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Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon

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Abstract

Staphylococcus aureus isolates from developed countries have been extensively analyzed with respect to their virulence patterns and clonal relatedness but there is only sparse information on the molecular diversity of *S. aureus* isolates from Africa. In particular, little is known about *S. aureus* isolates from asymptomatic carriers compared with isolates causing infections. From 2008 to 2010, we prospectively collected *S. aureus* isolates from asymptomatic carriers and infections in Lambaréné, Gabon, Central Africa. For these isolates, we determined major virulence factors, and performed multilocus sequence typing (MLST) and *spa* typing. Among 163 *S. aureus* isolates from asymptomatic carriers, we found the MLST clonal complexes (CCs) 5, 6, 7, 8, 9, 15, 25, 30, 45, 88, 101, 121 and 152; 3.7% were methicillin-resistant (MRSA). The clinical isolates were associated with CCs 5, 8, 9, 15, 88, 121 and 152; 11% were MRSA. Sequence types 1 and 88 were significantly associated with infection and sequence type 508 was associated with carriage. Remarkably, there was a high prevalence of Panton–Valentine leukocidin (PVL) -encoding genes both in disease-related isolates (57.4%) and in carrier isolates (40.5%). We found differences in the clonal structure and virulence pattern of Gabonese *S. aureus* isolates from asymptomatic carriers and infections. Of note, *S. aureus* isolates from Gabon show a very high prevalence of PVL-encoding genes, which exceeds the rates observed for developed countries.

Keywords: Carriage, infection, Panton–Valentine leukocidin, *Staphylococcus aureus*, typing, virulence

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Introduction

Staphylococcus aureus is a human pathogen causing a variety of diseases ranging from superficial skin and soft-tissue infections to life-threatening conditions such as necrotizing pneumonia [1,2]. The anterior nares are the main primary ecological reservoir of *S. aureus* in humans and about 25% of the world's population are persistently colonized [2,3]. Interestingly, nasal colonization was identified as a major risk factor for subsequent *S. aureus* infection [4]. However, the clonal lineages of *S. aureus* isolates and the distribution of virulence factors might differ between isolates derived from asymptomatic carriers and from infections as well as between different geographic regions [5]. For instance, carrier isolates in industrialized countries show a higher prevalence of genes of the enterotoxin gene cluster (*egc*), *seg*, *sei*, *sem*, *sen* and *seo* compared with disease-associated isolates [6–8]. In contrast to *egc*, the fixed gene combination *sed* and *sej* and the leukocidin *lukE-lukD* genes are more prevalent in clinical isolates than in carrier isolates [8–10]. Leukocidins are bi-component exoproteins forming pores in cellular membranes. The Panton-Valentine leukocidin (PVL), consisting of the two subunits LukS-PV and LukF-PV, is a potent cytocidal toxin that causes dermonecrosis and lysis of human granulocytes and enhances the adherence of *S. aureus* to extracellular matrix [11].

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Whereas the differences between *S. aureus* carrier and clinical isolates from developed countries have been extensively investigated, data from African countries are limited and mostly available for *S. aureus* isolates derived from infection. A few studies have indicated that African *S. aureus* isolates have completely different clonal structure and virulence patterns compared with isolates from industrialized countries: 25% of Malian carrier isolates belonged to the PVL-positive sequence type (ST) 152 as determined by multilocus sequence typing (MLST) [12], 42.7% of Nigerian isolates and 72% of methicillin-resistant *S. aureus* (MRSA) isolates from Algeria were PVL-positive [13,14], 57% of clinical *S. aureus* isolates in an African multi-centre study carried genes which encoded PVL [15].

So far, isolates from asymptomatic carriers and infections from the same African region have not been compared to address the question of whether there are differences in the distribution of clonal lineages and virulence factors between these two groups.

Therefore, this study aims to compare carrier-related and infection-related isolates from the same region in Gabon with respect to important virulence markers and genotypes to elucidate differences between carrier and disease isolates. The data will be used to assess the risk for certain toxin-mediated *S. aureus* infections such as toxic shock syndrome, diarrhoea or skin and soft-tissue infections associated with PVL.

Materials and Methods

Study population

Within the German–African network on staphylococci and staphylococcal diseases (DFG PAK 296), 552 participants living in Gabon, in the province 'Moyen-Ogooué' were screened for carriage of *S. aureus* from July 2008 to May 2010. Among these people, 163 were asymptomatic carriers of *S. aureus*. Swabs were taken from the anterior nares, the axilla and the groin. Only one isolate per patient was included in this study. If participants were colonized at different body sites, we gave priority to nasal isolates over axillary/inguinal isolates and axillary isolates over inguinal isolates. The carrier group comprised inpatients (n = 53), outpatients (n = 13), hospital personnel (n = 33) and participants without previous health-care contact (n = 64). The mean age (± SD) ranged from 25.8 years (± 20.0) in participants without previous health-care contact to 40.3 years (± 7.3) in hospital personnel. Males and females were equally distributed in all carrier groups except for the hospital personnel, who had a higher percentage of females (78.8%).

The *S. aureus* isolates from infection were collected in the routine laboratory of the Albert Schweitzer Hospital, Lambaréné, Gabon, from January 2009 to May 2010 (n = 54).

Written informed consent was obtained from all asymptomatic carriers before recruitment. Ethical approval for this study was obtained from the 'Comité d'Éthique Régional Indépendant de Lambaréné' (CERIL), Lambaréné, Gabon.

Bacterial isolates

Carriage and disease isolates were cultured on sheep blood agar plates and presumptive *S. aureus* colonies were identified by a positive catalase reaction, latex agglutination test (Pastorex Staph-Plus, Bio-Rad Laboratories, Marnes-la-Coquette, France) and rabbit coagulase test (Becton, Dickinson and Company, Erembodegem, Belgium). Species confirmation of *S. aureus* and detection of the *mecA* gene encoding methicillin resistance were performed for all isolates [16]. For one *nuc*-negative, coagulase-positive isolate, further species confirmation was carried out by sequencing the ribosomal 16S rRNA gene [17].

Virulence factors and capsular polysaccharides

Genes encoding PVL (*lukS*-PV/*lukF*-PV), toxic shock syndrome toxin (*tst*), enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*), exfoliative toxins (*eta*, *etb*, *etd*), members of the epidermal cell differentiation inhibitor (*edin-A*, *edin-B*, *edin-C*) and capsular polysaccharide type 5 and 8 were detected as published elsewhere [8,9,18,19].

Genotyping

Typing of the hypervariable region of protein A (*spa* typing) was performed as described previously [20]. We performed MLST exemplarily for each *spa* type [21]. All STs of our data set were compared with all allelic profiles of the MLST database using eBURST (version 3, http://eburst.mlst.net, 18 March 2011). We used the stringent group definition of a minimum of six out of seven shared alleles to assign the STs of this study to known clonal complexes.

Statistics

Genotypes and virulence factors of carrier and clinical *S. aureus* isolates were compared using the software 'R' (http://cran.r-project.org, Version: 2.10.1) and package EPICALC. Pearson's chi-square test or Fisher's exact test were used when appropriate to analyze the proportions of categorical data. The strength of association was calculated using OR and the 95% CI. The significance level was set at 5%. Simpson's index of diversity was calculated to assess diversity of genotypes within groups.

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Results

Study population

Fifty-four *S. aureus* isolates from clinical samples were compared with 163 *S. aureus* carrier isolates collected in the Lambaréné region in Gabon. Clinical isolates were recovered from patients with wound infection (*n* = 21), bacteraemia (*n* = 11), abscesses (*n* = 10), otitis (*n* = 4), adenitis without specification (*n* = 3), pyomyositis (*n* = 2), cerebrospinal infection (*n* = 1), vaginitis (*n* = 1), and phlegmon (*n* = 1). The prevalence of MRSA among clinical isolates was 11.1% (*n* = 6).

The isolates from asymptomatic carriers were obtained from the nose (*n* = 120), the axilla (*n* = 26) and the groin (*n* = 12). The carriage site of five carrier isolates was not recorded. Of all carrier isolates, 3.7% (*n* = 6) were positive for *mecA*.

Comparison of virulence factors

The virulence factors of clinical and carrier *S. aureus* isolates are shown in Table 1. Carrier isolates more frequently encoded at least one of the pyrogenic toxin superantigens (71.8% vs 64.8%, OR 0.73, 95% CI 0.36–1.49, p 0.33) or one of the exfoliative toxins tested (7.98% vs 3.7%, OR 0.45, 95% CI 0.05–2.07, p 0.283). Clinical isolates were significantly associated with the presence of PVL-encoding genes (OR 1.97, 95% CI 1.01–3.89, p 0.03), *seh* (OR 2.96, 95% CI 0.94–9.2, p 0.028) and *edin-A* (OR 9.41, 95% CI 0.74–501.7, p 0.049). The gene *see* was not detected in either carrier or clinical isolates. The fixed gene combination *seg-sei* was always co-detected in both clinical and carrier isolates (Table 1). In contrast, the linked gene loci *sed-sej* were only present in one carrier isolate. Three carrier isolates encoded *sej* alone. The distribution of capsular polysaccharide 5 and 8 (CP5 and CP8) is shown in Table 1. Except for one carrier isolate (ST8), all MRSA isolates were PVL-negative.

Disease-association of PVL

As the possession of PVL-encoding genes was significantly associated with isolates from infection, we analyzed the presence of PVL-encoding genes among isolates from different entities of infection (Table 2). We detected a significant association of PVL-encoding genes with isolates derived from abscesses (OR ∞, 95% CI 2.18–∞, p 0.003, Table 2). The STs related to abscesses were ST1 (*n* = 3), ST15 (*n* = 3), ST88 (*n* = 1), ST152 (*n* = 1) and ST1746 (*n* = 2).

Genetic background

The 217 isolates exhibited 67 different *spa* types (Table 3). In carrier isolates, t084 (33.7%) was the most prevalent, fol-

TABLE 2. Distribution of Panton–Valentine leukocidin (PVL) -encoding genes among *Staphylococcus aureus* isolates from clinical infection

Specimen source	No. PVL-positive isolates (%)	OR[a] (95% CI)	p-value
Abscess (*n* = 10)	10 (100)	∞ (2.18–∞)	0.003
Wound (*n* = 21)	12 (57.1)	0.98 (0.28–3.45)	0.975
Blood (*n* = 11)	5 (54.5)	0.57 (0.12–2.66)	0.402
Other[b] (*n* = 12)	4 (33.3)	0.28 (0.05–1.28)	0.096

[a]OR = odds ratio with 95% confidence interval (CI).
[b]including otitis (*n* = 4), adenitis without specification (*n* = 3), pyomyositis (*n* = 2), cerebrospinal infection (*n* = 1),vaginitis (*n* = 1), phlegmon (*n* = 1).

TABLE 1. Comparison of toxin genes in *Staphylococcus aureus* isolates from carriers and infected sites

	No. (%) of positive isolates							
	Carriage		Disease		Total			
Toxin gene	No.	%	No.	%	No.	%	OR[a] (95% CI)	p[b]
lukF-PV/lukS-PV	66	40.5	31	57.4	97	44.7	1.97 (1.01–3.89)	**0.03**
tst	16	9.82	3	5.56	19	8.76	0.54 (0.10–2.01)	0.42
sea	56	34.4	16	29.6	72	33.2	0.81 (0.38–1.63)	0.52
seb	16	9.82	1	1.85	17	7.83	0.17 (0–1.18)	0.078
sec	22	13.5	4	7.4	26	12.0	0.51 (0.12–1.62)	0.334
sed	1	0.61	0	0	1	0.46	0 (0–117.51)	1
seg	54	33.1	12	22.2	66	30.4	0.58 (0.26–1.23)	0.131
seh	9	5.52	8	14.8	17	7.83	2.96 (0.94–9.20)	**0.028**
sei	54	33.1	12	22.2	66	30.4	0.58 (0.26–1.23)	0.131
sej	4	2.45	0	0	4	1.84	0 (0–4.59)	0.574
eta	9	5.52	2	3.7	11	5.07	0.66 (0.07–3.33)	0.735
etb	1	0.61	0	0	1	0.46	0 (0–117.51)	1
etd	4	2.45	0	0	4	1.84	0 (0–4.59)	0.574
edin-A	1	0.61	3	5.56	4	1.84	9.41 (0.74–501.70)	**0.049**
edin-B	9	5.52	3	5.56	12	5.53	1.01 (0.17–4.24)	1
edin-C	1	0.61	0	0	1	0.46	0 (0–117.51)	1
cap5	42	25.8	14	25.9	56	25.8	1.01 (0.46–2.12)	0.98
cap8	119	73	40	74.1	159	73.3	1.06 (0.50–2.31)	0.87

[a]OR = odds ratio with 95% confidence interval (CI).
[b]Boldface p-values indicate significant association between the respective virulence factor and disease.

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TABLE 3. Distribution of multilocus sequence typing clonal complexes (CC), sequence types (ST), *spa* types, and Panton–Valentine leukocidin (PVL) encoding genes in clinical and carrier isolates from Gabon

			Carriage (*n* = 163)			Disease (*n* = 54)		
CC	ST	*spa* type	PVL	MRSA	MSSA	PVL	MRSA	MSSA
CC5	ST5	t002, t311, t653, t1215	0	1[a]	6	0	0	3
CC6	ST6	t304, t701	0	0	3	NA	0	0
CC7	ST7	t091	0	0	1	NA	0	0
CC8	ST8	t008, t121, t197, t1476	1	1[b]	5	0	0	3
	ST72	t148	0	0	6	0	0	1
	ST94	t024	NA	0	0	0	0	1
CC9	ST9	t1045, t2980, t4492	1	0	7	0	0	1
	ST27	t099	0	0	1	NA	0	0
	ST1292	t4236	0	0	1	NA	0	0
CC15	ST1	t127, t590, t693, t1407, t1931, t4832	3	0	8	7	0	8
	ST15	t084, t085, t094, t254,t279, t326, t491, t673, t774, t1711, t1877, t2636, t6240, t6318	46	0	75	13	0	17
	ST188	t189	1	0	2	0	0	1
CC25	ST25	t148, t3772, t4680	0	0	4	NA	0	0
CC30	ST30	t017,t253	0	0	2	NA	0	0
CC45	ST45	t939	0	0	2	NA	0	0
	ST508	t1113, t1510, t2784, t4576, t5575, t6241, t6243,	0	0	14	NA	0	0
	ST1745	t6242	0	0	1	NA	0	0
CC88	ST88	t186, t729, t2253, t2723, t3202, t4195	1	4[a]	3	1	6[a]	3
CC101	ST101	t056	0	0	1	NA	0	0
CC121	ST120	t645	1	0	1	NA	0	0
	ST121	t159	1	0	1	1	0	1
	ST1746	t314, t940	1	0	2	3	0	3
CC152	ST152	t355, t4235	9	0	10	6	0	6
Singleton	ST1822	t6331	0	0	1	NA	0	0

[a]PVL-negative.
[b]PVL-positive.
MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

lowed by t355 (5.5%) and t279 (4.3%). Similarly, t084 (25.9%) was the most prevalent in clinical isolates, followed by t355 (11.1%), t2723 and t4195 (5.6% each). Multilocus sequence typing resulted in 24 different STs in isolates from carriage and disease (Table 3). We detected new STs, designated ST1745 and ST1746. A third new ST was only found once in a carrier isolate which was *nuc*-negative (ST1822, Table 3). Most STs were equally distributed among both groups (Table 3). Only ST1 and ST88 were significantly associated with clinical infection (OR 3.35, 95% CI 1.03–10.86, p 0.016 and OR 5.18, 95% CI 1.55–18.7, p 0.001, Table 3, Fig. 1). In contrast, ST508 was associated with carriage (OR 0, 95% CI 0–0.87, p 0.024). Overall, seven of the thirteen CCs found among carrier isolates were also found in *S. aureus* derived from infection (CCs 5, 15, 8, 9, 88, 121, 152). Except for CC5, these CCs were also the only PVL-positive CCs comprising the following PVL-positive STs (percentage of all PVL-positive *S. aureus* isolates): ST1 (10.31%), ST8 (1.03%), ST9 (1.03%), ST15 (60.82%), ST88 (2.06%), ST120 (1.03%), ST121 (2.06%), ST152 (15.46%), ST188 (1.03%) and ST1746 (5.15%). When applying the Simpson's index of diversity, STs of clinical samples were more diverse than STs of the carrier

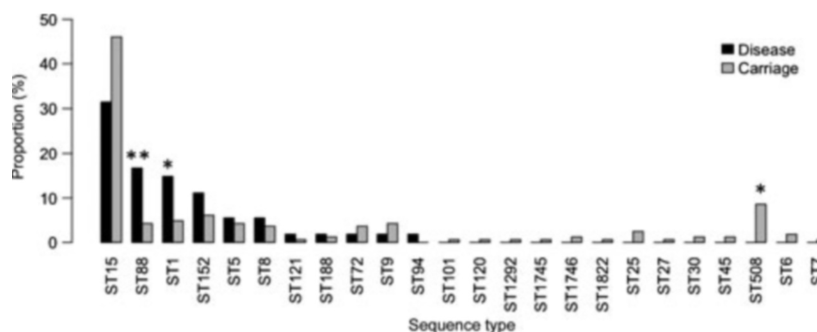

FIG. 1. Proportions of sequence types (STs) in samples from carriage and disease. Almost one-half of the carrier isolates belong to ST15. Most clinical isolates correspond to ST15, ST88 and ST1. Asterisks mark significant associations of the respective ST with carriage or disease according to Pearson's chi-square test and Fisher's exact test, respectively. *p <0.05, **p <0.005.

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isolates (0.84 vs 0.77). This is consistent with the finding that the quotient of the number of STs and the number of isolates was smaller in the group of carrier isolates (0.14 vs 0.22).

Discussion

We here present a first characterization of a collection of *S. aureus* isolates from clinical specimens and asymptomatic carriers in Gabon. Compared with European countries, we found high rates of PVL-positive isolates both in clinical and carrier isolates. This is consistent with reports from other African countries, where the prevalence of PVL ranged between 17 and 74% in clinical isolates from Cameroon, Nigeria, Madagascar, Morocco, Niger and Senegal [13,15]. In our study, we detected a significant association of PVL with clinical infection (p 0.03, Table 1). This was strongest for isolates recovered from abscesses as they were all PVL-positive (Table 2). This association was also shown in other studies [22,23]. However, it is not clear why African isolates have such a high prevalence of PVL and if this high prevalence has an impact on the incidence of disease. Further studies are necessary to assess the impact of PVL isolates on developing infection.

Other toxins, particularly the combination of different virulence factors, may also contribute to the incidence and severity of *S. aureus* infection. We found a significant association of *seh* and *edin-A* with clinical isolates (Table 1). Other studies comparing carrier isolates with clinical isolates from blood cultures did not show this finding [8]. Genes encoding other pyrogenic toxin superantigens were less prevalent among the Gabonese isolates compared with carrier and clinical isolates from industrialized countries: *tst* (8.8% vs 20.3–78%), *sed* (0.46% vs 7.0–13%), *seg/sei* (30.4% vs 55–90%), and *sej* (1.8% vs 7.0–10%). In contrast, *sea* and *eta* were more prevalent in our study (33.2% vs 15.9% and 5.1% vs 1.2%) [8,24]. The absence of *see* is not surprising because in European and American studies it was only detected in 0.5–3% of samples [8,24]. The enterotoxins *sed* and *sej* are located on one plasmid linked by an intergenic region [8]. Notably, *sed* and *sej* have not been exclusively co-detected in our study as three carrier isolates encoded *sej* alone. Such a disconnection of *sed* and *sej* has already been reported but is rare [25].

We found an unbalanced distribution of certain STs among carrier and clinical isolates. Isolates belonging to ST1 and ST88 were significantly associated with clinical infection (Table 3). This is consistent with a recent report from China, where ST88 among ST5 and ST7 was significantly over-represented in disease samples and was associated with an increased virulence [26]. Isolates belonging to ST88 rarely encode PVL but are related to community-associated MRSA [27], whereas ST1 (USA400) is a pandemic PVL-positive methicillin-susceptible *S. aureus* (MSSA) and MRSA clone [27,28]. Both ST88 and ST1 have been described in clinical isolates from different African countries [15,29].

In our study, ST508 was significantly associated with carrier isolates and is rarely found in Africa among clinical (2.6%) and carrier (1%) isolates [12,29].

Of note, ST15 was the predominant lineage in both disease-related and carriage isolates. This has also been reported from Malian carrier isolates and is consistent with a German study in which CC15 was the second most prevalent CC in asymptomatic carriers [12,30]. However, further pan-African studies comparing carrier with clinical isolates are warranted to verify the association of ST1 and ST88 with infection and ST508 with carriage.

The PVL-positive isolates of our study were mainly susceptible to methicillin and belonged to STs that are considered to be pandemic (STs 1, 30, 121) [27]. The PVL-positive STs 121 and 152 are more prevalent in Africa than on other continents [12,27].

Out of six clinical and six carrier isolates, we only found one PVL-positive MRSA. This is surprising, because STs of pandemic PVL-positive MSSA were present in our study and might function as a reservoir for PVL-positive MRSA as suggested by others [27]. However, our study argues against an intense inter-relation of PVL-positive MSSA and MRSA.

The STs of carrier isolates were less diverse compared with those of clinical isolates (Simpson's index of diversity 0.77 vs 0.84). This is surprising because one might expect that only a selection of carrier isolates possesses the potential to become invasive [4]. We only found 12 different STs among clinical isolates in contrast to 23 STs among carrier isolates. The higher Simpsons's index of diversity in clinical isolates is based on the fact that it is calculated from the total number of STs and the abundance of each ST. As the carrier isolates had a smaller quotient of STs per isolate and a strong dominance of ST15, they turned out to be less diverse than the group of clinical isolates.

Limitations of our study are the small sample size of clinical isolates. Furthermore, we only performed MLST exemplarily for each *spa* type. Although a high concordance of MLST and *spa* typing results has been shown [20], one *spa* type can correspond to several STs. Our strategy may therefore result in deviant proportions of STs within carriage and disease isolates. However, we judge this deviation as a minor limitation, as we recently performed MLST in all isolates from another Gabonese population and did not detect

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homoplasy of *spa* types (Schaumburg F., Köck R., Friedrich A.W., Soulanoudjingar S., von Eiff C., Issifou S., Kremsner P.G., Herrmann M., Peters G. and Becher K., unpublished data).

In conclusion, our study compares, for the first time, *S. aureus* isolates from infected patients and asymptomatic carriers in a defined Central African region and therefore controls the geographical bias. It shows a high prevalence of PVL in both groups, which was accentuated in clinical isolates and abscesses in particular.

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Transparency Declaration

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