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# Population structure of Staphylococcus aureus in the Lambaréné area, Gabon

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## Abbreviations

| | |
|---|---|
| ACME | arginine catabolic mobile element |
| *agr* | accessory gene regulator (gene) |
| AIP | auto-inducing peptide |
| aka | also known as |
| *arcC* | carbamate kinase (gene) |
| *aroE* | shikimate dehydrogenase (gene) |
| bp | base pair |
| BURP | based upon repeat pattern |
| CA-MRSA | community-associated methicillin-resistant *S. aureus* |
| cap | capsular polysaccharide type |
| CC | clonal complex |
| CERIL | Comité d'Ethique Régional Indépendent de Lambaréné |
| CERMEL | Centre de Recherches Médicales de Lambaréné |
| CI | confidence interval |
| *clfA* | fibrinogenreceptor (gene) |
| *coa* | coagulase (gene) |
| CP | capsular polysaccharide type |
| C-terminal region | carboxyl-terminus, carboxy-terminus |
| CV | core variable |
| DNA | deoxyribonucleic acid |
| Edin-A, -B, -C | epidermal cell differentiation inhibitor A, -B, -C |
| *edin-A, -B, -C* | epidermal cell differentiation inhibitor A, -B, -C (gene) |
| EMRSA | epidemic MRSA |
| ETA (ETB, ETD) | exfoliative toxin A (exfoliative toxin B, exfoliative toxin D) |
| *eta (etb, etd)* | exfoliative toxin A (exfoliative toxin B, exfoliative toxin D) (gene) |
| Fc part | fragment crystallizable part |

| | |
|---|---|
| GC | guanine and cytosine |
| GDP | gross domestic product |
| GISA | glycopeptide intermediately susceptible *S. aureus* |
| *glpF* | glycerol kinase (gene) |
| *gmk* | guanylate kinase (gene) |
| HAS | Hôpital Albert–Schweitzer, Albert–Schweitzer hospital |
| *hla* | alpha-hemolysin encoding gene (gene) |
| *hlg* | gamma-hemolysin encoding gene (gene) |
| IgG | immunoglobulin G |
| kb | kilobase |
| kDA | kilodalton |
| Ig | immunoglobulin |
| LPXTG-binding motif | Leu-Pro-(*any*)-Thr-Gly – binding motif |
| Mbp | megabases (millions of base pairs) |
| MDR | multi drug resistant |
| MGE | mobile genetic element |
| min | minute |
| MLST | multilocus sequence typing |
| MRSA | methicillin-resistant *S. aureus* |
| MSSA | methicillin-susceptible *S. aureus* |
| *n* | number |
| NCCLS | National Clinical and Labaratory Standard Institute |
| nt | non typable |
| N-terminal part | amino-terminus, $NH_2$-terminus |
| NUC | thermostable nuclease (protein) |
| *nuc* | thermostable nuclease (gene) |
| OR | odds ratio |
| PBP | penicillin-binding protein |
| PCR | polymerase chain reaction |
| PFGE | pulse-field electrophoresis |
| PNAG | poly-N-acetyl glucosamine |

| | |
|---|---|
| PIA | polysaccharide intercellular adhesin |
| PSSA | penicillin-susceptible *S. aureus* |
| *pta* | phosphate acetyltransferase (gene) |
| PTSAg | pyrogenic toxin superantigen |
| PVL | Panton-Valentine leukocidin |
| RIDOM | Ribosomal Differentiation of Microorganisms |
| RNA | ribonucleic acid |
| *sarA* | staphylococcal accessory regulator(gene) |
| *S. aureus* | *Staphylococcus aureus* |
| SEA (SEB, SEC, …) | staphylococcal enterotoxin A (staphylococcal enterotoxin B, staphylococcal enterotoxin C, …) |
| *sea (seb, sec…)* | staphylococcal enterotoxin A (staphylococcal enterotoxin B, staphylococcal enterotoxin C, …) (gene) |
| SpA | staphylococcal protein A |
| *spa* | staphylococcal protein A (gene) |
| SCC | staphylococcal cassette chromosome |
| SCC*mec* | staphylococcal cassette chromosome *mec* |
| SLV | single locus variant |
| ST | sequence type |
| *tpi* | triosephosphate isomerase (gene) |
| TSST-1 | toxic shock syndrome toxin 1 |
| *tst* | toxic shock syndrome toxin (gene) |
| vs. | versus |
| *yqiL* | acetyle coenzyme A acetyltransferase (gene) |

# 1 Introduction

## 1.1 *Staphylococcus aureus* – a major global human pathogen

The bacterium *Staphylococcus aureus (S. aureus)* is part of the family of Gram-positive *Staphylococcaceae.* It most commonly occurs as a commensal bacterium present on the human skin and the mucosae (epithelia), here primarily in the anterior nares [1, 2]. Nevertheless, under certain conditions, such as a host–bacterium disbalance [3], the commonly asymptomatic *S. aureus* carriage can lead to a wide spectrum of diseases [2,4].

### 1.1.1 History

Already in 1874 Theodor Billroth described micrococci in pus [5]. But it was only in 1882 that the Scottish surgeon Alexander Ogston first proposed the name *Staphylococcus* [6]. In 1880 [19], Ogston recovered bacteria in pus from abscesses and observed grapelike clusters under the microscope. Henceforward, the name *Staphylococcus* (from "*staphyle*", Greek for "*bunch of grapes*" and "*kokkos*", Greek for "*berry"*) [7, 6] became the accepted designation for this special kind of micrococci that had now been identified as the major cause of pus.

Two years later, in 1884, Anton J. Rosenbach devided the genus *Staphylococcus* into two species [8]. According to the pigmented appearance of their colonies, he called them *S. aureus* ("*aureum*", Latin for "*gold*") and *Staphylococcus albus* ("*albus*", Latin for "*white*"): the latter is referred to today as *Staphylococcus epidermidis*. These pigments were later classified as carotenoids, and in *S. aureus* the pertinent biosynthetic pathway has recently been identified [9].

### 1.1.2 Epidemiology

*S. aureus* is capable of colonizing multiple sites of the human skin and mucosae, but the anterior nares appear to be its primary ecological niche and reservoir [4, 1]. Extra-nasal sites that typically harbor the organism include the hands, perineum, pharynx and less frequently other carriage sites including the gastrointestinal tract, vagina, axillae [4, 10].

This colonization may act as an endogenous source of subsequent bacteremia and other infections [11]. For this reason, that is to detect variations in the risk of acquiring *S. aureus*-related infections, as well as for statistical reasons, the distinction in carriage types is useful. For several decades longitudinal studies of healthy individuals have shown three patterns of *S. aureus* carriage: noncarriage (~50% of individuals), intermittent carriage (~30% of individuals) and persistent carriage (~20% of individuals) of the same or different strains [4, 1,12, 13, 14, 15]. However, in 2009, van Belkum et al. presented a study in which they suggested a paradigm shift to be required. Van Belkum et al. propose that there are only two human types of nasal *S. aureus* carriers: persistent carriers and others [16]. This is based on findings that show that intermittent carriers and noncarriers share similar *S. aureus* nasal elimination kinetics and antistaphylococcal antibody profiles [16] and a low risk of infection as has been described in the literature earlier [15].

In general, a multifactorial genesis underlies *S. aureus* nasal carriage and, as a result, prevalence of *S. aureus* nasal carriage varies depending on an interaction of host factors (like age, gender, presence of chronic diseases such as HIV or Diabetes mellitus), environmental factors (like hospitalization, colonized household members) and bacterial virulence factors (like surface-associated proteins or secreted toxins) [4].

### 1.1.3 Bacteriology

In order to take account of recent results of molecular phylogenetic classification, the genus *Staphylococcus*, while formerly being part of the family *Micrococcaceae,* was proposed for reclassification into the family the *Staphylococcaceae* [17].

The complete lineage is:

Cellular organisms; Domain: Bacteria; Phylum: Firmicutes; Class: Bacilli; Order: Bacillales; Family: *Staphylococcaceae*; Genus: *Staphylococcus* [18].

The genus *Staphylococcus* consists of more than 35 species [69], of which *S. aureus* is the most virulent and in consequence also the most clinically relevant species [20].

*S. aureus* is a Gram-positive cocci and presents itself in clusters [2] with diameters of 0.5 – 1.5 µm [21]. Colonies of *S. aureus* can have β-hemolysis due to the production of the hemolysins α-toxin, β-toxin, γ-toxin, and δ-toxin. *Staphylococc*i can grow in a wide pH range (4.8 – 9.4), are able to resist drying, and can survive at temperature extremes as high as 60°C for up to 30 min [22]. In addition, *S. aureus* is capable to grow in a high-salt medium due to the production of osmoprotectants [23], and can tolerate 7.5% –10% sodium chlorid [22]. *S. aureus* is facultative anaerobic, non-motile, non-spore forming and possesses a number of virulence factors (e.g., coagulase, clumping factor) which play a role in the differentiation to other less virulent *Staphylococcus spp.*[5].

### 1.1.4 Molecular characteristics

#### 1.1.4.1 Genome structure

The first sequenced *S. Aureus* genomes were those of the two methicillin-resistant *S. aureus* (MRSA) strains N315 and Mu50 by Hiramatsu’s group in

2001 [24], followed by additional strains over the next ten years (among others MW2 [25], MRSA252 and MSSA476 [26], COL [27], USA300-IPR3737 [28], USA300-HOU-MR [29], NCTC8325 [30], ET3-1 [31], JH1 and JH9 [32], Newman [33], JKD6008 [34], and T0131 [35]).

Currently, there are about 24 annotated whole genome sequences of *S. aureus* in the public domain (www.ebi.ac.uk/genomes/bacteria.html). The number of sequenced genomes is growing at a fast rate as whole genome sequencing is becoming faster and less expensive.

All staphylococcal genomes consist of a circular chromosome, approximately 2.8 Mbp in size with a relatively low GC-content of 33% and several plasmids [5]. Comparative analysis revealed that most regions of the staphylococcal genome are well conserved, whereas several large parts of the genome display high variability [33].

Core genome and MGE

*S. aureus* genome is typically composed of a basic structure, which is a syntenous conserved core genome with all the genes vital to cell survival, and of a collection of mobile genetic elements (MGEs) that constitute about 25% of the whole genome [36, 37, 38]. MGEs are fragments of DNA integrated throughout the genome and may be classified as insertion sequences, transposons, phages, plasmids, pathogenicity islands, and chromosome cassettes. MGEs carry a variety of staphylococcal resistance and virulence genes: plasmids (e.g. *etb*, *sed*), phages (e.g. *sea*) or pathogenicity islands (e.g. *tst*, *sec*, *seg, sei*) [62,24]. By means of these MGEs, DNA transfer occurs horizontally between species and genera [36, 37] and vertically to daughter cells [39].

It is known that within the core genome there are core variable (CV) regions. These regions contain genes with a higher nucleoside substitution rate than core genes and often exhibit variations associated with lineage [36].

#### 1.1.4.2 Virulence factors

*S. aureus* expresses a wide array of virulence factors that facilitate attachment, colonization, cell–cell interactions, immune evasion, and tissue damage [26]. They act in concerted manner during the invasion processof infection [40]. Depending on whether the virulence factors remain associated with the cell surface or whether they are exported into the extracellular milieu, two groups (surface-associated virulence factors and extracellular virulence factors) can be distinguished [41]. Numerous of the*S. aureus'* putative and proven virulence factors are encoded in MGEs [37].

##### 1.1.4.2.1 Surface-associated virulence factors

Surface-associated virulence factors consists of structural components of the wall (e.g., wall teichoic acid, lipoteichoic acid, lipoprotein, peptidoglycan), surface exposed proteins (e.g., Protein A, fibronectin-binding proteins, fibrinogen-binding proteins) and extracellular polysaccharides (e.g., capsular polysaccharides (clinically relevant are serotypes 1,2,5 and 8) and polysaccharide intercellular adhesins (PIA, also referred to as poly-*N*-acetyl glucosamine/PNAG)) [42].

Protein A – an important virulence factor

Almost all isolates carry staphylococcal protein A (SpA) [40], a protein of 42 kDa encoded in the CV regions of the core genome [36] and consisting of several regions with distinct functions [43]. The N-terminal part is comprised of the signal sequence (S region) followed by four or five highly homologous immunoglobulin G (IgG)-binding domains in tandem (the E, D, A, B, and C regions) and is displayed on the cell surface [43,44, 45]. The C-terminal region, also referred to as X region, is anchored to the bacterial cell wall and "is divided into two domains: (i) the repeat region $X_R$, consisting of variable repeats with mostly octapeptide structures, which are used for *spa* typing, and (ii) the $X_C$

region, consisting of a conserved sequence, which confers anchoring to the cell wall via an LPXTG-binding motif.” [44]

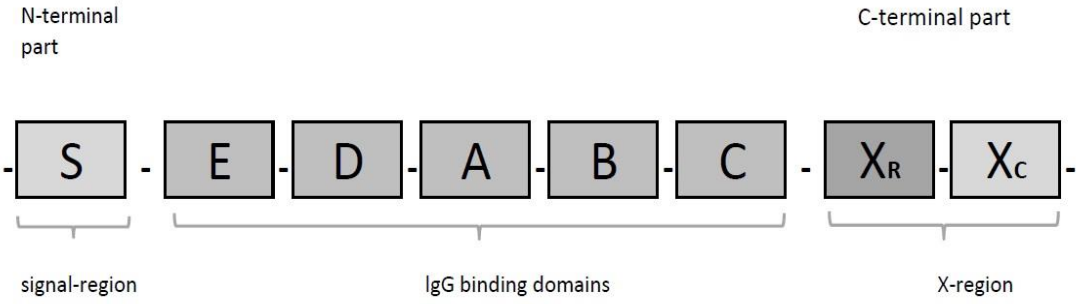


**Figure1:** *spa* region (from [44])

A well-studied function of SpA is its interaction with human IgG. SpA has the ability to bind with the Fc part of the immunoglobulins, so that neutrophils are unable to recognize the IgG molecules coating the cell surface. Thereby the host immune system is compromised through the protection of opsonization and phagocytosis of the bacterium [44, 46].

In addition, SpA can also interact with other host structures including the von Willebrand factor and the receptor gC1qR/p33 on platelets [47, 48, 49] and it plays a role in biofilm formation [50].

1.1.4.2.2 Extracellular virulence factors

The extracellular virulence factors include toxins, namely exfoliative toxins (eta, etb, etd), staphylococcal enterotoxins (sea, seb, sec, sed, see, seg, seh, sei, sej), toxic shock syndrome toxin 1 (TSST-1), heamolytic/cytolytic toxins (α-, β-, γ-, δ-toxin and PVL) as well as epidermal cell differentiation inhibitor exotoxins (Edin-A, Edin-B, Edin-C) and they also include exoenzymes (protease, thermonuclease (thermostable nuclease, NUC)).

Panton-Valentine leukocidin – its role in the pathogenesis and course of disease

Panton-Valentine leukocidin (PVL) is a poreforming cytotoxin that is produced by about 2% of methicillin-susceptible *S. aureus* n Europe [40] and belongs to the family of synergohymenotropic toxins [51]. This toxin consists of two separate synergistic protein subunits (referred to as LukS-PVL and LukF-PVL) encoded by two co-transcribed genes, *lukS-PV* and *lukF-PV*, which are carried on a temperate bacteriophage integrated in the *S. aureus* chromosome [52]. These toxins damage membranes of mononuclear and polymorphonuclear cells and cause cell death by necrosis or apoptosis [51]. It is to be noted that community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains are highly likely to be PVL positive [53] and there is some evidence indicating that PVL may contribute to the virulence of these strains [54] and may even lead to higher mortality among immunocompetent individuals through necrotizing pneumonia [55]. Characteristically, these CA-MRSA strains tend to carry staphylococcal cassette chromosome type IV and less frequently V [28, 56].

Another member of the same family of toxins – the synergohymenotropic toxins – is *y*-hemolysin encoded by *hlg* genes.

#### 1.1.4.3 Production control of virulence factors

The expression of *S. aureus'* wide array of virulence factors is influenced in a complex way by the accessory gene regulator (*agr*) locus and the staphylococcal accessory regulator (*sarA*) locus [57, 58].

*Agr* encodes a two-component signaling pathway whose activating ligand is a self-coded bacterial-density-sensing peptide (auto-inducing peptide [AIP]) binding to AgrC [59]. On the basis of a polymorphism of its AIPs, four major groups of *agr* (*agr* I to IV) in *S. aureus* strains can be distinguished [60]. The *agr* response is an increased expression of the regulatory RNA (RNAIII) which regulates the production of *S. aureus* virulence factors transcriptionally and posttranscriptionally. According to Jon S. Blevins et al., mutation in *agr* leads to an increased production of surface proteins, decreased production of

exoproteins, and reduced virulence [41]. However, they also point out that it has been reported that *agr* may influence regulatory strains differently [41].

*SarA* locus is the other important regulatory locus that also modulates the virulence production. Its protein (SarA) binds within promoter regions of genes encoding cell surface proteins (*spa*, encoding protein A), genes encoding exoproteins (*hla*, encoding alpha-hemolysin), and also *agr* [61]. SarA binding to *agr* promoter regions increases RNAIII transcription and therefore indirectly modulates virulence factor production but it has also been shown that SarA controls regulation of a number of virulence factors directly, in an *agr*-independent manner [62].

Expression of SarA is seen to promote biofilm formation whereas expression of agr is seen to limit biofilm formation [63]. Interestingly, Karen E. Beenken et al. demonstrated that *sarA* is epistatic to *agr* in regard to biofilm formation [63].

#### 1.1.4.4 Staphylococcal cassette chromosome

Staphylococcal cassette chromosome (SCC) is a MGE that is typically associated with methicillin resistance as many carry the *mecA* (SCC*mec*).The *mecA* or *mecC* encodes for a mutated penicillin binding protein (PBP) which confers resistance to almost all beta-lactam antimicrobial drugs by reducing affinity. SSC*mec* comprises the *mec* complex, with the regulatory genes and associated insertion sequences,and the *ccr* complex, which encodes for recombinases allowing integration and excision of SCC*mec* [64]. Additionally, the SCC*mec* elements may also contain other MGEs, e.g., plasmids or transposons which sometimes carry other resistance genes [65]. The acquisition/presence of the *mecA* gene results in methicillin resistance and resistance to all other β-lactam antibiotics by the production/output of its gene product PBP2a (aka PBP2'); PBP2a is an additional penicillin-binding protein, which has reduced affinity for β-lactam antibiotics [66, 67]. Normally, β-lactam antibiotics bind to PBPs in the cell wall, thereby stopping the biosynthesis of the cell wall and resulting in the death of the bacterium. Since PBP2a does not

allow this binding to occur, the biosynthesis of the cell wall is not disrupted and the bacterium lives on.

The origin of the *mecA* gene has not yet been defined. However, there is evidence that coagulase-negative staphylococci may act as its potential reservoir as it has been shown that horizontal transfer of SCC*mec* between staphylococci is likely to be possible [65].

By now, eleven major types (I to XI) of SCC*mec* have been described for *S. aureus* based on the combinations of the classes of the *mec* gene complex and the types of the *ccr* gene complex [68].

SCC*mec* types I, II or III are larger in size (34–67 kb) and are usually associated with hospital-acquired MRSA infections. The two smallest SCC*mec* types, either type IV (24 kb) or less frequently, SCC*mec* V, are associated with community-associated MRSA (CA-MRSA) [56].

### 1.1.5 Diagnostic

Phenotypic and genotypic methods

There are numerous methods to identify staphylococcus and staphylococcus subspecies, all varying in their sensitivity, specificity, standardization, reproducibility, time expenditures, costs and technical requirements. Classic phenotypic diagnostic methods are based on colony morphology, Gram straining, colony growth on sheep blood agar, catalase testing, rabbit plasma coagulase testing, clumping factor testing, a latex agglutination test as well as tests for biochemical characteristics such as enzymes.

Molecular species identification includes sequencing of universal target sequences (e.g., 16S RNA gene, *rpoB* [148] or the amplification of specific genes (e.g., thermonuclease (*nuc*), fibrinogenreceptor (*clfA*), coagulase (*coa*)) and genotyping (PFGE, *spa* typing, MLST) [69].

*Spa* typing

*Spa* typing is a sequence-based *S. aureus*-typing method which is fast, highly discriminatory, and reproducible [70]. It is based on single locus DNA-sequencing of the hyper-variable $X_R$-region of the *Staphylococcus*protein A gene (*spa*). This $X_R$-region consists of base pair (~ 24) repeats in different numbers (2 – 16). With the help of this polymorphism different *S. aureus* strains can be distinguished and named unambiguously as every repeat is given an alpha-numerical code and the *spa* type results from the kind and order of the specific repeats [71, 72]. This became possible with the development of databases that collate and harmonize data from different geographic areas such as the Ridom StaphType software (Ridom GmbH,Germany) [73]. The RIDOM (Ribosomal Differentiation of Microorganisms) web server is based on quality-controlled sequence entries and provides a sequence database that is comprehensive and validated [74]. It has been shown that the *spa* region is useful for short-term evolutionary events as well as for long-term phylogeny and it helps to determine clonal relatedness among different strains [75]. BURP–Algorithm, an algorithm that is based upon repeat pattern allows to form clusters of related *spa* types [76].

MLST

Multilocus sequence typing (MLST) is another “nucleotide sequence based approach for the unambiguous characterization of isolates of bacteria and other organisms via the internet” [77]. MLST is on the basis of the sequences of ~ 450-bp internal fragments of seven conserved housekeeping genes [78]. Initially, MLST had been developed 1998 by Martin Maiden, Dominique Caugant, Ian Feavers, Mark Achtman and Brian Spratt for *Neisseria meningitides* [78]. For *S. aureus*, the use of MLST has been described by Mark Enright in 2000 [100].

For each unique sequence of the fragments of the seven housekeeping genes a specific allele is assigned. A sequence type is then defined by allelic profile [100].

The following seven housekeeping genes are used for the*S. aureus* MLST scheme: *arcC* (carbamate kinase), *aroE* (shikimate dehydrogenase), *glpF* (glycerol kinase), *gmk* (guanylate kinase), *pta* (phosphate acetyltransferase), *tpi* (triosephosphate isomerase) and *yqiL* (acetyle coenzyme A acetyltransferase) [100]. These genes are located throughout the whole genome.

MLST data is freely available to anyone via the internet (http://saureus.mlst.net/) as is the eBURST software (http://eburst.mlst.net/) which allows to cluster clonal complexes (CC) of the sequence types (ST) of isolates (corresponding to groups of closely related strains) by grouping the STs with STs of the whole MLST database. The CCs predict “the founding genotype of each group, and displays the patterns of recent evolutionary descent of all other strains in the group from the founder.” [130, 131].

High concordance of results between *spa* typing, MLST and other widely-used typing methods as PFGE and microarray has been shown [79]. There are even some studies that have showed that *spa* typing sometimes offers greater resolution than MLST [73].

### 1.1.6 Clinical features

*S. aureus* mostly asymptomatically colonizes the skin, but provided the needed conditions, its pathogenic potential is versatile in causing infections of different degrees of severity. Infections may range from minor skin infections to life threatening invasive diseases and to toxin mediated disease (such as food poisoning, staphylococcal scalded skin syndrome, and toxic shock syndrome).

### 1.1.7 Treatment

The treatment of choice for *S. aureus* infection is the use of antibiotics topically or systemically and is sometimes accompanied with disinfectants.

#### 1.1.7.1 Antibiotics and their limits - the emerge of multi resistance

Over the past decades multidrug resistant (MDR) *S. aureus* strains have emerged and they are a major concern worldwide because of the diminishing therapeutic efficacy of known antimicrobial agents.

Even though the mortality rate of *S. aureus* bacteremia has been reduced, from 80% in times before the introduction of penicillin in the early 1940s to 20–40% nowadays, it remains a threat [80]. Already in 1942 penicillin-resistant strains were found first in hospitals and then in the community and in the 1960s about 80% of all *S. aureus* isolates showed a resistance to penicillin in Denmark [80, 149]. This resistance is due to β-lactamase (penicillinase), an enzyme that hydrolyzes the β-lactam ring resulting in its inactivation. High occurrence of β-lactamase (> 90%) is now common in the United States [80].

Additionally, with the introduction of the penicillinase-stable antimicrobial drug methicillin in 1961 resistance to this agent was reported shortly after. Methicillin resistant *S. aureus* (MRSA) was first detected in a British hospital and spread quickly in Europe, Australia, and the United States, while not restricting itself to hospital settings and causing community-acquired MRSA infection outbreaks [80]. Depending on the countries' infection control regulations, MRSA rates increased (USA) [1], stayed steady or dropped (Netherlands) over the years. In Africa the first MRSA cases were described in the literature only in 1988 by Peddie et al. [81].

Often resistance to various other antibiotics is associated with resistance to methicillin (so called MDR). The report of vancomycin-resistant strains in the USA in 2002 [82] has been the cause of new concern as the glycopeptide antibiotic vancomycin has been regarded as the drug of "last resort" for a long time. This resistance is developed by the acquisition of the *vanA* gene through transfer from an *Enterococcus faecalis* [80].

#### 1.1.7.2 Mupirocin for decolonization

Several studies showed that an effective way of reducing staphylococcal infections is to eliminate the nasal carriage of *S. aureus* [16]. Interestingly, this *S. aureus* eradication may only last for weeks or months before a recolonization of the anterior nares occurs [16]. For topical use a two percent mupirocin calcium ointment is available that successfully eliminates *S. aureus* skin colonization. Generally, mupirocin is used to decolonize nasal MRSA carriers and it is applied to the nose twice daily for five days. However, concerns regarding resistance exist and resistance has occurred especially when used unconfined.

### 1.1.8 Central Africa, Gabon and Lambaréné

The region of Central Africa includes Burundi the Central African Republic, Chad, the Democratic Republic of the Congo, Rwanda, Angola, Cameroon, the Central African Republic, Chad, the Republic of the Congo, the Democratic Republic of the Congo, Equatorial Guinea, São Tomé and Príncipe, and Gabon.

Gabon is located in the west of central Africa. It is equatorial and extends over an area of almost 268.000 km² [84]. Important Gabonese towns are Libreville, Franceville, Port-Gentil, Oyem, and Lambaréné, among others.

## 1.2 Objective and scope of this dissertation

*S. aureus* isolates have been analyzed in numerous studies but most of them examined *S. aureus* isolates from developed countries. In fact, the incidence of severe *S. aureus* infections causing mortality is much higher in developing countries than in developed countries [87,88]. Limited information exists of *S. aureus* from Africa, especially its carrier rate, virulence patterns, molecular diversity, and epidemiology. For obvious reasons, insight into the population structure of *S. aureus* is central to assess the pathogenicity/virulence of different strains of *S. aureus*. Hence this dissertation is an essential contribution to epidemiological surveillance and provides a much needed contribution to the

knowledge on which to base public health decisions to prevent staphylococcol related diseases in the Lambaréné area, and, more generally, in Gabon and Central Africa.

The main objective of this dissertation is to obtain a better understanding of the population structure of *S. aureus* in the study region. In order to do so, this dissertation documents the population structure of *S. aureus* isolated from the nose, the axilla and the groin of 500 study participants living in the city of Lambaréné or in villages in its surrounding rural environment (the “Lambaréné area”).The other important objective is to assess the *S. aureus* population structure in the Lambaréné area by putting it into comparison with the population structure found in other geographical regions.

## 1.3 Previous publication

The study population and analysis of this dissertation is closely related to that described in the following article: Ateba Ngoa U, Schaumburg F, Adegnika AA, Kösters K, Möller T, Gaus E, Fernandes JF, Alabi A, Issifou S, Becker K, Grobusch MP, Kremsner PG and Lell B (2012) Epidemiology and population structure of Staphylococcus aureus in various population groups from a rural and semi urban area in Gabon, Central Africa. Acta Trop 124: 42-47 [106].

For this reason some of the results included in this dissertation (results 3.1 to 3.3, part of results of 3.4.4, and most of the results in 3.5) have already been published in this article.

These results are included here in order to complement the original results of this dissertation and to thereby give a more complete overview of the characteristics of *S. aureus* in the study region.

# 2 Material and methods

## 2.1 Study design and study site

This cross-sectional, descriptive study was conducted in Lambaréné, Gabon and the rural environment of Lambaréné from February 2009 to July 2009. The study design and study site has been briefly described previously by Ateba-Ngoa et al. [106].

Gabon is covered to 80% with rain forest and has a coastline of over 800 km with the Atlantic Ocean (Gulf of Guinea) in the west. It borders on Equatorial Guinea in the northwest, Cameroon in the north and on the Republic of the Congo in the east and south. Gabon`s population is estimated at about 1.5 million with approximately 674 thousand living in Libreville, its capital and largest city situated in the northwest of the country [83].

Gabon's wealth of natural resources (oil, wood, and mineral reserves such as gold, uran, mangan, and ferum) contributes to its status as one of the wealthiest Sub-Saharan countries of Africa. Still, the majority of the population lives in poverty [84].

In Gabon, the climate is tropical with a high humidity of about 84% as a yearly average. The temperature stays high throughout the year at about 26°C with small seasonal variations. Seasonal variations in rainfall are higher with up to 490 mm per month in the rainy seasons and 7 mm per month in the dry seasons in Libreville, making for a total annual precipitation of about 2,850 mm [85].

The country is divided into nine provinces. Lambaréné is, with about 35,000 inhabitants, the capital of the province Moyen-Ogooué. It is located in the rain forest on the banks of the river Ogooué, which is ca. 1.200 km long and Gabon's largest river.

In Lambaréné in 2008 and 2009, three hospitals were responsible for the primary health care of the population and in the surrounding area basic medical care was carried out by dispensaries. One of the hospitals is the Hôpital Albert–

Schweitzer (HAS) which exists since 1913 and is a private institution, subsidized by the Gabonese state and the Albert Schweitzer Foundation. The following departments are present in HAS: surgery 1, surgery 2 (infectious cases), internal medicine, maternity ward, and pediatrics, including all together around 180 beds, as well as an outpatient clinic, an emergency ward, and a dental clinic. Most of the times, six to eight doctors are employed. In 2008, approximately 24,000 consultations took place, the emergency ward saw about 3,100 cases and around 6,000 patients were hospitalised, with the most frequent reason for admission being cited as infectious diseases (~1,500 cases) [86].

The Hôpital Régional de Lambaréné provides departements of internal medicine, surgery, gynecology, and pediatrics, as well as an emergency ward and a dental clinic.

Participants of this study were recruited in Lambaréné, its hospitals, and surrounding villages as far away as 40 km. Initial culture, species identification and antimicrobial susceptibility testing were all done in the Microbiology Laboratory of the Centre de Recherches Médicales de Lambaréné (CERMEL), Gabon. Further molecular characterization was carried out at the Institute of Medical Microbiology, University of Münster (Germany).

## 2.2 Study population

The study population comprised three major groups: one group was formed by inpatients of the Hôpital Albert–Schweitzer (HAS) and included patients from conservative wards (medical ward, pediatric ward), wards with invasive interventions (surgical ward, operation theatre) and wards with mixed specialties (maternity ward, emergency room). The second group consisted of health care workers from HAS and the Hôpital Régional de Lambaréné and included doctors, nurses, and cleaning staff. Inhabitants of Lambaréné (semi-urban area) and the surrounding villages (rural area) formed the third group and included individual from all social classes. All participants were asymptomatic

for *S. aureus*-related diseases. It is nearly the same study population as published previously by Ateba-Ngoa et al. [106].

## 2.3 Recruitment

The recruitment procedures of inpatients was done during regular morning rounds in the different wards as described previously [106]. The inpatients were chosen randomly, regardless of the length of their hospitalization with the intention to obtain similar numbers of participants from each ward. To recruit the health care workers, a list of all hospital staff was supplied by the hospital administration, so that everyone on the list could be visited by the study team during the morning rounds or on appointment. By the end of recruitment most of the hospital staff was enrolled in the study. Recruitment of the third group of inhabitants of Lambaréné and the surrounding villages was performed randomly by going from house to house in different districts and villages. As intended, we included about the same number of persons from the rural area and the semi-urban area. All persons present at the time of the visits were invited to participate in the study. Exclusion criteria were obvious *S. aureus*–related diseases and non-acceptance of the informed consent form. Recruitment of the study population was stopped after an enrollment of 500 participants.

## 2.4 Bacterial isolates – phenotypic characterization

The phenotypic characterisation was done as previously described [106]. Bacterial isolates were obtained from several body sites, namely the anterior nares, the axilla, and the groin. These body sites were swabbed with sterile cotton swabs. The swabs were then stored in Amies medium (BBL CultureSwab, Becton Dickinson, Belgium) and cultured on 5% sheep blood agar plates (Columbia Agar, BioMérieux) at 36±2°C for 48 hours.*S. aureus* identification was based on colony morphology, Gram-straining, colony growth on colistin and naladixic acid Agar (BioMérieux), catalase testing, rabbit plasma coagulase test (BD, Belgium), and Staphaurex Plus latex agglutination test (Remel, Germany). Species antibiotic susceptibility testing was performed by

the standardized agar disc diffusion method according to National Clinical and Laboratory Standard Institute (NCCLS) [89] using Müller-Hinton agar and the following panel of antibiotic discs: penicillin, cefoxitin, erythromycin, clindamycin, cotrimoxazole, and chloramphenicol.

Cefoxitin was used to screen for resistance to methicillin. Additionally, a penicillin binding protein latex test (PBP2a latex test, Oxoid, Germany) was performed on all cefoxitin resistant isolate to confirm methicillin resistance.

Subsequently, the samples were stored at -80 °C in Cryotubes until shipment to the Institute of Medical Microbiology, University of Münster (Germany) for molecular characterization.

Strains resistant to methicillin were further tested for a susceptibility to antibiotics including rifampicin, mupirocin, linezolid, gentamicin, levofloxacine, tobramycin, vancomycin, teicoplanin, fosfomycin, fusidic acid, tigecyclin, and nitrofurantoin, using a Vitek 2 automated system (BioMérieux, Marc l'Etoile, France).

## 2.5 Molecular characterization

Molecular characterization was performed as described previously [106]. Briefly, confirmation of *S. aureus* species was performed by detection of the thermonuclease (*nuc*) genes of *S. aureus* as described by Brakstad et al. [90].

The identification of one *nuc*-negative isolate was based on 16S RNA gene sequencing and a subsequent query of the sequences in GenBank BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [91].

Determination of methicillin-resistance was performed by the detecting the presence of *mecA* gene [92].

### 2.5.1 SCC*mec* type

SCC*mec* type was determined via a multiplex PCR approach [93].

### 2.5.2 *Agr* subtype

To determine the *agr* type (*agr* I – IV) of our *S. aureus* isolates a multiplex PCR was conducted as described previously by Lina et al. [94] with a slightly modified pan-*agr*-primer (agr1-4Sa-1) as published one year later by von Eiff et al. [95] (5_-ATGCACATGGTGCWCATGC-3_).

### 2.5.3 Virulence factors

The presence of *lukF-PV* and *lukS-PV* encoding the PVL toxin was performed by polymerase chain reaction, as described by Lina et al. [96].

In addition, genes for virulence factors were detected as described previously [95, 97, 98, 99]. These genes include the genes for the toxic shock syndrome toxin *(tst),* for enterotoxins *(sea, seb, sec, sed, see, seg, seh, sei, sej),* exfoliative toxins *(eta, etb, etd)*, for epidermal cell differentiation inhibitor *(edin-A, edin-B, edin-C),* gamma-hemolysin *(hlg)* and capsular polysaccharide type 5 and 8.

## 2.6 Genotyping

Genotyping procedure were described previously [106].

### 2.6.1 *Spa* typing und Cluster formation (BURP algorithm)

*Spa* typing was performed for all isolates as published [79] using the Ridom StaphType software (Ridom GmbH, Germany). The oligonucleotides used for amplification correspond to the 5' end (1113F, 5'-TGTAAAACGACGGCCAGTTAAAGACGATCCTTCGGTGAGC) and the 3' end (1514R, 5'-CAGGAAACAGCTATGACCCAGCAGTAGTGCCGTTTGCTT). PCR conditions were as follows: initial denaturation (95°C for 1 min), amplification (35 cycles of 95°C for 60 s, 60°C for 60 s and 72°C for 60 s) and final extension

(72°C for 5 min). DNA sequences were obtained with an ABI Prism Sequencer (Applied Biosystems) and analyzed with the StaphType software (Ridom GmbH, Germany).

BURP (Based Upon Repeat Pattern) – a grouping algorithm implemented by the StaphType software – was used to cluster (spa-CC) related *spa* types as described by Mellmann et al. [76]. The two user-defined parameters (*x* and *y*) as default parameters were preset as recommended: exclusion of *spa* types that are shorter than five repeats (*x* = *5*) and the maximum of four costs (*y* = *4*) for clustering *spa* types into the same group [76].

### 2.6.2 Multilocus sequence typing

For one isolate of each *spa* type MLST sequence types (ST) were determined as described by Enright et al. [100]. MLST clonal complexes were assigned to each ST running eBURST on the whole MLST database hosted by Imperial College London with a stringent group definition of at least 6/7 shared alleles (http://www.mlst.net) [77].

The seven genes included in the *S*. *aureus* MLST scheme are named *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL.*

**Table 1:** Sequences of primers used in the PCR (from [100])

| Gene | Primer | Sequence (5´ - 3´ ) |
|---|---|---|
| Carbamate kinase (*arcC*) | *arcC* -up | TTGATTCACCAGCGCGTATTGTC |
| | *arcC* -down | AGGTATCTGCTTCAATCAGCG |
| Shikimate dehydrogenase (*aroE*) | *aroE* -up | ATCGGAAATCCTATTTCACATTC |
| | *aroE* -down | GGTGTTGTATTAATAACGATATC |
| Glycerol kinase (*glpF*) | *glpF* -up | CTAGGAACTGCAATCTTAATCC |
| | *glpF* -down | TGGTAAAATCGCATGTCCAATTC |
| Guanylate kinase (*gmk* ) | *gmk* -up | ATCGTTTTATCGGGACCATC |
| | *gmk* -down | TCATTAACTACAACGTAATCGTA |
| Phosphate acetyltransferase (*pta*) | *pta* -up | GTTAAAATCGTATTACCTGAAGG |
| | *pta* -down | GACCCTTTTGTTGAAAAGCTTAA |
| Triosephosphate isomerase (*tpi*) | *tpi* -up | TCGTTCATTCTGAACGTCGTGAA |
| | *tpi* -down | TTTGCACCTTCTAACAATTGTAC |
| Acetyl coencyme A acetyl-transferase (*yqiL*) | *yqiL* -up | CAGCATACAGGACACCTATTGGC |
| | *yqiL* -down | CGTTGAGGAATCGATACTGGAAC |

We used an alternative primer pair for the amplification of *aroE* of the isolate with *spa* type t6331 (ST1822) as proposed by others [101]:

*aroE745-up, TTATCACCGTCGATGCATAGTGCA;*

*aroE255-down, CGGAGTAGTATTTATCACAATATC.*

## 2.7 Treatment and control

Participants who were colonized with MRSA were provided with Bactroban ointment, a Mupirocin containing ointment. Successful eradication of MRSA was controlled by repeated swabs and cultures after ten days of treatment.

## 2.8 Statistics

Statistical analysis was conducted by SPSS 16.0 and “R” software as well as by using the Windows Excel (XP-version) computer program. Pearson’s chi-square test was used to compare proportions. The significance level was set at p below 0.05. Odds ratios for potential risk factors of carriage were calculated by logistic regression.

## 2.9 Ethics

Ethical approval was obtained by the local ethics committee of Lambaréné (Comité d'Ethique Régional Indépendent de Lambaréné, CERIL). Before recruitment, and after clarification that participation was voluntary, all participants of the study signed a written informed consent form.

The Deutsche Forschungsgemeinschaft (DFG, El 247/8-1) supported this study.

# 3 Results

## 3.1 Study population

The study population consisted of 500 participants divided into three major groups: inpatients (*n* = 198), health care workers (*n* = 97), and the general public (*n* = 205). In total 283 (57%) females and 217 (43%) males participated. At the time of enrollment, the youngest participant was one month old and the oldest had a verified age of 87 years. The average age was 32 years. More detailed comparative characteristics of the study population are shown in Table 2. These results have been published previously [106].

**Table 2:** Characteristics of the study population groups (from [106])

| | | *n* | Female<br>*n* (%) | Age in years<br>mean (range) |
|---|---|---|---|---|
| **Inpatients** | Conservative wards | 87 | 38 (44%) | 27 (1-100) |
| | Mixed specialities | 45 | 45 (100%) | 24 (16-39) |
| | Invasive wards | 66 | 29 (44%) | 40 (1-100) |
| **Health care workers** | Doctors | 11 | 4 (36%) | 50 (36-59) |
| | Nurses | 75 | 52 (69%) | 38 (19-57) |
| | Cleaning staff | 11 | 11 (100%) | 41 (27-49) |
| **General public** | Rural | 102 | 57 (56%) | 29 (1-81) |
| | Semi urban | 103 | 47 (46%) | 29 (1-90) |

## 3.2 *S. aureus* carriage

From all 500 participants we found 146 to be carriers of *S. aureus*. The carriage rate differed only slightly between the three major groups, giving a total carriage rate of 29%. 53 (27%) inpatients, 60 (29%) of the general public, and 33 (34%) health care workers were carriers [p>0.05]).

For the general public there was a significant association between *S. aureus* carriage and living in the rural areas around Lambaréné. Subjects from rural areas had twice the risk of *S. aureus* carriage when compared to those from the semi-urban area of Lambaréné (OR = 2.2; 95% CI = 1.2–4.1; p = 0.01). For inpatients a significant increase of *S. aureus* carriage was found if they were staying in invasive or mixed wards. Table 3 displays more details of *S. aureus'* prevalence and risk factors for carriage. These results have been published previously [106].

**Table 3:** Risk factors for *S. aureus* carriage (from [106])

| | | Carriers *n* (%) | Odds Ratio (95% CI) | p-value |
|---|---|---|---|---|
| **Gender** | Male | 65 (30%) | Ref | - |
| | Female | 81 (29%) | 0.9 (0.6 - 1.4) | 0.8 |
| **Age in years** | <21 | 44 (28%) | Ref | - |
| | 21-39 | 54 (32%) | 1.2 (0.7 - 1.9) | 0.4 |
| | >39 | 48 (28%) | 0.97 (0.5 - 1.5) | 0.9 |
| **Study group** | Population | 60 (29%) | Ref | - |
| | Inpatients | 53 (27%) | 0.9 (0.6 - 1.4) | 0.4 |
| | Health care workers | 33 (34%) | 0.4 (0.7 - 2.1) | 0.6 |

| | | Carriers *n* (%) | Odds Ratio (95% CI) | p-value |
|---|---|---|---|---|
| **General puplic** | Semi urban area | 22 (21%) | Ref | - |
| | Rural area | 38 (37%) | 2.2 (1.2 - 4.1) | 0.01 |
| **Health care workers** | Doctors | 6 (56%) | Ref | - |
| | Nurses | 23 (31%) | 0.5 (0.2 - 1.4) | 0.2 |
| | Cleaning staff | 4 (36%) | 1.1 (0.3 - 4.2) | 0.8 |
| **Inpatients** | Conservative wards | 16 (18%) | Ref | - |
| | Mixed specialities | 15 (33%) | 2.4 (1.1 - 5.5) | 0.03 |
| | Invasive wards | 22 (33%) | 2.2 (1.1 - 4.6) | 0.03 |

## 3.3 Antimicrobial resistance

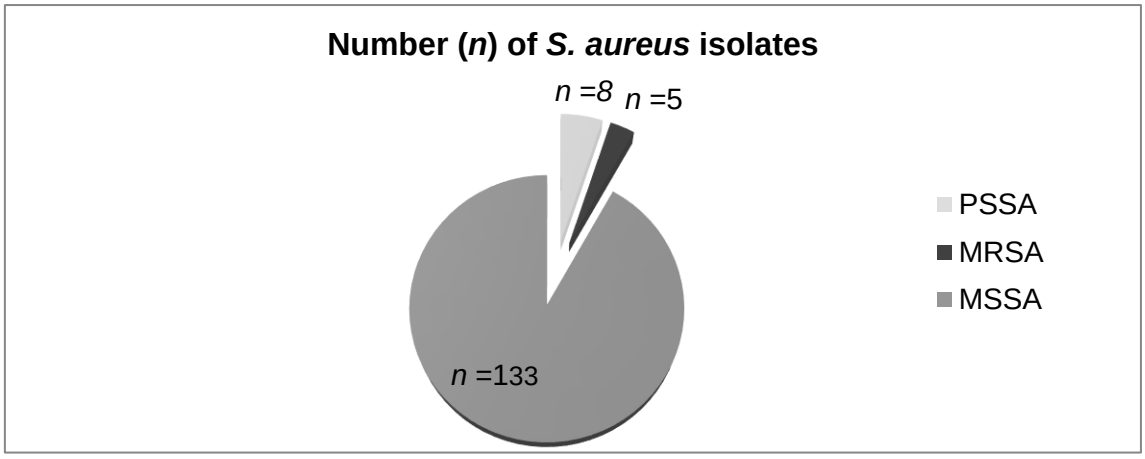


**Figure 2:** Antimicrobial resistance (adapted from [106])

Among a total of 146 *S. aureus* isolates, 133 isolates were methicillin-susceptible and resistant to penicillin (MSSA). All these strains were susceptible to cefoxitin or clindamycin, two strains were resistant to cotrimoxazol or

erythromycin (1.4%), respectively and one was resistant to chloramphenicol (0.7%).

Among the 146 *S. aureus* isolates 5% (*n* = 8) were detected to be susceptible to penicillin (PSSA) and 3% (*n* = 5) were methicillin resistant and positive for *mec*A (MRSA).

All the methicillin resistant isolates (MRSA; *n* = 5) were susceptible to rifampicin, vancomycin, teicoplanin, gentamicine, mupirocine, linezolid, fosfomycin and tigecycline. Three of the MRSA isolates were susceptible to cotrimoxazol and one MRSA isolate was intermediately susceptible to fusidic acid.

These results have been published previously [106].

## 3.4 Virulence factors

### 3.4.1 PVL and gamma-hemolysin

All 146 isolates were positive for either *pvl* or *hlg* genes. 143 isolates (98%) were positive for *hlg* and 61 isolates (42%) were positive for *pvl*. To go into further detail, 58 isolates (40%) were positive for both genes, 85 isolates (58%) were positive for *hlg* only and 3 isolates (2%) for *pvl* only.

### 3.4.2 Pyrogenic toxins

Overall, 107 (73.0%) isolates were positive for at least one of the pyrogenic toxin superantigens (PTSAgs) tested. These comprise toxic shock syndrome toxin 1 (*tst* (9%)) and the staphylococcal enterotoxins (SEs: *sea* (35%), *seb* (10%), *sec* (13%), *sed* (1%), *see* (0%), *seg* (34%), *seh* (6%), *sei* (34%), *sej* (3%)).

The most frequently found gene was the enterotoxin gene *sea* (*n* = 51; 35%), followed by *seg* and *sei* (*n* = 49; 34%). *Seg* and *sei* were always found in combination. Other PTSAgs were detected less frequently and gene *see* was not detected at all (Figure 3).

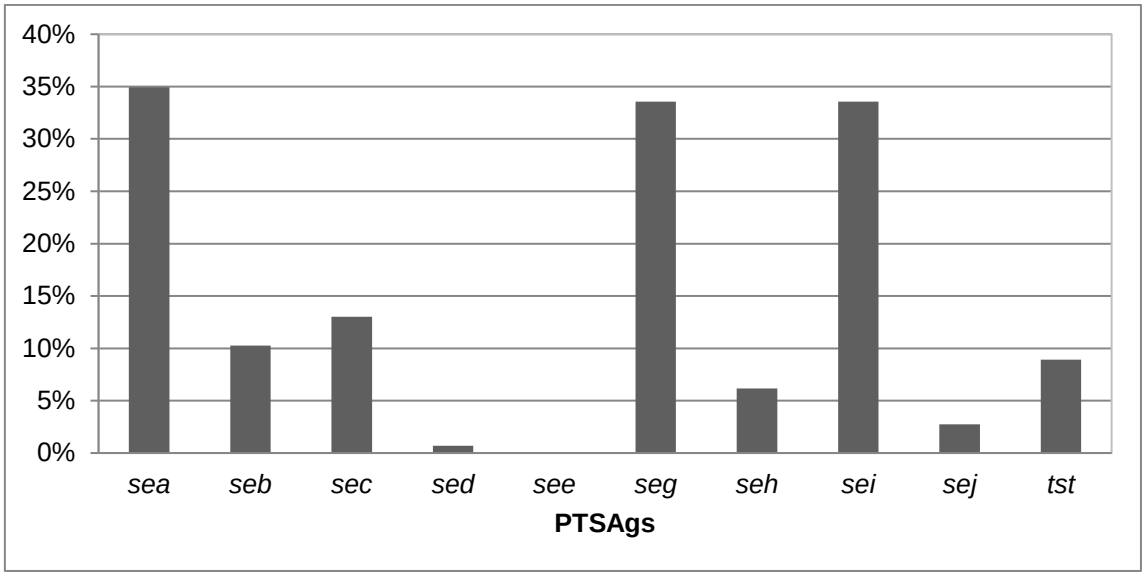


**Figure 3:** Distribution of pyrogenic toxin superantigens (PTSAgs) in %

Combinations of different PTSAg genes were observed. The fixed combinations of *seg* plus *sei* was the most frequent combination found (34%; *n* = 49). In total, 57 isolates (39%), possessed more than a single PTSAg gene - representing 53% of all PTSAg gene-positive isolates, whereas 50 (34%) possessed only one PTSAg gene (Figure 4). *Sea* was the gene that was found most commonly sole (*n* = 39).

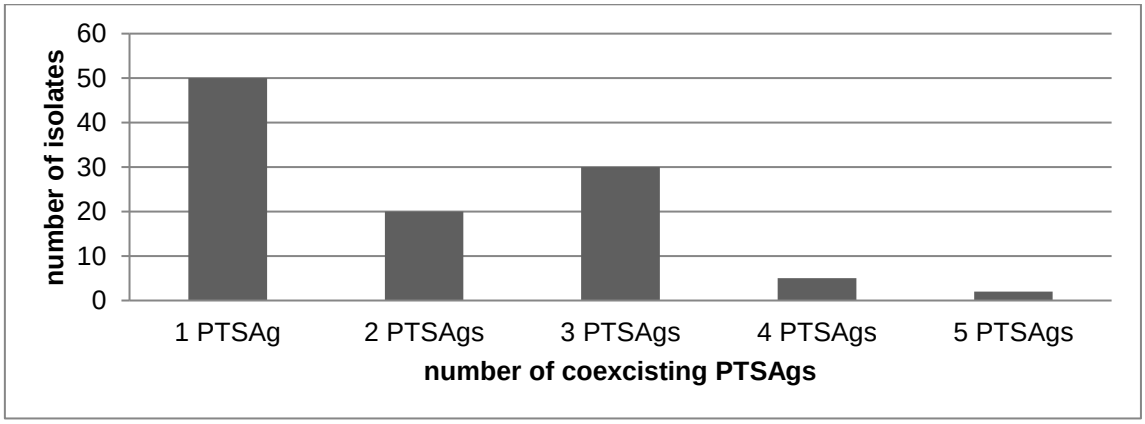


**Figure 4:** Multiplicity of pyrogenic toxin superantigens (PTSAgs)

The combination of *sed* plus *sej* was found once (0.7%) in an isolate together with the additional PTSAg genes *seb, seg* and *sei* (ST5, t002).

### 3.4.3 Exfoliative toxins, epidermal cell differentiation inhibitor and capsular polysaccharide types 5 and 8

Ten of the *S. aureus* isolates (7%) harbored a gene encoding an exfoliative toxin. In Figure 5 the distribution can be seen. One isolate possessed *eta* and *etb* (ST 15, t326).

Seven isolates possessed *edin-B* (5%) and one different isolate possessed *edin-C* (0.7 %) (ST 15, t326). *Edin-A* was not detected.

Genes encoding for capsular polysaccharide type 8 (*cap8*) were detected in 73% (*n* = 107) of the *S. aureus* isolates, whereas in only 25 % (*n* = 37) genes encoding for capsular polysaccharide type 5 (*cap5*) were detected. 2% (*n* = 2) did not harbor *cap5* or *cap8*.

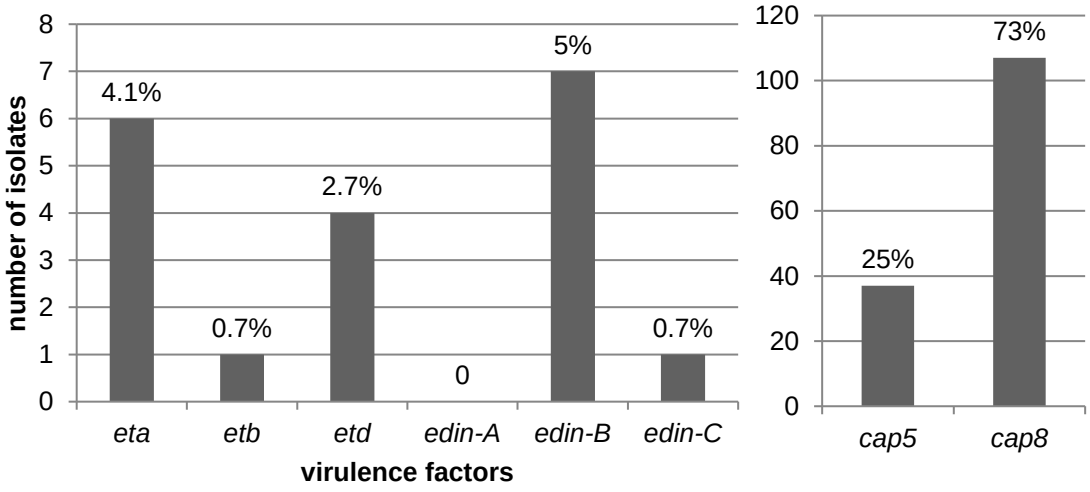


**Figure 5:** Distribution of exfoliative toxin antigens (*eta, etb,etd),* of the genes encoding for members of the epidermal cell differentiation inhibitor (*edin-A, edin-B, edin-C)* and for capsular polysaccharide (*cap5, cap8)*

### 3.4.4 *nuc* gene, *agr* subtype

One isolate showed no *nuc* gene expression (ST1822, t6331): its subsequent 16S RNA gene sequencing confirmed clearly its specie's affiliation to *S. aureus* [106].

Accessory gen regulator subtypes were distributed as follows among our *S. aureus* isolates: *agr* II was the most frequent subtype (*n* = 78; 53%) followed by *agr* I (*n* = 42; 29%), *agr* III (*n*= 16; 11%), and *agr* IV (*n* = 4; 3%), while two isolates did not harbor the *agr* subtypes I to IV (ST45, t939). Four isolates harbored two *agr* subtypes: *agr* I and *agr* II was co-detected in ST15 and ST188 and *agr* II and *agr* III in ST1 and ST15 (Figure 6). These results have been published previously [106].

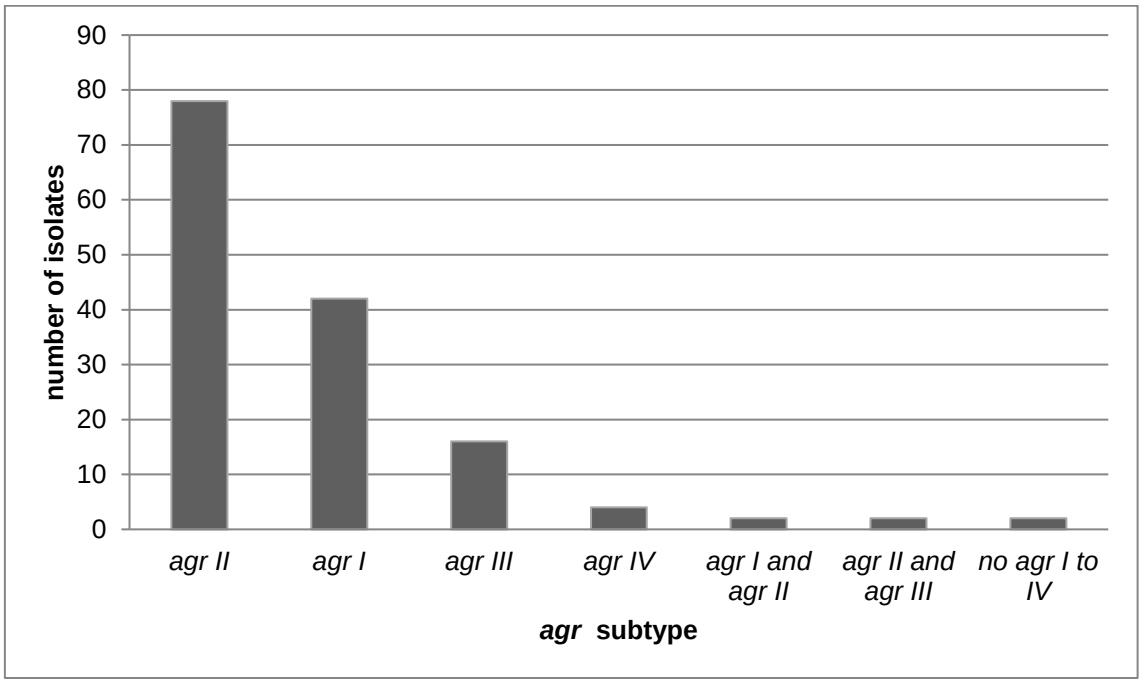


**Figure 6:** *agr* subtype distribution (from [106])

## 3.5 Genotyping

### 3.5.1 *spa* types

Among a total of 146 *S. aureus* isolates, 53 different *spa* types were found. Most of the isolates belonged to *spa* type t084 (*n* = 50, 34%) followed by those belonging to t355 (*n* = 9, 6%), t148 (*n* = 7, 5%), t279 (*n* = 6, 4%), t1510 (*n* = 6, 4%), t127 (*n* = 5, 3%), t1045 (*n* = 5, 3%), t311 (*n* = 3, 2%), and t2723 (*n* = 3, 2%).

Using the BURP algorithm *spa* clusters were formed and grouped into *spa*-clonal complexes [106] resulting in *spa*-CC084 (*n* = 66), *spa*-CC304 (*n* = 7), spa-CC1045 (*n* = 7),and *spa*-CC1510 (*n* = 10). Thirteen *spa* types were singletons (*n* = 27), seven clusters had no founder (*n* = 27) and two *spa* types were excluded in the *spa*-clonal complexe grouping (*n* = 2) (t1113, t2980).

These results have been published previously [106].

Sequence types (STs)

Using MLST, 21 different STs were revealed among our 146 *S. aureus* isolates. ST15 (*n* = 69; 47%) was the most prevalent followed by ST508 (*n* = 12; 8%) and ST152 (*n* = 9; 6%). Three STs were described for the first time in this population designated ST1745, ST1746, and ST1822. ST1745 and ST1746 were recovered from health care workers and ST1822 was recovered from a member of the general public.

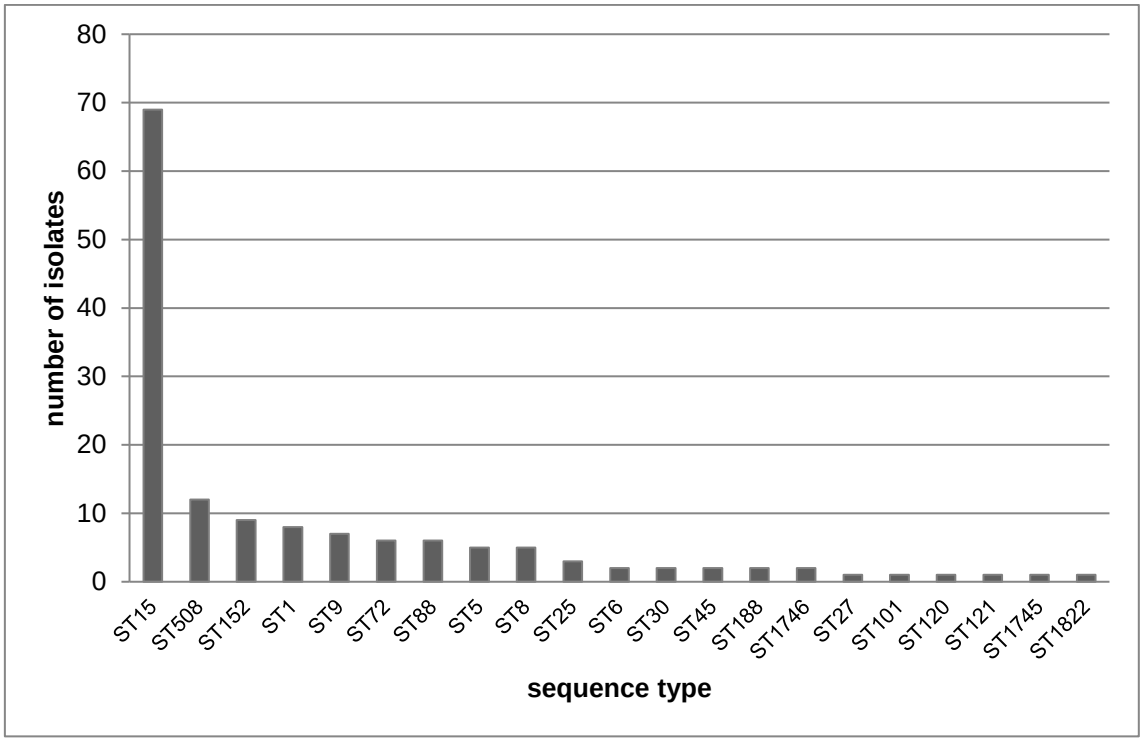


**Figure 7:** Number of *S. aureus* isolates per sequence type (ST) (from [106])

Ten clonal complexes (CC) assigned by MLST could be found among the 146 *S. aureus* isolates (Table 4). CC15 was the most frequent clonal complex, being detected 79 times. The other frequencies are shown in Table 4 as well as other characteristics of the complexes and their respective isolates.

**Table 4:** Distribution of multilocus sequence typing clonal complexes (CC), sequence types (ST), *spa* types, *agr* subtypes, SCC*mec* types, capsular polysaccharide types (CP), Panton-Valentine leukocidin (PVL) encoding genes and other antigens in all the 146 *S. aureus* isolates. *n* = number, nt = non typable(adapted from [106])

| CC (*n*; %) | ST | *spa* types | *agr* subtype (*n*) | SCC*mec* types | CP (*n*) | PVL (*n*) | other virulence factors (*n*) |
|---|---|---|---|---|---|---|---|
| CC15 (79; 54) | ST15, ST1, ST188 | t084, t085, t094, t127,t189, t254, t279, t326, t491, t590, t774, t1711, t1877, t1931, t2636, t6240, t6318 | I (2), II (66), III (7), II+III (2), I+II (2) | 0 | 8 (78), nt (1) | 45 | *sea* (48), *seb* (4), *sec* (3), *seg* (6), *seh* (8), *sei* (6), *tst* (5), *eta* (5), *etb* (1), *etd* (1), *hlg* (79), *edin-C* (1) |
| CC5 (15; 10) | ST5 | t653 | II (1) | IV | 5 (1) | 0 | *seg (1), sei (1), sej (1), hlg (1)* |
| | ST5, ST6, ST9, ST27 | t002, t099, t304, t311, t1045, t2980, t4492 | I (2), II (12), III (1) | 0 | 5 (11), 8 (3) | 1 | *sea* (3), *seb* (4), *sec* (2), *sed* (1), *seg* (11), *seh* (1), *sei* (11), *sej* (1), *tst* (1), *hlg* (15) |
| CC45 (15; 10) | ST45, ST508, ST1745 | t939, t1113, t1510, t2784, t4576, t5575, 6241, t6242, t6243 | I (13), nt (2) | 0 | 5 (2), 8 (13) | 0 | *seb* (1), *sec* (5), *seg* (15), *sei* (15), *tst* (5), *hlg* (15) |
| CC8 (11; 7) | ST8 | t121 | I (1) | IV | 5 (1) | 1 | *hlg (1)* |
| | ST8, ST72 | t008, t148, t197, t1476 | I (10) | 0 | 5 (10) | 0 | *sec* (6), *seg* (6), *sei* (6), *sej* (2), *tst* (1), *eta* (1), *hlg* (10) |
| CC152 (9; 6) | ST152 | t355 | I (9) | 0 | 5 (9) | 9 | *hlg* (6), *edin-B* (4) |
| CC25 (3; 2) | ST25 | t148, t3772, t4680 | I (3) | 0 | 5 (3) | 0 | *sec* (2), *seg* (3), *sei* (3), *etd* (3), *hlg* (3), *edin-B* (3) |
| CC30 (2; 1) | ST30 | t017, t253 | III (2) | 0 | 8 (2) | 0 | *sec* (2), *sei* (2), *tst* (1), *hlg* (2) |
| CC88 (6; 4) | ST88 | t186, t729 | III (3) | nt | 8 (3) | 0 | *sec* (1), *hlg* (3) |
| | ST88 | t2723 | III (3) | 0 | 8 (3) | 1 | *seb* (1), *hlg* (3) |
| CC101 (1; 0.7) | ST101 | t056 | I (1) | 0 | 8 (1) | 0 | *hlg* (1) |
| CC121 (4; 3) | ST120, ST121, ST1746 | t159, t314, t645 | IV (4) | 0 | 8 (4) | 4 | *seb* (3), *seg* (4), *sei* (4), *hlg* (4) |
| Singleton (1; 0.7) | ST1822 | t6331 | I (1) | 0 | nt (1) | 0 | *seg* (1), *sei* (1), *hlg* (1) |

MRSA isolates

A detailed comparative characterization of the MRSA isolates and their genotyping results are shown in Table 5.

SCC*mec* type:

Out of the five *mec*A positive isolates, two (40%) harbored the SCC*mec* type IV (ST5, t653 and ST8, t121). The others were non SCC*mec* I to IV (here referred to as “not typeable”).These results have been published previously [106].

**Table 5:** Clinical characteristics and genotypes of the 5 MRSA isolates (adapted from [106])
ST = sequence type, PVL =Panton-Valentine leukocidin

| ST | *spa*-CC | *spa* type | SCC *mec* type | PVL | *agr* type | Additional virulence factors | Study group | Gender | Age in years |
|---|---|---|---|---|---|---|---|---|---|
| **ST88** | cluster5, no founder | t186 | nt | negative | III | *hlg*, *cap8* | inpatient | male | 14 |
| **ST88** | cluster5, no founder | t186 | nt | negative | III | *sec, hlg, cap8* | health care worker | female | 38 |
| **ST88** | cluster5, no founder | t729 | nt | negative | III | *hlg*, *cap8* | general puplic | female | 11 |
| **ST8** | 304 | t121 | IV | positive | I | *hlg*, *cap5* | inpatient | male | 31 |
| **ST5** | singleton | t653 | IV | negative | II | *seg, sei, sej, hlg, cap5* | health care worker | male | 49 |

### 3.5.2 Virulence factors in MRSA isolates

#### 3.5.2.1 PVL and gamma-hemolysin

One of the five *mec*A positive isolates (20%) possessed the PVL-encoding genes *lukF-PV* and *lukS-PV* (ST8, t121) [106]. All 5 isolates were positive for the *hlg* gene.

#### 3.5.2.2 Pyrogenic toxins

Two of the 5 isolates (40%) were positive for at least one of the pyrogenic toxin superantigens (PTSAgs) tested. One isolate was positive for *sec* (ST88, t186) and one positive for *seg, sei* and *sej* (ST5, t653). No isolate was positive for *sea, seb, sed, see, she* and *tst*.

#### 3.5.2.3 Exfoliative toxins and epidermal cell differentiation inhibitor

No MRSA isolate harbored a gene encoding an exfoliative toxin and no MRSA isolate harbored *edin-B*, e*din-A* and *edin-C*.

#### 3.5.2.4 Capsular polysaccharide types 5 and 8

Genes encoding for capsular polysaccharide type 8 were detected in three of the MRSA isolates (60%) whereas in two MRSA isolates (40%) genes encoding for capsular polysaccharide type 5 were detected [106].

#### 3.5.2.5 *agr* subtype

*Agr* III subtype was detected in three isolates (60%) and both *agr* I only and *agr* II only in one isolate (20%), respectively [106].

### 3.5.3 Genotying of MRSA isolates: *spa* types and sequence types

The five MRSA had four different *spa* types. Two isolates belonged to the spa type t186 and one isolate to t121, t653 and t729 respectively. They clustered into different *spa*-CCs (*spa*-CC 5, *spa*-CC 304 and one isolate is a singleton). The MRSA isolates belonged to different lineages (ST5 (*n* = 1), ST8 (*n* = 1), and ST88 (*n* = 3)). These results have been published previously [106]..

# 4 Discussion

*S. aureus* infections and MRSA have become major public health problems worldwide. To obtain a better understanding of the population structure of *S. aureus,* we document the population structure of 146 *S. aureus* isolates from asymptomatic carriers in Gabon.

Among our study population a *S. aureus* carriage rate of about 29% was observed [106]. This prevalence is comparable to carriage rates reported in numerous studies worldwide ranging from 12% to 30% for persistent carriers of *S. aureus* [4].

Among our study population a MRSA carriage rate of 1% was observed [106]. This prevalence is as well comparable to carriage rates reported in numerous studies worldwide ranging from less than 1% to 24% for MRSA carriage [102, 103]. Notably, prevalences of MRSA carriage in Africa are reported to be higher ranging from 1.4% to 50% [104].

This study was not designed to reveal any possible difference between*S. aureus* carriage patterns in Gabon and those in other countries. In consequence, the sample taken for this study only allowed for inclusion of participants, depending on their momentary positive or negative sample results into either the group of carriers or noncarriers. As a result, intermittent carriers may be included in either one of the groups.

The discussion will focus on the population structure of *S. aureus* isolates. Carriage rate and antimicrobial resistance of nearly the same study population is discussed in more detail in another doctorial thesis and article [105, 106].

## 4.1 Virulence factors

### 4.1.1 PVL and gamma-hemolysin

In this study we a found high rate of PVL-positive (42%) *S. aureus* isolates. Similar or even higher rates for PVL-positive isolates have been found in other studies conducted in Gabon [150, 151] and other African countries such as Nigeria, Cameroon, Madagascar, Morocco, Niger, and Senegal [162, 107]. Comparably, Schaumburg et al. [150] showed a rate of 41% in isolates from persons asymptomatic for *S. aureus* related disease in Gabon. In their study [150] they could even show a significant association of PVL and *S. aureus* related infections by detecting a rate for PVL of 57% in this special group.

In the United States, PVL-positive *S. aureus* used to be rare, but as was demonstrated by a study of 1,055 *S. aureus* infection isolates [108], its frequency of occurrence has increased nearly threefold during the years 2004 through 2006 and was found at a rate of 40% in 2008.To go into further detail, in 2008, 12% of the MSSA strains and as much as 54% of the MRSA strains produced PVL, whereat 84% all PVL-producing isolates belonged to the so called “USA300” clone [108].

Interestingly, the prevalence of PVL in Europe is only 2% in MSSA in several studies [109, 95], whereas Monecke et al. described 30% PVL positive MSSA isolates in a study conducted in a German hospital [110]. In his study Monecke et al.analyzed isolates from diverse cases of skin and soft tissue infections.Contrarily, in our present study conducted in Gabon, only isolates from persons asymptomatic for *S. aureus* related disease were included.As PVL-producing *S. aureus* isolates have been linked in epidemiological studies to be more pathogenic, causing deep abscesses, severe necrotizing pneumonia, and severe bone and joint infections [51, 55] Monecke et al.´s result for the prevalence of PVL cannot be used for comparison with our study.

PVL is usually associated with community-acquired *S. aureus* [111, 112] and with skin and soft tissue infection [152, 51]. As the PVL genes are carried on mobile genetic elements (prophages), genes may be transferred from one strain to another via phage transduction. PVL genes may thus be transmitted both

vertically within the same clones or horizontally among different clones either before or after acquisition of the *mecA* gene [113].

The clinical relevance of PVL in human infections is controversial. However, a large meta-analysis and pathophysiological studies provide evidence of its clinical relevance [152, 153, 154].

Almost all isolates were positive for gamma-hemolysin (98%). This is consistent with other studies [95].

### 4.1.2 Pyrogenic toxins

Pyrogenic exotoxin genes are common in *S. aureus*, and are divergingly distributed among the different clonal types. As it is described elsewhere [97, 114] up to 73% of *S. aureus* isolates carry at least one of these genes.

Most of the pyrogenic exotoxin genes were observed less frequently in this study than in studies involving carrier isolates conducted in Europe [97, 155] and the United States [114]: *tst* (9% vs.22 - 78%), *sec* (13% vs. 14 - 27%), *sed* (1% vs. 3 - 23%), *seg* (34% vs. 57 - 90%), *seh* (6% vs. 6 - 15%), *sei* (34% vs. 57 - 89%) and *sej* (3% vs. 3 - 9%).

The prevalence of *sea, seb* and *see* were in the range of the prevalence described in these studies: *sea* (35% vs 14 - 58%), *seb* (10% vs 8 - 26%), *see* (0% vs 0 – 0.5%). Similar to the result of Becker et al. [97], in the present study we found that the possession of more than a single PTSAg gene was more frequent than the possession of only a single gene. In concurrence with Becker et al. [97], the combination that was found most often was the fixed combinations of *seg* plus *sei.* According to Zhang et al. [115], the enterotoxins *sed* and *sej* are located on the same plasmid linked by an intergenic region. However, in this study there were three *S. aureus* isolates that showed only *sej* genes. Morandi et al. [116] described a disconnection of *sed* plus *sej*. Unlike in our study, they found three isolates with *sed* genes only.

### 4.1.3 Exfoliative toxins and epidermal cell differentiation inhibitor

The prevalence of genes encoding for exfoliative toxins was comparable to the prevalence of these genes found in Europe: *eta* (4% vs. 2 – 4%) [97,117], *etb* (0.7% vs. 1%) [97] and *etd* (3% vs. 5%) [118].

It is known that, the gene encoding *edin-B* is located on the chromosome within a pathogenicity island and genes encoding *edin-A* and *edin-C* are carried on plasmids [119]. The prevalence of these genes in this study was also comparable to the prevalence of these genes found in Europe: *edin-A* (0% vs. 0%) [120], *edin-B* (5% vs.4 - 6%) [118, 120] and *edin-C* (0,7% vs. 0%) [120]. It should be noted, that the prevalence of *edin*-encoding genes is higher in clinical *S. aureus* isolates compared to that in *S. aureus* isolates from healthy carriers [120, 119]. In most studies on carrier or clinical isolates the predominant isoform found is *edin-B.* Interestingly, Munro et al. [119] reported a high prevalence of *edin-C* encoding toxin in clinical *S. aureus* isolates in France: 90% of all *edin*-carrying *S. aureus* isolates carried the *edin-C* encoding toxin. They concluded that *edin-C*-positive isolates were more frequently associated with deep-seated soft tissue infections than other types of infections. Nevertheless, the association of certain *S. aureus* infections with edin production is still to be explored more extensively.

### 4.1.4 Capsular polysaccharide types 5 and 8

International epidemiological studies have shown that isolates of *S. aureus* are usually capsulated with either type 5 (~25%) or type 8 (~50%) capsular polysaccharides (CPs) [121] deriving from both carrier isolates and clinical isolates. Similarly, in this study serotype 5 and 8 account for 25% and 73% respectively.

CP 5 and CP 8 have been reported to enhance virulence in a number of animal models of staphylococcal infection [122,123]. More precisely, Watts et al. [123] showed in a mouse model, that serotype 5 is more virulent than serotype 8.

Some authors see an option to use the major capsular polysaccharides type 5, type 8, and type 336 [124] as a target antigen for a vaccine to prevent *S. aureus* infections [121,125].

### 4.1.5 *agr* subtype

Certain *agr* subtypes are distributed according to international epidemiological studies in a manner broadly consistent with our study results: *agr* I (29% vs. 30 – 44%) and *agr* IV (3% vs. 0 - 6%) [117, 95, 126, 99]. However, *agr* II occurred more frequently (53% vs. 24 - 30%), whereas *agr* III occurred less frequently (11% vs. 25 - 34%) [117, 95, 126, 99].

Jarraud et al. [127] reported that the majority of exfoliative toxins-producing strains belong to *agr* IV. In contrast, all our exfoliative toxins-producing strains belonged to subtype I and II.

In this study four isolates harbored two *agr*-subtypes. This is uncommon and could possibly be explained by a nonspecific primer binding in the multiplex PCR used here [106].

As Jarraud et al. concluded "... though we cannot attribute a direct responsibility of the *agr* type in disease initiation, we can speculate that the preferential association between certain *agr* alleles, certain toxin genes, and a particular genetic background may make the activation of virulence factors more efficient …" [60].

## 4.2 Genotyping

In this study, the major human lineages were identified, namely clonal complexes CC5, CC8, CC9, CC15, CC25, CC30 and CC45 [132].

The 21 STs of the*S. aureus* carriers were distributed unevenly. ST15 (mainly *spa* type t084) was found to be predominant (47%). Similarly, ST15 was the predominant sequence type in Malian carrier isolates (27%) [134], was found in other African countries (Algeria, Nigeria, South Africa, Cape Verde

Islands(11%) [107] or Ghana (18%) [156]) and it is commonly recorded as a major MSSA clone in countries in Asia (9%) [157], America and Europe (18%) [158].

The other 20 STs we found in Gabon were distributed more evenly, with the most frequent ST accounting for 9%. Most of them have been recognized as internationally well-disseminated clones including ST1, ST5, ST8, ST25, ST30, ST45, ST120 and ST121 [128, 132].

The prevalence of ST508 in other African countries was lower when compared to this study. Among carriers a prevalence of 1% was found in Mali [134], among clinical isolates a prevalence of 2.6% was found in Nigeria [104]. ST508 associated with MRSA is described by Boyle-Vavra et al. [135]. In our study all *S. aureus* isolates of ST508 were positive of the fixed combination of *seg* plus *sei.* Three of the twelve ST508 isolats were additionally positive for *sec* and *tst*.

ST1 is a pandemic MSSA clone and, as has been demonstrated in this study, it can be either PVL-negative or positive [104]. ST1 is also found to be a MRSA clone that has been highly associated with MRSA outbreaks in the United States (USA400 strains: PVL-positive ST1 SCC*mec* IV) and it very frequently has been associated with skin and soft tissue infections [129].

Our PVL-positive MSSA isolates were classified in clonal complexes CC15, CC121, CC88, CC5 and CC152. This is partly consistent with other studies from all continents. Especially PVL-producing MSSA belonging to CC121 is present in different countries around the world including Africa (Nigeria, Togo, South Africa) [162]. It should be noted, as Breurec et al. pointed out, that the two genetic backgrounds CC121 and CC15 (which was found predominatly in our study, mainly ST15) may not present a genetically steady environment for SCCmec integration as they have never (CC121) or rarely (CC15) been identified in MRSA isolates [107].

PVL-positive MSSA ST152 however is commonly found in West Africa. It was the predominant clone in North-Eastern Nigeria [140] and the second most prevalent clone in Mali [134]. To date, ST152 seems to be mainly present in

West Africa [162], whereas it has also been reported that ST152 was linked to a CA-MRSA genetic background in some countries in Europe [140, 107].

## 4.3 MRSA

The MRSA isolates of this study carrying the methicillin resistance determinant *mec*A belonged to CC88 (*n* = 3, 60%), CC8 (*n* = 1, 20%), and CC5 (*n* = 1, 20%). These clonal complexes are reported to be commonly present among MRSA strains worldwide [159]. CC8 and CC5 are widespread in Europe, the Americas, and Asia [133]. MRSA clones belonging to CC8 are known to be either hospital-acquired or community-associated [133]. Community-associated MRSA (CA-MRSA) are MRSA that developed separately from the hospital MRSA clones. Contrary to hospital-acquired MRSA strains, CA-MRSA strains usually carry *lukS-PV* and *lukF-PV* and SCC*mec* types IV and V [111, 112]. CA-MRSA strains are known to be more virulent than HA-MRSA strains and they have begun to replace HA-MRSA in healthcare settings [111, 138]. Notably, ST8 is one of the most prevalent PVL-producing CA-MRSA clones in the United States [104]. More precisely, USA300 (named according to its PFGE pattern) is the predominant cause of infection in North America [53]. In Germany, USA300 is less widely disseminated than in the United States but its proportion among all MRSA is steadily increasing since the last decade [53, 160]. Characteristically, USA300 exhibits MLST ST8, has *spa* type t008, produces PVL, and carries SCC*mec* types IV and the arginine catabolic mobile element (ACME) [28, 136, 137]. For our MRSA clone having ST8 we did not do a PFGE profile but it was also found to produces PVL and to carry SCC*mec* types IV but it was found to belong to the *spa* type t121. The *spa* type t121 (repeat pattern: 11-19-21-17-34-24-34-22-25) is a single locus variant of t008 (repeat pattern: 11-19-12-21-17-34-24-34-22-25). Isolates belonging to *spa* type t121 were also ACME-positive can therefore be considered to be closely related to the USA300 clone although we did not perform PFGE.

ST5 is described to be a frequent MRSA clone worldwide, including in Africa,and is mostly associated with HA-MRSA infections [133, 145]. ST5 isolates with all four SCC*mec* types have been identified, but types I and II are most prevalent [139]. Our ST5–MRSA possessed type IV, as does the so-called Pediatric clone [141]. The Pediatric clone, a health-care associated MRSA clone, was first described in a pediatric hospital in Portugal in 1992 and subsequently in different countries on several continents [142]. The “classic” Pediatric clone goes along with *agr* II and cap5 [143]. Our ST5-MRSA-IV matches these characteristics but it has a different and rarely reported *spa* type (t653) [161] and it does not produce PVL. However, this clone was detected from a physician, who had been working in the pediatric ward for several years aboth in Gabon as well as in various other countries.

ST88, which was the major MRSA clone in this study, is well disseminated in Africa [107, 104, 145]. Beyond Africa, this clone seems to be rare but it has been isolated sporadically in Asia [144] and in Europe [145]. All our three ST88 clones were non-SCC*mec* I through IV, carried *agr* III and did not produce PVL. Two clones were *spa* type t186. This is the same *spa* type Breurec et al. reported in a multicenter study to be the most frequent *spa* type present in Africa [145]. One of the isolates was obtained from a nurse working on a surgical ward and the other isolate was obtained from a young male who was a patient on this surgical ward. The other *spa* type t729 was also reported by Breurec et al. [145]. ST88 clones were also found in a great number in Nigeria, with the notable difference being the production of PVL by Nigerian clones [104]. ST88 has been described both in community and hospital settings [145].

In contrast to Van Leeuwen et al.[146], who found the *agr* I to be the most prevalent *agr* group in MRSA isolates, in our study *agr* III was the most frequent group.

In accordance with Ghebremedhin el at.[104], our MRSA strains were less “toxigenic” than the MSSA strains according to the tested virulence factors. More MSSA isolates harbored superantigenic toxin genes than did MRSA isolates. It should be noted that this differs from the results of a Japanese study [147]. That study, however, examined both clinical MSSA and MRSA isolates.

Successful MRSA clones are usually associated with specific geographical areas. The factors underlying this observed fact are still being discussed. As Otter et al. summarize, important factors are most likely socioeconomic factors (such as hygienic habits and travel habits), antimicrobial prescribing policies and community/hospital outbreaks [53]. For instance, neither the main European CA-MRSA clone, ST80, nor one of the main MRSA clones found in Asia, ST30 [104], were detected in this study.

## 4.4 Limitations of this work

The main limitation of this work is the small number of MRSA observed in our study. The small number makes comparisons of MRSA characteristics difficult. Furthermore, the study was designed to only include*S. aureus* from asymptomatic carriers and no clinical isolates were included.

## 4.5 Conclusion

This dissertation analyzes and reports on the population structure of *S. aureus* in Gabon. In doing so it provides some valuable information on *S. aureus* carriage and the molecular diversity of *S. aureus* not only within Gabon but also in comparison with other geographical regions.Most notably, we found, firstly, a high rate of PVL-positive *S. aureus* isolates and, secondly, more “toxigenic” MSSA isolates than MRSA isolates in terms of prevalence of superantigenic toxin genes. Thirdly, we found ST15 to be the predominant sequence type

represented in this study. Finally, the MRSA clone predominant in our study, ST88, is a MRSA clone that is widely distributed in Africa.

# 5 Summary

Information about *S. aureus* in Africa is rare. This dissertation provides important information on both the *S. aureus* carriage rate and the *S. aureus* population structure in the Lambaréné area of Gabon. This cross-sectional study was conducted in 2009 in the semi-urban area of Lambaréné and its rural environment, in the Central African country of Gabon. Five hundred participants, all asymptomatic for *S. aureus*-related diseases were included in this study. Swabs were obtained from the anterior nares, the axilla, and the groin. Microbiological characterisation of the bacterial isolates was performed in the Microbiology Laboratory of the Centre de Recherches Médicales de Lambaréné, Gabon. Further analyses regarding molecular characterization were carried out at the Institute of Medical Microbiology, University of Münster (Germany). Some of the results have been published previously by Ateba-Ngoa et al. (2012).

In this study 146 participants were found to be carrier of *S.aureus*. Among the 146 *S.aureus* isolates five MRSA isolates were encountered. These prevalences are in accordance with the results of international studies. We found a high rate (42%) of PVL-producing *S. aureus* isolates. This is consistent with results from other African countries and the United States. In Europe, however, some studies show lower rates. PVL seems to contribute greatly to *S. aureus'* virulence. In total, 73% of our *S. aureus* isolates carried at least one pyrogenic exotoxin gene. This is consistent with other studies. However, some of the genes were found less often in this study than in other studies with carrier isolates. The prevalence of genes encoding for exfoliative toxins and for epidermal cell differentiation inhibitor were comparable to the prevalence of these genes found in Europe. Compared to the MRSA isolates of this study, the MSSA isolates carried more superantigenic toxin genes. In accordance with other studies, 25% were capsulated with serotype 5 and 73% with serotype 8. In this study *agr* II occurred more frequently (53%) and *agr* III less frequently (11%) than elsewhere. Among the *S. aureus* isolates 46 different *spa* types

were represented, with 35% of isolates belonging to *spa* type t084. Among the isolates 21 different sequence types (ST) were represented with a clear predominance of ST15 (47%). In other African countries ST15 has also been reported to be the predominant sequence type. ST508 seems to be less frequent in other African countries than in this study. Most of the STs have already been reported as MRSA clones. Our MRSA clones, ST88, ST8, and ST5, are well disseminated worldwide. ST88, our predominant MRSA clone, is especially prevalent in Africa. We detected only one PVL-producing MRSA clone, a clone which is closely related to the hyperviulent clone “USA300”.

# 6 German summary

Informationen über *S. aureus* in Afrika sind rar. Diese Doktorarbeit liefert wichtige Informationen sowohl über die Trägerrate von *S. aureus* als auch über die Populationstruktur von *S. aureus* im Gebiet um Lambaréné in Gabun. Diese Querschnittstudie wurde 2009 in der Stadt Lambaréné und ihrer ländlichen Umgebung durchgeführt. Lambaréné liegt in dem zentralafrikanischen Staat Gabun. In dieser Studie wurden 500 Teilnehmer aufgenommen, die alle asymptomatisch für *S. aureus* assoziierte Erkrankungen waren. Es wurden Abstriche vom Nasenvorhof, der Axilla und der Leiste genommen. Die mikrobiologischen Analysen der Bakterienisolate wurde im mikrobiologischen Labor der medizinischen Forschungseinheit des Albert Schweitzer Krankenhauses in Gabun durchgeführt. Weitere Analysen bezüglich molekularer Charakterisierung wurden am Institut für Mikrobiologie der Universität Münster in Deutschland ausgeführt. Einige der Resultate wurde bereits von Ateba-Ngoa et al. (2012) veröffentlicht.

In dieser Studie waren 146 Teilnehmer Träger von *S. aureus.* Unter den 146 *S. aureus* Isolaten gab es fünf MRSA Isolate. Diese Prävalenzen entsprechen den Resultaten von internationalen Studien. Wir fanden eine hohe Rate (42%) von PVL-produzierenden *S. aureus* Isolaten. Dies stimmt mit Ergebnissen von anderen akfrikanischen Ländern und den Vereinigten Staaten überein. In Europa jedoch zeigen einige Studien niedrigere Raten. PVL scheint bedeutend zur Virulenz von *S. aureus* beizutragen. Insgesamt trugen 73% unserer *S. aureus* Isolate zumindest ein pyrogenes Exotoxingen. Dies stimmt mit anderen Studien überein. Einige der Gene jedoch wurden in dieser Studie weniger häufig gefunden als in anderen Studien mit Trägerisolaten. Die Prävalenz der Gene, welche für exfoliative Toxine und für den epidermalen Zelldifferenzierungs-inhibitor kodieren, war vergleichbar mit der Prävalenz dieser Gene in Europa. Im Vergleich zu den MRSA-Isolaten dieser Studie, trugen die MSSA-Isolate mehr Superantigene für Toxine. In Übereinstimmung mit anderen Studien waren 25% mit Serotyp 5 und 73% mit Serotyp 8

bekapselt. In dieser Studie kam *agr* II häufiger (53%) und *agr* III weniger häufig (11%) vor als anderwärts. Unter den S*. aureus*-Isolaten waren 46 verschiedene *spa* Typen repräsentiert, wobei 35% der Isolate zu *spa* type t084 gehörten. Unter den Isolaten waren 21 verschiedene Sequenztypen (ST) repräsentiert, mit eindeutigem Überwiegen von ST15 (47%). In anderen afrikanischen Ländern wurde ST15 ebenfalls als der prädominante Sequenztyp beschrieben. ST508 scheint in anderen afrikanischen Ländern seltener zu sein als in dieser Studie. Die meisten STs wurden bereits als MRSA-Klone beschrieben. Unsere MRSA-Klone, ST88, ST8 und ST5 sind auf der ganzen Welt weit verbreitet. Unser prädominanter MRSA-Klon ST88 ist vor allem in Afrika prävalent. Wir wiesen nur einen PVL produzierenden MRSA-Klon nach, welcher zu dem hypervirulenten Klon „USA300“ nahe verwandt ist.

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# 8 Declaration of Originality

I hereby declare that this dissertation and the work specified in it are my own, except as specified below, and that I have used no other sources except those referenced.

The plan of the study and the topic of the dissertation was chosen in collaboration with physicians of the CERMEL, Gabon (Dr. med. U. Ateba Ngoa, Dr. med. B. Lell).

The recruitment of the study population was supported by several members of the CERMEL, Gabon (physicians, students and field workers). I was part of the recruitment team in Gabon.

The phenotypic characterization of the bacterial isolates was performed by laboratory technicians in the Microbiology Laboratory of the CERMEL, Gabon.

The molecular characterization of the bacterial isolates and genotyping was undertaken by the Institute of Medical Microbiology, University of Münster, Germany.

I received the data from the CERMEL and the Institute of Medical Microbiology, University of Münster.

I evaluated the data by myself except for results 3.1 to 3.3, part of results in 3.4.4, as well as most results in 3.5 (see below).

Some of the results included in this dissertation (results 3.1 to 3.3, part of results in 3.4.4, and most results in 3.5) have been published previously in this article: Ateba Ngoa U, Schaumburg F, Adegnika AA, Kösters K, Möller T, Gaus E, Fernandes JF, Alabi A, Issifou S, Becker K, Grobusch MP, Kremsner PG and Lell B (2012) Epidemiology and population structure of Staphylococcus aureus in various population groups from a rural and semi urban area in Gabon, Central Africa. Acta Trop 124: 42-47. The list of authors was corrected in 2018 by adding my name.

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