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Research Article

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Staphylococcus aureus in Rwandan dogs predominantly representing human-associated lineages

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Abstract

The present study aimed at examining the nasal and ear carriage of *Staphylococcus aureus* of Rwandan dogs and cats. Sixty-five *S. aureus* isolates were detected, all originating from the nostrils of dogs. Resistance to penicillin, penicillin/erythromycin/clindamycin, penicillin/tetracycline, and tetracycline solely was observed. The isolates were assigned to 23 different *spa* types, among them three novel (t21589, t21661, and t21662) variants, associated with eleven clonal complexes (CCs) (CC1, CC5, CC12, CC15, CC22, CC30, CC45, CC97, CC152, CC707, and CC834). Four isolates could not be assigned to any known CC. MLST revealed that one of them belonged to the new sequence type (ST) 9069. Panton–Valentine leukocidin (PVL) genes (*lukF-PV/lukS-PV*), the bovine leukocidin genes (*lukM/lukF-P83*), the toxic shock syndrome toxin gene *tst-1*, and various virulence-associated genes were detected. These findings demonstrate the dogs are colonized with human lineages of *S. aureus*. Coupled with the limited availability of *S. aureus* data from human medicine in Rwanda underscores the importance of hygiene measures and supports the need for a rigorous One-Health Surveillance program of the companion animals–human interface.

Impact Statement

This study substantially improved our knowledge on circulating *Staphylococcus aureus* in Rwandan dogs. The identification of human-associated clonal complexes (CCs) like CC1, CC5, CC12, CC15, CC22, CC30, CC45, and CC152 in the present study underscores the potential for bi-directional interspecies transmission, posing a significant public health risk.

Keywords: *Staphylococcus*; antibiotic resistance; veterinary; pathogens; microarray

Introduction

Widespread among humans and diverse animal species, *Staphylococcus* (*S.*) *aureus* can act as either a harmless colonizer of the skin and the mucosal membranes or often as a causative agent of infections in both humans and animals. The clinical signs range from mild skin infections to fulminant bacteremia and endocarditis (Haag et al. 2019, Laux et al. 2019). Abundant studies suggest that *S. aureus* can be bidirectionally transmitted between humans and animals (Haag et al. 2019). Companion animals, like dogs and cats, often live in close contact to humans and, therefore, the transmission of *S. aureus* is likely (Weese et al. 2006).

Staphylococcus aureus is of particular interest and is emerging as a major culprit in the ongoing silent pandemic of antibiotic resistance, as indicated by both present research and future projections (Antimicrobial Resistance Collaborators

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2022). A multilocus sequence typing (MLST) scheme based on seven housekeeping genes has been developed that provides the unambiguous nomenclature of *S. aureus* isolates to clonal complexes (CCs), and sequence types (STs) (Enright et al. 2000). Some CCs are more likely associated with specific hosts (Matuszewska et al. 2020). For example, the majority of *S. aureus* strains that primarily colonize or infect humans are within the following CCs: CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45, CC51, CC80, CC121, and CC152 (Haag et al. 2019, Matuszewska et al. 2020). Some of these CCs have also been identified in companion animals, pointing to the possibility of either zoonotic transmission or exposure to a common environmental source (Haag et al. 2019).

Data on *S. aureus* in companion animals across Africa is limited, with existing studies primarily focused on a limited number of countries and often lacking comprehensive details (Lozano et al. 2016, Akarsu et al. 2024). Currently, there is no available information from Rwanda on the presence *of S. aureus* in the population of companion animals, particularly dogs and cats. Analysis of the official data from the Rwanda Agricultural Board, demonstrates that information on the country's dog and cat population is inconsistently reported (Rwanda Agriculture Board, https://www.rab.gov.rw/publications/strategic-plan-and-reports; accessed April 2025), but it appears to be on the rise (Ntampaka et al. 2019). As the country's per capita income has been steadily growing for over two decades (World Bank Open Data, https://data.worldbank.org/; accessed April 2025), attitudes toward pet ownership are changing (National Geographic, https://www.nationalgeographic.com/photography/article/dogs-kigali-rwanda-pets-culture; accessed April 2025). While dogs in rural areas are primarily kept for security purposes (Ntampaka et al. 2019), a smaller number of dogs are kept in urban areas as domestic pets (Ntampaka et al. 2021). Finally, in our experience, cats are mainly kept as pet animals in both urban and rural regions. The rising population of companion animals is correlated with an increased potential for the bi-directional transmission of bacteria, including *S. aureus*.

This emphasizes the need to address the presence of *S. aureus* in dogs and cats in Rwanda. Given the paucity of research on *S. aureus* in companion animals, and considering its potential significance, the present study aimed at examining the nasal and ear carriage of *S. aureus* among companion animals in Rwanda. Furthermore, the study assessed the clonal diversity, antimicrobial resistance patterns, and virulence-associated genes in the *S. aureus* isolates.

Material and methods

Sampling and isolation of *S. aureus*

The study was communicated, and the sampling was approved by the institutional ethics and animal welfare committee of the Research Screening and Ethical Clearance Committee of the College of Agriculture, Animal Science, and Veterinary Medicine, University of Rwanda (UR-CAVM, 003/2022/DRI on 28.06.2022) in accordance with the Good Scientific Practice guidelines of the Rwandan national legislation.

Sample collections were conducted either at the New Vision Veterinary Hospital (NVVH) in Kigali, Rwanda, or at the NVVH in Musanze, Rwanda (https://nvvh.rw/). Animals were selected using convenience sampling, based on their owners' willingness to participate in the study. A total of 432 dogs (158 females, 246 males, and 28 missing data on sex) and 23 cats (11 females, 12 males) were examined. Animals originated from three regions: 268 dogs (62% of all dogs sampled) originated from Musanze District, 154 dogs (35.6%) and all cats originated from Kigali District, and 10 dogs (2.3%) from Akagera District. The mean age of the dogs was 3 years (±3.5 years, SD), while the cats had an average age of 3.4 years (±2.8 years, SD). Among dogs, 377 nasal and 55 ear swabs were collected, and 16 nasal and seven ear swabs were collected among cats. Ear swabs were usually collected in cases of aggressive animal behavior or when convenient for the veterinarian. Microbiological work was performed in the microbiological laboratory at the NVVH, Musanze. Swabs were incubated in tryptic soy broth (TSB) [Beckton Dickinson (BD); Heidelberg, Germany] with 6.5% (w/v) NaCl overnight at 37°C and subsequently streaked onto on mannitol salt (MS) agar (MS agar, Rabid Labs, UK). Presumptive *S. aureus* colonies grown on MS agar were re-cultivated on MS agar until a pure culture was obtained and were cryoconserved at −25°C. In addition, a 300 µl aliquot of each enriched TSB was cryoconserved at −25°C, and together with presumptive *S. aureus* sent to the Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria.

In Vienna, the 300 µl aliquot of cryoconserved biomass was re-cultivated in TSB overnight at 37°C and subsequently streaked onto on BD™ Baird-Parker Agar and BBL™ CHROMagar™ MRSA II (both from Becton Dickinson, Heidelberg, Germany). The colonies of *S. aureus* isolates that showed the typical colony appearance were pure cultured, cryoconserved at −70°C, and further characterized.

Identification of *S. aureus* and antimicrobial susceptibility testing

Identification to species level was confirmed by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik, Bremen, Germany).

Antimicrobial susceptibility testing (AST) was conducted by agar disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2024). The following antimicrobial agents were tested: penicillin (PEN 10 units), cefoxitin (FOX 30 µg), ciprofloxacin (CIP 5 µg), gentamicin (GEN 10 µg), tetracycline (TET 30 µg), erythromycin (ERY 15 µg), clindamycin (CLI 2 µg), chloramphenicol (CHL 30 µg), trimethoprim-sulfamethoxazole (SXT 1.25/23.75 µg), nitrofurantoin (NIT 300 µg), rifampicin (RIF 5 µg), and linezolid (LZD 30 µg) (BD; Heidelberg, Germany). *Staphylococcus aureus* ATCC® 25 923 served as a quality control strain.

Molecular characterization of *S. aureus*

Molecular characterization was performed using a DNA microarray-based technology (INTER-ARRAY Genotyping Kit *S. aureus*, Bad Langensalza, Germany) (Monecke et al. 2008), which is used for the detection of antimicrobial resistance and virulence-associated genes. For this purpose, DNA was extracted as previously described (Loncaric et al. 2019). DNA microarray results were visualized as a network tree using SplitsTree4 software (version 4.19.1) with default settings (Huson and Bryant 2006). Briefly, the hybridization profiles of the tested strains were transformed into "sequences," where

Table 1. Summarized molecular characterization, antimicrobial resistance, and toxins profile of the *S. aureus* isolates investigated.

Sector	CC*	*spa*	Resistance phenotype**	Resistance genes detected	Superantigens	Leukocidins
Kigali [13]	CC97 [5]	t267 [4]	PEN [1] PEN/TET [3]	*blaZ* [3] *blaZ*/*tet*(K) [1]	N.D.	*lukF/S*, *lukD/E*
		t3626 [1]	susceptible	N.D.	N.D.	*lukF/S*, *lukD/E*
	CC45 [4]	t21589 [2]	PEN	*blaZ*	*tst1*, *egc**** , *sec*, *sel*	*lukF*
		t333 [1]	susceptible	N.D.	*egc*	*lukF*
		t015[1]	susceptible	N.D.	*egc*	*lukF*
	CC30 [1]	t21329	PEN	*blaZ*	*egc*	*lukF/S*
	CC15 [1]	t084	susceptible	N.D.	N.D.	*lukF/S*, *lukD/E*
	CC5 [1]	t002	PEN	*blaZ*	egc	*lukF/S*, *lukD/E*
	CC834 [1]	t21661	susceptible	N.D.	N.D.	*lukF/S*, *lukD/E*
Musanze [52]	CC152[17]	t355 [16]	PEN [11], susceptible [5]	*blaZ* [11] N.D. [5]	*sea* [1] N.D.[15]	*lukF*, *lukF-PV/lukS-PV* [14] *lukF*, *lukD* [1] N.D. [1]
		t10904 [1]	PEN	*blaZ*	N.D.	N.D.
	CC97[8]	t267[6]	PEN [3], susceptible[3]	*blaZ* [3], N.D.[3]	N.D.	*lukF/S*, *lukD/E*
		t3626 [1]	susceptible	N.D.	N.D.	*lukF/S*, *lukD/E*
		t10008[1]	susceptible	N.D.	N.D.	*lukF/S*, *lukD/E*
	CC15 [9]	t774 [5]	PEN, ERY, CLI	*blaZ*, *erm*(C)	N.D.	*lukF/S*, *lukF-PV/lukS-PV*, *lukD/E*
		t360[1]	PEN, TET	*blaZ*, *tet*(K)	N.D.	*lukF/S*, *lukF-PV/lukS-PV*, *lukD/E*
		t084 [3]	PEN, TET [1] PEN [1] PEN, ERY, CLI [1]	*blaZ*, *tet(K)* [1] *blaZ* [1] *blaZ*, *erm*(C) [1]	N.D.	*lukF/S*, *lukD/E*
	CC30[4]	t1055[2]	susceptible	N.D.	*egc*	*lukF/S*, *lukF-PV/lukS-PV*
		t3651 [1]	PEN	*blaZ*	*egc*	*lukF/S*, *lukF-PV/lukS-PV*
		t021 [1]	TET	*tet*(K)	*egc*	*lukF/S*, *lukF-P83/lukM*
	CC45[3]	t2771 [2]	PEN	*blaZ*	*tst1*, *egc*, *sec*, *sel*	*lukF*
		t333 [1]	susceptible	N.D.	*tst1*, *egc*, *sec*, *sel*	*lukF*
	CC5 [3]	t002	PEN	*blaZ*	*egc* [2], *egc*, *sea* [1]	*lukF/S*, *lukD/E*[2] *lukF/S*, *lukF-PV/lukS-PV* [1]
	CC1 [1]	t127	PEN	*blaZ*	*egc*, *sea*, *seb*, *seh*, *sek*, *seq*	*lukF/S*, *lukD/E*
	CC12 [1]	t4252	susceptible	N.D.	*sec*, *sel*	*lukF/S*, *lukD/E*
	CC22 [1]	t223	PEN	*blaZ*	*tst1*, *egc*	*lukF*
	CC707 [1]	t1458	PEN	*blaZ*	*tst1*, *sek*, *seq*	*lukF/S*, *lukD/E*
	ST9069 [1]	t21662	susceptible	N.D.	*egc (sei, selm, seln, seu)****	*lukF*
	NP*** [3]	t21662	susceptible	N.D.	*egc (sei, selm, seln, seu)****	*lukF*

numbers in [] in each column is equal to the number of respective isolates
*CC, clonal complex, ST, sequence type
**PEN, penicillin, TET, tetracycline, ERY, erythromycin, CLI, clindamycin
***genes detected within egc comprising the enterotoxin genes seg, sei, sem, sen, seo, and seu

each probe's signal was represented as "positive" ("C"), "negative" ("G"), or "ambiguous" ("A"). These "sequences" were then used as input for SplitsTree4 to construct the network tree. The scale bar indicates the number of differences between signal strings, with 0.1 representing a 10% difference (Monecke et al. 2011). All isolates were subjected to *spa* typing (Loncaric et al. 2019). In one isolate, the CC could not be determined using DNA microarray, and 7-loci MLST was performed as previously described (Loncaric et al. 2014).

Estimation of carriage prevalence and confidence intervals

Carriage prevalence and exact 95% confidence intervals (CI) were calculated using the function *propCI*() in the package prevalence (Devleesschauwer 2022) in R v.4.4.1. (R Core Team 2023).

Results and discussion

Isolation and identification of *S. aureus*

Sixty-five *S. aureus* were isolated from nasal swabs of 62 dogs, 49 from Musanze and 13 from Kigali; the female:male ratio was 25:37, while all samples from cats tested negative, indicating a carriage prevalence of 13.6% (95% CI: 10.6–17.1) among companion animals (i.e. including dogs and cats) in Rwanda. The pooled prevalence of nasal *S. aureus* carriage has considerable geographical variation in dogs, with pooled prevalence rates of 6.7% (America), 7.3% (Europe), and 15.3% (Africa), as reviewed by Abdullahi et al. (2022).

However, a subsequent study (Scott et al. 2022) revealed an exceptionally high prevalence of 78% in Trinidadian dogs. The majority of dogs carrying *S. aureus* isolates ($n = 48$, 77.4%) were guard animals kept outside (Table 1, Supplementary Table S1).

Antimicrobial susceptibility testing and detection of resistance genes

None of the isolates was resistant to cefoxitin, and therefore all isolates were classified as methicillin-susceptible. On the other hand, methicillin-resistant *S. aureus* (MRSA) has been identified in all continents, with a pooled prevalence in dogs of 2.8% and 0.5% in cats, respectively, as reviewed by Abdullahi et al. (2022). Thirty-one isolates were solely resistant to penicillin, six were resistant to penicillin, erythromycin, and clindamycin, four were resistant to penicillin and tetracycline, and one was resistant exclusively to tetracycline. Twenty-four isolates were susceptible to all antimicrobial agents tested (Table 1, Supplementary Table S1). Phenotypic resistance correlated with the presence of resistance genes (Table 1, Supplementary Table S1). A limited number of studies have investigated phenotypic and genotypic antimicrobial resistance in parallel within the context of *S. aureus* nasal colonization in dogs and cats. Therefore, we discuss the studies that adopted this dual approach. Recently, Akarsu et al. (2024) studied canine *Staphylococcaceae* in Kenya and observed resistance to penicillin mediated by *blaZ* in 38 out of 47 isolated *S. aureus*. Three isolates were resistant to penicillin and trimethoprim-sulfamethoxazole (*dfrG*), and four to penicillin, trimethoprim-sulfamethoxazole (*dfrG*), and tetracycline [*tet*(K)]. Recently, Bezzi et al. (2024) investigated the presence of *S. aureus* in 138 nasal swabs of healthy pet cats, where from 23 *S. aureus* were detected, 11 methicillin-resistant *S. aureus* (MRSA) and 12 methicillin-susceptible *S. aureus* (MSSA). In this study, molecular characterization was conducted using whole-genome sequencing. Among MSSA, all but one isolate displayed resistance to at least one antibiotic. Different resistance profiles among MSSA were detected, and correlations between phenotypic and genotypic resistance for the following antibiotics were observed: penicillin resistance (*blaZ*), resistance to tetracycline [*tet*(L), *tet*(M)], erythromycin and inducible clindamycin [*erm*(T), *erm*(C), *msr*(A), *mph*(C)], aminoglycosides (*aadD*, *aadE*, *aphA3*, *sat4, str*), and fluoroquinolones (mutations in *grlA*: pS80F, *gyrA*: pS84L). Among 100 dogs and 34 cats Gharsa et al. (2015) investigated the presence of *S. aureus* in nasal swabs of 100 dogs and 34 cats in Tunisia. They did not observe cefoxitin resistance among isolates. One feline *S. aureus* was resistant to penicillin (*blaZ*), and another displayed combined penicillin (*blaZ*) and ciprofloxacin resistance. All four detected canine *S. aureus* isolates were resistant to penicillin (*blaZ*) solely ($n = 2$) or in addition to penicillin resistance to streptomycin (*aadE*) was observed in one isolate and resistance to tetracycline [*tet*(M)] and erythromycin [*erm*(A)]. In Spain, Gómez-Sanz et al. (2013a) investigated 98 kennel dogs for coagulase-positive staphylococci. They isolated 24 *S. aureus*, all of them being MSSA. The resistance rates among these MSSA are in approximate concordance with those from the present study. Twelve out of 24 isolates were susceptible and the remaining isolates were either solely resistant to penicillin *(blaZ*), tetracycline [*tet*(K)], or streptomycin (*str*). Five isolates were resistant to erythromycin [either erm(A) or *erm*(C)] and showed inducible clindamycin resistance. The same working group (Gómez-Sanz et al. 2013b) investigated *S. aureus* colonization in various households from humans and their pets (54 dogs and 12 cats). Eight MSSAs from pets were isolated. Two isolates were susceptible, four isolates (two from both species) were solely resistant to penicillin (*blaZ*), one canine to penicillin and erythromycin with inducible clindamycin resistance [*erm*(A)], and one canine to penicillin and tetracycline [*tet*(K)]. Contrary to the present study, Gómez-Sanz et al. (2013b) investigated animal owners as well and detected zooanthrophogenic or vice versa transmission of some MSSA. In the UK, Wedley et al. (2014) investigated the prevalence of nasal carriage of staphylococci in dogs using the same approach as in the present study (i.e. *spa*-typing and a DNA microarray-based technology). They examined 749 nasal swabs and detected 54 *S. aureus* (7 MRSA and 47 MSSA). They observed higher resistance rates to gentamicin, fusidic acid,and ciprofloxacin (all > 40%). DNA microarray-based technology from selected isolates revealed the ence of *mecA*, *blaZ*, and *erm*(C) in MRSA, whereas *blaZ* was detected in selected MSSA solely.

Genetic lineages of *S. aureus* and their virulence characteristic

Twenty-three *spa* types, including three new (t21589, t21661, t21662) were detected within twelve CCs with CC152 being predominant. Four isolates could not be assigned to any known CC. All of them belonged to the new *spa* type t21662, and MLST revealed that one of them belonged to the new ST 9069 (Table 1, Supplementary Table S1).

DNA microarray analyses revealed the presence of several virulence genes. The Panton-Valentine leukocidin (PVL) genes were detected in all isolates belonging to CC152, as well as in six isolates belonging to CC15, three to CC30, and in one to CC5. The toxic shock syndrome toxin 1 gene (*tst-1*) was observed in five CC45 isolates and in CC22 and CC707. Enterotoxin gene cluster *egc* comprising the enterotoxin genes *seg, sei, sem, sen, seo,* and *seu* as well as other enterotoxin genes, including *sea, seb, sec, seh, sek, sel*, and *seq* were detected in various isolates (Table 1, Supplementary Table S1). Two CC5-t002 isolates harbored the exfoliative toxin serotype A gene (*etA*). One CC30 isolate harbored the bovine-associated leukocidin genes *lukF-P83/lukM* (Table 1, Supplementary Table S1).

CC152 is a major human-associated CC from sub-Saharan Africa that commonly carries the PVL toxin (Ruffing et al. 2017, Baig et al. 2020). PVL-positive *S. aureus* belonging to CC152 and *spa* type t355 (CC152-t355) has already been associated with ruminants from Rwanda (Loncaric et al. 2024). Beyond Rwanda, CC152 has been identified in various animals across Africa, including clinical *S. aureus* from dogs in Côte d'Ivoire and Zambia (Youn et al. 2014, Schaumburg et al. 2015). CC15 is distributed among humans worldwide, encompassing both community-associated (CA) and hospital-associated (HA) *S. aureus* (Monecke et al. 2011, 2016). It is also present among various animal species in Africa (Lozano et al. 2016, El-Ashker et al. 2022, Akarsu et al. 2024), including from nostrils of dogs and cats in Tunisia, dogs in Kenya, and cats in Algeria (Gharsa et al. 2015, Akarsu et al. 2024, Bezzi et al. 2024). Outside of Africa, PVL-negative CC15 was detected in nostrils of dogs in UK and Spain (Wedley et al. 2014, Abdullahi et al. 2023). PVL genes were identified in other human-associated *S. aureus* lineages within the present study. CC30 is a pandemic CC that has a profound impact on

global human health and is frequently associated with PVL genes (McGavin et al. 2012, Madzgalla et al. 2016). This lineage is prevalent among *S. aureus* isolates of human origin but was also isolated among animals in Africa, including dogs (Lozano et al. 2016, Ruffing et al. 2017, El-Ashker et al. 2022). In three out of five CC30 isolates, the PVL genes were detected, and in one, the bovine-associated leukocidin genes *lukF-P83/lukM*. Even though recently detected PVL-positive CC30 from nasal swabs of sheep and cattle in Rwanda carried a similar set of virulence-associated genes, but they belonged to a distinct *spa* type (t318) (Loncaric et al. 2024). The *lukF-P83/lukM* genes are usually associated with animal *S. aureus* isolates, mainly involved in the pathogenesis of mastitis in ruminants. Their presence is a rare observation among isolates belonging to CC30 (Monecke et al. 2016). On the other hand, PVL-negative CC30 was detected in the nostrils of dogs in the UK (Wedley et al. 2014). CC5 is a globally disseminated *S. aureus* lineage, primarily associated with clinical manifestations in humans and poultry (Haag et al. 2019, Matuszewska et al. 2020). Humans are considered the original host of CC5, from which CC5 jumped to chicken, and underwent adaptation to the new host (Matuszewska et al. 2020). It is a common lineage among humans in Africa (Abdulgader et al. 2015, Ruffing et al. 2017). CC5 has also been associated with various other animals, including companion animals from Kenya, Tanzania, Zambia, and Tunisia (Shittu et al. 2011, Lozano et al. 2016, El-Ashker et al. 2022). So far, CC5 *S. aureus* has been isolated solely from the case of bovine mastitis in Rwanda (Ndahetuye et al. 2021). In two CC5-t002 isolates analyzed during the present study, the gene encoding exfoliative toxin serotype A (*etA*) was detected. Exfoliative toxins are exoproteins that can induce the separation of the epidermal layer of the skin (Dinges et al. 2000). The *etA* gene has already been identified in *S. aureus* from Africa in both human and animal isolates, but not associated with *S. aureus* belonging to CC5 or with companion animals (Kolawole et al. 2013, Shittu et al. 2021). PVL-positive CC5 was isolated from nasal swabs of cats from Tunisia, whereas PVL-negative from cats in Algeria and of dogs in Spain and the UK (Gómez-Sanz et al. 2013a,b, Wedley et al. 2014, Bezzi et al. 2024).

Five out of seven *S. aureus* isolates belonging to CC45, carried the toxic shock syndrome toxin 1 gene *tst-1*. CC45 is considered one of the most frequently reported clones among humans with substantial clinical relevance (Haag et al. 2019). Despite its global distribution, CC45 is less frequently reported in Africa (Ruffing et al. 2017). Toxic shock syndrome (TSS) is a rare but life-threatening condition characterized by fever, hypotension, a skin desquamation, and multiple organ dysfunctions (Stevens 1996). The primary cause of TSS is staphylococcal toxic shock syndrome toxin (Dinges et al. 2000). In Africa, *S. aureus* belonging to CC45 was rarely observed among animals (Lozano et al. 2016, Shittu et al. 2021). CC45 of animal origin was associated with primates and ruminants (Lozano et al. 2016, Shittu et al. 2021). *tst1*-negative CC45 was reported from nasal colonization of dogs in Spain and UK (Gómez-Sanz et al. 2013a, Wedley et al. 2014). The *tst-1* gene was present in two singletons belonging to CC22 and CC707. CC22 is primarily a human-associated lineage but has also been identified in companion animals with close contacts to humans (Monecke et al. 2016, Yebra et al. 2022). CC22 from companion animals was embedded within a more diverse human strain population, suggesting a recent anthroponotic origin of these animal isolates (Matuszewska et al. 2020). CC22 is scarcely reported from the African continent (Lozano et al. 2016). From the nostrils of companion animals, the *tst-1*-negative CC22 MSSA was observed in dogs from Spain (Gómez-Sanz et al. 2013a). CC707 has been sporadically isolated from humans, including those from the African continent and from wild animals in Europe (Ruffing et al. 2017, Luzzago et al. 2022). The CC707 isolate from the present study harbored the immune-evasion cluster (IEC) genes *sak*, *chp*, and *scn*, the genes *tst-1*, *sek*, *seq*, *ccrA/B2*, and the genes of the penicillinase operon (*blaZ/I/R*) that are commonly observed in CC707 isolates (Monecke et al. 2016, Luzzago et al. 2022).

A singleton belonged to CC1, the clonal lineage predominantly associated with humans and showing a worldwide distribution (Yebra et al. 2022). CC1 has been identified in several African countries in humans and animals (Lozano et al. 2016, Ruffing et al. 2017, Shittu et al. 2021), including dogs from Tanzania (Katakweba et al. 2016). In Rwanda, CC1-t127 was observed in *S. aureus* from cows with mastitis (Keinprecht et al. 2024) and has a similar set of resistance- and virulence-associated genes. The Rwandan ruminant CC1 isolates lack *sek*, *seq* genes, and *hlb*-converting phages. MSSA belonging to CC1 from nasal swabs was also detected in dogs from UK (Wedley et al. 2014).

Another *S. aureus* belonged to CC12, a sporadic human-associated clone rarely reported from the African continent (Ruffing et al. 2017). In animals, CC12 is usually associated with pigs and poultry (Monecke et al. 2016). There are reports about *S. aureus* belonging to CC12 isolated from European wildlife (Monecke et al. 2016), and from nasal colonization of dogs in UK (Wedley et al. 2014).

The second most prevalent CC was CC97. CC97 has evolved into the dominant ruminant *S. aureus* lineages, which is a primary clone associated with bovine mastitis worldwide. Originating from a spillover event (human-to-cattle transmission), it subsequently adapted to the cattle population (Yebra et al. 2022). Notably, this clone also exhibits the ability to re-infect humans (Matuszewska et al. 2020, Yebra et al. 2022). CC97 has already been detected among *S. aureus* isolates from bovine mastitis in Rwanda (Antók et al. 2019, Ndahetuye et al. 2021, Keinprecht et al. 2024) or from the nostrils of cattle, sheep, and goats (Loncaric et al. 2024). Unlike *S. aureus* isolates from ruminants, canine isolates from the present study harbored *scn* and *sak*. *hlb*-converting phages indicating possibly a very recent spillover from humans, or a pathogenetic role of these genes in dogs. Recently, CC97-carrying *hlb*-converting phages were detected from healthy cats in Algeria (Bezzi et al. 2024).

A singleton belonged to CC834 and new *spa* type t21661. CC834 is a very rare clonal lineage. Recently, *S. aureus* belonging to CC834 was detected in dogs from Kenya (Akarsu et al. 2024). Reports of CC834 *S. aureus* isolates from humans are scarce (Hisatsune et al. 2023).

Finally, for four isolates, CC could not be assigned. These isolates belonged to new *spa* type t21662, had a very similar array profile, and one of these isolates was subjected to MLST resulting in the new ST9069 without closely related STs (four or more identical alleles [PubMLST, https://pubmlst.org/bigsdb?db=pubmlst_saureus_seqdef&page=profiles, accessed August 2024)]. The presence of the IEC genes in these isolates suggests a possible human origin of these isolates.

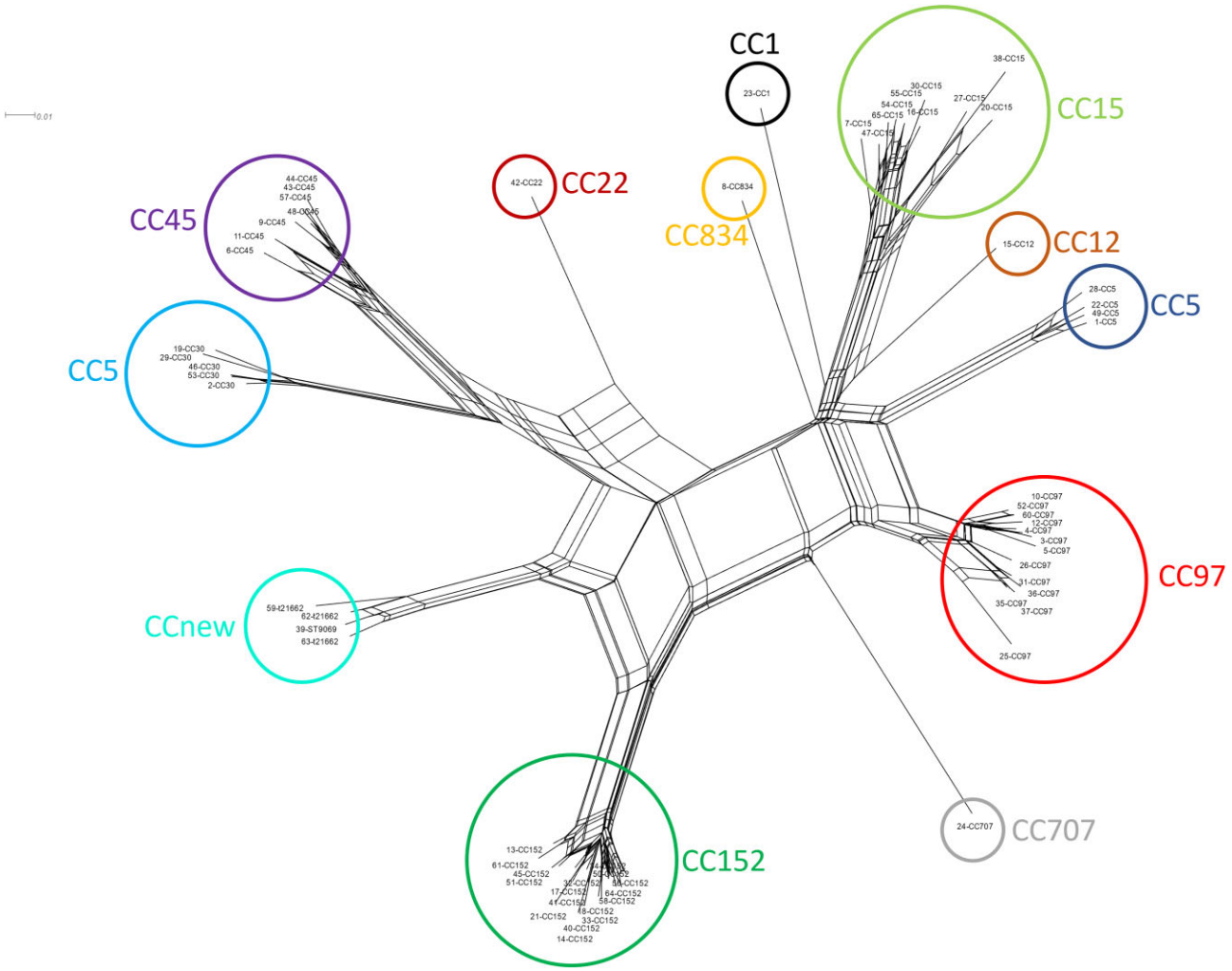

Figure 1. SplitsTree network of *S. aureus* isolates examined. ID alias was used. SplitsTree analysis of the microarray data indicated clonal clustering into seven groups and five singletons demonstrated high congruence with CC.

All but four isolates from the present study carried at least one of *hlb*-converting phages containing virulence genes, including *sea*, *scn*, *chp*, and *sak* (Supplementary Table S1).

SplitsTree analysis of the microarray data indicated clonal clustering into seven groups and five singletons. This clustering, based on similarities in their virulence and antimicrobial resistance profiles, demonstrated high congruence with CCs (Fig. 1).

The present study documents the presence of *S. aureus* in companion animals from Rwanda. So far, comprehensive studies (i.e. including molecular characterization) on *S. aureus* have only been conducted among humans (Masaisa et al. 2018) and ruminants in Rwanda (Antók et al. 2019, Ndahetuye et al. 2021, Keinprecht et al. 2024, Loncaric et al. 2024).

This study faced several limitations, primarily related to the challenges of conducting fieldwork and collecting data in a resource-limited environment. Furthermore, the samples were collected from only two sectors of Rwanda, limiting their representativeness of the overall situation in Rwanda. The lack of *S. aureus* isolates from cats also constitutes a limitation. Thus, a follow-up study is planned, including all provinces of Rwanda and a larger sample size of both canine and feline samples. This future research will also compare the *S. aureus* isolates obtained from these companion animals with those originating from other animals (e.g. ruminants) and humans.

Conclusion

The present study provides the first detailed comprehensive characterization of *S. aureus* from dogs in Rwanda. The majority of the isolates belonged to predominantly human-associated lineages like CC152, CC15, CC45, CC30, CC5, CC1, CC12, and CC22. Furthermore, the presence of the IEC cluster in CC97, a CC primarily associated with bovine hosts, suggests a human origin for these isolates. The identification of human-associated CCs in the present study highlights the potential for bi-directional interspecies transmission, posing a significant public health risk. Coupled with the limited availability of *S. aureus* data from human medicine in Rwanda, this underscores the importance of hygiene measures and supports the need for a rigorous One-Health Surveillance program on the companion animals–human interface.

Author contributions

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Supplementary data

Supplementary data is available at *LAMBIO* online.

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

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