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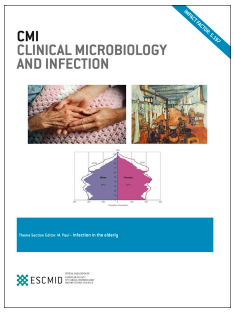

Staphylococcus aureus complex from animals and humans in three remote African regions

Frieder Schaumburg , Maude Pauly , Etile Anoh , Arsene Mossoun , Lidewij Wiersma , Grit Schubert , Arnaud Flammen , Abraham S. Alabi , Jean-Jacques Muyembe-Tamfum , Martin P. Grobusch , Stomy Karhemere , Chantal Akoua-Koffi , Emmanuel Couacy-Hymann , Peter G. Kremsner , Alexander Mellmann , Karsten Becker , Fabian H. Leendertz , Georg Peters

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Original article

Title

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Running title

S. aureus complex in remote African regions

Authors

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Abstract

Staphylococcus schweitzeri has been recently considered to be a highly divergent *Staphylococcus aureus* clade and usually colonizes non-human primates and bats in sub-Saharan Africa; yet, its transmissibility to humans remains unclear. We therefore investigated the transmission of *S. aureus* and *S. schweitzeri* between humans, domestic animals and wildlife in three remote African regions.

A cross sectional study on nasal and pharyngeal colonization in humans (n=1288) and animals (n=698) was performed in Côte d'Ivoire, Gabon and Democratic Republic of Congo (DR Congo). Isolates were subjected to *spa* typing and multilocus sequence typing. Antimicrobial susceptibility and selected virulence factors were tested.

S. schweitzeri was found in monkeys from all study sites but no transmission to humans was evident, despite frequent contact of humans to wildlife. In contrast, human associated *S. aureus* sequence types (ST1, ST6, ST15) were detected in domestic animals and non-human primates, pointing towards a human to monkey transmission in the wild. The proportion of methicillin resistant *S. aureus* (MRSA) among all *S. aureus* was 0% (Gabon), 1.7% (DR Congo) and 5.3% (Côte d'Ivoire). The majority of MRSA isolates belonged to the African clone ST88.

In conclusion, we did not find any evidence for a transmission of *S. schweitzeri* from animals to humans. However, such a transmission might remain possible due to the close phylogenetic relation of humans and non-human primates. The ST88-MRSA clone was widespread in Côte d'Ivoire but not in Gabon and DR Congo.

Key words

Staphylococcus aureus; Staphylococcus schweitzeri; Africa; transmission, animal; MRSA

Introduction

Staphylococcus aureus is part of the normal flora of animals and humans and can cause a multitude of infections ranging from superficial skin infections to invasive diseases. Colonization is a risk factor for subsequent infection of the homologous strain which is frequently found in the anterior nares [1;2].

Transmission of *S. aureus* and its methicillin resistant variant (MRSA) between animals and humans has been frequently reported in industrialized countries particularly in regions with high densities of livestock. Not only the livestock-associated MRSA (LA-MRSA, CC398) but also community-associated MRSA (CA-MRSA, CC97) can be transmitted from mammals (i. e. pigs, cattle) and poultry to humans where it is as pathogenic as other *S. aureus* lineages [3;4].

Recently, a highly-divergent *S. aureus* clade was described in bats, monkeys and great apes in sub-Sahara Africa and is now considered to be a new species termed *Staphylococcus schweitzeri* [5-7]. *S. schweitzeri* has similar biochemical properties as *S. aureus* (i. e. catalase and coagulase positive), can be genotyped by *spa* typing and multilocus sequence typing but differ from *S. aureus* at the whole genome level and the peptidoglycan type [5;7]. An isolate of a divergent *S. aureus* clade which has been retrospectively confirmed as *S. schweitzeri* was found once in a human carrier in Gabon [8]. Close contacts of humans to animals might facilitate the cross species transmission of *S. aureus* and *S. schweitzeri* for instance when preparing and consuming meat (e. g. monkeys). The extraction and consumption of wildlife ("bushmeat") is common in sub-Saharan Africa, particularly in remote regions [9]. However, the transmission of *S. aureus* and *S. schweitzeri* between animals and humans in remote African regions is unclear. The objective of this study was to investigate the population structure and transmission of *S. aureus* and *S. schweitzeri*

93 between humans and animals in three remote regions in Côte d'Ivoire, Gabon and
94 Democratic Republic of Congo (DR Congo).

Material and Methods

Ethical clearance

Ethical clearance was obtained from the ”Comité d’Éthique Institutionnel, Centre de Recherches Médicales de Lambaréné, Gabon” (CEI-MRU 001/2011), the “Comité National d’Éthique et de la Recherche (CNER), Ministère de la Santé et de l’Hygiène Publique, République de Côte d’Ivoire” (101 10/MSHP/CENR/P) and ”Comité d’Éthique, Ministère de l’Enseignement Supérieur et Universitaire, République Démocratique du Congo" (ESO/CE/018/11).

A written informed consent was signed or fingerprinted by each participant before enrolment. If the participant was illiterate or did not speak French, a local interpreter explained the study procedures. An independent witness signed additionally the consent form in these cases.

Study design

A population-based cross sectional study was performed in Côte d’Ivoire, Gabon and DR Congo to take nasal and pharyngeal swabs from humans and animals. The studied populations are characterized by a limited access to official documentation and health care and, with the exception of the Ivorian population, rare contacts to urban civilization. Subsistence farming and hunting are essential parts of their lifestyle.

In Côte d’Ivoire, participants were recruited and animals were sampled in eight villages (Daobly, Gahably, Gouliako, Keibly, Pauleoula, Ponan, Tai, Zaipobly) in close proximity to Taï National Park from 4/2012-10/2012 (Figure 1).

In DR Congo, samples were taken from humans and animals in seven villages (Bekombo, Bungosani, Ipopé, Iyoko, Lompolé, Lui Kotale, Nganda) at the border of “Salonga-Sud” National Park from 07/2011-9/2011 (Figure 1). In Côte d’Ivoire and

DR Congo, domestic animals were alive and lived in the same villages as the human participants. Wildlife was dead and hunted for consumption as “bushmeat” on the same day when samples were taken.

In Gabon, Babongo pygmies were recruited in six villages (Village tranquille, Tsibanga, Ossimba, Ndougou, Soga, Egouba) in Waka National Park in 10/2011. Samples from animals were collected from 2010-2013 in the provinces Moyen Ogooué and Ngounié where Waka National Park is located (Figure 1).

All participants were included if they consented to enrolment. No exclusion criteria were applied. Demographic data and data on contact to animals (e.g. history of animal bites) were recorded in standardized questionnaires.

Bacterial isolates

Swabs were taken by gently rubbing a sterile cotton tip in circular moves against the nasal septum of the anterior nares and the pharyngeal mucosa.

In Gabon, samples were stored in Amies transport medium (Transwabs, Medical Wire, Corsham, UK) until culture within a maximum of two days in the microbiology laboratory at the Albert Schweitzer Hospital, Lambaréné. Swabs from DR Congo and Côte d’Ivoire were stored in STTG medium in liquid nitrogen and shipped to Germany for culture.

All samples were cultured on Columbia blood agar plates, Columbia CAP selective agar plates (Oxoid, Wesel, Germany) and SAID agar plates (bioMérieux, Marcy l’Etoile, France) for 18-36 h at 36±1 ℃.

Presumptive colonies were screened for a positive catalase and agglutination test (Pastorex Staph-Plus, Bio-Rad, Marnes-la-Coquette, France). Species identification and susceptibility test (EUCAST breakpoints) were done using Vitek 2 automated systems (bioMérieux). Species of *S. aureus* was confirmed by the detection of the

nuc gene [10]. Identification of *S. schweitzeri* was based on the lack of *nuc* detection, its phylogenetic divergent position using multilocus sequence typing and a similar biochemical profile as *S. aureus* as assessed with GP ID Card (bioMérieux) [7].

Carriers were defined as a human or animal being colonized in the nose and/or throat. If an individual was colonized with an identical isolate at different body sites (based on *spa* typing), one isolate was included in the final analysis in order to report non-duplicate isolates only.

Molecular characterization

Genes encoding selected virulence factors were detected by multiplex PCRs [11]. Methicillin resistance was confirmed by the detection of *mecA*, all MRSA were tested for arginine catabolic mobile element (ACME) [12;13]. All isolates were *spa* typed and multilocus sequence typing (MLST) was done exemplarily for one isolate of each *spa* type in each country and each group (humans, animals) [14;15]. The concatenated sequences of MLST housekeeping genes were used to construct a Neighbour Joining Tree (MEGA5, http://www.megasoftware.net). The divergence between two groups was assessed using the Maximum Composite Likelihood model (MEGA5).

Statistics

Antimicrobial resistance rates were compared between animals and humans using Pearson's chi-square test or Fisher's exact test, the software 'R' (http://cran.r-project.org, Version: 2.13.1) and the package EPICALC.

Results

In total, 1288 participants and 698 animals were sampled in Côte d'Ivoire, Gabon and DR Congo (Figure 1, Table1). The median age of humans ranged from 15 years (Gabon) to 30 years (Côte d'Ivoire). Human *S. aureus* colonization rates were similar in Gabon (34.0%, n=35) and Côte d'Ivoire (32.4%, n=222) but lower in DR Congo (21.4%, n= 107). While animal bites were not recorded for participants from DR Congo, 3.9% (n=4, Gabon) and 20.7% (n=142, Côte d'Ivoire) had a history of animal injuries (Table 1). The majority reported snake bites (8.2%, n=65), followed by dog bites (4.6%, n=36) and bites by non-human primates (0.9%, n=7).

The distribution of sampled domestic animals (i. e. ruminants, fowls, dogs, cats) and wildlife (i. e. non-human primates, rodents, ruminants, reptiles) was heterogeneous among all study sites. The proportion of domestic animals among all animals was highest in Côte d'Ivoire (90.3%, n=501), while wildlife was predominant in Gabon (90.8%, n=116) and DR Congo (88%, n=11, Table 1). *S. aureus* colonization rates were highest in ruminants (19.6%, n=54) followed by non-human primates (19.0%, n=15), cats (7.1%, n=3) and dogs (3.0%, n=3). *S. schweitzeri* was only found in non-human primates which were colonized in 26.6% (n=21).

Nine human carriers had a culture-confirmed *S. aureus* skin infection. The same strain (based on *spa* typing) of the skin lesion was also found in the nose and/or throat of six participants. Three participants with a skin infection were not colonized.

The staphylococcal isolates were separated into two phylogenetic groups. Group 1 included 495 *S. aureus* isolates predominated by ST152 (17.4%, n=86), ST15 (16.8%, n=83) and ST5 (10.9%, n=54). Animal species which carried isolates belonging to group 1 were dogs (*Canis* sp.), goats (*Capra* sp.), guenons (*Cercopithecus* sp.), mangabeys (*Lophocebus* sp.), talapoins (*Miopithecus* sp.) and

194 sheep (*Ovis* sp., Table S1). The habitat regions of these species are terrestrial or
195 semi terrestrial.

196 The divergent group 2 consisted of 24 *S. schweitzeri* isolates and ST2295 (16.7%,
197 n=4), ST1872, ST1874 and ST2474 (each 8.3%, n=2) were predominant. All *S.*
198 *schweitzeri* isolates were isolated from non-human primates and were *nuc* PCR
199 negative. The mean distance between group 1 and 2 was 0.083 base substitutions
200 per site. Within group 1, we detected a cluster of monkey associated STs (ST1838,
201 ST1851, ST1854, ST1925, ST2721). Of these, only isolates belonging to ST1854
202 were *nuc* negative. Of note, two ST395 isolates from human carriers were also *nuc*
203 negative. Isolates from wildlife and domestic animals were distinctly scattered in
204 group 1 which is dominated by isolates from humans. In contrast, no isolates from
205 humans were found in group 2 (Figure 2).

206 *S. aureus* belonging to ST1, ST5, ST6, ST8, ST15, ST101, ST121, ST152, ST188,
207 ST567 and ST1472 were found in humans, domestic animals and/or wildlife. A
208 possible transmission of these isolates between humans and animals was assessed
209 by *spa* typing which has a higher discriminatory power than MLST (Table 2).
210 Transmission of *S. aureus* occurred more frequently between humans and domestic
211 animals than between humans and wildlife. *S. schweitzeri* isolates were not found
212 amongst humans or domestic animals (Table 2).

213 One early branching isolate (ST2353, *nuc* negative) from a 14 year-old female carrier
214 in DR Congo did not cluster with any group (Figure 2). The delineation of ST2353
215 from group 1, the closest related clade, was supported by a bootstrap value of 100%
216 (supporting material, Figure S1). The average distance of ST2353 from ST152 and
217 ST2022 (taken as reference STs from group 1 and 2, respectively) was 0.035 and
218 0.054 base substitution per site, respectively. ST2353 had an isolated position
219 regarding the MLST allelic profile; the closest relatives were ST1857, ST2022, and

ST2295 which were quadruple locus variants of ST2353. ST2353 was more similar to typical *S. aureus* STs than to *S. schweitzeri*, *Staphylococcus simiae* or the early branching ST1223 and ST1850 (Figure S1).

Antimicrobial resistance was only detected in *S. aureus* but not in *S. schweitzeri* (Table 3). Overall, the antimicrobial resistance rates were higher in *S. aureus* from humans compared to animals for penicillin (57.3 vs. 81.7%, p<0.005), cefoxitin (1.1 vs. 4.7%, p=0.2) and cotrimoxazol (23.6 vs. 31.4%, p=0.15).

Compared to the pygmy population in Gabon, *S. aureus* from Côte d'Ivoire and DR Congo showed higher resistance rates to penicillin, cotrimoxazol and tetracyclin (Table 3). The MRSA rate was 5.3% (Côte d'Ivoire) and 1.7% (DR Congo). No MRSA was detected in the Gabonese population. In Côte d'Ivoire, 94.1% (n=16) of all MRSA belonged to ST88 (t186, t786, PVL negative). One MRSA isolate was isolated from a sheep (ST2947, t186, PVL negative, Table 3). In DR Congo, the two MRSA were isolated from humans and belonged to ST8 (t1476, PVL negative, ACME negative).

In methicillin susceptible *S. aureus*, PVL was the most prevalent virulence factor (25-40.4%, Table 3). Only the enterotoxins encoding genes (*seb*, *sec*) and toxic shock syndrome toxin (*tst*) were found in *S. schweitzeri* isolates (Table 3).

Discussion

Our study assessed a possible transmission of *S. aureus* and *S. schweitzeri* between humans, domestic animals and wildlife in three remote African regions. We found evidence that transmission of *S. aureus* between humans and domestic animals could occur. *S. schweitzeri* was frequently found in non-human primates but was not detected in humans despite frequent exposure to bushmeat.

In human participants, 3.9-20.7% reported animal bites in the past which is in line with a report from Uganda where 19.3% reported injuries or close contact with primates [16]. Not only injuries by wild animals but also bushmeat hunting and consumption are risk factors for the transmission of pathogens between animals and humans [9]. Bushmeat trade and consumption is highest in West and Central Africa. Approximately 300 g bushmeat are consumed per person per day in the Congo Basin resulting in 4.5 million tons of extracted bushmeat per year [9]. Therefore, our remote studied populations can be considered as populations with a high risk to becoming exposed to zoonotic pathogens from wildlife.

We and others recently reported a highly divergent *S. aureus* clade in African non-human primates and bats which is now considered to be a new species *S. schweitzeri* [5;6]. *S. schweitzeri* harbours a *nuc* homologue with similar thermostable nuclease activity as *S. aureus* [17]. Despite frequent exposures to non-human primates we did not find *S. schweitzeri* in humans. In contrast, some isolates from monkeys clustered with typical human-associated isolates (i. e. ST1, ST6, ST15, Figure 2). This finding suggests that a transmission from humans to animals is more likely than vice versa. This transmission could be facilitated by overlapping habitats of humans (terrestrial), domestic animals (terrestrial) and wildlife (terrestrial and semi terrestrial, Table S1). It is also possible that animal-associated lineages were transmitted to humans where they clonally expanded. However, the polyclonal

264 population structure in humans argues against this scenario. As the monkeys have
265 been hunted and handled by humans, we cannot rule out that these animals were
266 contaminated with *S. aureus* after death and did not carry human-associated *S.*
267 *aureus* lineages during lifetime. We rank this possibility as low, as swabs were taken
268 from anterior nares and throat which are usually not touched by humans during
269 handling of bushmeat. However, easily accessible body sites of monkeys might
270 remain subject of contamination by humans.
271 A transmission of *S. aureus* from humans to great apes has been reported from
272 African sanctuaries and research centres in Uganda, Zambia and Gabon [18;19].
273 While direct skin contact of humans and animals might be the transmission route in
274 captive animals, other paths might apply for a transmission to wild non-human
275 primates. Contacts of wild animals with human faeces or secretions, either directly or
276 through soil or water could facilitate a transmission. One study from Uganda
277 confirmed a transmission of *Escherichia coli* between humans, livestock and gorillas
278 [20]. In contrast, a study from Gabon did not show any evidence for such a
279 transmission of *E. coli* from human faeces to wildlife through soil and surface waters
280 [21].
281 In a human carrier from DR Congo, we found an early branching ST2353 which was
282 unrelated to other early branching *S. aureus* isolates (ST1223, ST1850) [22;23]. To
283 the best of our knowledge, similar STs have not been reported yet. The closest
284 related STs are quadruple locus variants of ST2353 and cluster in group 2 (Figure 2).
285 To resolve the phylogenetic position of this ST, comparative whole genome analyses
286 are warranted.
287 The resistance rates to antimicrobial agents differed markedly between isolates from
288 the three studied populations and between *S. aureus* and *S. schweitzeri*. There
289 seemed to be a trend from low (Gabon), medium (DR Congo) and high (Côte

d'Ivoire) levels of antimicrobial resistance in *S. aureus* which might mirror the contact with healthcare institutions and access to antibiotics. The high resistance rates to penicillin (89.5%) and cefoxitin (5.3%) in *S. aureus* from Côte d'Ivoire correspond to resistance rates in urban populations in Gabon (penicillin resistance: 95%, methicillin resistance: 3%), Nigeria (methicillin resistance: 8%) or Kenya (penicillin resistance: 69.8%, methicillin resistance: 7%) [8;24;25]. Almost all MRSA isolates from Côte d'Ivoire belonged to ST88 (94.1%). The ST88-MRSA-III/IV is widely distributed in sub-Saharan Africa and termed the "African MRSA clone" [26]. We isolated a ST88-MRSA from a sheep in Côte d'Ivoire; others found this lineage in pigs from Senegal [27].

Although our study is valuable to understand the transmission of *S. aureus* and *S. schweitzeri* between animals and humans, some limitations need to be addressed. First, we were unable to include data on animal exposure and bites of the studied population in DR Congo. We assume that this population has similar exposures and contacts to wildlife as the Gabonese pygmies due to similar living conditions. Second, the cross sectional study design limits conclusion regarding the direction of transmission. Future longitudinal studies are therefore warranted. Third, since one *spa* type can belong to different MLST sequence types we might have underestimated the diversity of STs. Fourth, apart from the anterior nares and throat we did not sample other typical *S. aureus* colonization sites (e. g. hands, axilla, and groin) and might have missed additional *S. aureus* carriers.

In conclusion, we provide evidence for a transmission of human-associated *S. aureus* to domestic animals and to some extent also to wildlife in rural Africa. No transmission of the monkey associated *S. schweitzeri* to humans was detected, although the studied populations have frequent contact to wildlife through bushmeat

315 hunting and consumption. However, *S. schweitzeri* might have a zoonotic potential
316 due to the close phylogenetic relation of humans and non-human primates.

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Transparency declarations

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The authors declare no conflicts of interest.

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421 Table 1: Demographic Data of the study populations

		Côte d'Ivoire	Gabon	DR Congo
Humans	Total number (n)	686	103	499
	Median age in years (range)	30 (0-80)	15 (0.5-70)	22.5 (0-87)
	Proportion of females (%)	62.7	45.6	53.1
	Carrier rate (%)	32.4	34.0	21.4
	History of animal bites (%)	3.9	20.7	ND
Animal	Total number (n)	556	128	14
	Domestic animals (%)	90.1	21.4	9.4
	Wildlife (%)	9.9	78.6	90.6

422 Note: not done (ND)

423 Table 2: Transmission of *Staphylococcus aureus* between humans, domestic animals and wildlife

MLST sequence type	Human	domestic animal		wildlife		Country
	spa type (n of isolates)	*spa* type (n of isolates)	species	*spa* type (n of isolates)	species	
ST5	t002 (20)	-	-	t002 (1)	civet	DR Congo
	t311 (6)	t311 (1)	goat	-	-	Côte d'Ivoire
ST6	-	t1476 (5)	cat, dog, goat	t1476 (1)	monkey	Côte d'Ivoire
ST8	t008 (2)	t008 (1)	goat	-	-	Côte d'Ivoire
ST15	t084 (32)	t084 (12)	goat, sheep	t084 (1)	monkey	Côte d'Ivoire
	t346 (8)	t346 (2)	goat	-	-	Côte d'Ivoire

ST88/ST2947	**t186 (9)**	**t186 (1)**	sheep	-	-	Côte d'Ivoire
ST121	t314 (6)	t314 (2)	sheep	-	-	Côte d'Ivoire
ST152	t355 (55)	t355 (16)	chicken, dog goat, sheep	-	-	Côte d'Ivoire
	t4235 (1)	t4235 (2)	goat, sheep	-	-	Côte d'Ivoire
ST567	t13523 (1)	t13523 (1)	sheep	-	-	Côte d'Ivoire
ST1472	t318 (2)	t318 (1)	goat	-	-	Côte d'Ivoire

424 Note: MRSA isolates in bold

425 Table 3: Antimicrobial resistance and virulence factors of *Staphylococcus aureus* isolates

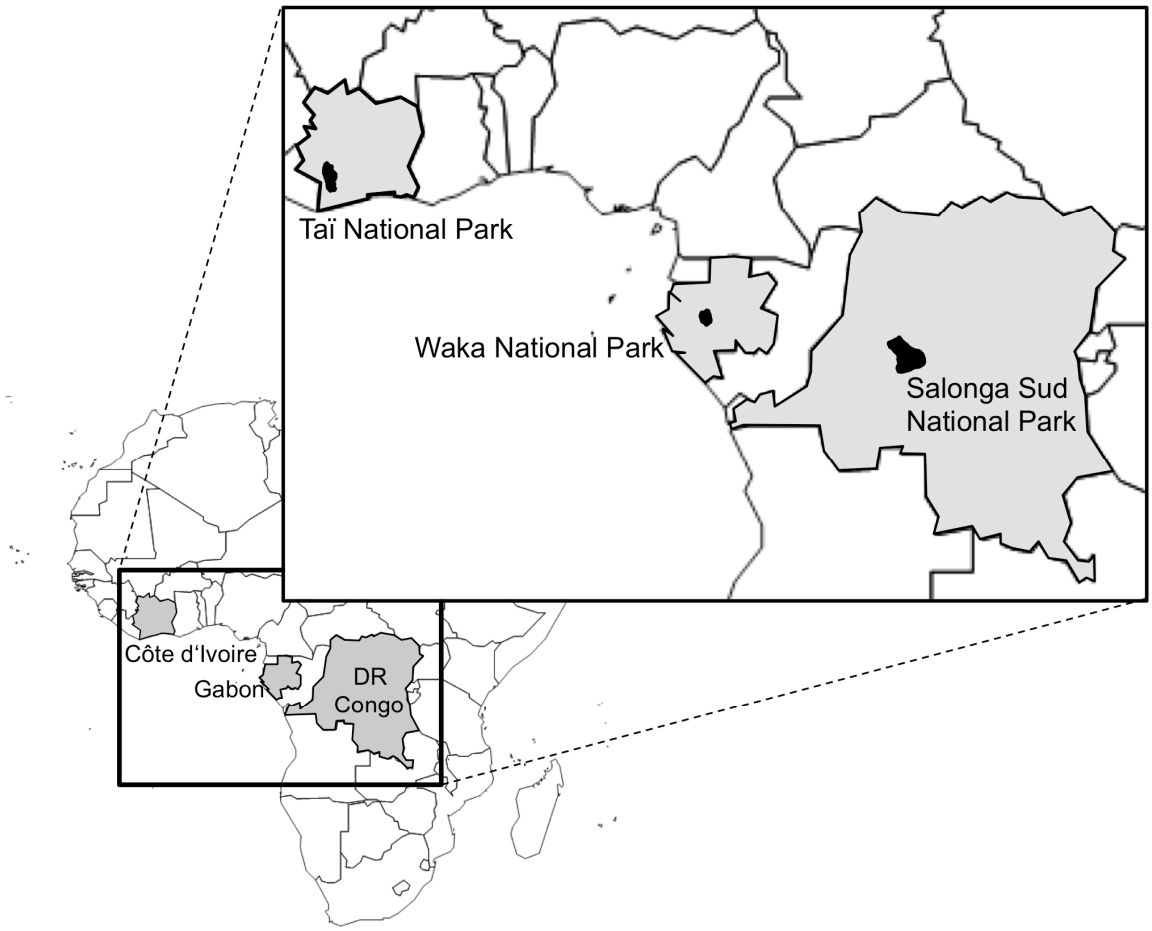
		Côte d'Ivoire		Gabon		DR Congo		Total	
		S. aureus (n=323)	*S. schweitzeri* (n=1)	*S. aureus* (n=52)	*S. schweitzeri* (n=17)	*S. aureus* (n=120)	*S. schweitzeri* (n=6)	*S. aureus* (n=495)	*S. schweitzeri* (n=24)
Antimicrobial resistance	Penicillin	289 (89.5)	0 (0)	13 (25)	0 (0)	81 (67.5)	0 (0)	383 (77.4)	0 (0)
	Cefoxitin	17 (5.3)	0 (0)	0 (0)	0 (0)	2 (1.7)	0 (0)	20 (4.0)	0 (0)
	Cotrimoxazole	110 (34.1)	0 (0)	1 (1.9)	0 (0)	37 (30.8)	0 (0)	148 (29.9)	0 (0)
	Tetracyclin	229 (70.9)	0 (0)	3 (5.8)	0 (0)	30 (25)	0 (0)	262 (52.9)	0 (0)
	Erythromycin	8 (2.5)	0 (0)	0 (0)	0 (0)	2 (1.7)	0 (0)	10 (2.0)	0 (0)
	Clindamycin	0 (0)	0 (0)	5 (9.6)	0 (0)	2 (1.7)	0 (0)	5 (1.4)	0 (0)
	Rifampicin	55 (17.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	55 (11.1)	0 (0)
Virulence factors	PVL	129	0 (0)	21	0 (0)	30 (25)	0 (0)	180	0 (0)

		(39.9)		(40.4)				(36.4)	
	SEA	67 (20.7)	0 (0)	2 (3.9)	0 (0)	30 (25)	0 (0)	99 (20.0)	0 (0)
	SEB	25 (7.7)	0 (0)	9 (17.3)	3 (17.6)	16 (13.3)	0 (0)	50 (10.1)	3 (12.5)
	SEC	33 (10.2)	0 (0)	6 (11.5)	2 (11.8)	12 (10)	1 (16.7)	51 (10.3)	3 (12.5)
	ETA	9 (2.8)	0 (0)	0 (0)	0 (0)	6 (5)	0 (0)	15 (3.0)	0 (0)
	ETB	2 (0.6)	0 (0)	32 (61.5)	0 (0)	3 (2.5)	0 (0)	37 (7.5)	0 (0)
	TSST	35 (10.8)	0 (0)	0 (0)	1 (5.9)	2 (1.7)	1 (16.7)	37 (7.5)	2 (8.3)
Three most frequent MLST sequence types (n)	1	ST152 (84)	ST2946 (1)	ST30 (9)	ST2295 (4)	ST5 (51)	ST2474 (2)	-	-
	2	ST15 (6)	NA	ST1 (8)	NT (2)	ST8 (16)	ST2475 (1)	-	-
	3	ST8 (26)	NA	ST1854 (4)	ST1872 (2)	ST15 (13)	ST2476 (1)	-	-

Note: Figures are n (%), unless indicated otherwise. NA: not applicable, NT: not typable. Isolates which were not typable by MLST were allocated to *S. aureus* if they were *nuc* PCR positive [10]. Isolates being *nuc* PCR negative were grouped with *S. schweitzeri*.

Figure 1: Map of the study sites. The national parks in Côte d'Ivoire (Taï National Park), Gabon (Waka National Park) and Democratic Republic of Congo (Salonga Sud National Park) are shaded in black.

Figure 2: Phylogenetic tree of *Staphylococcus aureus* and *Staphylococcus schweitzeri* Côte d'Ivoire, Gabon and DR Congo. Concatenated sequences of the seven MLST housekeeping genes were used to construct a Neighbor-Joining Tree. The countries of origin are marked by colors (yellow: Côte d'Ivoire, blue: DR Congo, green: Gabon). Black lines indicate isolates from non-human primates.


Taï National Park
Waka National Park
Salonga Sud
National Park
Côte d'Ivoire
Gabon
DR
Congo

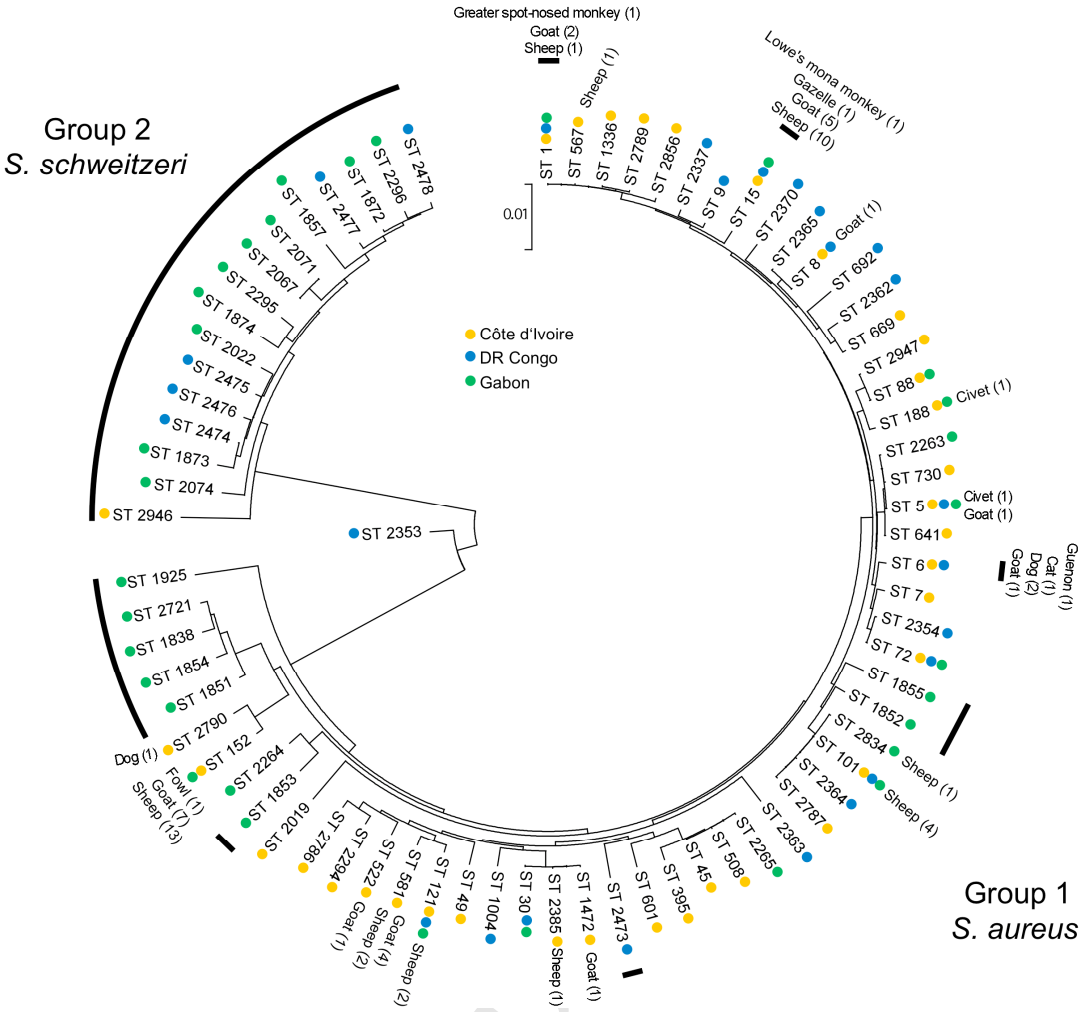


Table S1: Animal species and colonization rates

Species	Colonization rate (no. animals/no. carriers/no. human associated carriers)[a]			Habitat region
	Côte d'Ivoire	Gabon	DR Congo	
Canis sp.	91/3/3	7/0/0	3/0/0	terrestrial
Capra sp.	121/21/21	NA	NA	terrestrial
Cephalophus sp.	2/0/0	10/2/2	NA	terrestrial
Cercopithecus sp.	7/1/0	41/21/7	4/3/0	semi terrestrial
Lophocebus sp.	NA	3/3/3	NA	semi terrestrial
Mandrillus sp.	NA	4/1/1	NA	terrestrial
Miopithecus sp.	NA	4/1/1	NA	terrestrial
Ovis sp.	103/31/31	4/0/0	NA	terrestrial
Piliocolobus sp.	NA	NA	3/1/0	arboreal
Others	4/4/4[b]	2/2/1[c]	1/1/1[d]	terrestrial

Note: Not applicable (NA), the respective species was not sampled in this country.

Those animals, which carried isolates belonging to group 1 (Figure 2) were considered to be "human associated carriers".

[a]*S. aureus* and *S. schweitzeri*

[b]*Anatidae*, *Felidae*, Monkey (unknown species), animal (unknown species)

[c]*Nandinia binotata, Gorilla gorilla*

[d]*Felidae*