for *icaA* and *icaD* were present in virtually all isolates tested (≥99%), however, this was not the case with *icaC*, which was detected in 214 (83%) isolates only (Appendix 3).

4.1.3 Virulence genes profile of the Tanzanian *S. aureus* strains from Bagamoyo

The most relevant virulence genes detected in all CCs identified from the 258 Tanzanian *S. aureus* from Bagamoyo community is detailed in Appendix 3. A variable combination of virulence-associated genes were present in each CC with slight differences regarding their origins. Table 7 displays a selection of virulence factors of interest for this study including enterotoxin B (encoded by *seb*), hemolysins, leukocidins, immune evasion molecules, exfoliative toxins, and surface proteins.

DNA-MCA suggested the presence of *seb* in 46 isolates, with a higher proportion in isolates from infection (n=31; 25%) than from colonization (n=15; 11.2%). The *seb* toxin-encoding gene was identified in seven different CCs: the majority in CC121 isolates (n=24; 52%), and the remaining (n=22; 48%) isolates were from CC5 (n=7), CC8 (n=6), CC1 (n=4), CC25 (n=3), CC6 (n=1), and CC59 (n=1).

The DNA-MCA assays successfully identified 10 different leukocidin-conferring genes. These genes group comprises various components, such as the γ-hemolysin/leukocidin genes *lukF* and *lukS* (aka. *hlgB* and *hlgC*), the γ-hemolysin component A encoding gene *hlgA*, Panton-Valentine leukocidin F/S (PVL) components encoding genes *lukF-PV* and *lukS-PV*, a gene encoding a leukocidin S component from a hypothetical leukocidin in ruminants (*lukM*), leukocidin D component gene *lukD*, leukocidin E component gene *lukE*, and leukocidin/hemolysin toxin family proteins encoding genes *lukX* and *lukY*.

Of particular interest was the detection of the PVL genes: *lukF-PV/lukS-PV*, gene products which are very potent leukocidins, allowing for severe necrotizing infections even in healthy patients, and which its prevalence is known to vary globally (126). In the Tanzanian strain set, this gene pair was detected in 10 different CCs, namely CC152 (n=33/34), CC121 (n=23/30), C88 (n=18/23), and CC80 (n=10/10), CC30 (n=6/7), CC5 (n=5/23), CC1{ST573/772}(n=3/18), CC182 (n=2/2), CC59 (n=1/1), and CC8 (n=1/25) respectively. With respect to the sampling site, *lukF-PV/lukS-PV* were detected more in clinical infection isolates (62%) than in commensal isolates (20%).

The other hemolysin genes covered by the DNA-MCA were *hld* (encoding δ-hemolysin), *hla* (encoding α-hemolysin) and *hlb* (encoding β-hemolysin), with the latter gene being represented by four different probes (*hlb* probe-1, *hlb* probe-2, *hlb* probe-3, and undisrupted *hlb)* because this gene locus is a common insertion site for temperate phages in *S. aureus*. Accordingly, an undisrupted *hlb* was noticed in 40 isolates only (15.5% of all strains), while *hld* and *hla* were noticed in almost all isolates (99.7% and 98% respectively).

The three genes covered by the DNA-MCA encoding for the immune evasion molecules staphylokinase (*sak*), chemotaxis-inhibiting protein *(chp),* and staphylococcal complement inhibitor *(scin)* were found in varable frequencies. While presence of *sak* and *scn* was high (87.6% and 99%, respectively), *chp* was noticed at a much lower rate (49.6%), and its proportion was higher among infections-related strains than in commensals. Notably, *chp* was not detected in strains of the CC152 and CC121, respectively, the most frequent lineages found in this study.

The three genes covered by the DNA-MCA encoding exfoliative toxins are exfoliative toxin serotype A (*etA*), exfoliative toxin serotype B (*etB*), and exfoliative toxin serotype D (*etD*). With the exception of *etA,* which was detected in 15 strains (5.8%, mainly from isolates of infection origin), exfoliative toxin encoding genes were nearly not present in the Tanzanian *S. aureus* set.

An overview of the distribution of the above mentioned virulence factor-encoding genes in clinical samples and commensal samples is provided in Table 7.

Table 7: Distribution of the selected virulence factors in *S. aureus* isolates from clinical infections and colonization

Virulence gene (n/%)	Clinical infection (*n*=124)	Colonization (*n*=134)
seb (*n*=46/17.8%)	31 (25%)	15 (11.2%)
lukF-PV (*n*=102/39.5%)	76 (61.3%)	26 (19.4%)
lukS-PV (*n*=104/40.3%)	77 (62.1%)	27 (20.1%)
hla (*n*=234/90.7%)	121 (97.6%)	133 (99.3%)
hld (*n*=257/99.6%)	123 (99.2%)	134 (100%)
hlb (undisrupted) (*n*=40/15.5%)	26 (21%)	14 (10.4%)
sak (*n*=226/87.6%)	112 (90.3%)	114 (85%)
chp (*n*=128/49.6%)	76 (61.3%)	52 (38.8%)
scin (*n*=256/99.2%)	123 (99.2%)	133 (99.3%)
etA (*n*=15/5.8%)	14 (11.3%)	1 (0.75%)
etB (*n*=4/1.6%)	1 (0.8 %)	4 (3%)
etD (*n*=13/5%)	4 (3.2 %)	9 (6.7%)
cna (*n*=126/48.8%)	70 (56.5%)	56 (41.8%)

Utilization of the DNA- MCA also enabled to check for the presence/absence of 15 adhesion factors (surface proteins), namely *bbp, fnbB, ebh, fnbA, ebpS, vwb, clfB, clfA, eno, sasG, fib, map, sdrC, sdrD,* and *cna*. While most of the adhesion factor-encoding genes were detected with high frequencies (71-91%) and independent of their origin, this was not the case with *cna*, which was detected at a much lower rate (48.8%) with a higher proportion among infection than in commensal isolates. Notably, both *fib* and *map* were not detected in CC152 strains, suggesting gene polymorphisms for both genes in this lineage.

4.1.4 Antimicrobial resistance genes (ARGs) profile of the Tanzanian *S. aureus*

Profile of the ARGs among the identified lineages (CCs) from clinical infections'related and commensal *S. aureus* isolates are detailed in Appendix 4. The transport/efflux protein-encoding gene *sdrM* was the most frequently detected resistance determinant, which was found in almost all isolates (99.2%), except for two commensal isolates of the CC22 lineage. A very high prevalence was also found for the penicillinase encoding gene operon *bla*, composed of the beta lactamase repressor gene *blaI*, the beta-lactamase regulatory protein-encoding gene *blaR*, and the penicillinase-encoding gene *blaZ*. The complete operon was detected in about 95% of all strains, regardless of their origin. Genes encoding for fosfomycin resistance determinants were also found with high frequency, and detected in both clinical isolates and commensal isolates. In total, 156 isolates

were found being positive for a chromosomally localized *fosB* (encoding a bacillithiol transferase) and 33 isolates were positive for a plasmid-localized *fosB*. While the chromosomally localized *fosB* was found in comparable numbers in clinical and commensal isolates (72 and 84, respectively), a higher proportion of plasmid-localized *fosB* was found in commensal isolates (25) than in clinical isolates (8). For both *fosB* variants, a clear difference with respect to the CC was observed. While *fosB* was detected in all isolates of CC121, CC8, CC5, CC15, CC6, CC9, CC25, and CC30, it was not detected in all strains of CC152, CC88, CC45, and CC80 lineages. Genes associated with tetracycline resistance were regularly identified in the Tanzanian strain set (*n*=72; 28.3%), dominated by *tetK* (*n*=67; 26.4%). The *tetK* gene was mainly detected in CC152 (*n*=16), CC88 (*n*=12), and CC8 (*n*=10) isolates. The erythromycin and clindamycin resistance-associated gene, *ermC* was identified in 51 isolates (19.8%), and equally found in commensals and from clinical infection isolates. The *ermC* gene was mainly detected in CC152 (*n*=19) and CC5 (*n*=11) isolates. 26 of the 51 *ermC* positive isolates were also positive for the PVL associated genes (*lukF-PV/lukS-PV*), mostly being from CC152-MSSA (*n*=19; 73%). The energy-dependent efflux pump-encoding gene *msr*(*A*), which is also associated with erythromycin and clindamycin resistance, was detected in eight isolates including two from clinical infections and six from nasal carriers, and belonged to CC8 (*n*=4). CC45 (*n*=2), CC88 (*n*=1), and CC121 (*n*=1). The alternate penicillin-binding protein 2 encoding gene *mecA*, which defines MRSA, was identified in seven isolates (2.7% of the Tanzanian strain set), including three isolates from clinical infections (2.4%) and four from commensals (3%). With respect to the CC, *mecA* was found in CC88 (*n*=5), CC59 (*n*=1), and CC8 (*n*=1) isolates. All MRSA strains also contained at least one additional ARG (see Table 8).

Table 8: Distribution of Antimicrobial Resistance Genes among the MRSA identified in this study

MRSA strain type	**Origin**	**Antimicrobial Resistance Genes (ARGs) ***											
		sdrM	***fosB***	***tetK***	***ermC***	***ermB***	***msrA***	***dfrS1***	***aphA3***	***aacA-aphD***	***vga* (A)**	***cat***	***sat***
CC88-MRSA-IV	Commensal	+	-	-	+	-	-	+	-	+	-	+	-
CC88-MRSA-IV	Commensal	+	-	-	-	-	-	+	-	-	-	-	-
CC88-MRSA-IV	Commensal	+	-	+	-	-	-	+	-	-	-	-	-
CC59-MRSA	Commensal	+	-	-	-	+	-	-	+	-	+	+	+
CC88-MRSA-IV, MRSA-2	Clinical infection	+	-	+	-	-	-	+	-	-	-	-	-
CC88-MRSA-IV	Clinical infection	+	-	-	-	-	-	+	-	-	+	-	-
CC8-MRSA-V	Clinical infection	+	+	-	-	-	-	-	-	+	-	-	-

*: **+,** positive; **-**, negative

Out of the MRSA isolates identified among the Tanzanian strain set, one isolate, the CC59-MRSA commensal strain, popped out, as this isolate was negative for *bla*, but harbored five additional ARGs, *sdrM, erm(B), aphA3* (a gene associates with aminoglycoside resistance), *cat* (a gene encoding for a chloramphenicol resistance determinant), and *sat* (encoding for a strepto-thricine-acetyl-transferase conveying resistance to streptothricin. The *dfrS1* gene associated with co-trimoxazole resistance was detected in six isolates, with five of them being CC88-MRSA.

4.2 Phenotypic characterization of the Tanzanian *S. aureus*

Although the DNA-MCA is a strong tool to inform us about the gene content of a given isolate, it provides only little information on whether a gene encodes for a functional protein or represents a pseudogene. To account for this, a series of phenotypic assays was carried out, starting with AST of the whole isolates set.

4.2.1 Phenotypic AST results of the Tanzanian *S. aureus*

Mostly in line with the DNA-MCA-derived resistance-determinant data, a very high resistance rate against penicillin was observed (95% of the isolates tested), followed by erythromycin (29%), clindamycin (24%), tetracycline (19%), and co-trimoxazole (14%). Methicillin resistance, phenotypically detected by cefoxitin resistance, was suggested for 14 isolates (5.4%), and thus considerably higher than suggested by the DNA-MCA results. A detailed comparison of the phenotypic and DNA-MCA results is given in the discussion section.

With respect to the origin of the isolate, methicillin (cefoxitin) resistance was observed in seven infection-related and seven commensal isolates (Appendix 5). Penicillin resistance was detected in 98% of infection-related isolates and 93% commensals; erythromycin resistance was found in 27% infection-related isolates and 31% commensals; clindamycin resistance was detected in 25% infection-related isolates and 23% commenals; tetracycline resistance was noticed in 25% infection-related isolates and 14% commensals; and co-trimoxazole resistance was observed in 16% infection-related isolates and 13% commensals, respectively. Gentamycin and chloramphenicol were highly sensitive among the tested isolates of both infection and commensal origins.

4.2.2 Strain-specific phenotypic behaviour characterization of the Tanzanian *S. aureus*

Given that a full phenotypic characterization of the entire Tanzanian strain set was not possible for economic and time reasons, a representative set of 98 Tanzanian *S. aureus* isolates was chosen, which included 55 commensals and 43 clinical infection-related isolates (Table 9). This subset covered the seven predominant CCs found in the DNA-MCA analysis (CC152, CC6, and CC8 of *agrI;* CC15 and CC5 of *agrII*; CC88 of *agrIII*; and CC121 of *agrIV*), with each CC represented by 14 strains.

Table 9: The selected Tanzanian *S. aureus* strains for phenotypic activities characterization

CC type	Total	Commensal	Clinical infection
CC121	14	6	8
CC152	14	7	7
CC15	14	11	3
CC5	14	7	7
CC6	14	10	4
CC8	14	7	7
CC88	14	7	7
Total	98	55	43

4.2.2.1. Strain-specific hemolytic activity results of the Tanzanian *S. aureus* subset

The hemolytic activity of the selected 98 Tanzanian *S. aureus* strains was determined with supernatants of overnight cultures, which were serially diluted as outlined in the methods section before they were brought into contact with the human erythrocyte suspension. The hemolytic activity was recorded at a dilution titer with a complete lysis. Each strain was tested in three biological replicates, and mean values of strain-specific hemolytic potential are shown in Figure 6.

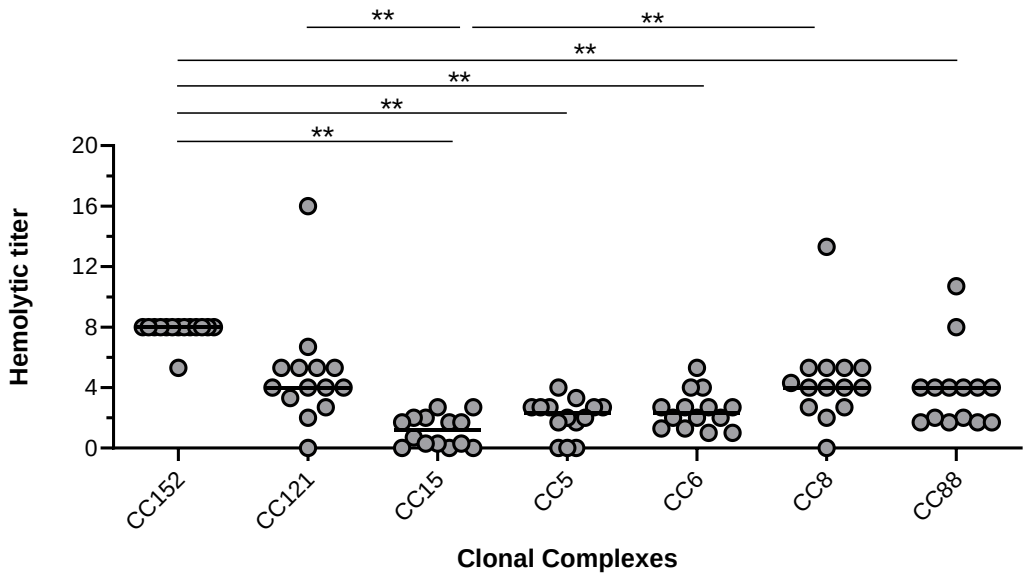

Figure 6: CC-specific hemolytic activity of the selected Tanzanian *S. aureus* subset
For the determination of the hemolytic potential of the CCs, isolates were grown overnight in TSB and supernatants normalized to a culture equivalent to an OD_{600} of 8. Normalized supernatants were either used undiluted or diluted up to 1:64 to challenge a human erythrocyte suspension for 30 min at 37°C followed by 16 h at 4°C. The highest dilution giving rise to full lysis of the erythrocytes was defined as the hemolytic titer. Isolates were tested in three biological replicates. Data represent the mean values of individual isolates (filled symbols) and the median per CC. **, $p<0.01$ (Kruskal Wallis test and Dunn's post hoc test).

The majority of the tested strains (*n*=73; 74.5%) displayed a haemolytic titer ≤4. Only 17 strains (17.3%) from four different CCs (CC152 {*n*=13}, CC121 {*n*=1}, CC8 {*n*=1}, and CC88 {*n*=2}) induced a complete hemolysis of the erythrocytes at dilution titers ≥8. No hemolytic activity was observed in supernatants of eight strains (8.2%) from four different CCs (CC5 {*n*=3}, CC15 {*n*=3}, CC121 {*n*=1}, and CC8 {*n*=1}).

On the CC level, isolates of CC152 peaked out of the strain subset. Here, 13 out of 14 tested isolates displayed a hemolytic titer of 8, which was significantly higher when compared to most of the other tested CCs (Figure 6). When checking for potential differences in the hemolytic behaviors of clinical and commensal isolates within a given CC, no clear differences were detected (Figure 7).

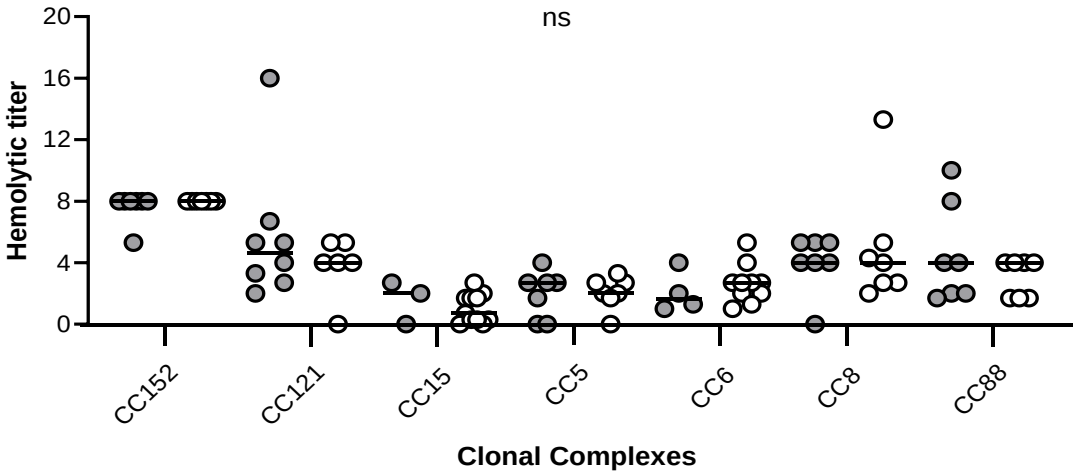

Figure 7: Origin-specific hemolytic activity of the Tanzanian *S. aureus* subset
Assays were performed as outlined in Figure 6. Data represent the mean values of individual clinical isolates (filled symbols) and commensal isolates (open symbols). The horizontal bar indicates the median per group; ns, not significant (Mann Whitney U test between clinical and commensal isolates of a given CC).

However, this type of comparison was done for this strain set only exemplary for the hemolytic activities, because for some CCs (i.e. CC15 and CC6), only a small number of clinical isolates was available to me, which presumably impairs the validity of the observations and the statistical analysis.

4.2.2.2 Strain-specific cytotoxic activity: lactate dehydrogenase (LDH) assay results of the selected Tanzanian *S. aureus* strains on epidermal keratinocytes

The capability of *S. aureus* to invade and induce the death of eukaryotic cells (various human cells) has been hitherto investigated. In this study, the cytotoxic activity of the Tanzanian *S. aureus* subset was investigated against HaCat, tested by the lactate dehydrogenase (LDH) release assay. Supernatants obtained from overnight cultures of the isolates were normalized to an OD_{600} of 8 and coincubated with the HaCaT cells in different dilutions (undiluted to 1:16; Figure 8).

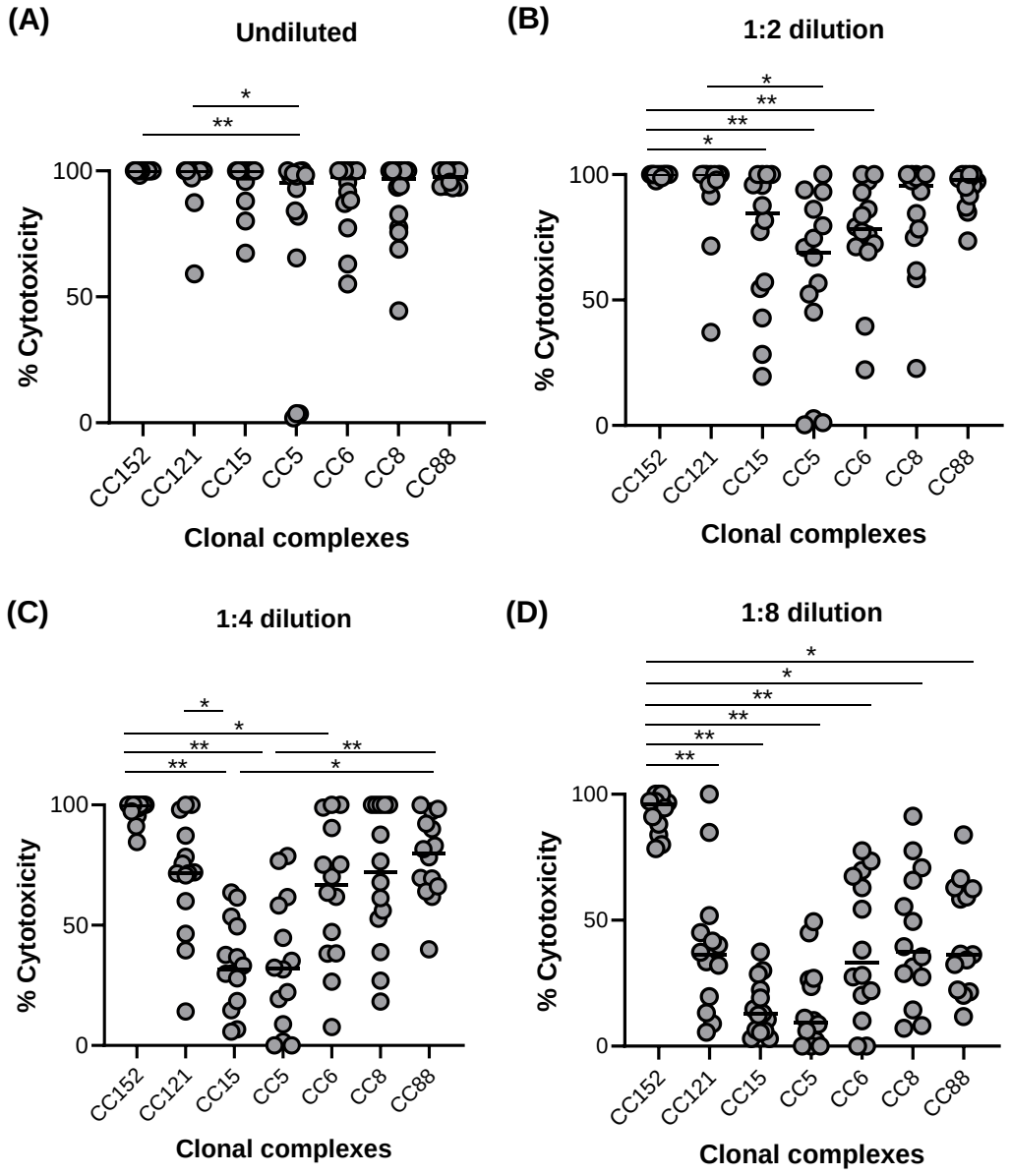

Figure 8: CC-dependent cytotoxic activities of overnight culture supernatants of the Tanzanian *S. aureus* subset

For the determination of the cytotoxic potential of the CCs, isolates were grown overnight in TSB and supernatants normalized to a culture equivalent to an OD_{600} of 8. Normalized supernatants were either used undiluted (A) or diluted 1:2 (B), 1:4 (C), and 1:8 (D) to challenge a confluent layer of HaCaT cells for 4 h. The cytotoxic potential of the supernatants was determined by an LDH release assay, and obtained values were normalized with the unchallenged and ethanol-treated controls as outlined in the methods section. Isolates were tested in three biological replicates. Data represent the mean values of individual isolates (filled symbols) and the median per CC. *, P<0,05; **, P<0.01 (Kruskal Wallis test and Dunn's post hoc test).

The undiluted supernatants of almost all tested strains (n=94; 96%) induced an LDH release >50% of the LDH release seen in 20% ethanol-treated HaCaT cells (which were set to 100%), except for four strains from

CC5 (*n*=3) and CC8 (*n*=1), respectively, which induced cytotoxic effects <50% of the ethanol control (Figure 8A). However, when diluted 1:16, only very few strains (*n*=6) still displayed a cytotoxic activity >50%, namely four CC152 isolates, one CC6 and one CC88 isolate, hence, in Figure 8, only the cytotoxic potential results of the undiluted, the 1:2, 1:4, and 1:8-diluted culture supernatants are presented. This serial dilution approach revealed that all of the CC152 strains maintained their high cytotoxic activity even when diluted 1:8, while most strains from other CCs (10%) started to display a reduced cytotoxic potential with increasing dilutions.

A comparison of cytotoxicity potential for each CCs at undiluted against its 1:8 dilution revealed significant differences ($p<0.05$, paired sample t-test) for all CCs except CC152. Similarly, at 1:8 dilution the cytotoxic potential of CC152 was significantly higher than those seen with the other CCs, indicating that isolates of this CC extrete larger amounts of cytolytic compounds into the medium than isolates of the other CCs.

4.2.2.3 Strain-specific phagocytosis evasion capacities of the selected Tanzanian *S. aureus*

S. aureus is known for its ability to modulate the host's innate immune response in many ways, including interference with the phagocytosis process by PMNs (3). Thus, the ability of the Tanzanian *S. aureus* subset was tested to prevent phagocytosis by PMNs in a whole blood phagocytosis assay (Figure 9).

By testing the *S. aureus* uptake rates by PMNs at different time intervals, an increased uptake of *S. aureus* by PMNs was observed for the first 30 min for all CCs. However, after 60 min of coincubation, for all CCs, a plateau was reached, indicating either a saturation of the PMN uptake capacities or a depletion of freely available bacterial cells. The detected ranges of the mean fluorescence intensity (MFI) values per PMN for each CCs' PMN uptake at 5 and 60 minutes were 232 and 642 RLU for CC152, 247 and 595 RLU for CC121, 280 and 627 RLU for CC8, 311 and 742 RLU for CC88, 332 and 802 RLU for CC5, 363 and 839 RLU for CC15, and 399 and 857 RLU for CC6. Overall, isolates from CC152 and CC121 presented the lowest uptake rates by PMNs, whereas isolates from CC15 and CC5 displayed the highest uptake rates. On the individual isolate level, a large variation was observed within a given CC. MFI values per PMN at 60 minutes post-challenge ranged from 362 RLU, presented by a CC8-MSSA isolate, to 1464 RLU, presented by a CC6-MSSA isolate. The 10 isolates giving rise to the lowest MFI values (≤500 RLU) at 60 minutes were detected among six different CCs: CC8 (*n*=4), CC152 (*n*=2), CC5 (*n*=2), CC121 *(n*=1), and CC15 *(n*=1). The 10 isolates giving rise to the highest MFI values (≥1000 RLU) at 60 minutes were detected among five different CCs: CC5 (*n*=3), CC15 (*n*=2), CC6 (*n*=2), CC88-MRSA (*n*=2), and CC8 (*n*=1), illustrating nicely that in one CC (e.g CC15), isolates with a very low and high phagocytosis prevention potential may be present.

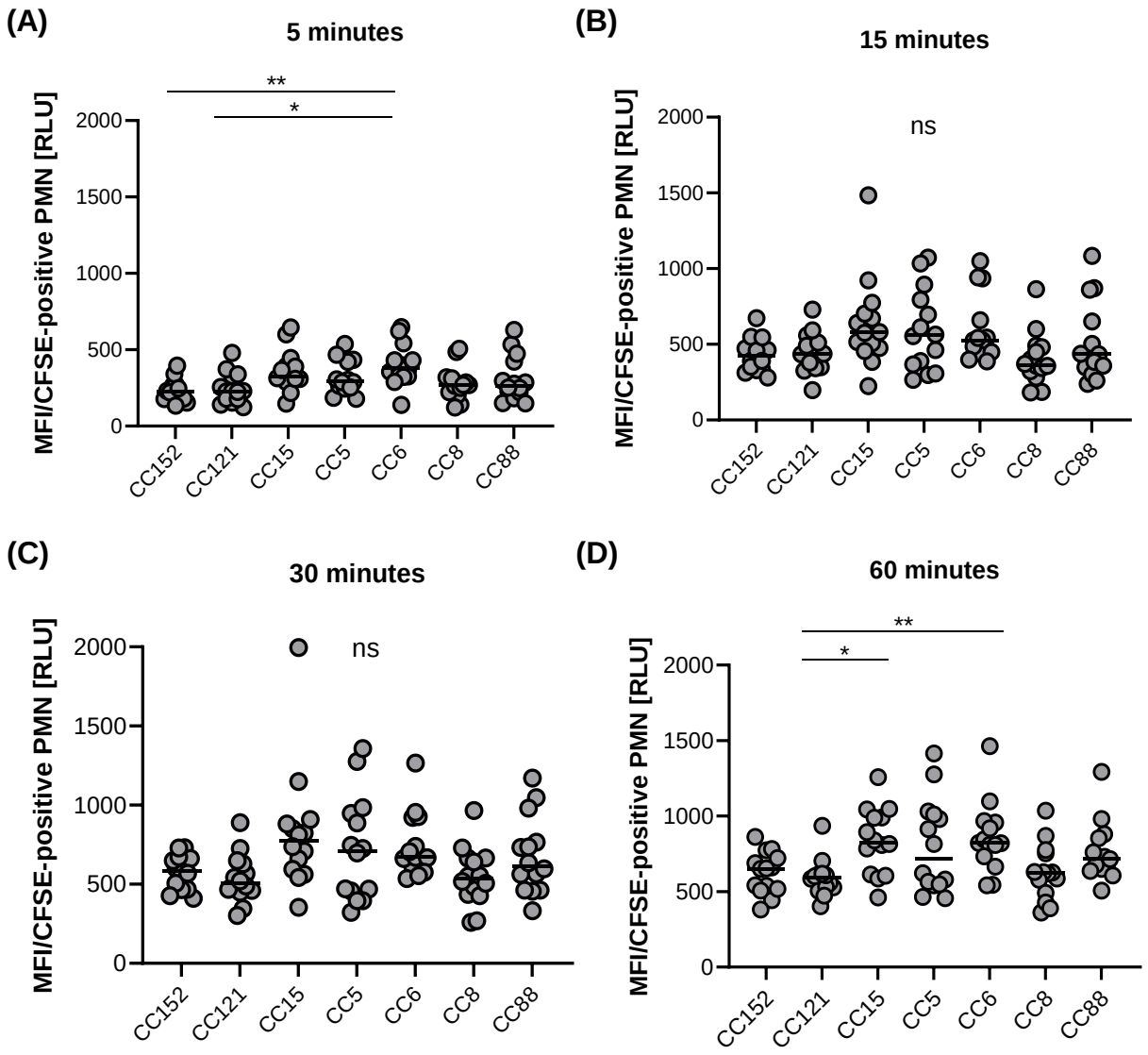

Figure 9: PMN phagocytosis evasion capabilities of the Tanzanian *S. aureus* subset
For the determination of the phagocytosis prevention potential, *S. aureus* isolates were CFSE-stained and subsequently co-incubated with freshly withdrawn whole blood for up to 1 h at 37°C and 1000 rpm. Samples were removed after 5 min (A), 15 min (B), 30 min (C) and 60 min (D). After lysis of erythrocytes and washing, samples were subjected to FACS analyses, gating for PMNs, and determining the fluorescence intensities of the gated PMNs. The data represent the mean values per isolate (filled symbols) determined in three biological experiments, and the horizontal bar indicates the median of all observations made in a given CC. ns, not significant; *, $p<0,05$; **, $p<0,01$ (Kruskal Wallis test and Dunn's post hoc test).

4.3 Strain-specific phenotypic behaviour characterization of the selected *S. aureus* from the African-German StaphNet Consortium

As a contributing partner of the African-German StaphNet consortium collection (119), I was substantially involved in the collection and genetic characterization of the Tanzanian isolates of this collection. In this

multinational network, 1200 isolates were collected, with 600 originating from African sites and 600 from Germany. Half of them were isolated from nasal swabs and the other half were isolated from infections. The genetic characterization of this strain collection revealed that some CCs are predominantly found in Africa (i.e. CC121 and CC152) and Germany (i.e. CC22 and CC30), respectively, while others (i.e. CC5 and CC8) were found with similar frequencies in both geographic areas. DNA-MCA-based genotyping of the isolates of this collection revealed that the "African clones" (i.e. CC121 and CC152) were particularly often positive for *lukF-PV/lukS-PV* (73% and 98%, respectively), while this leukocidin-encoding gene pair was if at all, only very rarely detected in the CCs predominantly found in Germany (119). As other important virulence factors of *S. aureus* such as *edinB, isaB, sasG, seb* and *splB* were found to be significantly enriched in the African isolates (119), it was tempting to speculate that isolates of the African and German-specific CCs may also differ in their phenotypic behavior. To address this hypothesis, a set of 150 isolates representing the 10 predominant CCs from the African-German StaphNet consortium collection was compiled for phenotypic behavior characterizations. The 10 CCs included five CCs (CC121, CC152, CC1, CC15, and CC88) that were found being overrepresented in the African strain set, three CCs (CC22, CC30, and CC45) that were predominantly found in the German strain set, and two CCs (CC5 and CC8) that were frequently detected at both geographic sites. Given the importance of CC121 and CC152 in Africa, a special focus was given in this strain set on isolates of these two CCs (60 CC121 isolates, 30 of them originating from infection and 30 originating from colonization, with half of them being positive for *lukF-PV/lukS-PV*; 30 CC152 isolates, 15 of them originating from infection and 15 originating from colonization, all being positive for *lukF-PV/lukS-PV*) to allow for a more in dept analysis of the origin and *lukF-PV/lukS-PV* status on the phenotypic behavior of these isolates, respectively. For the rest of the CCs included into this strain set, smaller isolate numbers were selected. The strains of this set were tested for their hemolytic capacity on human erythrocytes, cytotoxicity on keratinoctyes, and their PMN phagocytosis evasion capacities.

4.3.1 Hemolytic activity of the selected *S. aureus* strains from the African-German StaphNet Consortium

4.3.1.1 Hemolytic activity results of the selected 10 different *S. aureus* CCs from the African-German StaphNet Consortium

Results of strain-specific hemolytic activity of the 10 selected *S. aureus* CCs from the African-German StaphNet consortium are displayed in Figure 10: Cell culture supernatants of the majority of tested strains (n=109) induced a complete hemolysis at a dilution of <1:8 (i.e. a titer of <8), while only few strains (*n*=30) produced supernatants with hemolytic titers ≥ 8, of which 1/3 were contributed by CC152 (*n*=10). Other tested CCs with hemolytic titers ≥ 8 included C22 (*n*=6), CC45 (*n*=5), CC8 (*n*=3), CC121 (*n*=2), CC88 (*n*=2), CC30

(*n*=1), and CC5 (*n*=1). An overall high hemolytic potential of CC152 isolates is also suggested by the finding that the hemolytic titer of CC152 isolates was significantly higher than those of most other CCs tested here.

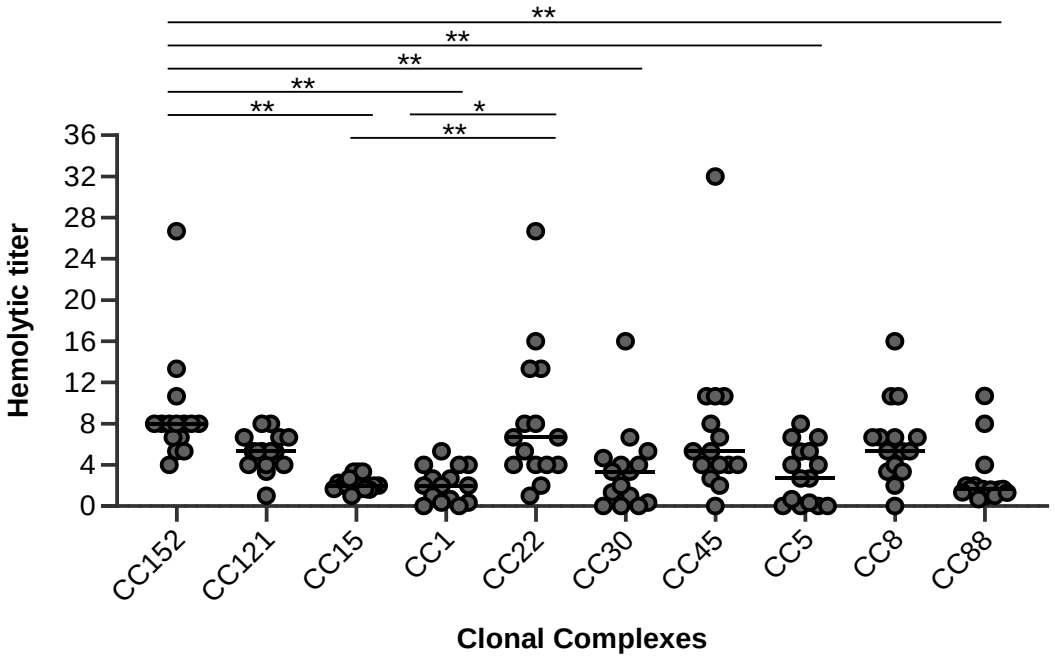

Figure 10: CC-specific hemolytic activity of the selected African-German StaphNet Consortium *S. aureus* subset
Hemolytic potentials of isolates of the African-German StaphNet consortium subset. Each CC was represented by 15 isolates. Assays were done as outlined in Figure 6, and isolates were tested in three biological replicates. Data represent the mean values of individual isolates (filled symbols) and the median per CC. *, *P*<0,05; **, *P*<0.01 (Kruskal Wallis test and Dunn's post hoc test).

From the 150 tested *S aureus* strains, non-hemolytic activity was observed in 11 (7%) strains belonging to five different CCs: CC5 (*n*=4), CC30 (*n*=3), CC1 (*n*=2), CC45 (*n*=1), and CC8 (*n*=1). These 11 non-hemolytic strains included four commensals and seven clinical infection-related isolates, of which four strains were from skin infections and three strains from blood born infections.

Checking for potential differences in the hemolytic behaviors of clinical and commensal isolates within a given CC revealed again no clear differences between both groups (Figure 11), suggesting that the origin of the isolate (clinical infection vs. colonization) is not a major factor for this virulence phenotype.

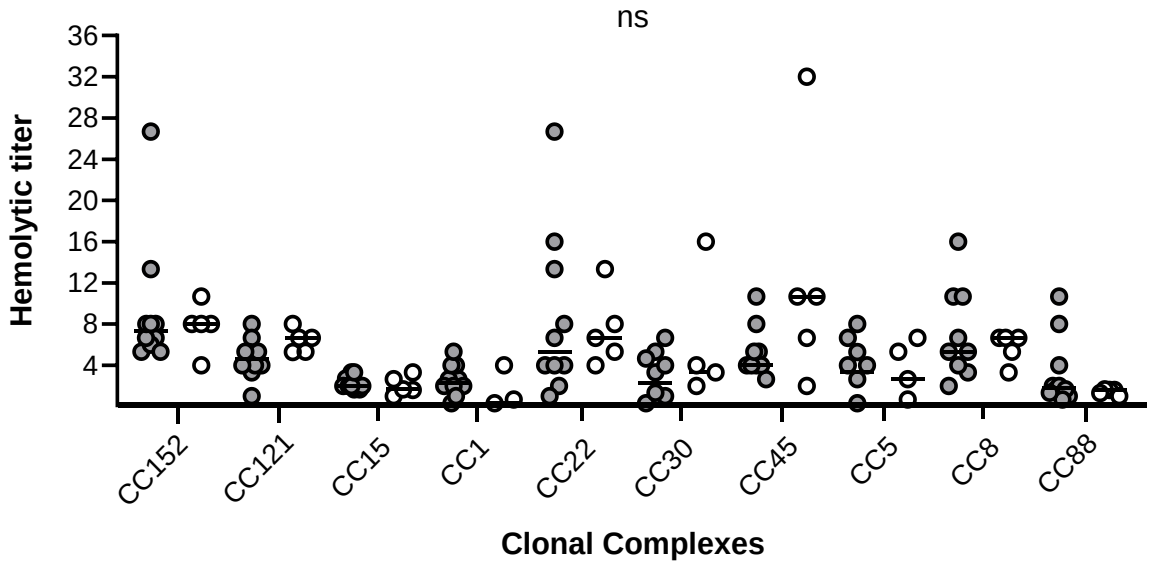

Figure 11: Origin-specific hemolytic activity of the African-German StaphNet Consortium *S. aureus* subset
Assays were performed as outlined in Figure 6. Data represent the mean values of individual clinical isolates (filled symbols) and commensal isolates (open symbols). The horizontal bar indicates the median per group; ns, not significant (Mann Whitney U test between clinical and commensal isolates of a given CC).

4.3.1.2 Hemolytic activity results of CC121 and CC152 *S. aureus* strains from the African-German StaphNet Consortium

To substantiate my hypothesis that the source of the isolate is only of minor importance for the hemolytic potential of a CC, I determined the hemolytic titers for the 60 CC121 strains and 30 CC152 strains from the African-German StaphNet *S.aureus* subset, which comprised equal numbers of clinical infection-related isolates and commensal isolates (Figure 12).

For CC121, the majority of the tested strains (52/60) displayed hemolytic titers <8 (25 infection-related and 27 commensal isolates). Only six strains (10%) produced hemolytic titers ≥8 (two commensals and four clinical infection-related isolates). Non-hemolytic activity was observed with two strains (one clinical isolate and one commensal). In contrast, the majority of tested CC152 strains (22/30) presented a hemolytic titer ≥8 (10 commensals and 12 clinical infection-related), suggesting CC152 isolates to be more hemolytic than CC121 isolates. However, for both CCs, no significant differences were observed between infection-related and commensal isolates, supporting the hypothesis that the body source is not a relevant factor for the virulence phenotype of the isolate within the CCs tested here.

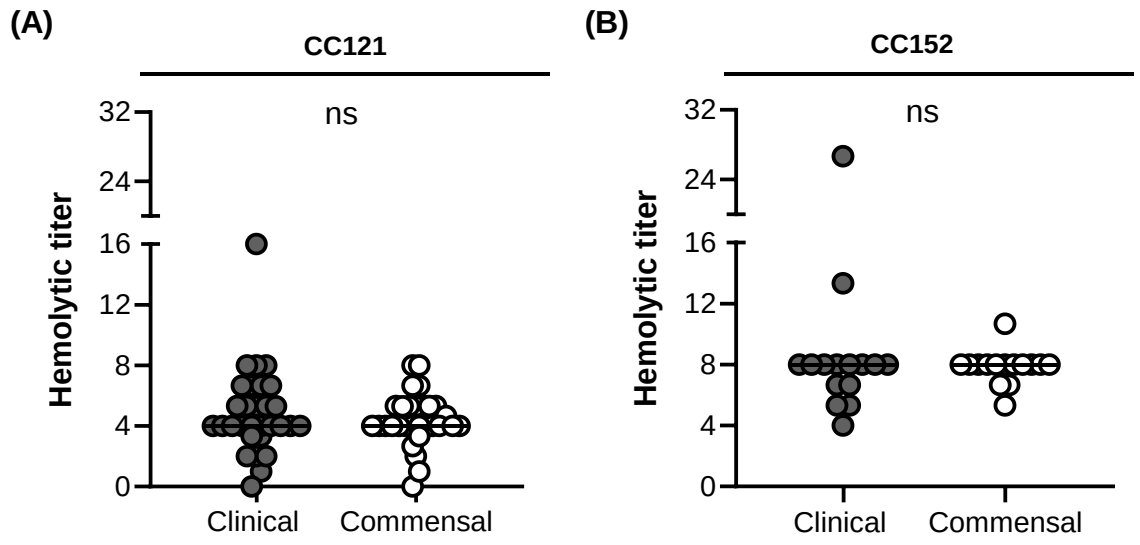

Figure 12: Hemolytic activities of CC121 and CC152 strains from the African-German StaphNet Consortium

Hemolytic potentials of 60 CC121 strains (A) and 30 CC152 strains (B) originating from infection (filled symbols) and colonization (open symbols), respectively. The hemolytic activity of each isolate was tested in three biological replicates as outlined in Figure 6. Data represent the mean values of individual isolates (symbols). The horizontal bar indicates the median per group; ns, not significant (Mann Whitney U test).

4.3.2 Cytotoxicity activity of the selected *S. aureus* strains from the African-German StaphNet Consortium on keratinocytes

4.3.2.1 Cytotoxicity activity results of the selected 10 different *S. aureus* CCs from the African-German StaphNet Consortium

For the determination of the cytotoxic potentials of the selected strains of the African-German StaphNet consortium, supernatants of the *S. aureus* strains were coincubated in different concentrations with epidermal keratinocytes, and the cytolytic potentials of the bacteria on this eukaryotic cell type determined by a lactate dehydrogenase (LDH) release assay in three biological replicates. Very few strains showed a cytotoxic activity at the 1:16 dilution, hence, in Figure 13 are only the results of the undiluted samples and three different dilutions (1:2, 1:4, and 1:8) presented. Almost all tested strains (96%) showed a cytotoxicity potential of ≥ 50% when the undiluted culture supernatants were used to harm the HaCaT cells (Figure 13A) with an exception of nine (6%) strains from CC30 (*n*=4), CC1 (*n*=3), CC5 (*n*=1), and CC8 (*n*=1). However, the cytotoxic effects of the cell culture supernatants markedly decreased when 1:8 dilutions were used (Figure 13D), allowing for a better discrimination of the cytotoxic potentials of the individual CCs.

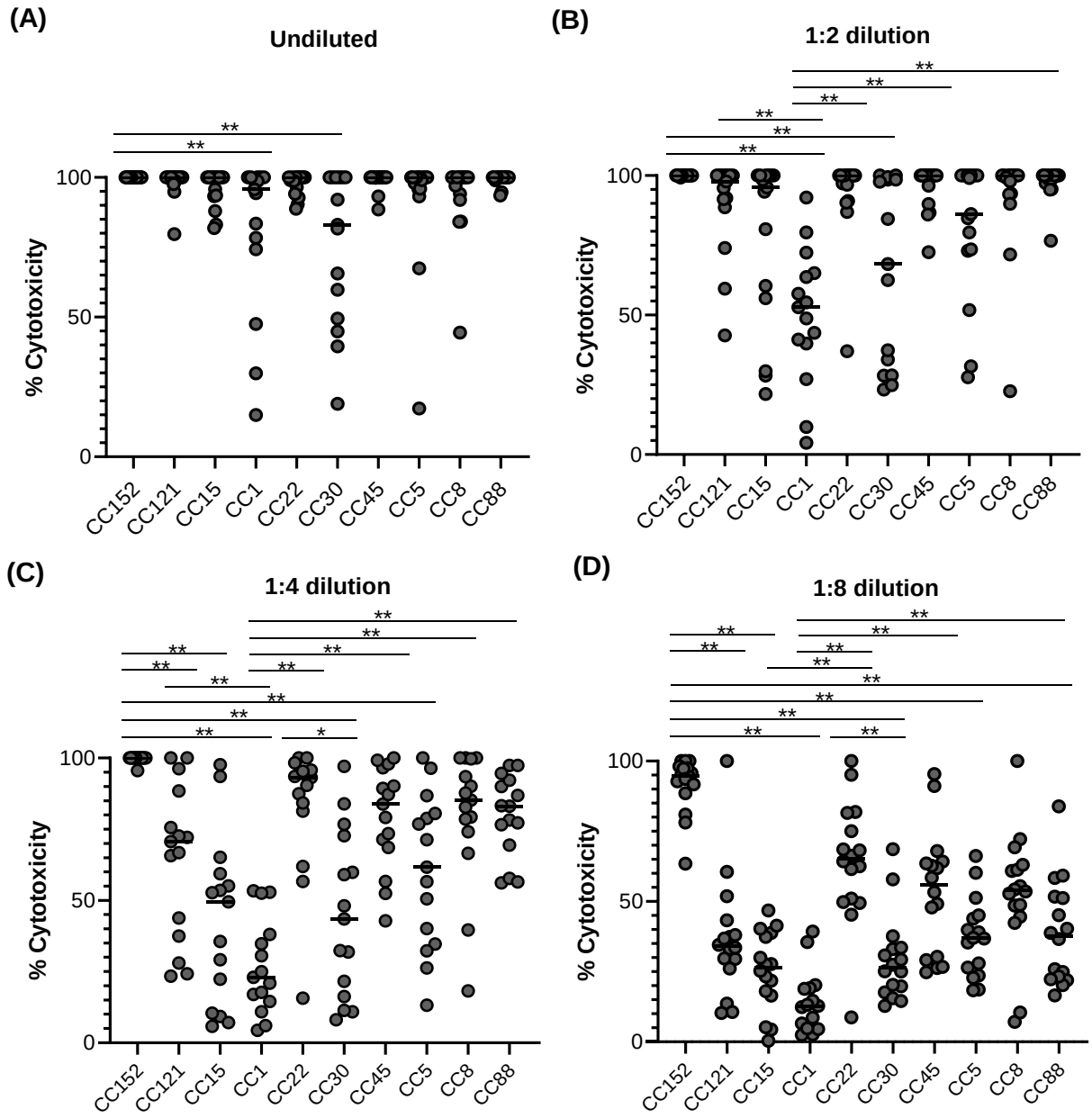

Figure 13: CC-dependent cytotoxic activities of overnight culture supernatants of the African-German StaphNet Consortium *S. aureus* subset

Assays were performed as outlined in Figure 8, included 150 African-German StaphNet Consortium *S. aureus* from 10 different CCs (each CC included 10 isolates from clinical infection and 5 commensal isolates) that were tested in three biological replicates at undiluted (A) and diluted 1:2 (B), 1:4 (C), and 1:8 (D). The data represent the mean values of individual isolates (filled symbols) and the median per CC. *, P<0,05; **, P<0.01 (Kruskal Wallis test and Dunn's post hoc test).

All of the CC152 isolates tested here still produced a cytotoxic potential of ≥50%. High cytotoxic potentials were also observed for the majority of isolates of CC22, CC45, and CC8, while all other CCs produced with

the majority of isolates cytotoxic potentials that were clearly below 50%. The lowest cytotoxic potential was observed with the CC1 isolates, which all displayed cytotoxic potentials <50%.

4.3.2.2 Cytotoxic activity of CC121 *S. aureus* from the African-German StaphNet Consortium

To get deeper insights into the impact of the origin or *lukF-PV/lukS-PV*-carriage on the cytotoxic potential of a given isolate, the cytotoxic potential of the 60 CC121 isolates on keratinocytes was tested next with the LDH release assay as outlined before (Figure 8). CC121 isolates were either grouped according to their origin (Figure 14A) or presence/absence of the *lukF-PV/lukS-PV* genes (Figure 14B).

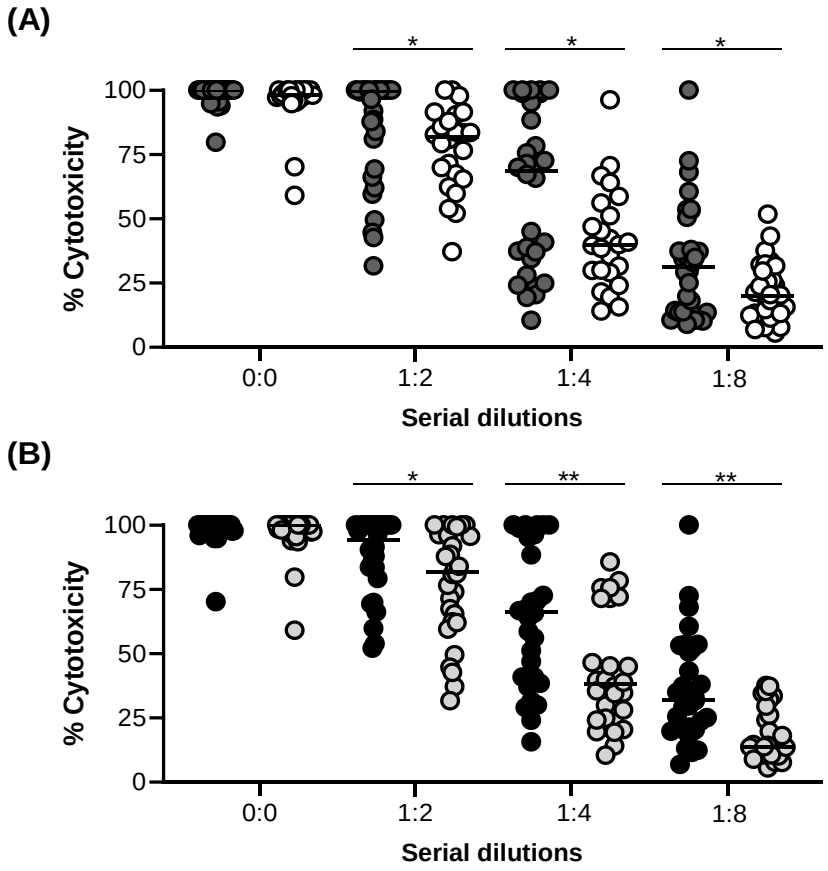

Figure 14: Impact on origin an *lukF-PV/lukS-PV*-carriage on the cytotoxic activity of CC121 isolates from the African-German StaphNet Consortium
Assays were performed as outlined in Figure 8. **A:** Data represent the mean values of individual clinical isolates (filled symbols) and commensal isolates (open symbols). **B:** Data represent the mean values of *lukF-PV/lukS-PV*-positive isolates (black symbols) and *lukF-PV/lukS-PV*-negative isolates (light grey-filled symbols). The horizontal bar indicates the median per group. *, $p<0.05$; **, $p<0.01$ (Mann Whitney U test between groups at a given dilution).

When undiluted culture supernatants were used to challenge the HaCaT cells, all CC121 strains caused strong cytotoxic effects on the keratinocytes (i.e. cytotoxic potentials >90%), in line with the observations made with the other CCs. However, when dilutions were used, clear differences between the groups were observed. Starting with a dilution factor of 2, supernatants of clinical infection CC121 isolates produced significantly stronger cytotoxic effects on HaCaT cells than those of commensal isolates, and this effect was visible in all three dilutions shown (Figure 14A). Basically the same trend was observed when supernatants of *lukF-PV/lukS-PV*-positive CC121 isolates were compared to *lukF-PV/lukS-PV*-negative isolates of this CC (Figure 14B), suggesting that both, a clinical origin and *lukF-PV/lukS-PV*-carriage, are associated with a higher cytotoxicity of CC121 isolates.

4.3.2.3 Cytotoxic activity of CC152 *S. aureus* from the African-German StaphNet Consortium

Since the CC121 strain set included *lukF-PV/lukS-PV*-positive and *lukF-PV/lukS-PV*-negative isolates, which might have influenced the impact of the sampling origin on cytotoxicity, I also tested supernatants of the CC152 strain set, which all were positive for *lukF-PV/lukS-PV*. In line with my findings presented in Figure 13 with a smaller number of CC152 isolates, I again observed a strong cytotoxic potential for all 30 isolates of CC152 at any concentration tested (Figure 15).

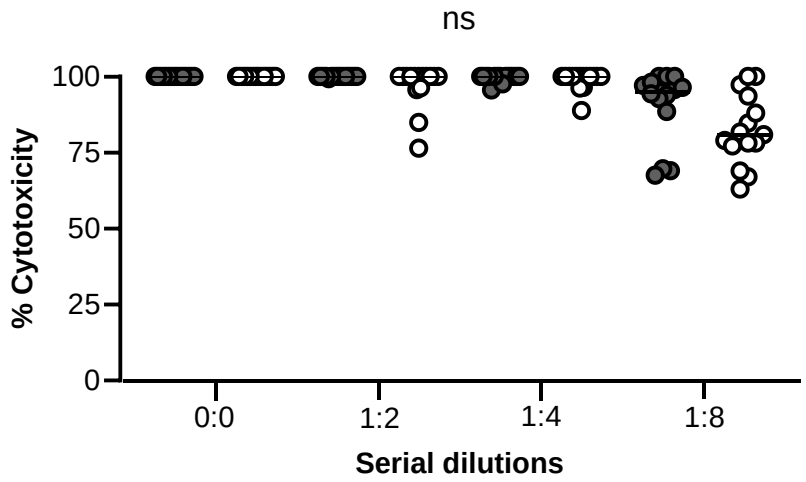

Figure 15: Impact of the origin on the cytotoxic potential of *lukF-PV/lukS-PV*-positive CC152 isolates from the African-German StaphNet Consortium
Assays were performed as outlined in Figure 8. Data represent the mean values of individual clinical isolates (filled symbols) and commensal isolates (open symbols). The horizontal bar indicates the median per group; ns, not significant (Mann Whitney U test between groups at a given dilution).

However, when dilutions of 1:8 were used, supernatants obtained from commensal isolates tended to produce a slightly lower cytotoxic potential on HaCaT cells than supernatants obtained from clinical infection-related isolates, although this effect did not reach statistical significance (p=0.068, Mann Whitney U test).

4.3.2.4 Cytotoxic activity of *lukF-PV/lukS-PV*-positive CC121 and CC152 *S.aureus* from the African-German StaphNet Consortium

My findings presented in Figures 14 and 15 indicated that *lukF-PV/lukS-PV*-carriage is a major contributor to the cytotoxic capacity of CC121 and CC152 isolates on HaCaT cells. However, since both CCs differed markedly in their capacity to harm the keratinocytes (Figure 13), *lukF-PV/lukS-PV*-carriage might not be the only determinate being responsible for the high cytotoxic potential seen with CC152 isolates. To corroborate this hypothesis, I directly compared in Figure 16 the cytotoxic activities of *lukF-PV/lukS-PV*-positive CC121 and CC152 isolates on HaCaT cells.

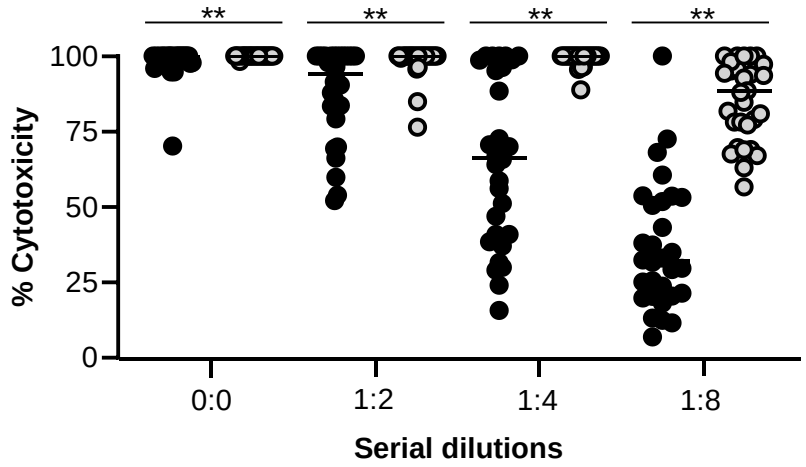

Figure 16: The cytotoxic potential of *lukF-PV/lukS-PV*-positive CC121 and CC152 *S. aureus* from the African-German StaphNet Consortium
Assays were performed as outlined in Figure 8. Data represent the mean values of *lukF-PV/lukS-PV*-positive CC121 isolates (black symbols) and *lukF-PV/lukS-PV*-positive CC152 isolates (light grey-filled symbols). The horizontal bar indicates the median per group. **, $p<0.01$ (Mann Whitney U test between groups at a given dilution).

Even when only *lukF-PV/lukS-PV*-positive isolates were compared to each other, supernatants of CC152 isolates produced in the median at all dilutions tested significant higher cytotoxic potentials than supernatants of CC121 isolates, supporting the idea that *lukF-PV/lukS-PV*-carriage is not the only factor contributing to the particularly high cytotoxic potential of CC152 isolates to harm HaCaT cells.

4.3.3 Phagocytosis evasion capabilities of the selected *S. aureus* strains from the African-German StaphNet Consortium

4.3.3.1 Strain-specific Phagocytosis prevention potential of the selected 10 different *S. aureus* CCs from the African-German StaphNet Consortium

The ability of isolates of the 10 different CCs to escape phagocytosis by PMNs was analyzed in whole blood phagocytosis assays over time (Figure 17).

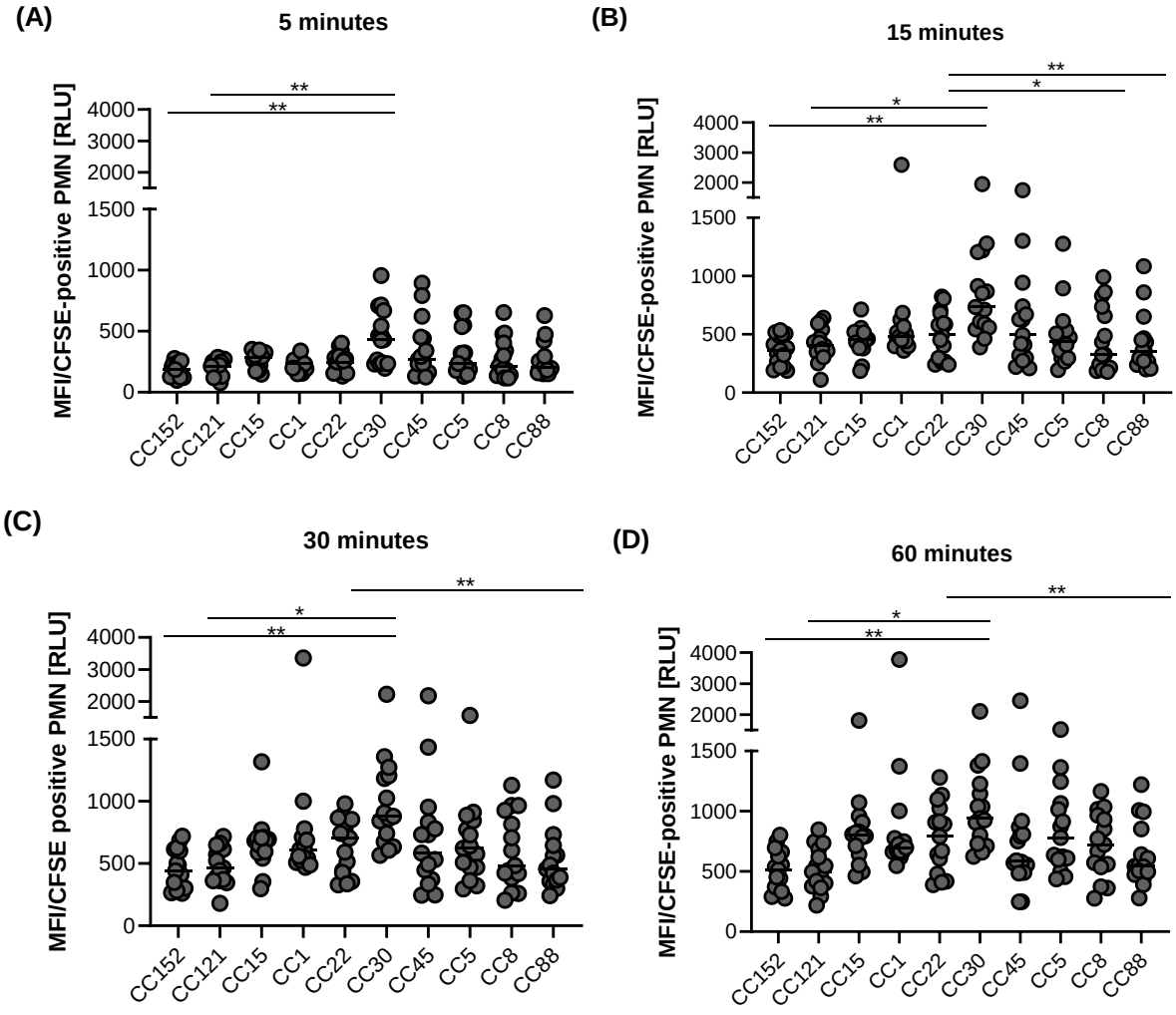

Figure 17: PMN phagocytosis evasion capabilities of the African-German StaphNet Consortium *S. aureus* subset

S. aureus isolates were CFSE-stained and subsequently co-incubated with freshly withdrawn whole blood as outlined in Figure 9 and samples were removed after 5 min (A), 15 min (B), 30 min (C) and 60 min (D), respectively. After lysis of erythrozytes and washing, samples were subjected to FACS analyses, gating for PMNs, and determing the fluorescence intensities of the gated PMNs. The data represent the mean values per isolate (filled symbols) determined in three biological experiments, and the horizontal bar indicates the median of all observations made in a given CC. *, $p<0,05$; **, $p<0,01$ (Kruskal Wallis test and Dunn's post hoc test).

Similar to my observations made with the Tanzanian strain set (Figure 9), large variations in uptake rates were detectable within a given CC, and after 60 min of coincubation, a plateau was reached for all CCs. Overall, isolates from CC152, CC121, and CC88 produced the lowest uptake rates by PMNs, whereas isolates from CC30 displayed the highest uptake rates. MFI values per PMN at 60 minutes post challenge ranged from 218 RLU, obtained with a CC121 isolate, to 3779 RLU, presented by a CC1 isolate. The eight isolates giving rise to the lowest MFI values (<300 RLU) at 60 minutes were detected among five different CCs: CC152 (*n*=2),

CC121 (*n*=2), CC45 (*n*=2), CC8 (*n*=1), and CC88 (*n*=1). The 10 isolates giving rise to the highest MFI values (>1300) at 60 minutes were detected among five different CCs: CC30 (*n*=3), CC1 (*n*=2), CC5 (*n*=2), CC45 (*n*=2), and CC15 (*n*=1), illustrating once again that in one CC (i.e. CC45), isolates with a very low and high phagocytosis prevention potential may be present. Overall, no clear differences in the phagocytosis evasion capacities were observable between the CCs tested, except for CC30, which was taken up more efficiently by PMNs than most of the other CCs.

4.3.3.2 Phagocytosis escaping of CC121 *S. aureus* from the African-German StaphNet Consortium

To test for the impact of the sampling site (infection or colonization) and *lukF-PV/lukS-PV*-carriage on the PMN phagocytosis evasion capacity, the CC121 isolate subset from the African-German StaphNet Consortium was tested with the whole blood phagocytosis assay, and results grouped according to the origin (Figure 18A) and *lukF-PV/lukS-PV*-carriage status (Figure 18B).

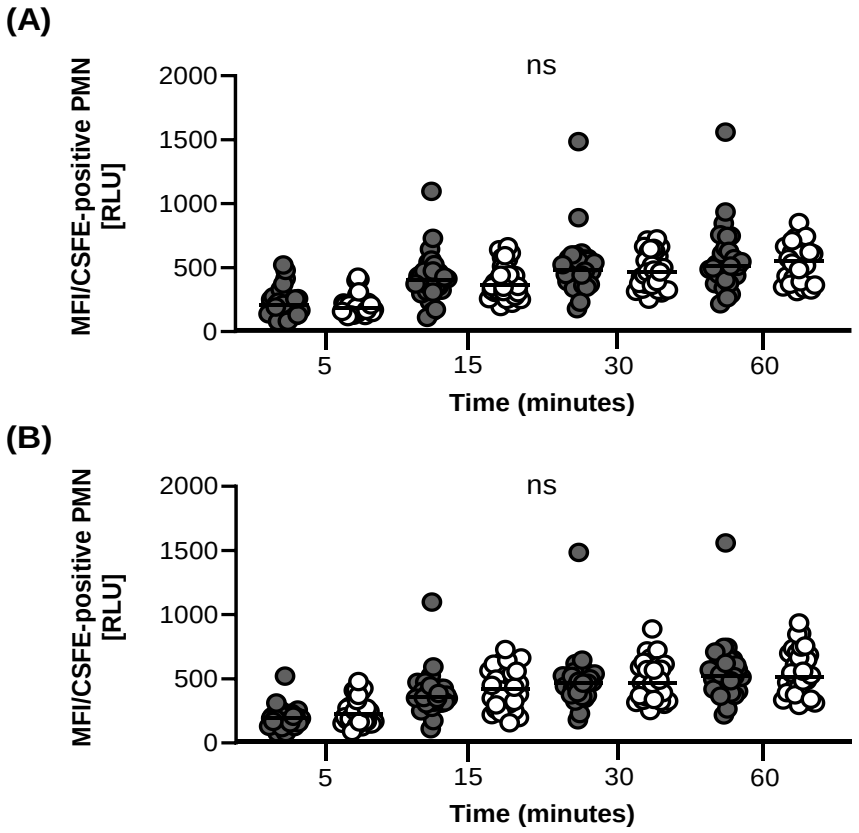

Figure 18: PMN phagocytosis evasion capabilities of CC121 *S. aureus* from the African-German StaphNet Consortium
CC121 isolates were subjected to the whole blood phagocytosis assay as outlined in Figure 9. Samples were removed after 5 min, 15 min**,** 30 min and 60 min**,** respectively. **A:** Data represent the mean values of individual

clinical isolates (filled symbols) and commensal isolates (open symbols). **B**: Data represent the mean values of *lukF-PV/lukS-PV*-positive isolates (black symbols) and *lukF-PV/lukS-PV*-negative isolates (light grey-filled symbols). The horizontal bar indicates the median per group. **ns**, not significant (Mann Whitney U test between groups at a given time point)

For both comparisons, no clear differences could be observed between groups for any time point studied, suggesting that neither the origin nor *lukF-PV/lukS-PV*-carriage is a major factor in CC121 isolates for this immune evasion phenotype.

4.3.3.3 Phagocytosis escaping of CC152 *S. aureus* from the African-German StaphNet Consortium

To substantiate my hypothesis that the sampling site is only of minor importance for the PMN phagocytosis evasion capacity of a CC, I also tested the CC152 isolate subset from the African-German StaphNet Consortium with the whole blood phagocytosis assay (Figure 19).

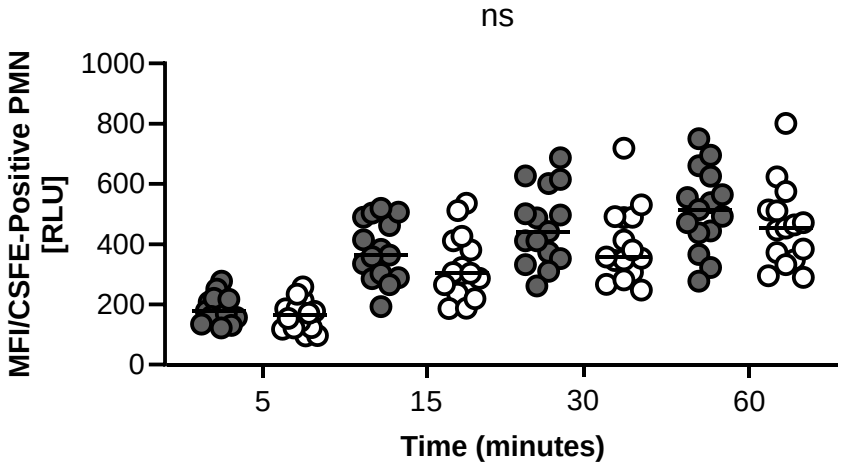

Figure 19: PMN phagocytosis evasion capabilities of CC152 *S. aureus* from the African-German StaphNet Consortium
CC152 isolates were subjected to the whole blood phagocytosis assay as outlined in Figure 9. Samples were removed after 5 min , 15 min, 30 min and 60 min respectively. Data represent the mean values of individual clinical isolates (filled symbols) and commensal isolates (open symbols). The horizontal bar indicates the median per group. ns, not significant (Mann Whitney U test between groups at a given time point).

Again, no clear differences between both groups were observed for all sampling time points studied, suggesting that, at least for CCs 152 and 121, the origin of the isolate is only of minor importance for the PMN phagocytosis evasion capacity.

5. DISCUSSION

5.1 Genetic Characteristics of the Tanzanian *S. aureus* isolates based on DNA-MCA analysis

Despite of the fact that *S. aureus* is also a major cause of infection in Africa, the landscape of *S. aureus* genotypes and phenotypes is still poorly described in many African nations, and this holds also truth for Tanzania (59,62). Hence, one of the major goals of this thesis was to provide genotypic and phenotypic information on *S. aureus* isolates circulating in the community, with a special focus on Bagamoyo, Tanzania.

5.1.1 Distribution of clonal complexes (CCs) in the Tanzanian *S. aureus* strain set

S. aureus strains are diverse from one place of origin to another, however, with certain strains having the propensity to spread globally. With the development of modern molecular typing methods such as DNA-MCA and WGS, a detailed description of the genetic composition of *S. aureus* strains became possible, which, however, were only rarely applied for African *S. aureus* isolates at the start point of this thesis. Collecting *S. aureus* isolates from normal colonization (commensals) and infections from the same area, Bagamoyo in Tanzania, and subsequent DNA-MCA-based genotypic analysis provided the basis for supplying information on the *S. aureus* strain composition in society and the healthcare system for Tanzania for the first time. These studies revealed the presence of a high diversity of *S. aureus* lineages in this geographic area with 24 different CCs being detected among the 258 Tanzanian *S. aureus* isolates (see Figure 4).

CCs 121 and 152 were the most frequently identified CCs in this study, being detected in both, clinical infection and colonization, however, with a clear dominance on the side of clinical infection. These study results agreed concordantly with findings from other sub-Saharan African countries (Gabon and Mozambique) that participated in the African-German StaphNet cohort, in which CC121 and CC152 strains were predominantly recognized in infections rather than colonization, and were found being highly enriched in African *S. aureus* isolate sets when compared to German isolate sets (119). A high prevalence of CC121 and CC152 strains in Tanzania has been also recognized by others in recent reports, which were recovered from nasal colonization, invasive (blood-born), and non-invasive (SSTIs) infections (63,64). Beside these two CCs, a couple of other CCs were found being enriched in clinical-derived isolates (i.e. CC25, CC30, and CC80), while other CCs such as CC6, CC8, CC15, and CC45 were significantly more often detected in colonization-related isolates.

The overall composition of CCs found in the Tanzanian strain set fits well with other studies originating from sub-Saharan Africa and other African countries, which consistently reported strains from CC121 and CC152, as well as CC1, CC5, CC15, CC30, CC8, CC80 and CC88 lineages being predominant in sub-Saharan Africa and Africa as a whole (119). The exclusive identification of CC45 isolates in commensal samples also fits well with the observations made by the African-German StaphNet consortium for the African sites, but is

in contradiction to the observations made at the German sampling sites, in which a roughly equal distribution of isolates of this CC was observed in clinical and commensal samples (119). However, earlier studies conducted in Europe and the USA also suggested a predominant occurrence of CC45 isolates in colonization-related samples, respectively (127–130).

Overall, clonal complexes such as CC30, CC45, CC15, CC5, CC121, and CC8 appear to be globally distributed (131–134). Among these CCs, the CCs 8 and 30 have been reported to be pandemic and predominantly detected in both nosocomial and community-associated infections (127,135–137). However, in sub-Saharan Africa, at least CC8 appears to be of more minor importance as cause of infection, as more isolates of this CCs were found in commensal samples than in clinical infection-related samples (see Figures 3 and 4).

5.1.2 DNA-MCA based *agr*/capsule typing of *S. aureus* from Tanzania

The DNA-MCA is applauded for being very good in identifying both, the clonal lineage (CC) and the *agr* subtype of a *S. aureus* strain (117). In the present study, DNA-MCA analyses successfully assigned 99% of the analyzed Tanzanian *S. aureus* strains into *agr groups I, II, III,* and *IV*. *agr group I* comprised the largest number of the identified strains (44.5%), with about 1/3 of strains belonging to CC152. The dominance of *agr* group *I* in the Tanzanian *S. aureus* strains observed in this study is in agreement with the results from another study conducted in Mozambique (138). However, the general conclusion that *agr* group *I* is the prevalent *agr* type in African isolates cannot be drawn, as earlier reports from Africa reported a dominance of *agr* group *IV* in their *S. aureus* strain sets (139–142). Notably, in my investigations, *agr* group *IV* was the *agr* type found least frequently (14%), and was found in the majority of CC121 isolates (83%), a finding that is in agreement with Monecke *et al*, (124), who reported that *agr* group *IV* is indicative for the CC121 lineage. However, in my studies, hybridization signals for both *agr I* and *agr IV* were observed in 30 strains, which were mainly from the CC152 lineage (n=28: 93%), indicating that the overall number of *agr* group *IV* isolates in my strain collective might be larger as indicated above. This kind of cross-hybridization was already noted before as the alleles for these two *agr* groups are closely related (124).

DNA-MCA-based typing of the capsular polysaccharide (*cap*) genes content of my strain collection detected almost exclusively two serotypes: capsular polysaccharide 5 (*cap5*) and capsular polysaccharide 8 (*cap8*). The dominance of *cap 8* was revealed in more than half of the strains, a finding being in agreement with data from Mozambique (138), Gabon (137), but also fit with data from European countries such as Norway (143) and Sweden (132,133), suggesting that this capsule type is distributed all over the world.

The combination of *agr* and capsule typing has been suggested as a good tool for the characterization of *S. aureus* lineages (144). In line with this, in my study, 256 out of 258 (99%) strains could be assigned to seven different groups (Figure 5). The most frequently detected CCs, CC121 and 152, which were mainly derived

from clinical isolates in this study, were assigned into 2 different groups. CC121 strains were assigned to the *agrIV*/*cap*8 group, and in this group, only strains from CC121 were found. In contrast, CC152 strains were assigned to the *agrI*/*cap*5 group, however, in this group strains from 8 different CCs were included (Table 5), indicating that *agr* and capsule typing alone does not provide sufficient power to discriminate between CCs found in Africa.

5.1.3 Profile of virulence factors in the Tanzanian *S. aureus* strains

Clinical reports document that *S. aureus* infections differ in their clinical presentation and course of diseases between tropical/(so-called) developing and temperate/industrialized regions (119). Hence, it was of great interest to determine the virulence factor repertoire of the Tanzanian strain set. *S. aureus* expresses a large number of cell surface-associated and extracellular proteins that act as virulence factors, which are often of relevance for both colonization and disease pathogenesis. For the majority of diseases caused by this bacterium, pathogenesis is multifactorial. Therefore, it is challenging to define the role of a specific factor for disease progression, as most factors occur in more than one variant (3,22). My study confirmed that *S. aureus* isolates possess a wide range of virulence factors (Appendix 3)**,** which were acknowledged to have a substantial influence on the bacteriums capacity to colonize and/or infect humans (10,87). Concerning the enterotoxin gene repertoires of the Tanzanian *S. aureus* isolates, in this investigation, the DNA-MCA analysis identified different enterotoxin encoding genes of both the classical (*sea-see*) and the newer (*seg-se/y*) enterotoxin groups (Appendix 3). The identification of the enterotoxin gene repertoire was of particular interest, as previous studies indicated that the enterotoxin gene content of a strain can be linked with specific CCs (123,133). Among all of the detected classical enterotoxin gens, the *seb* gene was detected at the highest frequency (18%). It was predominatly found in clinical infection-related isolates dominated by CC121 (52%), and to a lesser extent in CC8 and CC5 isolates, which were all among the predominant lineages detected in this study. The frequent occurrence of *seb* in CC121 was also reported previously in Nigerian *S. aureus* isolates (142). Staphylococcal enterotoxins (SEs) in general are fever-inducing pyrogenic toxin superantigens (PTSAgs) that stimulate T-lymphocytes to cause toxic shock-like syndromes and food poisoning (145,146). The *seb*-encoded Staphylococcal enterotoxin B (SEB) is the most potent staphylococcal enterotoxin and classified as a category B select agent because of its potential to yield a toxic effect even at a low doses (147).

Another group of virulence factors of particular interest for this study were the leukocidin-encoded genes, particulary the Panton Valentine Leukocidin-encoding genes: *lukF-PV* and *lukS-PV*. Their gene products LukS and LukF act synergistically to stimulate and destroy leukocytes by creating lytic pores, however, *lukF-PV* and *lukS-PV*-positive *S. aureus* isolates have a predilection of causing severe, deep, often recurrent and necrotic infections of the skin as well as lung, and to cause severe bone and joint infections (89,91,148). While *lukF-PV* and *lukS-PV*-positive *S. aureus* strains are only rarely found in Europe, they are frequently reported in many

African studies with a frequency of 24-60%: Mali (136), Gabon (149), Nigeria (150,151), Ghana(152,153), Mozambique (138,154), Algers (155), Congo (156), Burkinafaso (131), and Senegal (140).

In line with these reports, in my study, *lukF-PV* and/or *lukS-PV* were deteceted by DNA-MCA analyses in about 40% of all Tanzanian isolates tested (Table 10).

Table 10: List of *lukF-PV and/or lukS-PV*-positive *S. aureus* strains (CCs) from Bagamoyo, Tanzania

CC	PVL encoding gene	
	lukF-PV	***lukS-PV***
CC152 (*n*=34)	33	33
CC121 (*n*=30)	23	24
CC88 (*n*=23)	18	19
CC80 (*n*=10)	10	10
CC30 (*n*=7)	6	6
CC5 (*n*=23)	5	5
CC1 (*n*=18)	3	3
CC182 (*n*=2)	2	2
CC8 (*n*=25)	1	1
CC59 (*n*=1)	1	1
All (*n*=258)	**102 (39.5%)**	**104 (40%)**
Commensal (*n*=134)	**26 (19.4%)**	**27 (20.1%)**
Clinical (*n*=124)	**76 (61.3%)**	**77 (62.1%)**

In the Tanzanian strain set, *lukF-PV/lukS-PV*-carriage was a feature presented by almost all CC152 (97%) isolates except for one commensal isolate. This high carriage rate was not unexpected, as a high prevalence rate of *lukF-PV/lukS-PV*-positive isolates was reported for the CC152 clade before (136,137,140,149,157). Besides CC152, *lukF-PV/lukS-PV*-carriage was also found in all CC80 isolates and most of the CC30, CC121, and CC88 isolates tested, indicating a frequent presence of these genes in these CCs in Tanzania. Although the two CC182 and the one CC59 isolate of this strain set were also positive for *lukF-PV/lukS-PV*, the small numbers of isolates for these CCs makes it impossible to draw any conclusions about how frequently these two genes actually occur in these CCs in Tanzania. With respect to the sample origin (nasal swab or clinical infection), *lukF-PV/lukS-PV*-carriage was detected significantly more often in isolates obtained from clinical infection than colonization, suggesting that these two toxin genes are highly beneficial for *S. aureus* to cause skin and soft tissue infections (SSTIs), which was the major disease type covered by the isolates of this study. However, as a positive hybridization signal for *lukF-PV/lukS-PV* in the DNA-MCA assay is per se not

nescessarily indicative for functional genes and their expression, a certain degree of caution is required when correlating the presence of genes and their significance for the infection process.

A third group of genes known to be of importance for *S. aureus* virulence are the hemolysin-encoding genes. In the Tanzanian strain set, the genes encoding for delta (δ) hemolysin (*hld*) and alpha (α) hemolysin *(hla)* were very common across the identified clonal lineages, and were detected at frequencies of 99.7% and 98%, respectively, a finding in line with a previous report from Mozambique (138). The *hld* gene encodes for small 26 amino acid peptide spanning toxin and at the same time for a regulatory RNA, RNAIII (79,158). δ-hemolysin has been for a long time acknowledged in the vast majority of *S. aureus* isolates obtained from humans; it can cause lysis of erythrocytes, and harm a range of mammalian cells and sub-cellular structures such as membrane-bound organelles, protoplasts and spheroplasts (159,160). The *hla* gene was also previously acknowledged to be commonly present in most of the *S. aureus* isolates from clinical infection (161). In this study, *hla* was detected at equal proportions in clinical infection-related (98%) and colonization-related isolates (99%). The *hla* gene product plays an important role in the pathogenesis of *S. aureus* diseases. α-hemolysin is a small β-barrel pore-forming cytotoxin that lyses erythrocytes, particularly rabbit erythrocytes (161,162). Beside of this, *hla* is one of the exotoxins known to affect many other different human cell types such as epithelial cells, endothelial cells, T-lymphocytes, macrophages, and monocytes (83), but not neutrophils (163,164). *Hla* causes host cells damage mainly through two pathways, either by producing pores in the cell membrane of the target cell or by inducing the release of cytokines and chemokines (161,162). The produced pores on susceptible host cell membranes induce changes in ion gradients and consequently damage the membranes, leading to cell death (161). The gene *hlb* is represented on the DNA-MCA by 4 different probes: *hlb probe-1, hlb probe-2, hlb probe-3,* and an undisrupted *hlb*. The reason for this is that *hlb* serves as an integration site for prophages such as *Sa3int* phages, a temperate phage type commonly found in human isolates (165). In line with this, an undisrupted *hlb* was detected in the Tanzanian strain set at the lowest rate (15.5%) when compared to *hla* and *hld.* Notably, an undisrupted *hlb* was found in all CC152 strains of this study, which is in line with data reported by another study in Germany (124). The gene itself encodes for β-hemolysin (*Hlb*), also known as sphingomyelinase-C, which hydrolyzes sphingomyelin in the cell membrane, especially in eukaryotic cells (166,167). Similar to *hla*, *hlb* is cytotoxic to several human cells including erythrocytes, keratinocytes, skin dermal cells, and white blood cells (83). *Hlb* inhibits interleukin-8 (IL-8) secretion by endothelial cells, which protects *S. aureus* from phagocytic cells and promotes biofilm development (83,168,169). Irrespective of its great significance for virulence of *S. aureus*, it is only rarely produced by human isolates, as carriage of *Sa3int* phages provides an even higher benefit for *S. aureus* during infection of humans, as most of these prophages encode for a number of immune evasion molecules such as the staphylococcal complement inhibitor (SCIN), staphylokinase (SAK), and chemotaxis-inhibiting protein

(CHIP). These immune-modulatory proteins may act together to repel the innate immune response by interferring with complement activation and the phagocytosis processes (170,171).

The SCIN encoding gene *scn* was the most frequently detected gene of this cluster and found in 98% of the strains tested, evenly distributed across all CCs except for two strains from the CC45 and CC25 clades. The staphylokinase encoding gene *sak* was also widely distributed across all identified CCs, but overall found at a slightly lower rate than *scn* (88%). The few strains of the Tanzanian strain set (12%) that lacked *sak* were mainly isolates belonging to CC15 (75% of the CC15 isolates were negative for *sak*), a finding contrary to the observation made in a previous report from Nigeria (117). Notably, the chemotaxis-inhibiting protein encoding gene *chp* was detected only in 50% of all Tanzanian *S. aureus* strains studied, and found being slightly enriched in clinical infection-related isolates (59%). Notably, all strains from CC152, C121, CC6, and CC80 were negative for *chp*, an observation expected for CC152 strains that was also reported recently in Kenya (172), but unexpected for the CC121 and CC6 isolates that were mostly positive for *scn* and *sac*, suggesting that these isolates harbor a *Sa3int* prophage with a truncated immune evasion cluster.

My DNA-MCA analysis revealed a remarkably low presence rate of all exfoliative toxin (ET) genes: *etA*, *etB*, and *etD* were found being present in only 6%, 1.6%, and 5%, respectively. Compared to the other ET encoding genes, most of the *etA*-positive isolates were derived from infection (93%), mostly by CC121 isolates, which is in concordance with a previous study (173).

The overall low rate of ET-encoding genes in the Tanzanian strain set is in agreement with findings from other studies, which reported even lower rates <1.5% (133,174,175). ETs are also known as epidermolytic toxins, serine proteases that are similarly important elements in staphylococcal skin infections (78,83). ETs cause intra-epidermal blisters at the granular cell layer (78,176) by destroying junctions between keratinocytes and epidermis cells resulting in exfoliation of the skin (83,177).

As mentioned before, nearly all of the *S. aureus* isolates of the Tanzanian strain set were positive for the capsular polysaccharide gene clusters *cap8* and *cap5*, respectively. Earlier studies reported the presence of 11 capsule serotypes in *S. aureus* and that about half of the tested *S. aureus* isolates produced a capsule or microcapsule, whereby the two serotypes, *cap5* and *cap8*, were produced by most of the clinical *S. aureus* strains (178,179). Later work demonstrated that capsular polysaccharide is an important virulence factor expressed by *S. aureus* to inhibit the process of phagocytosis by acting as an anti-opsonin (86).

The capacity of *S. aureus* to form a biofilm on artificial and biological surfaces is another important virulence feature of this bacterium. *S. aureus* may form these multicellular aggregates either by producing large quantities of proteinacous adhesins (the mechanism used mostly by MRSA) or by producing a poly-β(1-6)-N-acetylglucosamine-based macromolecule called polyintercellular adhesin (PIA), whose synthesis is

mediated by the *ica* locus (180,181). In the Tanzanian strain set, both *icaA* (99.7%) and *icaD* (99.2%) were found with a very high frequency, in accordance with previous studies (182,183). However, *icaC* was detected only in 82% of the isolates tested and found being absent in all CC152 strains. The latter observation suggest that the Tanzanian CC152 isolates are probably not capable of producing PIA, or harbor an *icaC* variant that does not hybridize with the DNA-MCA used here.

A major factor for the success of *S. aureus* as human pathogen is its large reservoir of adhesion factors. The bacterium produces adhesins for a large number of human cell surface components including collagen, fibrinogen, human cell types, including epithelial and endothelial cells, fibroblasts, and osteoblasts (184).

In my studies, DNA-MCA analyses successfully detected the adhesin-encoding genes *bbp, fnbB, ebh, fnbA, ebpS, vwb, clfB, clfA,* and *eno* in almost all isolates fibronectin and others (185). With the help of these surface proteins, *S. aureus* can adhere to and invade a wide range of (95-100%). However, the *fib* gene, encoding for an alternative fibronectin binding protein, was not detected in all strains from CC152, CC45, and CC30. Additionally, the genes *sdrC* and *map/eap* were not found in CC152 isolates. An absence of hybridization signals for *map*/*eap* and *sdrC* in CC152 clones was already noted by Ruffing *et al* (119) when analyzing the virulence gene repertoire of the African-German StaphNet consortium strain set, suggesting that the respective gene regions differ substantially between CC152 isolates and the other CCs tested.

Collagen adhesin (CNA) is another important virulence factor of *S. aureus* that binds to collagen and was shown to block stimulation of complement in a similar fashion as SCIN (86). The corresponding gene, *cna,* was the the least frequently identified adhesin gene in the Tanzanian strain set. The gene was detected in all strains beong to CC152, CC121, CC1, CC6, CC45, and CC30, a finding pretty much in line with a study report that tested *S. aureus* isolates from Nigeria, which identified *cna* in strains assigned to CC152, CC121, CC1, CC45, and CC30 (117).

Taken together, a notable observation of my study was the different distribution of virulence genes among CC152 and CC8 strains, which both belong to *agr* group *I*. While CC8 strains were prevalently recovered from colonization and found to harbor most of the virulence genes tested, the situation was different for CC152 strains. The latter strain type was mostly isolated from infection, lacked a couple of virulence genes found in most of the other CCs, but particularly harbored an undisrupted *hlb* and the *lukF-PV/lukS-PV* genes, suggesting that the latter gene products are particularly important for infectivity of this CC in humans.

5.1.4 Profile of Antimicrobial Resistance Genes (ARGs) in the Tanzanian *S. aureus*

The evolution of bacterial isolates harboring one to multiple antibiotic resistance determinants is a major challenge for our healthcare systems. *S. aureus* is one of the bacterial species considered as a priority pathogen for which new antibiotics are needed (186). Thus, it was of particular interest to identify the ARG profiles of

the isolates of the Tanzanian *S. aureus* strain set. My DNA-MCA-based genotypic analyses revealed a very high prevalence of the transport/efflux protein-encoding gene *sdrM* in 100% and 98% of the Tanzanian clinical and commensal isolates, respectively. Its gene product, *sdrM*, allows for the transportation of different substrates from the bacterial cell to the external environment, including antibiotics (187). The chromosomally-encoded multidrug efflux pump has been reported to escalates the frequency of resistance development (188).

A worldwide important class of antibiotics are β-lactam antibiotics such as penicillin or oxacillin. In Tanzania, β-lactam antibiotics are by far the most commonly used antibiotics (189). The accurate detection of penicillin- and methicillin resistance in *S. aureus* is thus of highest importance in diagnostic microbiology to guide the treatment of infections and ratify the presence of MRSA pathogens, which are linked with challenges in antibiotic therapy. Reliable and accurate detection of penicillinase encoding genes responsible for penicillin resistance is essential because penicillin is considered superior to β-lactam stable antibiotics such as cefoxitin, methicillin or oxacillin against isolates that do not produce a penicillinase. An incorrect report of penicillin susceptibility, on the other hand, may lead to possibly insufficient therapy for *S. aureus* infections, hence augmenting the development of MRSA (190). Important genetic markers for β-lactam resistance genes in *S. aureus* are *blaZ*, encoding for a penicillinase, and *mecA/mecC*, encoding for an alternative penicillin binding protein with highly reduced affinities for β-lactam antibiotics (101). In the present study, genes associated with penicillinase production (i.e. *blaZ*, *blaI*, and *blaR*) were detected in 94% of all isolates of the Tanzanian strain set, and equally found among strains originating from colonization and infection, respectively. The high prevalence of genes associated with penicillinase production was also reported elsewhere in Africa (150,191–193), and backed-up by phenotypic data obtained from Tanzania (62).

The phosphonic fosfomycin is a commonly used first-line antibiotic for the treatment of uncomplicated urinary tract infections. The drug is also considered a valuable antibiotic for the treatment of MRSA infections, particularly when other antibiotics might be ineffective due to the resistance gene repertoire of the MRSA (194). Worryingly, genes encoding for resistance determinants against fosfomycin such as *fosB* were detected at high proportions in both clinical infection-related isolates (62%) and commenal isolates (79%), albeit of the fact that this phosphonic antibiotic is not frequently used in Tanzania (189). However, these findings are in line with a recent study reporting that 55.7 % of the tested *S. aureus* strains harbored *fosB*, highlighting its global prevalence and the successful spread of this resistance gene to several continents including Africa (195).

In Tanzania, other frequently used non-β-lactam antibiotics are the nitroimidazole metronidazole, the tetracyclines tetracycline and doxycycline, the quinolones ciprofloxacin and levofloxacin, the macrolide erythromycin, and the lincosamide clindamycin (189). In line with this, resistance genes for tetracycline (*tetK*), and erythromycin/clindamycin (*ermC*) were observed in the Tanzanian strain set at considerable rates of 26% and 19.8%, respectively. *tetK* was detected in both clinical infection-related isolates (30%) and commensal

isolates (24.6%) at comparable rates. Of all *tetK*-positive strains, more than half (55%) were from CCs 152, 88, and 8. A higher rate for *tetK* in *S. aureus* isolates belonging to CC152, CC88, and CC8 was also reported for Gabon (149). Generally, the presence of *tetK* in *S. aureus* from Africa has been reported in several studies (150,172,193), indicating the importance of this antibiotic for antibacterial therapy on this continent. The *tetK* gene product guards' bacteria against tetracycline by a resistance mechanism known as active efflux. This mechanism inhibits the accumulation of tetracycline within bacterial cells by the synthesis of a cytoplasmic-membrane protein which pumps tetracycline out of the cell at a quicker rate than it enters (49,196). The *ermC* gene encodes for an rRNA adenine N-6-methyltransferase that conveys resistance to the macrolide erythromycin, the lincosamide clindamycin, and streptogramin B. Similar to *tetK, ermC* was found both in clinical infection-related and commensal isolates at comparable rates. The mechanism of action of *ermC* is the methylation of the 23S subunit of the bacterial ribosome; it is the most frequently identified resistance determinant in human-associated *S. aureus* (107). On the CC level, *lukF-PV/lukS-PV*-positve isolates of the CC152 lineage were the main attributes for the *ermC* and *tetK* genes in this study. Both *ermC* and *tetK* are predominantly encoded by plasmids, while *lukF-PV/lukS-PV* are encoded by bacteriophages (specifically prophage *ϕSa2*), however, they are both easily transmissible (197,198). Phages and plasmids are considered as distinctive forms of mobile genetic components features that motive bacterial evolution and facilitate antibiotic resistance (199).

Other non-β-lactam antibiotic resistance encoding genes occasionally found in the Tanzanian strain set were *msr*(A), *dfrS1,* and *tetM,* which were detected at proportions of 3.1%, 2.3%, and 2%, respectively. *tetM* encodes for a protein that counteracts the inhibitory effect of tetracyclin on protein synthesis by a non-covalent modification of the ribosomes. Unlike *tetK, tetM* is commonly in *S. aureus* within the chromosome. The *msr*(A) gene, encoding for a macrolide efflux protein, confers resistance to erythromycin and streptogramin B (200). *dfrS1* encodes for a type S1 dihydrofolate reductase that is insensitive to the antibiotic trimethoprim (201). In the Tanzanian strain set, 83% of the detected *dfrS1* genes were derived from CC88-MRSA strains. Importantly, other trimethoprim resistance determinant encoding genes such as *dfrB, dfrG,* and *dfrK* are not covered by the DNA-MCA used and were thus not detectable in the Tanzanian strain set. The lack of representation of the latter *dfr* genes by the DNA-MCA is a limitation, as a previous study by Nurjadi and colleagues (202) identified *dfrG* as the predominant trimethoprim resistance determinant encoding gene in their African *S. aureus* strain set, and did not find any *dfrS1*-positive isolate. Essentially, the same was reported by another study conducted in Africa (193).

Other commonly in *S. aureus* observed resistance genes such as *cat* (encoding for a chloramphenicol acetyltransferase conveying resistance to chloramphenicol), *aacA-aphD* (encoding for a bifunctional 6'-aminoglycoside N-acetyltransferase/aminoglycoside 2"-phosphotransferase conveying resistance to

gentamicin, tobramycin, and kanamycin), *aphA3* (encoding for an aminoglycoside-3'- phosphotransferase conveying resistance to kanamycin), and *ermB* (encoding for an rRNA adenine N-6-methyltransferase conveying resistance to macrolide-lincosamide-streptogramin B antibiotics) were all detected at rates <1%, and only obtained from MRSA. Thus, although MRSA were detected at a low proportion of less than 3%, they were confirmed in this study as a major source for multiple drug resistance (MDR) in *S. aureus*, in agreement with other reports showing that MDR in *S. aureus* evolved more in MRSA than MSSA strains (51,203–205).

Taken together, the most frequently detected ARGs from the present study were *blaZ*, *tetK*, and *ermC*, which all are predominatly plasmid-encoded, thus being easily transmissible (206). Both *tetK* and *ermC* were acknowledged to be frequently found in African isolates (119), in agreement with other reports from Africa (138,193,207,208), and in Tanzania as well (62). Importantly, vancomycin-associated resistance genes were not detected in all Tanzanian *S. aureus* isolates tested. This indicates that vancomycin is still effective in treating *S. aureus* infections in the Bagamoyo community.

5. 2 Phenotypic behaviors of the tested *S. aureus* strains

5.2.1 Phenotypic antimicrobial resistance patterns of the Tanzanian *S. aureus*

One of the major disadvantages of the DNA-MCA technology is that only a small part of a respective gene is tested. Thus, a positive signal for a certain gene is not nescessarily indicative for an intact gene allowing for the expression of a functional protein. Thus, it was very tempting to test the phenotypic behavior of the Tanzanian strain set and to correlate the genotypic and phenotypic findings made with this strain set. In order to do this, phenotypic antimicrobial susceptibility testing was done for all 258 Tanzanian *S. aureus* isolates against seven antimicrobial agents: penicillin, tetracycline, erythromycin, clindamycin, co-trimoxazole (a combination of the antibiotics trimethoprim and sulfamethoxazole), and chloramphenicol. Additionally, a cefoxitin disc was used for MRSA screening. The results of this comparison are depicted in Table 11.

Table 11: Comparison between phenotypic AMR and ARGs of *S. aureus* from Bagamoyo, Tanzania

Antimicrobial agent	Phenotypic resistance n/(%)	Presence of associated ARGs (n/%)	Concordance (%)
Penicillin	245/(95%)	*blaZ* (*n*=244/95%)	99.6%
Cefoxitin	14/(5%)	*mecA* (*n*=7/2.7%)	50%
Erythromycin	75/(29%)	*ermC* (*n*=51/20%) *ermB* (*n*=1/0.4%) *msrA (n*=8/3%) *vatB* (*n*=1/0.4%),	82,7%

		v*agA* (*n*=2/0.8%) **Total (*n*= 62/24%)**	
Tetracycline	50/(19%)	*tetK* (*n*=67/26%), *tetM* (*n*=5/1.9%) **Total (72/28%)**	69%
Co-trimoxazole	37/(14%)	*dfrS1* (*n*=6/2.3%)	16%
Chloramphenicol	2/(0.78%)	*cat* (*n*=2/0.8%)	100%
Gentamycin	6/(2.3%)	*aacA-aphD* (*n*=1/0.4%) *aphA3* (*n*=1/0.4%) **Total (*n*=2/ 0.8%)**	33%
Clindamycin (const.) Clindamycin (ind.) Clindamycin (const. + ind.)	6/(2.3%) 56/(22%) **62/(24%)**	*ermC* (*n*=52/20%) *ermB* (*n*=1/0.4%) *msrA* (*n*=8/3.1%) **Total (*n*=61/24%)**	98.4%

This genotypic and phenotypic comparison of the resistance profiles of the Tanzanian *S. aureus* strains revealed a number of surprising observations. While for some of the antibiotics tested, a high concordance rate between resistance phenotype and genotype was observed (for instance for chloramphenicol resistance and presence of *cat* or penicillin resistance and presence of *blaZ*), this was not the case for other groups (i.e. co-trimoxacole and *dfrS1* or cefoxitin and *mecA*). Genotypic and phenotypic resistance perfectly matched for penicillin and *blaZ*, indicating that nearly all of the *S. aureus* isolates circulating in the Bagamoyo area of Tanzania are resistant to this clinically important antibiotic. The one isolate found to be resistant against penicillin that lacked *blaZ* was an MRSA, which produces *mecA* that also conveys resistance to penicillin. Similarly, the two strains found to be insensitive against chloramphenicol in the antimicrobial resistance testing were also positive for *cat*. However, a much weaker correlation was found for cefoxitin resistance and *mecA/mecC* carriage. In the present study, MRSA was phenotypically detected in 5% of all isolates tested by the cefoxitin disc diffusion method. The percentage of *mecA/mecC* observed in this study, however, was clearly low and found only in 2.7% of the isolates tested, indicating the presence of cefoxitin resistance in some non-MRSA isolates. Overall, the percentage of MRSA in the Tanzanian strain set is lower than the ones observed in other studies reporting on Tanzanian *S. aureus* isolates (64,209–213). This might be explained by the differences between the populations and settings studied. As mentioned before, in my study, *S. aureus* recovered from nasal swabs obtained either from healthy carriers (commensal) or isolates from outpatients with SSTIs (community-acquired infections) in the Bagamoyo community were tested. Bagamoyo is a suburban area in Tanzania. However, most of the other Tanzanian studies mentioned above that reported higher

rates of MRSA mainly obtained their *S. aureus* strains from tertiary hospitals' inpatients and in Tanzanian urban areas with a higher population density and mobility of people (214–216).

For tetracycline, phenotypic AST detected fewer resistant isolates (19%) than the DNA-MCA analyses suggested by the ARGs identified (28%). Conversely, for the antibiotics erythromycin, gentamycin, and co-trimoxazole, resistance was phenotypically detected at a higher proportion than detectable corresponding resistance genes were found. The latter observation might be explained by the fact that not all known resistance genes conveying resistance to an antibiotic were included in the DNA-MCA. For example, the *dfrS1(dfrA)* gene conferring trimethoprim resistance was the only *dfr* gene covered by the DNA-MCA used. It is very likely that at least some of the phenotypically co-trimoxazole-resistant isolates harbored genes such as *dfrB, dfrG,* and *dfrK,* which gene products all convey trimethoprim resistance, especially in the light of the findings made by Nurjadi and colleagues (202), who reported *dfrG* to be the prevalent *dfr* gene in African *S. aureus* strains. The inconsistencies between phenotypic and genotypic tetracycline resistance observed in my studies might be explained by technical issues. The Kirby-Bauer disc diffusion AST method used in my study has been reported to have intrinsic limitations (217,218). Results obtained with the disc diffusion technique results are affected by factors such as the inoculum density, the composition of the medium, the quality of antibiotic discs, time length between application of the disc and incubation, and the temperature of incubation (217). However, the technique continues to be a feasible approach, particularly in resource-limited settings, and it is the most commonly used AST in Tanzania (62).

The remarkably high resistance proportions of *S. aureus* against penicillin, erythromycin, and tetracycline reported in this study (219), have been confirmed in other studies (62). These high resistance rates might be linked to the frequent use of these antibiotics in veterinary medicine, antibiotics'pollution in the environment, and/or their availability "over the counter", as most of the antibiotics can be purchased in Tanzania without proper prescription documents (220–222).

5.2.2 Hemolytic activity of the tested *S. aureus* from Tanzania and the African-German StaphNet Consortium

The hemolytic behaviour of a bacterial colony on blood agar plates is one of the phenotypic criteria by which S. aureus can be identified. In this study, the hemolytic potential of the *S. aureus* isolate was typed by challenging human-derived erythrocytes with serial dilutions of a broth culture supernatant from a given *S. aureus* strain, which allows for the determination of a hemolytic titer (i.e. the highest dilution yielding in a complete lysis of the erythrocyte cell pool). Overall, hemolysis was confirmed to be a common feature of the tested *S. aureus* strains. When using undiluted bacterial cell culture supernatants, >90% of the tested *S. aureus* strains from Tanzania and the African-German StaphNet consortium caused a complete lysis of the

erythrocytes being present in the asssay. However, when dilutions of the supernatants were used on the erythrocytes, differences between the CCs emerged. For most of the *S. aureus* isolates tested here, a hemolytic titer below 1:8 was observed with only a few exceptions showing a hemolytic titer ≥ 8, which were mainly strains from CC152 lineage (Figures 6 and 10). *S. aureus* is known to produce at least five different types of proteins/protein pairs with hemolytic activity: α-hemolysin (aka. α-toxin, encoded by *hla*), β-hemolysin (aka. β-toxin, sphingomyelinase C or phospholipase C, encoded by *hlb*), δ-hemolysin (encoded by *hld*), and the bicomponent leukocidins HlgAB (aka. γ-hemolysin, encoded by *hlgABC*) (9,68) and LukED (encoded by *lukED*), respectively (223). As all relevant hemolysin encoding genes were represented on the DNA-MCA (see Appendices 6 and 7), a direct comparison between genotype and phenotype was possible. Based on the DNA-MCA results, some interesting differences in the genotypic hemolysin/leukocidin profiles were detected among the Tanzanian and African-German Staphnet consortium *S. aureus* isolates. While virtually all *S. aureus* isolates of this study were positive for *hla*, *hld*, and *hlgA* (≥ 97%), irrespective of the CC, this was neither the case for *hlb* nor *lukED*. In the Tanzanian strain set, *lukE* was only detected in 70% of the isolates, and was completely missing in all CC152 isolates and about half of the CC15 and CC6 isolates tested. In the African-German Staphnet consortium isolate set, an even lower presence of *lukE* was observed (55%). Here, all CC152, CC22, CC30, and CC45 were negative for this gene. For *hlb*, four different targets were present on the DNA-MCA to discriminate between an undisrupted *hlb* and disrupted *hlb* variants that served as internalization sites for prophages. In both strain sets, for most of the isolates positive signals for the disrupted *hlb* variants were observed (≥ 81%). An undisrupted *hlb* gene, which is needed for the expression of a functional Hlb, was mainly detected with CC152 isolates (which all were positive for this *hlb* marker), and about 1/3 of the CC30 isolates of the African-German StaphNet consortium isolate set. When correlating the hemolysin gene profiles with the phenotypic hemolysis data, one might conclude that a functional Hlb is of particular importance for the hemolytic pontential of *S. aureus* against human erythrocytes, while *LukED* seems to be dispensible. Carriage of *lukF-PV/lukS-PV*, another characteristic feature of the Tanzanian CC152 isolates, can also not explain the high hemolytic activities observed for these strains, as PVL (*LukSF-PV*) itself is not hemolytic (223) and may even reduce the hemolytic activities of *HlgAB* and *LukED* by non-cognate pairing of the F-type subunit *LukF-PV* with the respective S-type subunits *HlgA* and *LukE*, respectively (223). The hypothesis that Hlb is an important factor for the capacity of *S. aureus* to lyse human erythrocytes is supported by the findings of Jung and colleagues (125), who reported that integration of bacteriophage Sa*int3* into the genomes of *S. aureus* CC398 strains yielded in *S. aureus* variants displaying a reduced hemolytic potential against human-derived erythrocytes. Notably, no significant differences were observed when the hemolytic potentials of infection-related and commensal strains from all tested CCs were compared, suggesting that the hemolytic potential of an isolate is not correlated with infection (see Figures 11 and 12).

5.2.3 Cytotoxicity activity of the tested *S. aureus* from Tanzania and the African-German StaphNet Consortium

The capability of *S. aureus* to (i) be internalized by, (ii) survive within, and (iii) be cytotoxic to different types of human cells is a major contributor to the capacity of the bacterium to cause superficial and deeper tissue infections that may lead to the development of persistent or chronic infections (224). In the present study, I investigated the cytotoxic capacities of culture supernatants of the Tanzanian and African-German StaphNet Consortium strain sets on human epidermal keratinocytes (i.e. HaCaT cells). Epidermal keratinocytes comprise 90% of all cells found in the epidermis, the outermost layer of skin (225,226). As *S. aureus* is known to cause skin infections in particular, it was interesting to investigate the in-vitro strain-specific cytotoxic capacities of my test strains on HaCaT cells. When cell culture supernatants of overnight cultures of the isolates were used undiluted to challenge the HaCaT cells, the vast majority of the tested *S. aureus* isolates from Tanzania (96%) and the African-German StaphNet consortium (94%) were able to induce an LDH release by the HaCaT cells equivalent to a 50% killing rate of the keratinocyte cell population and higher. These data demonstrated that nearly all *S. aureus* isolates tested were in principle capable of producing and secreting factors that were cytotoxic for HaCaT cells, which is in line with earlier studies reporting that *S. aureus* can invade and kill non-professional phagocytes including keratinocytes (168,227,228). In order to identify potential differences in the cytotoxic capabilities between the tested strains, supernatants were serially diluted 1:2 to 1:8 with PBS before the supernatants were used to challenge the HaCaT cells. Diluting the bacterial cell culture supernatants 1:8 with PBS prior to challanging the HaCaT cells indeed allowed to identify larger differences in the cytotoxic potentials of the tested lineages. While the cytotoxic activities of the CC152 isolates-derived supernatants were still above a 75% killing rate for all isolates tested (in both strain sets), this was not the case with the other CCs (see Figures 8 and 13). In the Tanzanian strain set, isolates of the CC152 lineage induced a much stronger LDH release by the HaCaT cells than all other CCs tested, which all produced a median cytotoxicity <40%. Essentially the same trend was seen with the African-German StaphNet Consortium *S. aureus* subset in this assay, in which 1:8 dilutions of the supernatants obtained from CC152 isolates produced a median cytotoxicity of 95%, while the majoriy of the CCs tested produced median cytotoxicities <40% (i.e. CC121, CC15, CC1, CC30, CC5, and CC88). However, isolates of CC22, a CC more commonly found in Germany than Africa, also displayed a comparably high cytotoxic potential against HaCaT cells (median cytotoxicity of 65%), albeith not on the same level as CC152.

One of the cardinal features of *S. aureus* is its ability to secrete a number of virulence factors capable of damaging host cells; among others Hla, Hlb, PVL, and phenol-soluble modulin α (PSMα) that all have been reported to have a cytotoxic effect on keratinocytes (168,229). As virtually all tested lineages were positive for *hla*, the simple presence of the hemolyin-encoding gene cannot account for the differences in cytotoxic

activities seen between the CCs tested, leaving Hlb and PVL as first hits. The genes encoding for the bicomponent leukocidin PVL (*lukF-PV/lukS-PV*) were found in virtually all CC152 isolates tested phenotypically, and was also commonly found in other African CC isolates (see appendices 1 and 2). To explicitly test the impact of *lukF-PV/lukS-PV*-carriage on the cytotoxic potential of a given isolate, I determined the cytotoxic potentials of 60 CC121 strains (30 *lukF-PV/lukS-PV*-positive and 30 *lukF-PV/lukS-PV*-negative isolates obtained from infection and colonization, respectively) on keratinocytes with the LDH release assay. This investigation allowed me to identify a highly significant difference in the cytotoxic potential between *lukF-PV/lukS-PV*-positive and *lukF-PV/lukS-PV*-negative isolates (see Figure 8), strongly suggesting that PVL contributes positively to the cytotoxic potential of *S. aureus* towards keratinocytes, and is one of the factors contributing to the high cytotoxic potential of CC152 isolates. However, the simple presence/absence of *lukF-PV/lukS-PV* in the genome of *S. aureus* cannot serve as sole explanation for a high cytotoxic potential against keratinocytes, as 1:8 dilutions of the supernatants obtained from *lukF-PV/lukS-PV*-positive CC121 isolates still displayed a significantly lower cytotoxicity on HaCaT cells than equally diluted supernatants obtained from CC152 isolates (see Figure 16), suggesting that either additional factors are likely contributing to the high cytotoxic potential of CC152 isolates towards keratinocytes, or that expression of *lukF-PV/lukS-PV* is regulated differentialy in CC152 isolates than in *lukF-PV/lukS-PV*-positive isolates of the other CCs tested. The other factor that likely contributes to the high keratinocyte-harming capacity of CC152 isolates is *hlb*, as an undisrupted *hlb* was found almost exclusively in the CC152 lineage, while in all other CCs, *hlb* was disrupted by prophage integration. This hypothesis is in line with the observations made by Katayama and colleagues (168), who demonstrated that *hlb* is cytotoxic for human primary keratinocytes. A correlation between *psma* carriage (encoding PSMα) and the cytotoxic potential of an isolate/CC was unfortunately not possible based on the DNA-MCA data, as this gene locus was not covered by the DNA-MCA, leaving the question open, to which content this group of amphiphilic, membrane-destabilizing peptides contributed to the high cytotoxic activity of CC152 isolates towards keratinocytes. Another factor that should be considered for the differences in cytotoxicity observed between the CCs are differences in the composition of the regulators present in these isolates and their activity. *S. aureus* is known to produce a complex network of regulatory factors to control the expression of its virulon (79). Differences in the composition of the regulatory network and/or the expression/activity of specific regulators might well account for at least some of the differences in cytotoxicity observed between the CCs. A perfect example for such a scenario is the CC8 sublineage USA300, which is known for its high cytotoxic potential that is among others caused by a high expression of *agr*, leading to an unusally high production of Hla and PSMs (230).

The determination of the cytotoxic potentials of the 60 CC121 strains also allowed me to address the question, whether the origin (infection or colonization) of the isolate has an impact on the cytotoxic potential of the isolate. Notably, starting at a dilution of 1:2, cell culture supernatants obtained from infection-related

isolates induced a higher LDH release by HaCaT cells upon co-culture than supernatants obtained from nasal swab isolates (see Figure 14A). As a similar trend was seen with 1:8 dilutions of supernatants obtained from CC152 cultures (see Figure 15), which, however, did not reach statistical significance (probably due to the lower number of isolates tested (15 CC152 isolates each compared to the 30 CC121 isolates each), one can conclude that infection-related isolates are likely to exhibit a higher cytotoxicity on human keratinocytes than commensal isolates.

5.2.4 Immune evasion capacity of the tested *S. aureus* from Tanzania and the African-German StaphNet Consortium

Phagocytosis of invading pathogens by different types of immune cells is a major pilar of our innate immune system. Major immune cell types that have the ability to engulf and remove microbial pathogens from the host include polymorphonuclear neutrophils (PMNs), macrophages, monocytes, and dendritic cells (231). *S. aureus*, as a colonizer of human nares and a frequent cause of infection, has evolved a large repertoire of immune modulating factors allowing it to interfere with almost any component of our innate immune system (170). Many of these immune evasion proteins target neutrophils, the most prominent leukocyte subpopulation in blood, which are considered to play a major role in clearing *S. aureus* infections (170). Although significant progress has been made over the past few decades to reveal the essential role of neutrophils as human innate immune cells for defense against *S. aureus* (170), the knowledge on strain-specific uptake of *S. aureus* by neutrophils in the process of phagocytosis is scanty. Thus, it was also of great interest to investigate the uptake of the *S. aureus* strains collected from Tanzania and the African-German StaphNet consortium by neutrophils in blood. To do this, I utilized a whole blood assay, in which bacteria were stained with the fluorescent dye CFSE, subsequently incubated in freshly whithdrawn whole blood for up to one hour, and aliquots of the bacteria-blood mixture were FACS-analyzed for CFSE-positive PMNs (125). These experiments revealed that isolates of CC152 and CC121 were particularly well suited to prevent phagocytosis by PMNs in this environment, while isolates of CC6 in the Tanzanian strain set and CC30 in the African-German StaphNet consortium strain set presented with the lowest PMN phagocytosis escape capacities (see Figures 9 and 19). Comparisons of the PMN uptake rates of the 60 CC121 isolates and 30 CC152 isolates suggested that neither the origin (infection or colonization) nor *lukF-PV/lukS-PV*-carriage (absence/presence) is a major factor for this immune evasion phenotype.

As already outlined above, *S. aureus* produces a large panel of virulence factors interfering with the phagocytic activity of neutrophils (170). Some of these factors inhibit neutrophil extravasation (i.e. staphylococcal superantigen-like proteins [SSLs], staphylococcal enterotoxin-like X [SelX], and Eap), others

inhibit priming, chemotaxis, and activation of neutrophils (i.e. CHIPS, FPR2 inhibitory protein [FLIPr], staphopain A [ScpA], and SSLs), and yet others interfere with opsonization and phagocytosis (i.e. aureolysin [Aur], capsuluar polysaccharides, *S. aureus* collagen adhesin [Cna], extracellular complement-binding protein [Ecb], extracellular fibrinogen-binding protein [Efb], SAK, SCIN, staphylococcal binding of IgG [Sbi], and protein A [Spa]) reviewed in (170). Since the presence/abscence of these factors did not vary greatly within a given CC and between CCs (see Appendices 6-8), a direct correlation of the phenotype with the presence of certain genes/gene combinations was difficult. On the genotypic level, CC152 isolates were assigned to *agr* group *I*, contained the genes for capsular polysaccharide type 5, harbored the hemolysis-associated genes *hla, hlb, hld,* and *hlgA*, were positive for *aur* (encoding Aur), *cna* (encoding Cna), *sak* (encoding SAK), *sbi* (encodning Sbi), *scn* (encoding SCIN), and *ssl* (encoding SSL), but were negative for *chp* (encoding CHIPS) and *eap*. CC121 isolates were assigned to *agr* group *IV*, contained the genes for capsular polysaccharide type 8, harbored the genes *aur, cna, hla, hld, hlgA, sak , sbi, scn*, and *ssl*, but were negative for *chp* and the intact *hlb*. The gene for *Eap* was present in 57% of the tested CC121 isolates. Surprisingly, a rather comparable immune evasion molecule pattern was seen for the CC6 isolates studied here (positive for *aur, cna, hla, hld, hlgA, sak, sbi, scn*, and *ssl*, negative for *chp,* and the undisrupted *hlb*), however, CC6 isolates were assigned to *agr* group *IV*. Similarly, the vast majority of the tested CC30 isolates (6/7) were found being positive for *aur, cap5, cna, hla, hld, hlgA, sak sbi, scn, ssl*, and negative for *hlb,* however, unlike the CC152, CC121 and CC6 isolates mentioned above, were also positive for *chp* (see Appendix 3). All CC30 isolates were assigned to *agr* group *III*. As the genes coding for Ecb (*ecb*), Efb (*efb*), FLIPr (*flr*), ScpA (*scpA*), and Spa (*spa*) were not covered by the DNA-MCA, no conclusions between the immune evasion phenotype and the genetic repertoire could be drawn for the latter genes. The finding that the gene repertoires encoding for immune evasion factors is rather comparable between well phagocytosed CCs (i.e. CC6 and CC30) and weak phagocytosed CCs (i.e. CC152 and CC121) suggests that differences in the expression of the respective gene products might be causative for the differences observed for these CCs with respect to PMN uptake in whole blood.

5.3 Limitations

In this thesis, I described for the first time the genetic repertoire of *S. aureus* isolates obtained from Bagamoyo, a sub-urban area in Tanzania, and determined for a subset of these strains (covering the seven most frequently identified CCs) some phenotypic properties (i.e. their antibiotic resistance pattern, hemolytic activity, cytotoxicity for keratinocytes and antiphagocytotic properties in whole blood), which allowed me for the first time to correlate genotypic and phenotypic data obtained with the same strain set for the African country, Tanzania. A second major goal of this thesis was to test, whether and how the sampling site of the isolate (derived from infection or colonization) might influence the genotype and/or phenotype of the isolate. Especially for the second goal, I encountered some difficulties, as for some of the CCs, such as CC15 and CC6,

only a small number of clinical isolates were available (3 an 4 out of the 14 isolates tested phenotypically). Additionally, the majority of clinical isolates (98.4%) of the Tanzanian strain set were obtained from non-invasive-skin and soft tissue infections (SSTIs), and only 2 isolates (1.6%) were from blood-borne infections. Both characteristics were likely to restrict the informative value of the findings described here. In order to address these issues, I utilized part of the *S. aureus* isolate set from the African-German StaphNet Consortium that has been genotypically characterized by DNA-MCA technology (119), and phenotypically characterized 15 isolates each of CCs predominantly found in Africa and Germany, respectively, and CCs found in both geographic areas. However, although with this strain set, I encountered difficulties to balance the numbers of colonization-related and infection-related isolates. As a putative consequence, I failed to identify significant differences in the hemolytic activities between commensal and infection-related isolates for all 10 CCs tested, albeith of the fact that for some of the CCs such as CC45 and CC121, a clear difference in the median hemolytic titers were observed between the commensal and infection-related isolate sets (see Figure 11). Making use of a larger set of CC121 isolates (30 isolates obtained from nasal swabs and 30 isolates from infection) finally allowed me to conclude that at least for CC121, no clear correlation between the origin of the isolate (colonization or infection) and the hemolytic titer can be found (see Figure 12). In combination with the findings made with the extended CC121 strain set in the LDH release-based cytotoxicity assay, in which for both, the clinical origin and *lukF-PV/lukS-PV*-carriage, associations with a higher cytotoxicity of the CC121 isolates were observed (see Figure 14), while for the extended CC152 strain set (covering 15 isolates each from infection and colonization), only a trend was observable that did not reach statistical significance (see Figure 15), I come to the conclusion that a larger number of isolates derived from a condition of interest is needed (i.e. ≥30 isolates per CC and condition) to allow for a reliable statement as to whether this condition is relevant for a particular phenotype of the CC.

In my study, a DNA-MCA was utilized for the genotypic characterization. By covering probes for 191 unique staphylococcal genes, the method is sutable for a thorough genotypic characterization of a *S. aureus* isolate, and was proven to be a useful tool for identifying clonal lineages and *agr* groups (118). However, especially when compared to whole genome sequencing, the DNA-MCA method has its drawbacks: (i) it is based on hybridization reactions and covers only one to few small parts of the gene of interest, and (ii) a positive signal does not nescessarily indicate that the strain is able to produce the respective gene product (e.g., protein), and thus may not reflect its actual phenotypic function. In line with this, my phenotypical AST revealed some discordant results for certain antibiotics with respect to the resistance determinants identified by DNA-MCA anlysis. A telling example for such a discordant result are my findings for tetracycline, for which the DNA-MCA analyses indicated the presence of a tetracycline resistance gene in 72 isolates, however, a phenotypic resistance was only detected in 50 isolates (concordance rate of 69%). Potential reasons for this

might be technical errors with the Kirby-Bauer disc diffusion test or the inability of DNA-MCA technology to predict the expression of a functional resistance determinant.

5.4 Conclusion and Recommendations/Outlook

Staphylococcus aureus is both a human commensal and a feared human pathogen capable of causing a variety of illnesses, from minor skin infections to more serious, life-threatening wound and bloodstream infections. Conversely, the bacterium is an expert in rapidly acquiring or generating numerous forms of antibiotic resistance, acknowledging Methicillin-resistant and Vancomycin-resistant variants of *S. aureus* as a major public health concern on a global scale (14).

Earlier work demonstrated that the distribution of strain types and overall characteristics of the *S. aureus* population may differ geographically, information that until recently remained largely elusive for emerging nations. For Tanzania, like for most other African countries, there is a narrow knowledge on *S. aureus* characteristics, leading to an out-of-date and incomplete understanding of the bacterium's epidemiology. This study was conducted to fill the gap by determining the genotypic and phenotypic characteristics of Tanzanian *S. aureus* of both commensal and clinical infection-related origins.

My study revealed a high diversity of the MSSA strains from the Bagamoyo area, with a high prevalence of *lukF-PV/lukS-PV*-positive strains, especially from CCs 152, 121, 88, and 80. *lukF-PV/lukS-PV*-positive strains were predominantly observed in MSSA strains obtained from clinical skin and soft tissue infections, indicating that the gene product encoded by this locus, the bi-component leukocidin PVL, is a driver for infection. However, as *lukF-PV/lukS-PV* were also identified in a number of commensal (nasal carriage) isolates, adequate measures should be implemented to restrict their spread in the community, especially in easily infectable groups of people in our society, such as the elderly and people with limited immune status. It is well documented that the majority of invasive *S. aureus* infections arosed from either subsequent skin infections or colonization (nasal carriage)(3).

A second major finding of my study is the high resistance proportion of the Tanzanian *S. aureus* strain set to antibiotics such as penicillin and fosfomycin. The high resistance rates for penicillin in the Tanzanian *S. aureus* strain set is in line with the high usage rate of this antibiotic in this country, as penicillins are by far the most commonly used antibiotics in Tanzania (189). The finding of *fosB* in 62% of the clinical infection-related isolates and 79% of the commenal isolates, however, is alarming, because the phosphonic antibitic is not frequently used in Tanzania (189), highlighting that some resistance determinants might spread easily within the *S. aureus* population circulating in the Bagamoyo area even without an antibiotic pressure. The findings that MRSA are only rarely found and VRSA are absent in the Bagamoyo community area, on the other hand, very encouraging, as this circumstance allows the treating physicians to use these antibiotics for treatment of

community-acquired *S. aureus* infection even without prior resistance testing. However, as the MRSA prevalence may change quickly in certain geographic areas, the timely surveillance of the antimicrobial resistance pattern of *S. aureus* is recommended in order to understand emerging resistance trends, and to adapt the overall management of *S. aureus* infections, if needed.

By correlating the genotypic and phenotypic observations made with the Tanzanian and African-German StaphNet Consortium subsets, I could last not least identify β-hemolysin (Hlb) and Pantone-Valentine leukocidin (PVL) as important features for the epidemiological success of CC152 as human pathogen in Tanzania.

Overall, the *S. aureus* strain-specific phenotypic and genotypic characterization in this study provided some valuable information about the clonal-phenotypic behavior of a different set of *S. aureus* CCs as the ones usually circulating in the Western hemisphere.

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7. OWN PUBLICATIONS

List of own publications related to this dissertation:

1. Mzee T, **Kazimoto T**, Madata J, Masalu R, Bischoff M, Matee M, et al. Prevalence, antimicrobial susceptibility and genotypic characteristics of *Staphylococcus aureus* in Tanzania: a systematic review. Bulletin of the National Research Centre. 2021;45(1):162. . DOI: 10.1186/s42269-021-00612-z

2. Kazimoto T, Abdulla S, Bategereza L, Juma O, Mhimbira F, Weisser M, et al. Causative agents and antimicrobial resistance patterns of human skin and soft tissue infections in Bagamoyo, Tanzania. Acta Tropica. 2018;186:102–6. DOI: 10.1016/j.actatropica.2018.07.007

3. Alabi A, **Kazimoto** T, Lebughe M, Vubil D, Phaku P, Mandomando I, Kern WV, Melmann A et al. Management of superficial and deep-seated of *Staphylococcus aureus* skin and soft tissues infections in sub-Saharan Africa: a post hoc analysis of the Staphnet cohort. Infection. 2018;46:395. DOI: 10.1007/s15010-018-1140-6

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9. CURRICULUM VITAE

10. APPENDICES

Appendix 1: List of the Tanzanian *S. aureus* isolates from colonization and clinical infection

SN	CC	Strain type	Sample source	Sample type
1	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
2	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
3	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
4	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
5	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
6	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
7	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
8	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
9	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
10	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
11	CC152	CC152-MSSA [PVL+]	Commensal	Nasal swab
12	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
13	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
14	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
15	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
16	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
17	CC152	CC152-MSSA	Clinical	Wound swab
18	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
19	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
20	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
21	CC152	CC152-MSSA [PVL+]	Clinical	Wound swab
22	CC152	CC152-MSSA [PVL+]	Clinical	Blood
23	CC152	CC152-MSSA [PVL+]	Clinical	Deep skin abscess
24	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
25	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
26	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
27	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
28	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
29	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
30	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
31	CC152	CC152-MSSA [PVL+]	Clinical	Deep skin abscess
32	CC152	CC152-MSSA [PVL+]	Clinical	Deep skin abscess
33	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
34	CC152	CC152-MSSA [PVL+]	Clinical	Superficial skin swab
35	CC121	CC121-MSSA	Commensal	Nasal swab
36	CC121	CC121-MSSA	Commensal	Nasal swab
37	CC121	CC121-MSSA	Commensal	Nasal swab
38	CC121	CC121-MSSA	Commensal	Nasal swab

39	CC121	CC121-MSSA	Commensal	Nasal swab
40	CC121	CC121-MSSA [PVL+]	Commensal	Nasal swab
41	CC121	CC121-MSSA [PVL+]	Clinical infection	Ear pus
42	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
43	CC121	CC121-MSSA [PVL+]	Clinical infection	Deep skin abscess
44	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
45	CC121	CC121-MSSA [PVL+]	Clinical infection	Deep skin abscess
46	CC121	CC121-MSSA [PVL+]	Clinical infection	Deep skin abscess
47	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
48	CC121	CC121-MSSA	Clinical infection	Superficial skin swab
49	CC121	CC121-MSSA [PVL+]	Clinical infection	Deep skin abscess
50	CC121	CC121-MSSA [PVL+]	Clinical infection	Deep skin abscess
51	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
52	CC121	CC121-MSSA [PVL+]	Clinical infection	Deep skin abscess
53	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
54	CC121	CC121-MSSA [PVL+]	Clinical infection	Deep skin abscess
55	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
56	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
57	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
58	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
59	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
60	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
61	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
62	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
63	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
64	CC121	CC121-MSSA [PVL+]	Clinical infection	Superficial skin swab
65	CC8	CC8-MSSA	Commensal	Nasal swab
66	CC8	CC8-MSSA	Commensal	Nasal swab
67	CC8	CC8-MSSA	Commensal	Nasal swab
68	CC8	CC8-MRSA-V, WA MRSA-115/-132	Clinical infection	Wound swab
69	CC8	ST72-MSSA	Clinical infection	Wound swab
70	CC8	ST72-MSSA [PVL+]	Clinical infection	Wound swab
71	CC8	CC8-MSSA	Commensal	Nasal swab
72	CC8	CC8-MSSA	Commensal	Nasal swab
73	CC8	CC8-MSSA	Commensal	Nasal swab
74	CC8	CC8-MSSA	Commensal	Nasal swab
75	CC8	CC8-MSSA	Commensal	Nasal swab
76	CC8	CC8-MSSA	Commensal	Nasal swab
77	CC8	CC8-MSSA	Commensal	Nasal swab
78	CC8	CC8-MSSA	Clinical infection	Superficial skin swab
79	CC8	CC8-MSSA	Clinical infection	Superficial skin swab
80	CC8	CC8-MSSA	Clinical infection	Wound swab
81	CC8 (ST72)	ST72-MSSA	Commensal	Nasal swab

82	CC8 (ST72)	ST72-MSSA	Commensal	Nasal swab
83	CC8 (ST72)	ST72-MSSA	Commensal	Nasal swab
84	CC8 (ST72)	ST72-MSSA	Commensal	Nasal swab
85	CC8 (ST72)	ST72-MSSA	Commensal	Nasal swab
86	CC8 (ST72)	ST72-MSSA	Commensal	Nasal swab
87	CC8 (ST72)	ST72-MSSA	Commensal	Nasal swab
88	CC8 (ST72)	ST72-MSSA	Clinical infection	Wound swab
89	CC8 ST72	ST72-MSSA	Commensal	Nasal swab
90	CC15	CC15-MSSA	Commensal	Nasal swab
91	CC15	CC15-MSSA	Commensal	Nasal swab
92	CC15	CC15-MSSA	Commensal	Nasal swab
93	CC15	CC15-MSSA	Commensal	Nasal swab
94	CC15	CC15-MSSA	Commensal	Nasal swab
95	CC15	CC15-MSSA	Commensal	Nasal swab
96	CC15	CC15-MSSA	Commensal	Nasal swab
97	CC15	CC15-MSSA	Commensal	Nasal swab
98	CC15	CC15-MSSA	Commensal	Nasal swab
99	CC15	CC15-MSSA	Commensal	Nasal swab
100	CC15	CC15-MSSA	Commensal	Nasal swab
101	CC15	CC15-MSSA	Commensal	Nasal swab
102	CC15	CC15-MSSA	Commensal	Nasal swab
103	CC15	CC15-MSSA	Commensal	Nasal swab
104	CC15	CC15-MSSA	Commensal	Nasal swab
105	CC15	CC15-MSSA	Commensal	Nasal swab
106	CC15	CC15-MSSA	Commensal	Nasal swab
107	CC15	CC15-MSSA	Clinical infection	Superficial skin swab
108	CC15	CC15-MSSA	Clinical infection	Wound swab
109	CC15	CC15-MSSA	Clinical infection	Superficial skin swab
110	CC15	CC15-MSSA	Clinical infection	Superficial skin swab
111	CC15	CC15-MSSA	Clinical infection	Superficial skin swab
112	CC15	CC15-MSSA	Commensal	Nasal swab
113	CC15	CC15-MSSA	Commensal	Nasal swab
114	CC88	CC88-MSSA [PVL+]	Commensal	Nasal swab
115	CC88	CC88-MSSA [PVL+]	Commensal	Nasal swab
116	CC88	CC88-MSSA [PVL+]	Commensal	Nasal swab
117	CC88	CC88-MSSA [PVL+]	Commensal	Nasal swab
118	CC88	CC88-MSSA	Clinical infection	Wound swab
119	CC88	CC88-MSSA [PVL+]	Clinical infection	Wound swab
120	CC88	CC88-MSSA	Commensal	Nasal swab
121	CC88	CC88-MSSA [PVL+]	Commensal	Nasal swab
122	CC88	CC88-MSSA [PVL+]	Commensal	Nasal swab
123	CC88	CC88-MSSA [PVL+]	Commensal	Nasal swab
124	CC88	CC88-MRSA-IV [etA+]	Commensal	Nasal swab

125	CC88	CC88-MRSA-IV [PVL+]	Commensal	Nasal swab
126	CC88	CC88-MRSA-IV [etA+]	Commensal	Nasal swab
127	CC88	CC88-MSSA [PVL+]	Clinical infection	Wound swab
128	CC88	CC88-MSSA [PVL+]	Clinical infection	Wound swab
129	CC88	CC88-MSSA [PVL+]	Clinical infection	Wound swab
130	CC88	CC88-MRSA-IV, WA MRSA-2	Clinical infection	Superficial skin swab
131	CC88	CC88-MSSA [PVL+]	Clinical infection	Superficial skin swab
132	CC88	CC88-MSSA [PVL+]	Clinical infection	Wound swab
133	CC88	CC88-MRSA-IV [PVL+]	Clinical infection	Superficial skin swab
134	CC88	CC88-MSSA [PVL+]	Clinical infection	Superficial skin swab
135	CC88	CC88-MSSA [PVL+]	Clinical infection	Superficial skin swab
136	CC88	CC88-MSSA [PVL+]	Clinical infection	Superficial skin swab
137	CC5	CC5-MSSA	Commensal	Nasal swab
138	CC5	CC5-MSSA	Commensal	Nasal swab
139	CC5	CC5-MSSA	Clinical infection	Wound swab
140	CC5	CC5-MSSA	Commensal	Nasal swab
141	CC5	CC5-MSSA	Commensal	Nasal swab
142	CC5	CC5-MSSA	Commensal	Nasal swab
143	CC5	CC5-MSSA	Commensal	Nasal swab
144	CC5	CC5-MSSA	Commensal	Nasal swab
145	CC5	CC5-MSSA	Commensal	Nasal swab
146	CC5	CC5-MSSA	Commensal	Nasal swab
147	CC5	CC5-MSSA	Commensal	Nasal swab
148	CC5	CC5-MSSA	Commensal	Nasal swab
149	CC5	CC5-MSSA [PVL+]	Commensal	Nasal swab
150	CC5	CC5-MSSA	Commensal	Nasal swab
151	CC5	CC5-MSSA	Clinical infection	Superficial skin swab
152	CC5	CC5-MSSA	Clinical infection	Wound swab
153	CC5	CC5-MSSA	Clinical infection	Superficial skin swab
154	CC5	CC5-MSSA	Clinical infection	Wound swab
155	CC5	CC5-MSSA	Clinical infection	Superficial skin swab
156	CC5	CC5-MSSA [PVL+]	Clinical infection	Superficial skin swab
157	CC5	CC5-MSSA [PVL+]	Clinical infection	Superficial skin swab
158	CC5	CC5-MSSA [PVL+]	Clinical infection	Superficial skin swab
159	CC5	CC5-MSSA [PVL+]	Clinical infection	Superficial skin swab
160	CC6	CC6-MSSA	Commensal	Nasal swab
161	CC6	CC6-MSSA	Commensal	Nasal swab
162	CC6	CC6-MSSA	Commensal	Nasal swab
163	CC6	CC6-MSSA	Commensal	Nasal swab
164	CC6	CC6-MSSA	Commensal	Nasal swab
165	CC6	CC6-MSSA	Commensal	Nasal swab
166	CC6	CC6-MSSA	Commensal	Nasal swab
167	CC6	CC6-MSSA	Commensal	Nasal swab

168	CC6	CC6-MSSA	Commensal	Nasal swab
169	CC6	CC6-MSSA	Commensal	Nasal swab
170	CC6	CC6-MSSA	Commensal	Nasal swab
171	CC6	CC6-MSSA	Commensal	Nasal swab
172	CC6	CC6-MSSA	Commensal	Nasal swab
173	CC6	CC6-MSSA	Clinical infection	Wound swab
174	CC6	CC6-MSSA	Clinical infection	Wound swab
175	CC6	CC6-MSSA	Clinical infection	Superficial skin
176	CC6	CC6-MSSA	Clinical infection	Superficial skin
177	CC6	CC6-MSSA	Clinical infection	Superficial skin
178	CC1	CC1-MSSA	Commensal	Nasal swab
179	CC1	CC1-MSSA	Commensal	Nasal swab
180	CC1	CC1-MSSA	Commensal	Nasal swab
181	CC1	CC1-MSSA	Commensal	Nasal swab
182	CC1	CC1-MSSA	Commensal	Nasal swab
183	CC1	CC1-MSSA	Commensal	Nasal swab
184	CC1	CC1-MSSA	Commensal	Nasal swab
185	CC1	CC1-MSSA	Clinical infection	Superficial skin swab
186	CC1	CC1-MSSA	Clinical infection	Wound swab
187	CC1	CC1-MSSA	Clinical infection	Wound swab
188	CC1	CC1-MSSA	Clinical infection	Wound swab
189	CC1	CC1-MSSA	Clinical infection	Wound swab
190	CC1	CC1-MSSA	Clinical infection	Wound swab
191	CC1	CC1-MSSA	Clinical infection	Superficial skin
192	CC1 (ST573/772)	ST573/772-MSSA	Commensal	Nasal swab
193	CC1 (ST573/772)	ST573/772-MSSA [PVL+]	Clinical infection	Superficial skin
194	CC1 (ST573/772)	ST573/772-MSSA [PVL+]	Clinical infection	Superficial skin
195	CC1 (ST573/772)	ST573/772-MSSA [PVL+]	Clinical infection	Superficial skin swab
196	CC9	CC9-MSSA	Commensal	Nasal swab
197	CC9 (ST834)	ST834-MSSA	Commensal	Nasal swab
198	CC9 (ST834)	ST834-MSSA	Commensal	Nasal swab
199	CC9 (ST834)	ST834-MSSA	Commensal	Nasal swab
200	CC9 (ST834)	ST834-MSSA	Commensal	Nasal swab
201	CC9 (ST834)	ST834-MSSA	Commensal	Nasal swab
202	CC9 (ST834)	ST834-MSSA	Clinical infection	Superficial skin swab
203	CC9 (ST834)	ST834-MSSA	Clinical infection	Wound swab
204	CC9 (ST834)	ST834-MSSA	Clinical infection	Superficial skin swab
205	CC9 (ST834)	ST834-MSSA	Clinical infection	Wound swab
206	CC9 (ST834)	ST834-MSSA	Clinical infection	Superficial skin swab
207	CC9 (ST834)	ST834-MSSA	Clinical infection	Wound swab
208	CC9 (ST834)	ST834-MSSA	Clinical infection	Superficial skin swab
209	CC9 (ST834)	ST834-MSSA	Commensal	Nasal swab
210	CC45	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab

211	CC45	CC45-MSSA	Commensal	Nasal swab
212	CC45	CC45-MSSA	Commensal	Nasal swab
213	CC45	CC45-MSSA	Commensal	Nasal swab
214	CC45	CC45-MSSA	Commensal	Nasal swab
215	CC45	CC45-MSSA	Commensal	Nasal swab
216	CC45	ST45	Commensal	Nasal swab
217	CC45 (agr IV)	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab
218	CC45 (agr IV)	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab
219	CC45 (agr IV)	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab
220	CC45 (agr IV)	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab
221	CC80	CC80-MSSA [PVL+]	Commensal	Nasal swab
222	CC80	atypical CC80-MSSA [ORF CM14/PVL+]	Commensal	Nasal swab
223	CC80	CC80-MSSA [PVL+]	Commensal	Nasal swab
224	CC80	atypical CC80-MSSA [ORF CM14/PVL+]	Commensal	Nasal swab
225	CC80	atypical CC80-MSSA [ORF CM14/PVL+]	Clinical infection	Superficial skin swab
226	CC80	atypical CC80-MSSA [ORF CM14/PVL+]	Clinical infection	Superficial skin swab
227	CC80	atypical CC80-MSSA [ORF CM14/PVL+]	Clinical infection	Wound swab
228	CC80	CC80-MSSA [PVL+]	Clinical infection	Superficial skin swab
229	CC80	CC80-MSSA [PVL+]	Clinical infection	Superficial skin swab
230	CC80	atypical CC80-MSSA [ORF CM14/PVL+]	Clinical infection	Wound swab
231	CC30	CC30-MSSA [PVL+]	Clinical infection	Wound swab
232	CC30	CC30-MSSA [PVL+]	Clinical infection	Nasal swab
233	CC30	CC30-MSSA [PVL+]	Clinical infection	Nasal swab
234	CC30	CC30-MSSA [PVL+]	Clinical infection	Blood
235	CC30	CC30-MSSA [PVL+]	Clinical infection	Wound swab
236	CC30	CC30-MSSA [PVL+]	Clinical infection	Superficial skin swab
237	CC30	CC30-MSSA [PVL+]	Clinical infection	Superficial skin swab
238	CC25	CC25-MSSA	Commensal	Nasal swab
239	CC25	CC25-MSSA	Clinical infection	Wound swab
240	CC25	CC25-MSSA	Clinical infection	Wound swab
241	CC25	CC25-MSSA	Clinical infection	Wound swab
242	CC25	CC25-MSSA	Clinical infection	Nasal swab
243	CC25	CC25-MSSA	Clinical infection	Wound swab
244	CC25	CC25-MSSA	Clinical infection	Wound swab
245	CC182	CC182-MSSA [PVL+]	Clinical infection	Superficial skin swab
246	CC182	CC182-MSSA [PVL+]	Clinical infection	Superficial skin swab
247	CC101	CC101-MSSA	Commensal	Nasal swab
248	CC101	CC101-MSSA	Commensal	Nasal swab
249	CC22	CC22-MSSA	Commensal	Nasal swab
250	CC22	CC22-MSSA	Commensal	Nasal swab
251	CC97	CC97-MSSA	Clinical	Superficial skin

252	ST2370		Clinical	Superficial skin
253	ST2744	new MLST	Clinical	Superficial skin
254	CC395	CC395-MSSA	Commensal	Nasal swab
255	CC59	>ST59/952-MRSA-V(T) [PVL+], Taiwan Clone	Commensal	Nasal swab
256	ST2734	new MLST	Commensal	Nasal swab
257	ST580		Commensal	Nasal swab
258	CC1290	CC1290-MSSA	Commensal	Nasal swab

Appendix 2: List of the African-German StaphNet *S. aureus* subset for phenotypic behaviours characterization

(A) First Batch: A set of 150 *S .aureus* selected from 10 different clonal complexes(CCs)				
SN	**CC/Strain type**	**Sample Source**	**Sample type**	**Place of origin**
1	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Germany-Freiburg
2	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Germany-Freiburg
3	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa-Tanzania
4	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa-Tanzania
5	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa-Tanzania
6	CC152-MSSA [PVL+]	Clinical infection	Blood	Africa-Garbon
7	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa-Garbon
8	CC152-MSSA [PVL+]	Clinical infection	Blood	Africa-Mozambique
9	CC152-MSSA [PVL+]	Clinical infection	Blood	Africa-Mozambique
10	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa-Mozambique
11	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa-Mozambique
12	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa-Mozambique
13	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa-Garbon
14	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa-Garbon
15	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa-Garbon
16	CC121-MSSA	Clinical infection	Skin swab	Africa-Tanzania
17	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa-Tanzania
18	CC121-MSSA	Clinical infection	Skin swab	Germany-Freiburg
19	CC121-MSSA	Clinical infection	Skin swab	Germany-Freiburg
20	CC121-MSSA	Clinical infection	Skin swab	Africa-Mozambique
21	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa-Mozambique
22	CC121-MSSA	Clinical infection	Skin swab	Germany-Muenster
23	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Germany-Muenster
24	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Homburg/Saar
25	CC121-MSSA [PVL+]	Clinical infection	Blood	Africa - Garbon
26	CC121-MSSA	Commensal	Nasal swab	Germany - Freiburg
27	CC121-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
28	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Garbon
29	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
30	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Tanzania
31	CC15 -MSSA	Clinical infection	Blood	Africa - Mozambique
32	CC15-MSSA	Clinical infection	Blood	Africa - Mozambique
33	CC15-MSSA	Clinical infection	Blood	Germany - Freiburg
34	CC15-MSSA	Clinical infection	Blood	Germany - Homburg/Saar
35	CC15-MSSA	Clinical infection	Skin swab	Africa - Tanzania
36	CC15-MSSA	Clinical infection	Skin swab	Africa - Gabon
37	CC15-MSSA	Clinical infection	Skin swab	Africa - Tanzania
38	CC15-MSSA	Clinical infection	Skin swab	Germany - Muenster

39	CC15-MSSA	Clinical infection	Skin swab	Germany - Freiburg
40	CC15-MSSA	Clinical infection	Blood	Germany - Freiburg
41	CC15-MSSA	Commensal	Nasal swab	Germany - Muenster
42	CC15-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
43	CC15-MSSA	Commensal	Nasal swab	Africa - Tanzania
44	CC15-MSSA	Commensal	Nasal swab	Africa - Gabon
45	CC15-MSSA	Commensal	Nasal swab	Germany - Muenster
46	CC1-MSSA	Clinical infection	Skin swab	Africa - Tanzania
47	CC1-ST573/772-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Tanzania
48	CC1-MSSA	Clinical infection	Skin swab	Germany - Freiburg
49	CC1-ST573/772-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Freiburg
50	CC1-MSSA	Clinical infection	Skin swab	Germany - Hoburg/Saar
51	CC1-MSSA	Clinical infection	Skin swab	Africa -Tanzania
52	CC1-MSSA [PVL+]	Clinical infection	Skin swab	Africa -Tanzania
53	CC1-ST573/772-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Muenster
54	CC1-MSSA	Clinical infection	Skin swab	Germany - Muenster
55	CC1-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
56	CC1-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
57	CC1-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
58	CC1-MSSA	Commensal	Nasal swab	Africa -Tanzania
59	CC1-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
60	CC1-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
61	CC22-MRSA-IV, UK-EMRSA-15/Barnim EMRSA	Clinical infection	Blood	Germany - Freiburg
62	CC22-MRSA-IV, UK-EMRSA-15/Barnim EMRSA	Clinical infection	Skin swab	Germany - Freiburg
63	CC22-MRSA-IV, UK-EMRSA-15/Barnim EMRSA	Clinical infection	Skin swab	Germany - Freiburg
64	CC22-ST22-MRSA-V	Clinical infection	Blood	Germany - Freiburg
65	CC22-MSSA	Clinical infection	Blood	Germany - Freiburg
66	CC22-MRSA-IV, UK-EMRSA-15/Barnim EMRSA	Clinical infection	Skin swab	Germany - Homburg/Saar
67	CC22-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Homburg/Saar
68	CC22-MRSA-IV, UK-EMRSA-15/Barnim EMRSA	Clinical infection	Skin swab	Germany - Muenster
69	CC22-MRSA-IV, UK-EMRSA-15/Barnim EMRSA	Clinical infection	Skin swab	Germany - Muenster
70	CC22-MSSA	Clinical infection	Blood	Germany - Muenster
71	CC22-MSSA	Commensal	Nasal swab	Germany - Muenster
72	CC22-MSSA	Commensal	Nasal swab	Germany - Freiburg
73	CC22-MSSA	Commensal	Nasal swab	Africa -Tanzania
74	CC22-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
75	CC22-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique

76	CC30-MSSA	Clinical infection	Blood	Germany - Freiburg
77	CC30-MSSA	Clinical infection	Skin swab	Germany - Freiburg
78	CC30-MSSA	Clinical infection	Blood	Germany - Homburg/Saar
79	CC30-MSSA	Clinical infection	Skin swab	Germany - Homburg/Saar
80	CC30-MSSA	Clinical infection	Skin swab	Africa - Mozambique
81	CC30-MSSA [PVL+]	Clinical infection	Blood	Africa -Tanzania
82	CC30-MSSA [PVL+]	Clinical infection	Skin swab	Africa -Tanzania
83	CC30-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Gabon
84	CC30-ST34-MSSA	Clinical infection	Skin swab	Germany - Muenster
85	CC30-MSSA	Clinical infection	Skin swab	Germany - Muenster
86	CC30-MSSA	Commensal	Nasal swab	Germany - Muenster
87	CC30-MSSA [lukFP83/M+]	Commensal	Nasal swab	Germany - Muenster
88	CC30-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
89	CC30-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
90	CC30-MSSA [PVL+]	Commensal	Nasal swab	Africa -Tanzania
91	CC45-MRSA-IV, Berlin EMRSA	Clinical infection	Skin swab	Germany - Muenster
92	CC45-MRSA-V	Clinical infection	Skin swab	Africa - Mozambique
93	CC45-MSSA	Clinical infection	Blood	Germany - Freiburg
94	CC45-MSSA	Clinical infection	Skin swab	Germany - Freiburg
95	CC45-MSSA	Clinical infection	Skin swab	Germany - Homburg/Saar
96	CC45-MSSA	Clinical infection	Skin swab	Germany - Homburg/Saar
97	CC45-MSSA	Clinical infection	Skin swab	Germany - Homburg/Saar
98	CC45-MSSA	Clinical infection	Skin swab	Africa - Mozambique
99	CC45-MSSA	Clinical infection	Skin swab	Germany - Muenster
100	CC45-MSSA	Clinical infection	Skin swab	Germany - Muenster
101	CC45-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
102	CC45-MSSA	Commensal	Nasal swab	Africa -Tanzania
103	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab	Africa -Tanzania
104	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab	Africa - Gabon
105	CC45/agrIV-MSSA [capsule type 5]	Commensal	Nasal swab	Africa - Gabon
106	CC5-(ST5/ST225)-MRSA-II, Rhine-Hesse EMRSA/New York-Japan Clone	Clinical infection	Skin swab	Germany - Freiburg
107	CC5-MRSA with atypical SCCmec elements	Clinical infection	Skin swab	Germany - Freiburg
108	CC5-MRSA-II [ACME+], WA MRSA-125	Clinical infection	Skin swab	Germany - Homburg/Saar
109	CC5-MSSA	Clinical infection	Skin swab	Germany - Homburg/Saar
110	CC5-MSSA	Clinical infection	Blood	Africa - Gabon
111	CC5-MSSA	Clinical infection	Skin swab	Africa - Gabon

112	CC5-MSSA	Clinical infection	Skin swab	Africa -Tanzania
113	CC5-MSSA [PVL+]	Clinical infection	Skin swab	Africa -Tanzania
114	CC5-ST5/ST225-MRSA-II, Rhine-Hesse EMRSA/New York-Japan Clone	Clinical infection	Skin swab	Germany - Muenster
115	CC5-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
116	CC5-MSSA	Commensal	Nasal swab	Africa - Mozambique
117	CC5-MRSA-IV, Paediatric clone [sed/j/r+]	Commensal	Nasal swab	Germany - Freiburg
118	CC5-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
119	CC5-MSSA	Commensal	Nasal swab	Africa -Tanzania
120	CC5-MRSA-IV [fusC+], New Zealand AK3/WA MRSA-39	Commensal	Nasal swab	Africa - Gabon
121	CC8-(ST8)-MRSA-IV [PVL+/ACME+], USA300	Clinical infection	Skin swab	Africa - Gabon
122	CC8-(ST8)-MRSA-IV [PVL+/ACME+], USA300	Clinical infection	Skin swab	Africa - Gabon
123	CC8-MRSA-IV [sea+], Lyon Clone/UK-EMRSA-2	Clinical infection	Blood	Africa - Gabon
124	CC8-(ST72)-MSSA	Clinical infection	Blood	Africa - Mozambique
125	CC8-MRSA-IV [sea+], Lyon Clone/UK-EMRSA-2	Clinical infection	Skin swab	Africa - Mozambique
126	CC8-MSSA	Clinical infection	Blood	Africa - Mozambique
127	CC8-MSSA	Clinical infection	Skin swab	Germany - Freiburg
128	CC8-MSSA	Clinical infection	Skin swab	Africa -Tanzania
129	CC8-MSSA	Clinical infection	Skin swab	Africa -Tanzania
130	CC8-MSSA	Clinical infection	Skin swab	Germany - Muenster
131	CC8-(ST72)-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
132	CC8-(ST72)-MSSA	Commensal	Nasal swab	Africa -Tanzania
133	CC8-(ST72)-MSSA	Commensal	Nasal swab	Africa - Gabon
134	CC8-MRSA-V	Commensal	Nasal swab	Germany-Muenster
135	CC8-MSSA	Commensal	Nasal swab	Germany - Freiburg
136	CC88-MRSA-IV [PVL+]	Clinical infection	Skin swab	Africa -Tanzania
137	CC88-MRSA-IV, WA MRSA-2	Clinical infection	Skin swab	Africa -Tanzania
138	CC88-MSSA [PVL+]	Clinical infection	Skin swab	Africa -Tanzania
139	CC88-MSSA [PVL+]	Clinical infection	Skin swab	Africa -Tanzania
140	CC88-MSSA [PVL+]	Clinical infection	Skin swab	Africa -Tanzania
141	CC88-MSSA [PVL+]	Clinical infection	Blood	Africa - Mozambique
142	CC88-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
143	CC88-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
144	CC88-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
145	CC88-MRSA-IV, WA MRSA-2	Clinical infection	Skin swab	Africa - Gabon
146	CC88-MRSA-IV [etA+]	Commensal	Nasal swab	Africa - Mozambique

147	CC88-MRSA-IV [PVL+]	Commensal	Nasal swab	Africa -Tanzania
148	CC88-MRSA-IV [etA+]	Commensal	Nasal swab	Africa -Tanzania
149	CC88-MSSA	Commensal	Nasal swab	Africa - Gabon
150	CC88-MSSA	Commensal	Nasal swab	Africa - Mozambique
(B) Second batch: List of CC121 *S. aureus* (n=60) and CC152 *S. aureus* (n=30)				
1	CC121-MSSA	Clinical infection	Skin swab	Germany - Freiburg
2	CC121-MSSA	Clinical infection	Skin swab	Germany - Freiburg
3	CC121-MSSA	Clinical infection	Skin swab	Africa - Tanzania
4	CC121-MSSA	Clinical infection	Skin swab	Africa - Mozambique
5	CC121-MSSA	Clinical infection	Skin swab	Africa - Mozambique
6	CC121-MSSA	Clinical infection	Skin swab	Africa - Mozambique
7	CC121-MSSA	Clinical infection	Skin swab	Africa - Mozambique
8	CC121-MSSA	Clinical infection	Skin swab	Germany - Muenster
9	CC121-MSSA	Clinical infection	Skin swab	Germany - Muenster
10	CC121- MSSA	Clinical infection	Skin swab	Germany - Homburg/Saar
11	CC121-MSSA	Clinical infection	Skin swab	Africa - Tanzania
12	CC121-MSSA	Clinical infection	Skin swab	Africa - Tanzania
13	CC121-MSSA	Clinical infection	Skin swab	Africa - Tanzania
14	CC121-MSSA	Clinical infection	Skin swab	Africa - Gabon
15	CC121-MSSA	Clinical infection	Skin swab	Africa - Mozambique
16	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Homburg/Saar
17	CC121-MSSA [PVL+}	Clinical infection	Skin swab	Germany - Homburg/Saar
18	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Homburg/Saar
19	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Tanzania
20	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Tanzania
21	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Gabon
22	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Gabon
23	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
24	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
25	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Muenster
26	CC121-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Muenster
27	CC121-MSSA [PVL+}	Clinical infection	Skin swab	Africa - Tanzania
28	CC121-MSSA [PVL+}	Clinical infection	Skin swab	Africa - Tanzania
29	CC121-MSSA [PVL+]	Clinical infection	Blood	Africa - Gabon
30	CC121-MSSA [PVL+]	Clinical infection	Blood	Africa - Mozambique
31	CC121-MSSA	Commensal	Nasal swab	Germany - Freiburg
32	CC121-MSSA	Commensal	Nasal swab	Germany - Freiburg
33	CC121-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
34	CC121-MSSA	Commensal	Nasal swab	Germany - Homburg/Saar
35	CC121-MSSA	Commensal	Nasal swab	Africa - Tanzania
36	CC121-MSSA	Commensal	Nasal swab	Africa - Tanzania
37	CC121-MSSA	Commensal	Nasal swab	Africa - Tanzania

38	CC121-MSSA	Commensal	Nasal swab	Africa - Tanzania
39	CC121-MSSA	Commensal	Nasal swab	Africa - Mozambique
40	CC121-MSSA	Commensal	Nasal swab	Africa - Mozambique
41	CC121-MSSA	Commensal	Nasal swab	Africa - Mozambique
42	CC121-MSSA	Commensal	Nasal swab	Africa - Mozambique
43	CC121-MSSA	Commensal	Nasal swab	Africa - Mozambique
44	CC121-MSSA	Commensal	Nasal swab	Africa - Mozambique
45	CC121-MSSA	Commensal	Nasal swab	Africa - Mozambique
46	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Tanzania
47	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
48	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
49	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
50	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
51	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
52	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
53	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
54	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
55	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
56	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
57	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
58	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
59	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
60	CC121-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
61	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Freiburg
62	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Germany - Freiburg
63	CC152-MSSA[PVL+}	Clinical infection	Skin swab	Africa - Tanzania
64	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Tanzania
65	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Tanzania
66	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Tanzania
67	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Gabon
68	CC152-MSSA[PVL+}	Clinical infection	Skin swab	Africa - Gabon
69	CC152-MSSA[PVL+}	Clinical infection	Skin swab	Africa - Gabon
70	CC152-MSSA[PVL+}	Clinical infection	Skin swab	Africa - Gabon
71	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Gabon
72	CC152-MSSA [PVL+]	Clinical infection	Skin swab	Africa - Mozambique
73	CC152-MSSA [PVL+]	Clinical infection	Blood	Africa - Mozambique
74	CC152-MSSA [PVL+]	Clinical infection	Blood	Africa - Mozambique
75	CC152-MSSA [PVL+]	Clinical infection	Blood	Africa - Mozambique
76	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
77	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
78	CC152-MSSA[PVL+}	Commensal	Nasal swab	Africa - Mozambique
79	CC152-MSSA[PVL+}	Commensal	Nasal swab	Africa - Mozambique
80	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique

81	CC152-MSSA[PVL+}	Commensal	Nasal swab	Africa - Mozambique
82	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
83	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
84	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Mozambique
85	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
86	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
87	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
88	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
89	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon
90	CC152-MSSA [PVL+]	Commensal	Nasal swab	Africa - Gabon

Appendix 3: Virulence genes profile of the Tanzanian *S. aureus* strains

Gene/CC	CC152 (*n*=34)	C121 (*n*=30)	CC8 (*n*=25)	CC15 (*n*=24)	CC88 (*n*=23)	CC5 (*n*=23)	CC1 (*n*=18)	CC6 (*n*=18)	CC9 (*n*=14)	CC45 (*n*=11)	CC80 (*n*=10)	CC30 (*n*=7)	CC25 (*n*=7)	Others (*n*=14) *	All Strains (*n*=258)	Commensal (*n*=134)	Clinical (*n*=124)
A. Toxic shock syndrome toxin 1 *(tst1)* and enterotoxins profile																	
tst1	0	0	5	0	1	1	0	3	0	2	0	0	0	2	**13 (5%)**	**10**	**3**
sea	0	0	5	0	0	5	11	17	0	0	0	0	0	0	**38 (15%)**	**21**	**17**
seb	0	24	6	0	0	7	4	1	0	0	0	0	3	1	**46 (18%)**	**15**	**31**
sec	0	0	4	0	1	1	3	4	0	3	2	0	5	1	**24 (9%)**	**11**	**13**
sed	3	1	1	1	0	5	1	0	1	0	0	0	1	2	**16 (6%)**	**10**	**6**
see	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0 (0%)**	**0**	**0**
seg	3	30	12	3	2	23	6	0	2	11	1	7	6	4	**110 (43%)**	**56**	**54**
seh	0	0	0	0	0	0	14	0	0	0	0	0	0	0	**14 (5%)**	**7**	**7**
sei	1	28	11	0	0	23	4	0	1	11	0	7	7	2	**95 (37%)**	**45**	**50**
sej	0	0	9	0	0	5	0	0	0	0	0	0	0	1	**15 (6%)**	**11**	**4**
sek	0	0	4	0	2	4	2	0	0	0	0	0	0	2	**14 (5%)**	**8**	**6**
sel	0	0	4	0	1	1	3	5	0	3	2	0	6	1	**26 (10%)**	**12**	**14**
selm	0	30	11	0	0	23	4	0	1	11	0	7	7	2	**96 (37%)**	**46**	**50**
seln	0	30	11	0	0	23	4	0	1	11	0	6	6	2	**94 (36%)**	**46**	**48**
selo	0	30	11	0	0	23	4	0	1	11	0	6	6	2	**94 (36%)**	**46**	**48**
egc	3	30	12	0	1	23	4	0	2	11	0	7	7	2	**102 (40%)**	**50**	**52**

seq	0	0	4	0	2	4	2	0	0	0	0	0	0	2	**14 (5%)**	**8**	**6**
ser	0	0	7	0	0	5	0	0	0	0	0	0	3	1	**16 (6%)**	**9**	**7**
selu	0	30	11	0	0	23	4	0	1	11	0	6	6	2	**94 (36%)**	**46**	**48**
No entero-toxin gene	30	0	1	20	11	0	0	0	12	0	2	0	0	7	**83 (32%)**	**41**	**42**
B. Hemolysin gamma, component A *(hlgA),* and leukocidin genes profile																	
lukF	27	30	25	24	23	23	18	18	14	11	10	6	6	13	**248 (96%)**	**131**	**117**
lukS	0	30	25	24	23	23	18	18	14	3	10	7	7	9	**211 (82%)**	**112**	**99**
hlgA	33	30	25	23	23	23	18	18	14	11	10	6	6	13	**253 (98%)**	**133**	**120**
lukF-PV	33	23	1	0	18	5	3	0	0	0	10	6	0	3	**102 (39.5%)**	**26**	**76**
lukS-PV	33	24	1	0	19	5	3	0	0	0	10	6	0	3	**104 (40%)**	**27**	**77**
lukM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**	**0**
lukD	17	16	25	24	23	23	14	18	13	0	10	0	6	4	**193 (75%)**	**109**	**84**
lukE	0	30	25	13	21	23	14	11	13	0	10	0	6	9	**175 (68%)**	**90**	**85**
lukX	10	27	23	21	20	22	14	17	11	6	8	6	6	10	**201 (78%)**	**113**	**88**
lukY	33	30	25	24	22	23	18	17	14	0	10	0	6	10	**232 (90%)**	**117**	**115**
C. Haemolysin genes profile																	
hld	34	30	25	24	23	23	18	18	14	11	10	7	6	14	**257 (99.7%)**	**134**	**123**
hla	33	30	25	24	23	23	18	18	13	11	10	6	6	14	**254 (98%)**	**133**	**121**
hlb_1	0	30	25	0	23	21	15	17	14	0	8	7	6	9	**175 (68%)**	**89**	**86**
hlb_2	3	30	25	0	23	23	15	18	14	1	10	7	7	10	**186 (72%)**	**94**	**92**
hlb_3	32	30	25	10	23	19	14	16	14	0	7	7	6	10	**213 (83%)**	**106**	**107**

undisrupted hlb	34	0	0	0	0	0	0	0	0	2	0	0	0	4	**40 (15.5%)**	**14**	**26**
D. Immune evasion molecule (Hlb converting phages) profile																	
sak	33	30	25	0	23	23	14	18	14	10	10	7	6	13	**226 (87.6%)**	**114**	**112**
chp	0	0	14	24	19	23	4	0	11	10	0	7	7	9	**128 (49.6%)**	**52**	**76**
scn	34	30	25	24	23	23	18	18	14	10	10	7	6	14	**256 (99.2%)**	**133**	**123**
E. Exfoliative toxin genes profile																	
etA	0	6	0	1	2	0	1	4	0	0	0	0	0	1	**15 (5.8%)**	**1**	**14**
etB	0	4	0	0	0	0	0	0	0	0	0	0	0	0	**4 (1.6%**	**3**	**1**
etD	0	0	2	0	0	0	0	0	0	0	4	0	6	1	**13 (5%)**	**9**	**4**
F. Epidermal cell differentiation inhibitor genes profile																	
edinA	0	0	0	0	0	9	0	1	0	0	0	0	0	0	**10 (3.4%)**	**5**	**5**
edinB	34	0	2	0	0	0	0	0	0	0	4	0	7	3	**50 (19.4%)**	**35**	**15**
edinC	0	4	0	0	0	0	0	0	0	0	0	0	0	0	**4 (1.6%)**	**3**	**1**
G. Proteases genes profile																	
aur	32	29	25	22	23	23	16	18	13	4	9	6	6	12	**238 (92.2%)**	**123**	**115**
splA	0	11	25	24	22	23	14	18	13	0	10	0	7	9	**176 (68%)**	**104**	**72**
splB	0	30	25	24	23	23	14	18	13	0	10	0	7	8	**195 (76%)**	**105**	**90**
splE	0	0	21	24	0	0	5	14	13	1	0	6	6	9	**99 (38%)**	**63**	**36**
sspA	32	30	24	23	23	23	18	18	14	11	10	7	7	14	**254 (98%)**	**133**	**121**
sspB	33	30	25	24	23	23	18	18	14	11	10	7	7	14	**257 (99.7%)**	**134**	**123**
sspP	34	30	24	23	22	23	18	18	14	11	10	7	7	14	**255 (99%)**	**133**	**122**
H. Capsule and biofilm-associated genes profile																	

cap 1	0	0	0	1	0	0	0	0	0	1	0	0	0	**0**	**2 (0.8%)**	**2**	**0**
cap 5	34	0	25	0	0	23	4	0	1	6	0	0	6	**8**	**107 (41.5%)**	**56**	**51**
cap 8	0	30	0	24	23	0	14	18	13	5	10	7	0	**6**	**150 (58%)**	**78**	**72**
icaA	33	30	25	24	23	23	18	18	14	11	10	7	7	**14**	**257 (99.7%)**	**134**	**123**
icaC	0	30	20	23	23	23	18	18	14	11	10	6	6	**12**	**214 (83%)**	**119**	**95**
icaD	33	30	25	23	22	23	18	18	14	11	10	7	7	**14**	**255 (99%)**	**133**	**122**
bap	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0 (0%)**	**0**	**0**
I. Adhesion factors (surface proteins) profile																	
bbp	32	30	25	24	23	23	18	16	8	10	10	7	7	**12**	**245 (95%)**	126	119
clfA	34	30	25	24	23	23	18	18	14	11	10	7	7	**14**	**258 (100%)**	134	124
clfB	34	30	25	24	22	23	18	18	14	11	10	7	7	**14**	**257 (99.7%)**	134	123
cna	34	30	0	1	0	0	18	18	0	11	0	7	0	**7**	**126 (49%)**	56	70
ebh	34	30	25	24	23	23	18	18	14	11	10	7	6	**12**	**255 (99%)**	132	123
ebpS	32	30	25	24	23	23	18	18	14	11	10	7	6	**14**	**256 (99%)**	134	122
eno	34	30	25	24	23	23	18	18	14	11	10	7	7	**14**	**258 (100%)**	134	124
fib	0	30	25	24	23	23	18	18	14	0	10	0	7	**6**	**198 (77%)**	105	93
fnbA	32	30	25	24	23	23	18	18	14	11	10	7	6	**13**	**255 (99%)**	132	123
fnbB	32	29	25	23	23	23	18	18	14	8	10	7	7	**11**	**249 (97%)**	128	121
map	0	30	25	24	23	23	9	18	14	11	10	7	7	**12**	**213 (82.5%)**	117	96
sasG	10	5	25	24	23	23	18	18	13	6	10	0	0	**8**	**183 (71%)**	107	76

sdrC	1	30	25	24	23	23	18	18	14	11	10	7	7	**12**	**223 (86%)**	123	100
sdrD	33	30	25	23	23	14	18	17	8	9	10	7	7	**11**	**235 (91%)**	124	111
vwb	33	30	25	24	23	23	18	18	14	11	10	7	7	**14**	**257 (99.7%)**	134	123

*, Others (*n*=14) included 11 different CCs with a low number of strains: two strains from each of CC22, CC101, CC182, and one strain from each of ST1290, ST580, ST2744, ST2734, ST2370, CC59, CC97, and CC395

Appendix 4: Distribution of Antimicrobial Resistance Genes (ARGs) in the Tanzanian *S. aureus* strains (CCs) from clinical infections and commensals

ARG * CC/ST	*mecA*	*sdrM*	*blaZ*	*blaI*	*blaR*	*fosB*	*fosB* (plasmid)	*tet (K)*	*tet (M)*	*erm (C)*	*erm (B)*	*msr (A)*	*dfrS1*	*vat (B)*	*vga (A)*	*aacA-aphD*	*aphA3*	*sat*	*cat*
CC152-MSSA	0	34	34	33	33	0	5	16	0	19	0	0	0	0	0	0	0	0	0
CC121-MSSA	0	30	30	30	30	30	3	5	0	3	0	0	0	0	0	0	0	0	0
CC8-MSSA	0	13	13	13	13	13	1	10	0	1	0	0	0	0	0	0	0	0	0
CC8 [ST72]-MSSA	0	11	11	11	11	11	1	0	0	0	0	4	0	0	0	0	0	0	0
CC8-MRSA-V	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
CC15-MSSA	0	24	22	22	22	24	3	2	0	5	0	0	0	0	0	0	0	0	0
CC88-MSSA	0	18	18	18	18	0	3	10	0	1	0	1	0	0	0	0	0	0	0
CC88-MRSA	2	5	5	5	5	0	0	2	0	1	0	0	5	0	2	0	0	0	0
CC5-MSSA	0	23	23	23	23	23	5	7	2	11	0	0	0	0	0	0	0	0	0
CC6-MSSA	0	18	17	17	17	18	3	0	1	0	0	0	0	0	0	0	0	0	0
CC1-MSSA	0	18	16	16	16	4	0	3	1	2	0	0	0	0	0	0	0	0	0
CC9 [ST834] -MSSA	0	13	11	11	11	13	2	4	1	1	0	0	1	0	0	0	0	0	0
CC9-MSSA	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
CC45-MSSA	0	10	9	9	9	0	1	1	0	0	0	2	0	0	0	0	0	0	0
ST45-MSSA	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CC80-MSSA	0	4	0	1	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0

Atypical CC80-MSSA	0	6	6	6	6	0	1	0	0	1	0	0	0	0	0	0	0	0	0
CC25-MSSA	0	7	7	7	7	5	0	1	0	4	0	0	0	0	0	0	0	0	0
CC30-MSSA	0	7	7	7	7	7	0	0	0	0	0	1	0	0	0	0	0	0	0
CC182-MSSA	0	2	2	2	2	1	1	1	0	1	0	0	0	1	0	0	0	0	0
CC101	0	2	2	2	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0
CC22	0	0	2	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
CC97-MSSA	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
ST2370	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
ST2744	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
ST59/952-MRSA	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1
CC395	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
ST2734	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ST580	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CC1290	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
All No All (%)	**7 (2.7)**	**256 (99.2)**	**244 (94.6)**	**244 (94.6)**	**243 (94.1)**	**156 (60.5)**	**33 (12.8)**	**67 (26.0)**	**5 (1.9)**	**51 (19.8)**	**1 (0.4)**	**8 (3.1)**	**6 (2.3)**	**1 (0.4)**	**2 (0.8)**	**1 (0.4)**	**1 (0.4)**	**1 (0.4)**	**2 (0.8)**
Clinical infection (n=124)	**3**	**124**	**118**	**117**	**117**	**72**	**8**	**37**	**3**	**27**	**0**	**2**	**2**	**1**	**1**	**1**	**0**	**0**	**0**
Commen-sal (n=134)	**4**	**132**	**126**	**127**	**126**	**84**	**25**	**30**	**2**	**24**	**1**	**4**	**4**	**0**	**1**	**0**	**1**	**1**	**2**

*, *mecA*, alternate penicillin binding protein 2, defining MRSA; *sdrM*, Transport/Efflux protein gene, *blaZ*, β-lactamase; *blaI*, β-lactamase repressor (inhibitor) gene; *blaR*, β-lactamase regulatory protein gene. *fosB*/*fosB* (plasmid): metallothiol transferase gene; *tet*, tetracycline-resistance encoding gene; *dfrS1*, dihydro-folate reductase type 1 gene; *ermC* rRNA adenine N-6-methyl-transferase gene; *msrA*, energy-dependent efflux protein gene; *vatB*, acetyltransferase gene; *vga*(A), *vgaA* (BM 3327) ATP binding protein gene; *aacA-aphD*, aminoglycoside acetyltransferase A/aminoglycoside phosphotransferase D gene.

Appendix 5: Antimicrobial susceptibility testing (AST) results of the Tanzanian *S. aureus*

Antibiotics	Clinical infection isolates (*n*=124)			Commensal isolates (*n*=134)			All (*n*=258)		
	[S]	[I]	[R]	[S]	[I]	[R]	[S]	[I]	[R]
Penicillin	3 (2.4%)	0 (0%)	121 (97.6%)	9 (6.7%)	0 (0%)	124 (92.5%)	12 (4.7%)	0 (0%)	245 (95%)
Cefoxitin	117 (94.4%)	0 (0%)	7 (5.7%)	125 (93.3%)	1 (0.8)	7 (5.2%)	242 (94%)	1 (0.4%)	14 (5.4%)
Co-trimoxazole*	100 (80.7%)	3 (2.4%)	20 (16.1%)	114 (85%)	2 (1.5%)	17 (12.7%)	214 (83%)	5 (2%)	37 (14.3%)
Tetracycline	87 (70.2%)	6 (4.8%)	31 (25%)	109 (81.3)	5 (3.7%)	19 (14.2%)	196 (76%)	11 (4.3%)	50 (19.4%)
Chloramphenicol*	121 (97.6%)	1 (0.8%)	1 (0.8%)	124 (92.5%)	2 (1.5%)	7 (5.2%)	245 (95%)	3 (1.2%)	8 (3.1%)
Gentamicin	118 (95.2%)	2 (1.6%)	4 (3.2%)	131 (97.8%)	0 (0%)	2 (1.5%)	249 (96%)	2 (0.8%)	6 (2.3%)
Erythromycin	79 (63.7%)	11 (8.9%)	34 (27.4%)	87 (64.9%)	5 (3.7%)	41 (30.6%)	175 (68%)	19 (7.4%)	75 (29%)
Clindamycin	116 (93.6%)	4 (3.2%)	4 (3.2%)	120 (89.6%)	11 (8.2%)	2 (1.5%)	237 (92%)	15 (5.8%)	6 (2.3%)
Induced Clindamycin Resistant (ICR)	97 (78.2%)	NA	27 (21.8%)	104 (77.6%)	NA	29 (21.6%)	201 (78%)	NA	56 (21.7%)
Clindamycin (constitutive+ ICR)	93 (75%)	NA	31 (25%)	102 (76.1%)	NA	31 (23.1%)	195 (76%)	NA	62 (24%)

[S], susceptible; [I], intermediate resistant; [R], resistant

*, One clinical infection isolate was not tested for co-trimoxazole and chloramphenicol. One commensal isolate was not tested for all antimicriobial agents. NA, Not applicable.

Appendix 6: Virulence factors profile of the selected Tanzanian *S. aureus* strains; seven different clonal complexes (CCs) for phenotypic behaviours characterization

Gene/CC	CC152 (*n*=14)	CC121 (*n*=14)	CC15 (*n*=14)	CC5 (*n*=14)	CC6 (*n*=14)	CC88 (*n*=14)	CC8 (*n*=14)	All (*n*=98)	Commensal (*n*=55)	Clinical (*n*=43)
A. Toxic shock syndrome toxin 1 (*tst1*) and enterotoxins profile										
tst1	0	0	0	0	3	1	2	**6 (6%)**	3	3
sea	0	0	0	5	13	0	5	**23 (23.5%)**	13	10
seb	0	9	0	4	1	0	6	**20 (20%)**	8	12
sec	0	0	0	0	4	1	4	**9 (9%)**	5	4
sed	1	0	1	3	0	0	1	**6 (6%)**	5	1
see	0	0	0	0	0	0	0	**0 (0%)**	0	0
seg	1	14	2	14	0	0	7	**38 (39%)**	20	18
seh	0	0	0	0	0	0	0	**0 (0%)**	0	0
sei	0	13	0	14	0	0	7	**34 (35%)**	16	18
sej	0	0	0	3	0	0	2	**5 (5%)**	2	3
sek	0	0	0	3	0	2	4	**9 (9%)**	5	4
sel	0	0	0	0	5	1	4	**10 (10%)**	6	4
selm	0	14	0	14	0	0	7	**35 (36%)**	17	18
seln	0	14	0	14	0	0	7	**35 (36%)**	17	18
selo	0	14	0	14	0	0	7	**35 (36%)**	17	18
egc	1	14	0	14	0	0	7	**35 (36%)**	18	18
seq	0	0	0	3	0	2	4	**9 (9%)**	5	4
ser	0	0	0	3	0	0	2	**5 (5%)**	2	3
selu	0	14	0	14	0	0	7	**35 (36%)**	17	18
No Enterotoxin genes	13	0	11	0	0	9	1	**34 (35%)**	19	15
B. Hemolysin gamma component A *(hlgA),* and leukocidin genes profile										
lukF	12	14	14	14	14	14	14	**96 (98%)**	54	42
lukS	0	14	14	14	14	14	14	**84 (86%)**	48	36
hlgA	14	14	13	14	14	14	14	**97 (99%)**	55	42
lukF-PV	14	8	0	5	0	10	1	**38 (39%)**	13	25
***lukS*-PV**	14	9	0	5	0	11	1	**40 (41%)**	14	26
lukM	0	0	0	0	0	0	0	**0 (0%)**	0	0
lukD	11	8	14	14	14	14	14	**89 (91%)**	53	36
lukE	0	14	7	14	8	12	14	**69 (70%)**	37	32
lukX	7	12	12	13	13	12	12	**81 (83%)**	49	32
lukY	14	14	14	14	13	13	14	**96 (98%)**	54	42
C. Hemolysin genes profile										
hld	14	14	14	14	14	14	14	**98 (100%)**	55	43
hla	14	14	14	14	14	14	14	**98 (100%)**	55	43

hlb_probe 1	0	14	0	14	14	14	14	**70 (71%)**	37	33
hlb_probe 2	2	14	0	14	14	14	14	**72 (73.5%)**	39	33
hlb_probe 3	14	14	**6**	14	13	14	14	**89 (91%)**	49	40
undisrupted hlb	14	0	**0**	**0**	**0**	**0**	**0**	**14 (14%)**	7	7
D. Immune evasion molecule (HLB Convage phages) profile										
sak	14	14	0	14	14	14	14	**84 (86%)**	44	40
chp	0	0	14	14	0	12	7	**47 (48%)**	27	20
scin	14	14	14	14	14	14	14	**98 (100%)**	55	43
E. Exfoliative toxin genes profile										
etA	0	5	0	0	3	1	0	**9 (9%)**	9	0
etB	0	3	0	0	0	0	0	**3 (3%)**	3	0
etD	0	0	0	0	0	0	2	**2 (2%)**	1	1
F. Epidermal cell differentiation inhibitor genes profile										
edinA	0	0	0	6	1	0	0	**7 (7%)**	2	5
edinB	14	0	0	0	0	0	2	**16 (16%)**	8	8
edinC	0	3	0	0	0	0	0	**3 (3%)**	3	0
G. Protease genes profile										
aur	14	14	13	14	14	14	14	**97 (99%)**	55	42
splA	0	7	14	14	14	13	14	**76 (77.5%)**	47	29
splB	0	14	14	14	14	14	14	**84 (86%)**	48	36
splE	0	0	14	0	11	1	11	**37 (38%)**	24	13
sspA	14	14	14	14	14	14	13	**97 (99%)**	55	42
sspB	14	14	14	14	14	14	14	**98 (100%)**	55	43
sspP	14	14	14	14	14	13	13	**96 (98%)**	55	41
H. Capsule and biofilm-associated genes profile										
cap 1	0	0	1	0	0	0	0	**1 (1%)**	1	0
cap 5	14	0	0	14	0	0	14	**42 (43%)**	21	21
cap 8	0	14	14	0	14	14	0	**56 (57%)**	34	22
icaA	14	14	14	14	14	14	14	**98 (98%)**	55	43
icaC	0	14	14	14	14	14	13	**95 (97%)**	48	34
icaD	14	14	14	14	14	14	14	**97 (99%)**	55	42
bap	0	0	0	0	0	0	0	**0 (0%)**	0	0
I. Adhesion factor (surface protein) genes profile										
bbp	13	14	14	14	14	14	14	**97 (99%)**	54	43
clfA	14	14	14	14	14	14	14	**98 (100%)**	55	43
clfB	14	14	14	14	14	14	14	**98 (100%)**	55	42
cna	14	14	0	0	14	0	0	**42 (43%)**	23	19
ebh	14	14	14	14	14	14	14	**98 (100%)**	55	43
ebpS	14	14	14	14	14	14	14	**98 (100%)**	55	43
eno	14	14	14	14	14	14	14	**98 (100%)**	55	43
fib	0	14	14	14	14	14	14	**84 (86%)**	48	36
fnbA	14	14	14	14	14	14	14	**98 (100%)**	55	43
fnbB	14	14	14	14	14	14	14	**98 (100%)**	55	43

map	0	14	14	14	14	14	14	**84 (86%)**	48	36
sasG	5	1	14	14	14	14	14	**76 (77.5%)**	46	30
sdrC	0	14	14	14	14	14	14	**84 (86%)**	48	36
sdrD	14	14	14	9	14	14	14	**93 (95%)**	52	41
vwb	14	14	14	14	14	14	14	**98 (100%)**	55	43

Appendix 7: Virulence factors profile of the selected African-German StaphNet Consortium *S. aureus* from 10 different clonal complexes (CCs) for phenotypic behaviours characterization

Gene/CC	CC121	CC152	CC15	CC1	CC22	CC30	CC45	CC5	CC8	CC88	Commensal (n=50)	Clinical (n=100)	All (n=150)
A. Toxic shock syndrome toxin 1 (*tst1*) and enterotoxins profile													
tst1	0	0	0	3	1	5	1	1	2	1	5 (10%)	9 (9%)	**14 (9%)**
sea	0	0	0	11	0	1	0	3	3	0	5 (10%)	13 (13%)	**18 (12%)**
seb	5	0	0	2	0	0	0	2	2	1	6 (12%)	6 (6%)	**12 (8%)**
sec	0	1	0	2	6	1	7	0	2	1	2 (4%)	18 (18%)	**20 (13%)**
sed	0	0	3	0	0	0	0	5	2	1	5 (10%)	6 (6%)	**11 (7%)**
see	0	0	0	0	0	0	0	0	0	0	0 (0)	0 (0%)	**0 (0%)**
seg	15	0	2	3	15	14	14	15	4	1	29 (58%)	54 (54%)	**83 (55%)**
seh	0	0	1	12	0	1	0	0	0	0	5 (10%)	9 (9%)	**14 (9%)**
sei	14	0	0	3	15	14	15	15	4	0	27 (54%)	53 (53%)	**80 (53%)**
sej	0	0	0	0	0	0	0	8	2	0	3 (6%)	7 (7%)	**10 (7%)**
sek	0	0	0	7	0	0	0	2	3	5	7 (14%)	10 (10%)	**17 (11%)**
sel	0	1	0	2	6	1	7	0	2	1	2 (4%)	18 (18%)	**20 (13%)**
selm	15	0	0	3	15	14	15	15	4	0	27 (54%)	54 (54%)	**81 (54%)**
seln	15	0	0	3	15	14	15	15	4	0	27 (54%)	54 54%)	**81 (54%)**
selo	15	0	0	3	15	15	15	15	4	0	28 (56%)	54 (54%)	**82 (55%)**
egc	15	0	0	3	15	15	15	15	4	0	28 (56%)	54 (54%)	**82 (55%)**
seq	0	0	0	7	0	0	0	2	3	5	7 (14%)	10 (10%)	**17 (11%)**
ser	0	0	0	0	0	0	0	8	1	0	3 (6%)	6 (6%)	**9 (6%)**
selu	15	0	0	3	15	14	15	15	4	0	27 (54%)	54 (54%)	**81 (54%)**
No Enterotoxin genes	0	14	12	0	0	0	0	0	3	8	11 (22%)	26 (26%)	**37 (25%)**
B. Hemolysin gamma component A *(hlgA),* and leukocidin genes profile													
lukF	15	13	15	15	14	15	15	15	15	15	48 (96%	99 (99%)	**147 (98%)**
lukS	15	0	15	15	11	15	10	15	15	15	42 (84%)	84 (84%)	**126 (84%)**
hlgA	15	15	14	15	13	15	15	15	15	15	49 (98%)	98 (98%)	**147 (98%)**
lukF-PV	7	15	0	8	3	5	0	2	2	9	15 (30%)	36 (36%)	**51 (34%)**
***lukS*-PV**	8	15	0	8	3	5	0	2	2	9	16 (32%)	36 (36%)	**52 (35%)**
lukM	0	0	0	0	0	1	0	0	0	0	1 (2%)	0 (0%)	**1 (0.7%)**
lukD	13	1	15	12	0	0	0	15	15	15	29 (58%)	57 (57%)	**86 (57%)**
lukE	15	0	11	12	0	0	0	15	14	15	29 (58%)	53 (53%)	**82 (55%)**
lukX	12	0	13	13	14	13	15	14	12	14	43 (86%)	77 (77%)	**120 (80%)**
lukY	15	15	15	15	15	0	0	15	15	15	40 (80%)	80 (80%)	**120 (80%)**
C. Hemolysin genes profile													
hld	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**
hla	15	15	15	15	15	15	15	14	15	15	50 (100%)	99 (99%)	**149 (99%)**
hlb_probe 1	15	2	0	13	15	12	2	12	15	15	35 (70%)	66 (66%)	**101 (67%)**

hlb_probe 2	15	2	0	13	15	14	4	15	15	15	37 (74%)	71 (71%)	**108 (72%)**
hlb_probe 3	15	15	5	13	15	9	0	8	15	15	39 (78%)	71 (71%)	**110 (73%)**
undisrupted hlb	0	15	0	0	1	6	1	3	2	0	10 (20%)	18 (18%)	**28 (19%)**
D. Immune evasion molecule (HLB Convage phages) profile													
sak	15	15	0	12	14	10	14	13	14	15	40 (80%	82 (82%)	**15**
chp	0	0	15	3	14	8	14	10	8	12	26 (52%)	58 (58%)	**0**
scin	15	15	15	15	14	10	14	13	14	15	45 (90%	95 (95%)	**15**
E. Exfoliative toxin genes profile													
etA	7	0	1	1	0	0	0	0	0	2	5 (10%)	6 (6%)	**11 (7%)**
etB	3	1	1	0	0	0	0	0	0	0	1 (2%)	4 (4%)	**5 (3%)**
etD	0	0	0	0	0	0	0	0	1	0	0 (0%)	1 (1%)	**1 (0.7%)**
F. Epidermal cell differentiation inhibitor genes profile													
edinA	0	0	0	0	0	0	0	3	0	0	1 (2%)	2 (2%)	**3 (2%)**
edinB	0	14	0	0	0	0	0	0	1	0	5 (10%)	10 (10%)	**15 (10%)**
edinC	3	0	1	0	0	0	0	0	0	0	0 (0%)	4 (4%)	**4 (2.7%)**
G. Protease genes profile													
aur	15	15	15	14	15	15	6	15	14	14	48 (96%)	90 (90%)	**138 (92%)**
splA	13	0	15	12	0	0	0	15	14	15	27 (54%)	57 (57%)	**84 (56%)**
splB	15	0	15	12	0	0	0	15	14	15	29 (58%)	57 (57%)	**86 (57%)**
splE	0	0	15	11	0	12	0	0	14	0	16 (32%)	36 (36%)	**52 (35%)**
sspA	15	14	15	15	15	15	15	15	15	15	50 (100%)	99 (99%)	**149 (99%)**
sspB	15	15	15	15	15	15	15	15	15	15	50 (100%)	100(100%)	**150 (100%)**
sspP	14	15	15	15	15	14	15	15	14	15	50 (100%)	97 (97%)	**147 (98%)**
H. Capsule and biofilm-associated genes profile													
cap 1	0	0	1	0	0	0	0	0	1	0	1 (2%)	1 (1%)	**2 (1.3%)**
cap 5	0	15	0	3	15	0	3	15	15	0	23 (46%)	43 (43%)	**66 (44%)**
cap 8	15	0	15	12	0	15	12	0	0	15	27 (54%)	57 (57%)	**84 (56%)**
icaA	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**
icaC	15	0	15	15	15	14	15	15	14	15	43 (86%)	90 (90%)	**133 (89%)**
icaD	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**
bap	0	0	0	0	0	0	0	0	1	0	0(0%)	1(1%)	**1 (0.7%)**
I. Adhesion factor (surface protein) genes profile													
bbp	15	15	14	12	15	13	14	14	15	15	49 (98%)	93 (93%)	**142 (100%)**
clfA	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**
clfB	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**
cna	15	15	0	15	15	14	15	0	0	0	30 (60%)	59 (59%)	**89 (59%)**
ebh	15	15	15	15	0	15	15	15	14	15	45 (90%)	89 (89%)	**134 (89%)**
ebpS	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**
eno	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**
fib	15	0	15	15	0	0	0	15	15	15	30 (60%)	60 (60%)	**90 (60%)**
fnbA	14	15	15	15	15	15	15	15	15	15	50 (100%)	99 (99%)	**149 (99%)**
fnbB	15	15	15	15	8	11	15	15	14	15	48 (96%)	90 (90%)	**138 (92%)**
map	15	0	15	9	15	15	15	14	15	15	41 (82%)	87 (87%)	**128 (85%)**

sasG	1	2	15	15	15	0	3	15	13	15	33 (66%)	61 (61%)	**94 (63%)**
sdrC	14	0	15	15	15	15	15	14	15	15	44 (88%)	89 (89%)	**133 (89%)**
sdrD	14	15	13	15	15	14	14	12	14	15	48 (96%)	93 (93%)	**141 (94%)**
vwb	15	15	15	15	15	15	15	15	15	15	50 (100%)	100 (100%)	**150 (100%)**

Appendix 8: Virulence factors profile of the selected CC121 and CC152 *S. aureus* from the African-German StaphNet Consortium for phenotypic behaviors characterisation

Gene/CC	CC121				CC152			
	Commensal (*n*=30)	Clinical (*n*=30)	All (*n*=60)	All (%)	Commensal (*n*=15)	Clinical (*n*=15)	All (*n*=30)	All (%)
A. Toxic shock syndrome toxin 1 (*tst1*) and enterotoxin genes profile								
tst1	0	0	**0**	**0.0**	0	0	**0**	**0.0**
sea	0	0	**0**	**0.0**	0	0	**0**	**0.0**
seb	15	14	**29**	**48.3**	0	0	**0**	**0.0**
sec	0	0	**0**	**0.0**	0	1	**1**	**0.0**
sed	2	3	**5**	**8.3**	0	2	**2**	**6.7**
see	0	0	**0**	**0.0**	0	0	**0**	**0.0**
seg	30	30	**60**	**100.0**	0	2	**2**	**6.7**
seh	0	0	**0**	**0.0**	0	0	**0**	**0.0**
sei	27	27	**54**	**90.0**	0	0	**0**	**0.0**
sej	0	0	**0**	**0.0**	0	0	**0**	**0.0**
sek	0	2	**2**	**3.3**	0	0	**0**	**0.0**
sel	0	0	**0**	**0.0**	0	1	**1**	**3.3**
selm	30	30	**60**	**100.0**	0	0	**0**	**0.0**
seln	30	30	**60**	**100.0**	0	0	**0**	**0.0**
selo	29	30	**59**	**98.3**	0	0	**0**	**0.0**
egc	30	30	**60**	**100.0**	0	0	**0**	**0.0**
seq	0	2	**2**	**3.3**	0	0	**0**	**0.0**
ser	0	0	**0**	**0.0**	0	0	**0**	**0.0**
selu	30	30	**60**	**100.0**	0	0	**0**	**0.0**
No enterotoxin genes	0	0	**0**	**0**	15	12	**27**	**90**
B. Hemolysin gamma component A (*hlgA*) and leukocidin genes profile								
lukF	30	30	**60**	**100.0**	15	13	**28.0**	**93.3**
lukS	30	30	**60**	**100.0**	0	0	**0.0**	**0.0**
hlgA	29	30	**59**	**98.3**	15	14	**29.0**	**96.7**
lukF-PV	14	18	**32**	**53.3**	15	15	**30.0**	**100.0**
***lukS*-PV**	15	18	**33**	**55.0**	15	15	**30.0**	**100.0**
lukM	0	0	**0**	**0.0**	0	0	**0.0**	**0.0**
lukD	24	24	**48**	**80.0**	0	1	**1.0**	**3.3**
lukE	30	30	**60**	**100.0**	0	0	**0.0**	**0.0**
lukX	26	26	**52**	**86.7**	0	0	**0.0**	**0.0**
lukY	29	30	**59**	**98.3**	15	14	**29.0**	**96.7**
C. Hemolysin genes profile								

hld	30	29	**59**	**98.3**	15	15	**30**	**100.0**
hla	30	29	**59**	**98.3**	15	15	**30**	**100.0**
hlb_probe 1	30	30	**60**	**100.0**	1	1	**2**	**6.7**
hlb_probe 2	30	30	**60**	**100.0**	2	0	**2**	**6.7**
hlb_probe 3	30	29	**59**	**98.3**	14	15	**29**	**96.7**
undisrupted hlb	0	2	**2**	**3.3**	15	15	**30**	**100.0**
D. Immune evasion molecules (*hlb*-converting phages) profile								
sak	28	30	**58**	**96.7**	15	15	**30**	**100.0**
chp	0	0	**0**	**0.0**	0	0	**0**	**0.0**
scin	28	30	**58**	**96.7**	15	15	**30**	**100.0**
E. Exfoliative toxin genes profile								
etA	12	11	**23**	**38.3**	0	0	**0**	**0.0**
etB	9	7	**16**	**26.7**	1	0	**1**	**3.3**
etD	0	0	**0**	**0.0**	0	0	**0**	**0.0**
F. Epidermal cell differentiation inhibitor genes profile								
edinA	0	0	0	6	1	0	0	**7 (7%)**
edinB	14	0	0	0	0	0	2	**16 (16%)**
edinC	0	3	0	0	0	0	0	**3 (3%)**
G. Protease genes profile								
aur	0	0	**0**	**0.0**	0	0	**0**	**0.0**
splA	0	0	**0**	**0.0**	15	14	**29**	**96.7**
splB	8	6	**14**	**23.3**	0	0	**0**	**0.0**
splE	0	0	**0**	**0.0**	0	0	**0**	**0.0**
sspA	0	0	**0**	**0.0**	15	14	**29**	**96.7**
sspB	8	6	**14**	**23.3**	0	0	**0**	**0.0**
sspP	0	0	**0**	**0.0**	0	0	**0**	**0.0**
H. Capsule and biofilm.associated genes profile								
cap 1	0	0	**0**	**0**	0	0	**0**	**0**
cap 5	0	0	**0**	**0**	15	15	**30**	**100**
cap 8	30	30	**60**	**100**	0	0	**0**	**0**
icaA	30	30	**60**	**100**	15	15	**30**	**100**
icaC	30	30	**60**	**100**	0	0	**0**	**0**
icaD	30	30	**60**	**100**	15	15	**30**	**100**
bap	0	0	**0**	**0**	0	0	**0**	**0**
I. Adhesin (surface proteins) genes profile								
bbp	30	30	**60**	**100.0**	15	15	**30**	**100.0**
clfA	30	30	**60**	**100.0**	15	15	**30**	**100.0**
clfB	30	30	**60**	**100.0**	15	15	**30**	**100.0**
cna	30	30	**60**	**100.0**	15	15	**30**	**100.0**
ebh	30	30	**60**	**100.0**	15	15	**30**	**100.0**
ebpS	30	30	**60**	**100.0**	15	15	**30**	**100.0**
eno	30	30	**60**	**100.0**	15	15	**30**	**100.0**
fib	30	30	**60**	**100.0**	0	0	**0**	**0.0**

fnbA	30	29	**59**	**98.3**	15	15	**30**	**100.0**
fnbB	27	30	**57**	**95.0**	15	15	**30**	**100.0**
map	30	30	**60**	**100.0**	0	0	**0**	**0.0**
sasG	5	2	**7**	**11.7**	4	5	**9**	**30.0**
sdrC	29	30	**59**	**98.3**	0	0	**0**	**0.0**
sdrD	26	30	**56**	**93.3**	15	15	**30**	**100.0**
vwb	30	30	**60**	**100.0**	15	15	**30**	**100.0**