

Aus dem Department für Biologische Wissenschaften und Pathobiologie
der Veterinärmedizinischen Universität Wien

(DepartmentleiterIn: O. Univ.-Prof. Dr. med. vet. Mathias Müller)

Zentrum für Pathobiologie – Institut für Mikrobiologie

(LeiterIn: Univ.-Prof. Dr. rer. nat. Dipl. Ing. agr. Monika Ehling-Schulz)

**Diversity of *Staphylococcus aureus* associated with mastitis from
dairy cows in Rwanda**

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von

BSc. Jennifer Zöchbauer

Wien, im Oktober 2025

Betreuer:in:

Priv.-Doz. Dr.med.vet. Igor Loncaric

Institut für Mikrobiologie

Veterinärplatz 1, 1210 Wien Österreich

Igor.Loncaric@vetmeduni.ac.at

Dr. Adriana Cabal Rosel

Institut für Surveillance & Infektionsepidemiologie, Geschäftsfeld Öffentliche Gesundheit

AGES - Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH

Traisengasse 5, 1200 Wien

Österreich

adriana.cabal-rosel@ages.at

Gutachter:in

Dr.med.vet. Beatrix Stessl

Institut für Lebensmittelsicherheit, Lebensmitteltechnologie und öffentliches
Gesundheitswesen

Veterinärplatz 1, 1210 Wien Österreich

Beatrix.Stessl@vetmeduni.ac.at

Table of contents

1. Introduction and hypothesis	1
1.1. Dairy sector in Rwanda.....	1
1.2. Background Mastitis	2
1.3. <i>Staphylococcus</i> spp.....	2
1.3.1. <i>Staphylococcus aureus</i>	3
1.3.1.1. Virulence factors and genes of <i>Staphylococcus aureus</i>	5
1.3.1.2. Antimicrobial resistance.....	6
1.4. Hypothesis.....	7
2. Diversity of <i>Staphylococcus aureus</i> associated with mastitis from dairy cows in Rwanda	8
3. Discussion	18
4. Summary	22
5. Zusammenfassung	23
6. List of abbreviations.....	25
7. Eigenständigkeitserklärung.....	26
8. References	27

1. Introduction and hypothesis

1.1. Dairy sector in Rwanda

The production of milk plays an essential role in providing people in Rwanda with food of animal origin (1). Such foods, including milk, contribute to poverty reduction, improved nutrition and food security and livelihoods (2, 3).

Rwanda's national cattle population amounted to around 1.64 million animals in 2023 (4) and has developed well since the mid-1990s, when an estimated 80 % of the herd was lost (5). This is based on projects and investments initiated by the Rwandan government in recent decades, such as the Rwanda Dairy Development Project (RDDP), which aims to increase cattle productivity and improve milk quality (6), or the “One cow per poor family” program (Girinka program), which has been supporting families living in poverty since 2006 (7, 8). Additionally, the National Dairy Strategy (NDS) was launched in 2013, aiming to improve the development of the dairy industry. The NDS includes guidelines for the development of the dairy industry by integrating livestock into a healthy ecosystem, increasing livestock productivity and improving milk quality (9). As a result of such strategies, dairy farming has been one of the fastest growing sectors within Rwanda agriculture. However, despite significant progress, there were still major challenges to overcome, including access to animal health services, limited financial resources for smallholder farmers, (5) as well as livestock-related issues, such as insufficient numbers of improved dairy cows, inadequate feed composition or seasonal fluctuations in water availability (10).

Ankole is the predominant cattle breed in Rwanda, followed by crossbreeds and even pure breeds, which are less common (3, 11). Indigenous breeds are well adapted to the harsh environmental conditions in Rwanda. This is partly due to genetic traits such as trypanosome tolerance found in some local breeds – most notably the N'Dama – and genetic factors contributing to the evolution of heat tolerance of indigenous populations (12). Surveys conducted at local smallholder farms also show that farmers rely on local breeds such as Ankole cattle due to their adaptations to the environment, but the future trend is towards crossbreeds, as these achieve a higher milk yield (13) and are expected to be better adapted to the environment than exotic pure breeds (14). According to one study, about 21 % of the cows given away under the Girinka program were exotic purebreds or crossbreeds, with a majority of exotic traits (e.g. imported Holstein or Jersey). These are less able to cope with environmental fluctuations and require better feeding strategies to maintain their milk yield

(14). Unfavorable conditions or stress factors, as described above, contribute to the development of diseases such as mastitis.

1.2. Background Mastitis

Mastitis is an inflammation of the mammary gland and a worldwide problem in dairy cows. It can be subclinical or clinical, although subclinical mastitis is much more common (15, 16). While clinical mastitis is accompanied by visible symptoms such as changes in the milk appearance (clots, flecks, changes in colour or consistency), inflammation of the udder (pain, swelling, redness), loss of appetite or fever, subclinical mastitis usually progresses without any obvious changes (16, 17).

Various studies show a high prevalence of subclinical mastitis in Rwanda, ranging from approximately 52 % (18) to 76 % (19). Purebred cows in Rwanda, such as Friesian or Jersey, are affected significantly more often (74 %) than cross-bred (52 %) or Ankole (36 %) (18). A lack of training on hygiene practices during milking or inadequate cleaning and disinfection, as well as a lack of knowledge about animal health, leads to contamination right at the beginning of the milk chain (20). Udder health, such as the condition of the teat ends, has the greatest influence on the prevalence of mastitis, followed by the stage of lactation, the breed and the degree of soiling of the cows (18). Furthermore, a study shows that 84 % of farmers in Rwanda cite poor sanitation and hygiene as the main factor in the spread of subclinical mastitis (21). The consequences of mastitis include a drop in milk yield, which varies depending on the causative pathogen, such as non-*aureus* staphylococci (NAS), *Staphylococcus aureus* (*S. aureus*) or *Escherichia coli* (*E. coli*) (22). This decline in productivity leads to economic losses, as studies have shown (21, 23). Mastitis can be caused by various pathogens. According to some studies, infections with NAS are the most common (40.3-46 %), followed by *S. aureus* (23-25.5 %), *E. coli* (5.6 %), *Streptococcus (Sc.) uberis* (8.4 %) and *Sc. dysgalactiae* (7.9 %) (16, 22). Staphylococci, and in particular *S. aureus*, have been the subject of various research projects for many decades (24–27) due to their importance in both veterinary and human medicine.

1.3. *Staphylococcus* spp.

Staphylococci are gram-positive, non-motile, facultatively anaerobic bacteria whose cell wall contains peptidoglycan and teichoic acid (28). The genus *Staphylococcus* currently contains 94 child taxa. Of these, 70 are validly published with correct names and 76 are validly published

including synonyms (<https://lpsn.dsmz.de/genus/staphylococcus>, last accessed 14.09.2025). As catalase-positive bacteria and often occurring in clumps, they are subdivided into coagulase-negative *staphylococci* (CoNS) and coagulase-positive *staphylococci* (CoPS) based on their coagulase reaction (29). CoNS are often commensals of the skin and mucosa (30). In contrast, of the major representative within CoPS is *S. aureus*, which is usually associated with pathogenicity (31). Staphylococci can cause a wide variety of diseases in both humans and animals. In human medicine, staphylococci play a significant role and can cause urinary tract infections, endocarditis, life-threatening foreign body-associated bloodstream infections (30) and septicemia, particularly in newborns (52.86 %) (32). In veterinary medicine, staphylococci are isolated, for example, in mastitis, dermatitis, rhinitis, or wound infections in various animals, including cows (33). Closer contact associated with the domestication and globalization of livestock increased the possibility of bacterial bidirectional transmission between humans and animals. It is described that the adaptation of bacteria from one host species to another usually occurs through the loss of non-functional virulence factors in the new host and the acquisition of new genetic elements (34, 35).

1.3.1. *Staphylococcus aureus*

S. aureus can be a commensal bacterium by colonizing the skin of both humans and animals (36, 37) but it can also act as a pathogen causing a wide range of serious diseases as described above. The prevalence of *S. aureus* in carriers can vary depending on the species and location. For example, the prevalence of *S. aureus* on human skin can be approximately 11 %, while skin colonization in pigs was detected at approximately 42 % (37). Another study, however, describes approximately 38 % prevalence in human nasal carriers and 39 % in cows with mastitis (26).

The strains of *S. aureus* can be divided into sequence types (STs) by Multilocus Sequence Typing (MLST), which makes it possible to analyse clonal complexes (CC). STs assigned to a CC match the central genotype at four or more loci. This applies unless they match more closely with another central genotype (<https://pubmlst.org/organisms/staphylococcus-aureus>, last accessed 17.05.2025). Various phenotypic and molecular typing methods, such as antimicrobial susceptibility testing, Multi-locus sequence typing (MLST) or whole genome sequencing (WGS), are available for the characterization of *S. aureus* (38). MLST allows assignment to CC based on STs (39). Common CCs associated mainly with humans are, CC5, CC8, CC22, CC30 or CC45 (34, 40). CC152 is also frequently associated with humans, mainly

in Africa, but sporadically also in Europe and on the Caribbean islands (41). Among other clonal complexes, one frequently CC detected in the animal kingdom is CC398, which is found in pigs, horses, poultry and cattle, among others, but also frequent in humans. This CC is quite well-studied, as it is frequently associated with methicillin-resistant *Staphylococcus aureus* (MRSA) (34).

CCs mainly associated with humans or animals differ in the expression of virulence factors, antibiotic resistance, among other traits, and thus determine the distribution of strains into CCs. To describe evolutionary changes and epidemiological relationships, the *S. aureus* genome can be divided into three parts: the core genome, the core variable genome (CV), and the mobile genetic elements (MGE). While the core genome, which makes up approximately 75 % of the genome, is essential in *S. aureus* isolates and shows minimal variation within the species, the CV genome can vary (approximately 15 % of the genome). Thus, each CC possesses a unique and conserved combination of CV genes (42, 43).

MGEs (approximately 10 % of the genome) can be exchanged between bacteria via horizontal transfer and include, for example, bacteriophages, plasmids, or staphylococcal chromosomal cassettes (SCCs), which may vary within and between CCs (42, 44). Recently, it was described that the acquisition of such MGEs often accompanies host-specific adaptation, and *S. aureus* strains are adapted to local host species as host specialists. Furthermore, it is described that the same animal CC possesses MGEs that are not present in the same human CC and vice versa (45).

Typical ruminant-associated CCs described often in goats (46, 47) as well as in sheep are CC133 and CC522 (48). In cows, the most frequently occurring CC causing bovine mastitis is CC97 (26, 49–51), while the predominant CC in poultry (including turkey and chicken) is CC5 (27, 52). Both are known to be of human origin and crossed over to animals years ago, where they have ever since become widespread (26, 53). The assignment of *S. aureus* isolates obtained from cows to typically-human CCs, especially the same clone line (both the same ST and the same *spa* type), also suggests transmission of *S. aureus* from humans to animals in close proximity (54, 55). This was also observed in horses and to a lesser extent in goats (55). As already mentioned, *S. aureus* strains can be divided into clonal complexes by MLST, WGS, DNA microarray and other methods. Isolates can be further identified and classified using typing methods such as *spa* typing. The resulting *spa* types serve to differentiate between different strains and are used in epidemiology (56). There are currently 22,334 *spa* types described, which can be freely downloaded from a public database ([Ridom SpaServer](#), last accessed 14.08.2025).

S. aureus is usually transmitted indirectly from cow to cow through milking, but humans also play a role as vectors, for example, during the milking process through poor milking hygiene such as contaminated hands of staff, milking utensils, or udders due to inadequate cleaning (57).

Genotypically indistinguishable isolates from cattle and humans advocates for the need for surveillance of *S. aureus* populations in certain areas, such as dairy farms or similar facilities where a close animal-human contact is likely to occur in order to prevent zoonotic transmission (51).

It is known that certain virulence genes and resistance genes are associated with certain CC (58, 59). Due to the close contact between humans and animals, the potential transmission of *S. aureus* strains between animals and humans, and in particular, the transmission of virulence genes present between different plays an essential role in the context of One Health.

1.3.1.1. Virulence factors and genes of *Staphylococcus aureus*

S. aureus possesses several virulence factors that determine its pathogenicity. As a coagulase-positive pathogen, it has the ability to produce coagulase, an enzyme that converts serum fibrinogen into fibrin (60). This leads to the formation of a fibrin wall that surrounds bacterial colonies, making phagocytosis more difficult (29).

Many cytolytic toxins, such as hemolysins or leukotoxins, increase pathogenicity. One of the best known toxins is alpha-toxin, which has a lytic effect on red blood cells and is involved in the destruction of focal adhesion (61).

Leukotoxins such as Pantone-Valentine leucocidin (PVL) or the Luk toxins LukED and the mainly bovine LukMF' can form pores in the cell membrane of leukocytes, especially neutrophils, and destroy them, thereby reducing host defenses. PVL also leads to tissue necrosis, which impedes the healing of the infection. In cattle with mastitis, LukMF' is one of the most potent toxins, playing a key role in faster colonization of the udder (62, 63).

There are also hyaluronidases, lipases and DNAses, which act locally and facilitate the local spread of the pathogen in the tissue. In addition, *S. aureus* can produce exogenous superantigens, which can cause food poisoning as enterotoxins or toxic shock syndrome toxin-1 (TSST-1), which stimulates the production of large amounts of cytokines, ultimately leading to shock symptoms (29).

As several reviews show, *S. aureus* is also able to secrete an extracellular polymeric substance (EPS), which is defined as a so-called biofilm (64–66). As a structured embedded bacterial community located in the depths of the biofilm, they are largely protected from the immune

system and antibiotics (29). This makes biofilm control more difficult and increases the development of antimicrobial resistance (65).

1.3.1.2. Antimicrobial resistance

In Rwanda, there is a high level of antibiotic use in cattle farming (67, 68). According to a study by Manishimwe and coworkers (68), around 97 % of respondents stated that they used antibiotics in their livestock. All respondents in the study reported using antibiotics to treat diseases, often without prescription (at up to 56 %), but also as growth promoters (~ 27 %). Such improper antibiotic administration as prophylaxis, growth promoters or non-prescribed antibiotics can lead to the development of antibiotic resistance (68).

Common resistances in dairy cows worldwide are to penicillin, erythromycin, tetracycline, oxacillin, gentamicin, trimethoprim and others (69, 70). According to a study from 2021, the highest global prevalence of antibiotic-resistant *S. aureus* in bovine mastitis was seen for penicillin, followed by clindamycin, erythromycin and gentamicin. Over a period from before 1999 to 2020, almost all antimicrobial substances studied showed an increasing trend, but clindamycin or gentamicin, for example, showed a greater increase in antimicrobial resistance (AMR) prevalence globally. Over the same period, the global AMR prevalence for cefoxitin decreased 1.63 times. Within Africa, there was high AMR of *S. aureus* to cefoxitin, clindamycin, penicillin, erythromycin, and gentamicin, among others, and is one of the countries with higher AMR of the drugs studied, according to the same study (71). Tetracycline was not considered in the study just mentioned.

According to a preliminary report, tetracycline was the most imported antibiotic in Rwanda from 2019 to 2021, followed by sulfonamides and aminoglycosides. Due to its low cost, tetracycline is widely used in livestock farming in Rwanda (72). Resistance to penicillin or tetracycline are frequently observed in Rwanda, ranging from 6.66-16 % (only tetracycline resistance), 33.33-84 % (only penicillin resistance), and 12.0-33.33 % (a combination penicillin and tetracycline) (63, 73).

The development of resistance in bacteria can be based on the formation of so-called resistance genes or through de novo mutation, such as point mutations. Antibiotic resistance genes (ARG) encode proteins that modify the metabolism or structure of the cell so that it becomes insensitive to certain antibiotics. They can be localized on non-mobilizable and mobilizable genetic elements like plasmids, integrons (In), transposons (Tn), or staphylococcal chromosomal cassette *mec* (SCC*mec*), among others. ARGs can be rapidly transferred

between bacteria – even of different genera – through ARG-carrying mobilizable elements, as mentioned above, by horizontal gene transfer (HGT). HGT can occur through intracellular conjugation (e.g., plasmids), transformation (through uptake of cellular DNA), or transduction (through bacteriophages) (74–76). Some of the most well-known ARGs of *S. aureus* in previously mentioned antibiotics and their mechanisms are described below.

Resistance of *S. aureus* to β -lactam antibiotics is due to several genes like *blaZ*, *mecA*, *mecB* or *mecC* which are characterized by different mechanisms. While resistance in *blaZ* occurs through enzymatic inactivation through the synthesis of β -lactamase, in *mecA/mecC*, which is located within the *SCCmec*, resistance occurs through target site replacement by synthesis of a new protein called PBP2a (penicillin binding protein), which appears only in resistant strains (77, 78). Additionally, beta-lactam resistance can also be caused by mutations in PBP genes. Mutations of PBP2 and PBP4 genes, which cause oxacillin resistance, occur however less frequently (77). Tetracycline resistance is encoded on genes such as *tet(K)* or *tet(L)*, which ensure the active transport of an antibiotic out of the cell through efflux pumps. Furthermore, tetracycline resistance can arise through target site protection by ribosome protection proteins, which supports the mechanism of *tet(M)*. The genes *aacA-aphD* are responsible for gentamicin resistance, which use the mechanism of enzymatic inactivation by acetyltransferase and phosphotransferase. Trimethoprim resistance may be due to genes such as *dfrA*, *dfrG*, or *dfrK*, which use the target replacement mechanism (synthesis of dihydrofolate reductase) (77, 78). The mechanisms of resistance development to macrolides such as erythromycin include target site modification (rRNA methylase), target site protection (ribosome-protecting ABC-F protein), or enzymatic inactivation (phosphotransferase or esterase). Genes involved may include *erm(A)*, *erm(B)*, *erm(C)*, *mph(C)*, or *ere(A)* (78).

1.4. Hypothesis

There is a paucity of studies on cows with mastitis and their association with *S. aureus* and characterization of this bacterium from Rwanda.

The aim of this study was to investigate the diversity of *Staphylococcus aureus* from cows with mastitis at the national level in Rwanda, i.e. all five provinces.

The hypothesis of this study was:

- The majority of *Staphylococcus aureus* isolates belong to the CCs typically found in East Africa, like CC97, CC3666, CC3591, and CC152.

2. Diversity of *Staphylococcus aureus* associated with mastitis from dairy cows in Rwanda

Journal of Global Antimicrobial Resistance 36 (2024) 326–335



Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar



Diversity of *Staphylococcus aureus* associated with mastitis from dairy cows in Rwanda



Helga Keinprecht^{a,b}, Emmanuel Irimaso^c, Adriana Cabal Rosel^d, Beatrix Stessl^e, Christophe Ntakirutimana^b, Lydia Marek^{a,b}, Otto W. Fischer^b, Michael P. Szostak^a, Jennifer Zöchbauer^a, Thomas Wittek^f, Elke Müller^{g,h}, Amelie Desvars-Larrive^{i,j}, Andrea T. Feßler^{k,l}, Sascha D. Braun^{g,h}, Stefan Schwarz^{k,l}, Joachim Spergser^a, Monika Ehling-Schulz^a, Stefan Monecke^{g,h,m}, Ralf Ehrlich^{g,h,n}, Werner Ruppitsch^d, Tom Grunert^a, Igor Lončarić^{a,*}

^a Institute of Microbiology, University of Veterinary Medicine Vienna, Vienna, Austria

^b New Vision Veterinary Hospital (NVVH) Northern Province, Musanze District, Rwanda

^c School of Veterinary Medicine- CAVM, University of Rwanda, Nyagatare Campus, Nyagatare, Rwanda, Rwanda

^d Austrian Agency for Health and Food Safety (AGES), Institute of Medical Microbiology and Hygiene, Vienna, Austria

^e Unit of Food Microbiology, Institute of Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

^f Clinical Unit of Ruminant Medicine, University of Veterinary Medicine, Vienna, Austria

^g Leibniz Institute of Photonic Technology (IPHT), Jena, Germany

^h InfectoGnostics Research Campus, Jena, Germany

ⁱ Unit of Veterinary Public Health and Epidemiology, Institute of Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

^j Complexity Science Hub Vienna, Vienna, Austria

^k Institute of Microbiology and Epizootics, Centre for Infection Medicine, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

^l Veterinary Centre for Resistance Research (TZR), School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

^m Institut für Medizinische Mikrobiologie und Hygiene, Universitätsklinik Dresden, Dresden, Germany

ⁿ Institute of Physical Chemistry, Friedrich Schiller University Jena, Jena, Germany

ARTICLE INFO

Article history:

Received 13 September 2023

Revised 24 January 2024

Accepted 25 January 2024

Available online 1 February 2024

Editor: Stefania Stefani

Keywords:

Bovine mastitis

Staphylococcus aureus

Antimicrobial resistance

DNA-microarray

Spa typing

MLST

WGS

ABSTRACT

Objectives: The objective of the present study was to examine the diversity of *Staphylococcus aureus* from mastitis milk samples of cows in Rwanda.

Methods: A total of 1080 quarter milk samples from 279 dairy cows were collected in 80 different farms from all five provinces of Rwanda. In total, 135 *S. aureus* isolates were obtained and subjected to genotyping (spa typing, DNA microarray, whole-genome sequencing (WGS)), antimicrobial susceptibility testing (AST) and phenotypic profiling by Fourier Transform Infrared (FTIR) spectroscopy (including capsular serotyping).

Results: Resistance to penicillin and/or tetracycline was most frequently observed. Ten sequence types (STs) (ST1, ST151, ST152, ST5477, ST700, ST7110, ST7983, ST7984, ST8320, ST97) belonging to seven clonal complexes (CCs) (CC1, CC130, CC152, CC3591, CC3666, CC705, CC97) were detected. The Pantone–Valentine leukocidin (PVL) genes (*lukF-PV/lukS-PV*), the bovine leukocidin genes (*lukM/lukP-P83*) and the human and bovine toxic shock syndrome toxin gene *tst-1* variants were detected. FTIR-based capsular serotyping showed CC-specific differences. Most CC97 (*cap5* allele) isolates were primarily nonencapsulated (82%), whereas isolates of CC3591 and CC3666 (*cap8* allele) were mostly encapsulated (86.4% and 57.8%, respectively). Our results underline the widespread global distribution of cattle-adapted CC97.

* Corresponding author; Igor Lončarić, Institute of Microbiology, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria.
E-mail address: igor.loncaric@vetmeduni.ac.at (I. Lončarić).

<https://doi.org/10.1016/j.jgar.2024.01.017>

2213-7165/© 2024 The Author(s). Published by Elsevier Ltd on behalf of International Society for Antimicrobial Chemotherapy. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Conclusion: The presence of CC3591 and CC3666 in bovine mastitis suggests an important role in cattle health and dairy production in Rwanda. The results of the present study support the need for a rigorous One-Health Surveillance program of the bovine–human interface.

© 2024 The Author(s). Published by Elsevier Ltd on behalf of International Society for Antimicrobial Chemotherapy.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

Bovine mastitis is one of the most prevalent diseases in dairy cattle and influences dairy production worldwide by decreasing the quantity and quality of milk [1,2]. Interaction between animals, environment, microorganisms, and physical or chemical trauma can be the origin of this multifactorial inflammatory disease. A distinction is made between clinical mastitis and subclinical mastitis (SCM). While cows with clinical mastitis show the typical symptoms of an inflammation of the udder together with changes in the composition and physical properties of their milk, cows with SCM do not present any apparent clinical symptoms or changes of their milk. However, cows suffering from SCM and those suffering from clinical mastitis have a lower milk yield [2]. The California Mastitis Test (CMT) on-site can easily detect the increased somatic cell count [3] of such animals.

Clinical mastitis and SCM can be due to *Staphylococcus aureus*, typically causing recurrent or chronic mastitis even in dairy cows from farms with good milking practices [4]. The primary route of transmission of *S. aureus* is cow-to-cow, but also via contaminated equipment used during the milking process. The prevalence of SCM in Rwanda is more than 50% [5]. Aside from reduced milk yields and increased veterinary costs, contaminating bacteria and antimicrobial residues also significantly impact the farmer's economic losses [6]. Although veterinarians and farmers in Rwanda must follow laws and regulations concerning the purchase and usage of veterinary medicine, more than 55% of the farmers reported having purchased and used over-the-counter medication, including antimicrobial agents. Most farmers in this study justified the use of antimicrobial agents to prevent disease and increase the growth of the animals [7].

Bovine mastitis is an important disease in Rwanda. Due to the historical and cultural significance of cows and milk, the economic losses caused by mastitis have even greater importance [8]. Rwanda Vision 2020, a development program recently launched by the government of Rwanda, aimed at transforming the country into a knowledge-based middle-income country by improving agriculture and livestock production [9].

In Rwanda, milk is often consumed and processed raw which increases the risk of milk as a source of diverse infections in humans [8,10,11].

A recent study by our working group characterised antimicrobial resistance, biocide resistance, and virulence-associated genes of *Staphylococcus* species associated with bovine mastitis in the Northern Province of Rwanda [12]. However, information about *S. aureus*-associated bovine mastitis at a national level is still scarce. *S. aureus* is one of the main causes of bovine mastitis worldwide [2]. The aim of the present study was to examine the diversity of *S. aureus* isolates that originated from mastitis milk samples of cows living in all five Provinces in Rwanda.

2. Materials and methods

2.1. Isolation and identification of *S. aureus*

The study was discussed, and the sampling was approved by the institutional ethics and animal welfare committee of the Re-

search Screening and Ethical Clearance Committee of the College of Agriculture, Animal Science and Veterinary Medicine, University of Rwanda (UR-CAVM, 003/2021/DRI 23.08.2021) in accordance with the Good Scientific Practice guidelines of the Rwandan national legislation.

The study was conducted from November 2021 to August 2022 at the New Vision Veterinary Hospital, Northern Province, Rwanda. A total of 1080 quarter milk samples from 279 dairy cows (mix breed) were collected at 80 different farms throughout the country. Milk samples originated from Northern Province ($n = 380$), Eastern Province ($n = 264$), Western Province ($n = 324$), Southern Province ($n = 100$), and Kigali ($n = 12$). Out of 1080 quarter milk samples, 732 were CMT-positive and were used for the isolation of *S. aureus* with the following distribution: in 55 cows, one quarter, in 61 cows, two quarters, in 45 cows, three quarters, and in 105 cows all quarters were CMT-positive. They were collected as previously described by Antók et al. [12]. The milk samples were transferred to the microbiological laboratory of New Vision Veterinary Hospital (NVVH, <https://nvvh.rw/>), where bacteriological examinations were performed. Milk samples were cultivated on blood agar (Blood Agar Base, Rapid Labs, UK) supplemented with 5% defibrinated cow blood and on mannitol salt (MS) agar (Mannitol Salt Agar, Rabid Labs, UK). Typical *S. aureus* colonies grown on blood agar and MS agar were re-cultivated on MS agar until a pure culture was obtained. *S. aureus* was afterwards identified by classical bacteriological methods, cryoconserved at $-25\text{ }^{\circ}\text{C}$, and sent to the Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria, for species identification by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik, Bremen, Germany) and further analyses.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed by agar disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) [13]. The following antimicrobial agents were tested: penicillin (PEN 10 units), cefoxitin (FOX 30 μg), ciprofloxacin (CIP 5 μg), amikacin (AMK 30 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) (Beckton Dickinson (BD); Heidelberg, Germany). *S. aureus* ATCC® 25,923 served as a quality control strain.

2.3. Molecular characterisation of *S. aureus*

DNA was extracted as previously described by Lončarić et al. [14]. Detection of antimicrobial resistance and virulence genes in all isolates was conducted using a DNA microarray-based technology (INTER-ARRAY Genotyping Kit *S. aureus*, Bad Langensalza, Germany) [15], and visualization of the results was performed as previously described [14]. The *dfcG* gene present in *S. aureus* belonging to CC152 was detected by PCR [16]. All isolates were genotyped by *spa* typing [14]. Nineteen isolates were selected for whole-genome sequencing (WGS) [14] based on their assignment to different

clonal complexes and/or because isolates of the respective CCs have been poorly characterised until now. In addition, the whole genome sequenced isolates were typed with ABRicate (v1.1.0) [17]. The genomes of the isolates subjected to WGS were deposited to the BioSample database under the SubmissionID: SUB13788009 in the NCBI database.

Fourier Transform Infrared (FTIR) spectroscopy was used to subtype all isolates phenotypically, primarily based on their surface glycostructural composition that included the determination of the capsular polysaccharide (CP) expression/capsule serotype [18,19]. The *S. aureus* Reynolds prototype strain CP5 and its corresponding mutants, Reynolds CP8 and Reynolds CP- (nonencapsulated) (REF), were employed as reference samples for FTIR spectroscopic biotyping [20].

3. Results

3.1. *S. aureus* isolates and antimicrobial susceptibility testing

A total of 135 *S. aureus* isolates were obtained from 81 different cows (Table 1, Table S1), including 47 isolates from the Northern Province (26/107 cows, 24.3%), 39 from the East Province (24/66 cows, 36.4%), 37 from the West Province (24/81 cows, 29.6%), 11 from the South Province (6/25 cows; 24.0%), and one *S. aureus* isolate from Kigali (1/3 cows; 33.3%).

All isolates were susceptible to cefoxitin. The most frequently observed resistance property was combined resistance to penicillin and tetracycline ($n = 49$, 36.3%), followed by resistance to penicillin ($n = 46$, 34.1%), and resistance to tetracycline ($n = 4$, 3.0%). Thirty-six isolates were susceptible to all antimicrobial agents tested (26.7%). Genetic resistance matched the phenotypic resistances of the respective isolates. The *bla_Z* gene was observed in penicillin-resistant isolates, whereas resistance to tetracycline was mediated by the *tet(K)* gene. In addition, the fosfomycin resistance gene *fosB* gene was detected in three isolates (Table 1). The phenotypic fosfomycin resistance was not examined due to the lack of clinical breakpoints. The *str* gene conferring resistance to streptomycin was detected in five isolates selected for WGS: one assigned to CC3591, one to CC3666 and the remaining three to the new sequence type (ST) 7984. The trimethoprim resistance gene *dhfrG* was detected in five isolates belonging to clonal complex 152 (Table 1).

3.2. Molecular characterization of isolates and FTIR-based capsule serotyping

Clonal complex (CC) 97 was the most common CC ($n = 50$, 37.0%), followed by CC3666 ($n = 45$, 33.3%), CC3591 ($n = 22$, 16.3%), CC152 ($n = 8$, 5.9%), CC1, ($n = 5$, 3.7%), CC130 and CC705 (each $n = 1$, 0.7%).

Within CC97, 11 different *spa* types were identified. The *spa* type t9432 ($n = 14$) was predominant, followed by t2421 ($n = 7$), t10103 ($n = 6$), t3992 ($n = 5$), t7753 ($n = 4$), t1236 ($n = 4$), t2112 ($n = 3$), novel *spa* type t20843 ($n = 2$), t380 ($n = 2$), t9298 ($n = 2$), and t6280 ($n = 1$). Two CC97 isolates, chosen for WGS, belonged to ST97 or the new ST8320, respectively.

The second most common CC was CC3666. Within this CC, three different *spa* types were detected: t18853 ($n = 40$), novel *spa* type t20842 ($n = 4$) and novel *spa* type t20844 ($n = 1$). Two of the whole genome sequenced isolates belonged to ST5477.

All 22 CC3591 isolates represented *spa* type t458. WGS revealed that five examined isolates belonged to the novel sequence types ST7983 ($n = 3$) or ST7110 ($n = 2$). Eight isolates belonged to CC152 and were indistinguishable by *spa* typing (t355), and four sequenced isolates represented ST152. Five isolates were assigned to CC1 and *spa* type t127. In addition, ST1 was detected in one

isolate selected for WGS. Finally, two singletons belonged to either CC705, *spa* type t529 and ST151, or CC130, *spa* type t3569 and ST700. Three sequenced isolates represented ST7984 and an independent CC (Table 1, Table S1).

DNA-based microarray analyses, as well as WGS, revealed the presence of several virulence-associated genes. Within CC97, five isolates carried the toxic shock syndrome toxin 1 gene *tst-1* (human variant). All but one CC3666 isolate harboured the toxic shock syndrome toxin 1 gene *tst-1* (bovine variant). All CC3666 isolates carried the enterotoxin gene cluster (*egc*) comprising the enterotoxin genes *sei*, *sem*, *sen* (other than RF 122), *seo*, and *seu*. In addition, three isolates carried the enterotoxin B gene (Table 1, Table S1). The presence of the bovine leukocidin genes *lukF-P83/lukM* was observed in all isolates belonging to CC3591. The Pantone-Valentine leukocidin (PVL) genes *lukF-PV/lukS-PV* were detected in 3 of 7 CC152 isolates. All five isolates assigned to CC1 carried the gene for enterotoxin H, and three also had the gene for enterotoxin B. The single CC130 isolate harboured the bovine leukocidin genes *lukF-P83/lukM*. The same genes were detected in the single CC705 isolate. In addition to the *lukF-P83/lukM* genes, this CC705 isolate also harboured the *tst-1* gene (bovine variant), the *egc* cluster, as well as the enterotoxin C and enterotoxin L genes. All isolates belonging to CC1 harboured *seh*, whereas three CC1 isolates carried additional *seb* genes coding for the staphylococcal enterotoxins H and B, respectively (Table 1, Table S1). Isolates belonging to all CCs harboured hemolysin *hla*, *hlb* and *hld* genes. Splits tree analysis of the microarray data showed clonal clustering into eight groups based on their virulence and antimicrobial resistance profile similarities (Fig. 1).

Core-genome MLST (cgMLST)-based typing, including the 19 isolates selected for WGS, was performed, and a minimum spanning tree was generated (Fig. 2). Distance calculation between all isolates revealed an allelic distance ranging between 0 and 1509 allelic differences. Based on the defined cluster threshold (CT) of 24 allelic differences, three different clusters were detected. Two clusters comprised isolates within CC152 and the other cluster isolates within CC3591.

All isolates were phenotypically subtyped for capsule polysaccharide (CP) expression using FTIR spectroscopy (Fig. 3). In total, more than half of the isolates had no capsule ($n = 75$; 55.6%), and the remaining isolates were categorised into two serotypes: serotype 8 (CP8, $n = 45$; 33.3%) and serotype 5 (CP5, $n = 12$; 8.9%). Most isolates with the *cap5* allele were nonencapsulated (78.3%), whereas isolates with *cap8* were often encapsulated CP8 (60.0%). Notably, the distribution of CP expression varied depending on the CC. CC3591 and CC3666 isolates harbouring the *cap8*-allele predominantly expressed the capsule 86.4% ($n = 22$) and 57.8% ($n = 45$), respectively. In contrast, CC97 isolates ($n = 50$) were primarily nonencapsulated (82.0%). Hierarchical cluster analysis of the FTIR spectral data revealed seven main clusters. Clusters 1 to 3 contain encapsulated isolates and can be assigned to specific CCs (Cluster 1: CC3591, CP8; Cluster 2: CC3666, CP8; Cluster 3: CC151, CP5). The two CP8-producing CCs (CC3591 and CC3666) can be clearly distinguished using FTIR spectroscopy. The remaining clusters (4 to 7) comprise mainly nonencapsulated strains, each with a mixture of various CCs, except for one cluster (cluster 7) assigned to CC97. Interestingly, the three isolates of the newly described ST7984 cluster were closely related to CC97 isolates (Fig. 3).

4. Discussion

The present study describes the characterization of *S. aureus* isolated from mastitis milk samples in Rwanda. Even though cattle play an essential role in the life of the people in Rwanda, comprehensive studies on *S. aureus*-associated mastitis considering the pheno- and genotypic characterization of the causative isolates and

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

Isolates	ID alias	Province ^a	CC ^{b,c}	ST ^{b,c,d}	spa	Antimicrobial resistance profile	Superantigens	Leucocidins
151FL, 168FR		W	CC97		t380	nr ^{***}		lukF/S, lukD/E
167FL, 167FR, 167RL, 168FL, 169FL		W	CC97		t3952	nr		lukF/S, lukD/E
188RR		W	CC97		t6280	nr		lukF/S, lukD/E
219RR		N	CC97		t7753	nr		lukF/S, lukD/E
220FR, 227FL, 227FR		W	CC97		t7753	nr		lukF/S, lukD/E
111HL	510,580–22	S	CC97	ST8320		nr	tst-1 (human)	lukF/S, lukD/E
111RR, 111FR, 115RL, 116BR		S	CC97		t9432	PEN	tst-1 (human)	lukF/S, lukD/E
128FL		E	CC97		t2421	PEN		lukF/S, lukD/E
12RRb		E	CC97		t9432	PEN		lukF/S, lukD/E
134RR		E	CC97		t9432	PEN		lukF/S, lukD/E
135RL		E	CC97		t2112	PEN		lukF/S, lukD/E
14FL		N	CC97		t1236	PEN		lukF/S, lukD/E
144FR		W	CC97		t1236	PEN		lukF/S, lukD/E
151RL, 151RR		W	CC97		t9298	PEN		lukF/S, lukD/E
84FL, 84RL, 88FL		E	CC97		t10103	PEN		lukF/S, lukD/E
123FR, 123RL, 125RR, 127FL, 127RL, 129RR		E	CC97		t2421	PEN, TET		lukF/S, lukD/E
137FL		E	CC97		t9432	PEN, TET		lukF/S, lukD/E
140FL, 142RL		E	CC97		t2112	PEN, TET		lukF/S, lukD/E
147RL, 251FR		W	CC97		t10103	PEN, TET		lukF/S, lukD/E
149FL, 149RL		W	CC97		t20843	PEN, TET		lukF/S, lukD/E
150FL, 15RR		W	CC97		t9432	PEN, TET		lukF/S, lukD/E
153RR		W	CC97		t10103	PEN, TET		lukF/S, lukD/E
191RL		N	CC97		t1236	PEN, TET		lukF/S, lukD/E
225RR		W	CC97		t1236	PEN, TET		lukF/S, lukD/E
7FL2, 7FRa		N	CC97		t9432	PEN, TET		lukF/S, lukD/E
7RL	510,581–22	N	CC97	ST97	t9432	PEN, TET		lukF/S, lukD/E
9FL1		N	CC97		t9432	PEN, TET		lukF/S, lukD/E
35FR, 15FL, 15RL		N	CC3666		t18853	nr		lukF/S, lukD
185FR, 192RR, 210FL, 12FL, 15RR, 12RRa		N	CC3666		t18853	PEN	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
186RL		N	CC3666		t20844	PEN	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
222RL		W	CC3666		t18853	PEN	egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
226RL, 254RR		W	CC3666		t18853	PEN	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
227RR	510,578–22-WH	W	CC3666	ST5477	t18853	PEN	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
65FR, 87FL		E	CC3666		t18853	PEN	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
70FL, 70FR, 72FL, 72FR		E	CC3666		t20842	PEN	tst-1 (bovine), seh, egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
85RR		E	CC3666		t18853	PEN	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
107FL, 117FL, 117FR, 117RR		S	CC3666		t18853	PEN, TET	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
136RL2		E	CC3666		t18853	PEN, TET	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
152RL, 152RR/FL		W	CC3666		t18853	PEN, TET	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD
16FR	510,577–22-WH	N	CC3666	ST5477	t18853	PEN, TET	tst-1 (bovine), egc, sei, selm, sehn (other than RF122), selo, selu	lukF/S, lukD

(continued on next page)

Table 1 (continued)

Isolates	ID alias	Province*	CC**	ST***	spa	Antimicrobial resistance profile	Superantigens	Leukocidins
207FL, 207RL, 207RR, 208RL, 50FR, 6RR, 7FL1, 8FRb, 9RR		N	CC3666	t18853	PEN, TET	bla _Z , tet(K)	<i>ist-1</i> (bovine), <i>egc</i> , <i>sei</i> , <i>selm</i> , <i>seln</i> (other than RF122), <i>selo</i> , <i>selu</i>	lukFJS, lukD
56FR, 56RR, 58FR, 65FL, 76FR, 90FL		E	CC3666	t18853	PEN, TET	bla _Z , tet(K)	<i>ist-1</i> (bovine), <i>egc</i> , <i>sei</i> , <i>selm</i> , <i>seln</i> (other than RF122), <i>selo</i> , <i>selu</i>	lukFJS, lukD
69FL		Kigali	CC3666	t18853	PEN, TET	bla _Z , tet(K)	<i>ist-1</i> (bovine), <i>egc</i> , <i>sei</i> , <i>selm</i> , <i>seln</i> (other than RF122), <i>selo</i> , <i>selu</i>	lukFJS, lukD
131FR, 133FL, 133RR, 135RR		E	CC3591	t458	nr			lukFJS, lukF-P83/lukM
150RL, 172RR		W	CC3591	t458	nr			lukFJS, lukF-P83/lukM
200FL, 207FLa		N	CC3591	t458	nr			lukFJS, lukF-P83/lukM
220FL		W	CC3591	t458	nr			lukFJS, lukF-P83/lukM
254FL		W	CC3591	ST7983	nr			lukFJS, lukF-P83/lukM
27FL		N	CC3591	ST7983	nr			lukFJS, lukF-P83/lukM
27RR, 29FL, 29RR, 7RR, 8FRa, 7FRb		N	CC3591	t458	nr			lukFJS, lukF-P83/lukM
10RL		N	CC3591	t458	nr			lukFJS, lukF-P83/lukM
6FR		N	CC3591	t458	nr			lukFJS, lukF-P83/lukM
116aFR		S	CC3591	t458	TET	ret(K)		lukFJS, lukF-P83/lukM
133RL		E	CC3591	ST7110	TET	ret(K)		lukFJS, lukF-P83/lukM
134FR		E	CC3591	ST7110	TET	ret(K)		lukFJS, lukF-P83/lukM
100FL		S	CC152	t355	PEN	bla _Z		lukF
218RL		W	CC152	t355	PEN	bla _Z		lukF
240RL		W	CC152	t355	PEN	bla _Z , <i>dfgC</i>		lukF, lukF-PV/lukS-PV
240RR		W	CC152	ST152	PEN	bla _Z , <i>dfgC</i>		lukF, lukF-PV/lukS-PV
247RL		W	CC152	t355	PEN	bla _Z , <i>dfgC</i>		lukF, lukF-PV/lukS-PV
34FL		N	CC152	ST152	PEN	bla _Z , <i>dfgC</i>		lukF, lukF-PV/lukS-PV
35RL		N	CC152	ST152	PEN	bla _Z , <i>dfgC</i>		lukF, lukF-PV/lukS-PV
248FR		W	CC152	ST152	TET	ret(K), <i>dfgC</i>		lukF, lukF-PV/lukS-PV
183FL		N	CC1	ST1	PEN	bla _Z	<i>seb</i> , <i>seh</i>	lukFJS, lukD/E
183FR, 183RL		N	CC1	t127	PEN	bla _Z	<i>seb</i> , <i>seh</i>	lukFJS, lukD/E
184FL, 188FL		N	CC1	t127	PEN	bla _Z	<i>seh</i>	lukFJS, lukD/E
136RL1		E	CC (in.) [§]	ST7984	PEN	bla _Z , tet(K), <i>str</i>		lukFJS, lukD/E
127FR/RR		E	CC (in.)	ST7984	PEN, TET	bla _Z , tet(K), <i>str</i>		lukFJS, lukD/E
76FL		E	CC (in.)	ST7984	PEN, TET	bla _Z , tet(K), <i>str</i>		lukFJS, lukD/E
211RL		N	CC130	ST700	nr			lukFJS, lukF-P83/lukM, lukD
58FL		E	CC705	ST151	nr		<i>ist-1</i> (bovine), <i>sec</i> , <i>sel</i> , <i>egc</i> , <i>seg</i> , <i>sei</i> , <i>selm</i> , <i>seln</i> , <i>seln</i> (other than RF122), <i>selo</i> , <i>selu</i>	lukFJS, lukF-P83/lukM, lukD/E

* W, Western; N, Northern; E, Eastern; S, Southern.
 ** CC, clonal complex.
 *** ST, sequence type.
 **** nr, nonresistant; PEN, penicillin; TET, tetracycline.
 § (in.), independent.

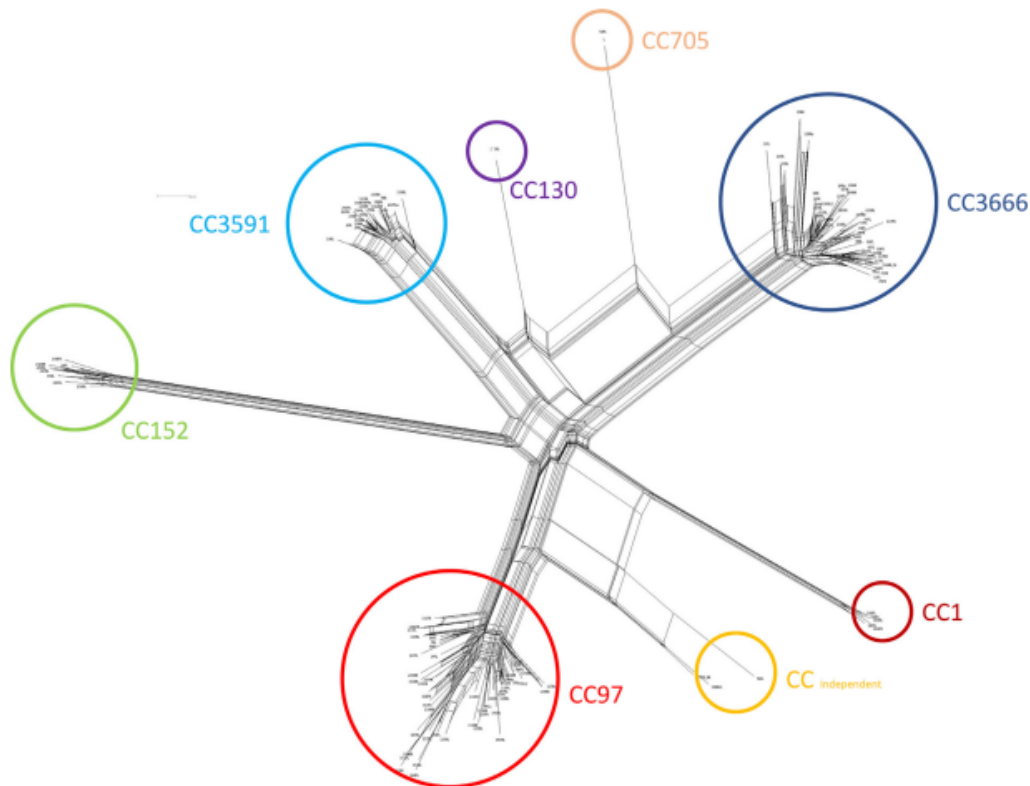


Fig. 1. SplitsTree network of *Staphylococcus aureus* isolates examined.

their antimicrobial resistance properties are still rare. In 2019, our working group (Antok et al., 2019) identified *S. aureus* belonging to four CCs (CC97, CC3591, CC3666, CC152). *S. aureus* isolates belonging to these CCs were also detected in a recent study dealing with the genetic characterization of isolates using WGS originating from dairy cows in Rwanda [21].

CC97 was found to be the predominant clonal lineage comprising 50 isolates. Humans were presumably the original host of this CC [22]. After the human-to-cattle host jump, CC97 became the most prevalent bovine *S. aureus* clone globally [22], but it is also known to switch back into humans potentially [22,23]. CC97 is strongly associated with bovine mastitis [22]. The majority of the CC97 isolates in the present study were resistant to penicillin (*blaZ*) in combination with tetracycline (*tet(K)*), which is in accordance with our previous study (Antok et al., 2019) and the recent study of Ndahetuye et al. [21]. Resistance to penicillin and/or tetracycline is also common in CC97 isolates originating from bovine mastitis in other countries [24,25]. Five CC97 isolates belonging to *spa* type t9432 harboured a human variant of the toxic shock syndrome toxin 1 gene *tst-1*. The presence of the *tst-1* gene in CC97 of bovine origin is uncommon [25,26]. The *tst-1* genes in CC97 isolates were not associated with superantigen genes, such as the staphylococcal enterotoxin C and L genes, which are part of the pathogenicity island SaPI_{bov} carrying *sec/tst-1/sel* toxin genes [27]. All CC97 isolates harboured the leukocidin genes *lukD/E*, which are prevalent in CC97 of bovine origin [24,26,28], but their role in the pathogenesis of bovine mastitis is still not entirely known [2,28]. Confirming previous studies, we could not detect capsular expression in most CC97 isolates. CC97 strains (*cap5-agrI*) are

considered to be able to survive and persist in the intracellular niche [29].

The second most prevalent clonal complex was CC3666. To the author's best knowledge, *S. aureus* belonging to CC3666 was isolated solely from bovine mastitis cases in Rwanda (Antok et al., [21]) and in the neighbouring country Tanzania [30]. As for CC97, most CC3666 isolates were resistant to penicillin or the combination of penicillin and tetracycline due to the presence of *blaZ* and *tet(K)* genes. This is in accordance with previous studies (Antok et al., [21,30]). In contrast to CC97, isolates belonging to CC3666 are much less diverse. All but five isolates belonged to the same *spa* type t18553, and amongst the remaining five, two novel *spa* types, t20842 and t20844, were observed. The main characteristic of the CC3666 isolates is that all but one isolate carried a bovine variant of the toxic shock syndrome toxin 1 gene *tst-1*, which is common amongst mastitis isolates [12,31]. In addition, all isolates harboured the enterotoxin genes *sei*, *sem*, *sen*, *seo*, and *seu*, which belonged to the *egc* cluster, and all isolates were positive for the *lukD* gene but negative for the *lukE* gene. These characteristics were observed in CC3666 from our previous study as well as those from Tanzania (Antok et al., [30]). Ndahetuye et al. [21] did not report virulence-associated genes, but after comparing their whole genome sequences, the same characteristics could be detected. Data on the prevalence of capsular expression of the clonal complexes CC3666 and CC3591 have not yet been published. We found the majority of CC3666 isolates were encapsulated. Since this clonal complex can be assigned to the *agr* type II (*agrII*), it is tempting to speculate that CC3666 isolates, like CC705, are better adapted to the extracellular niche in the udder environment

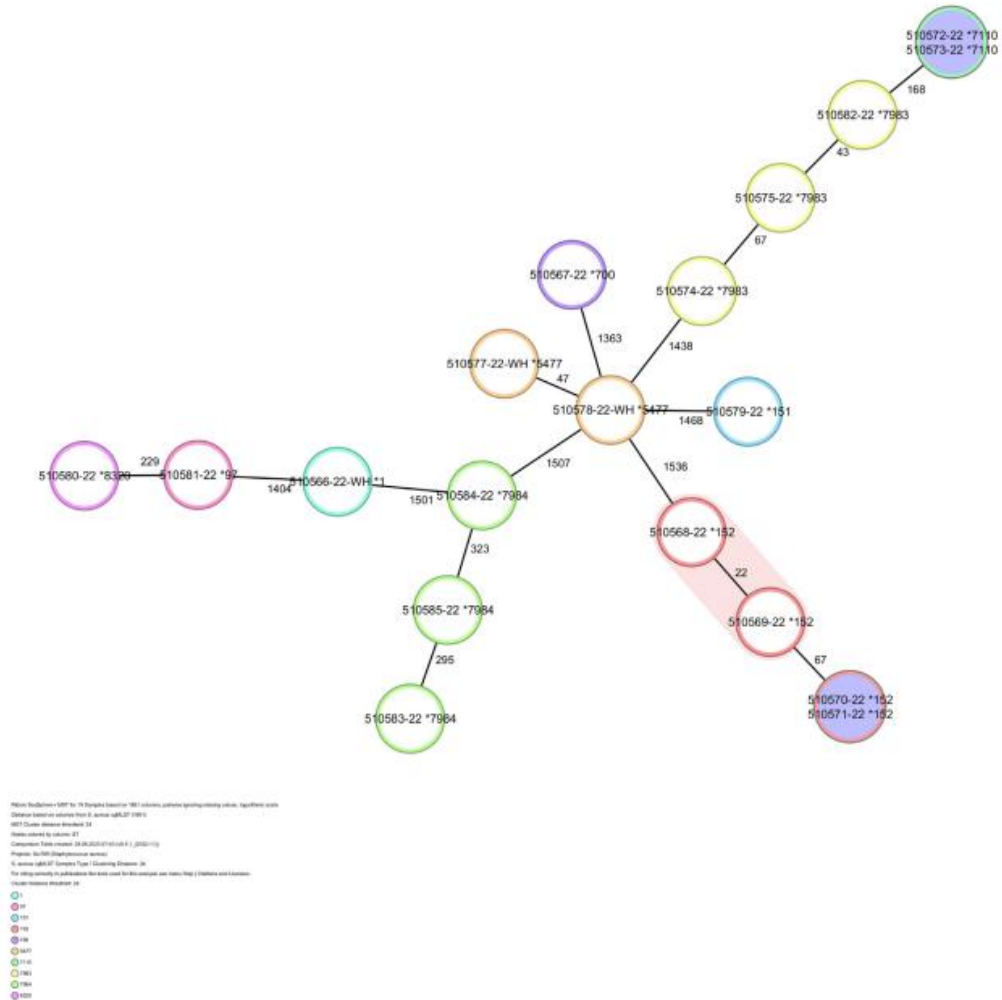


Fig. 2. Minimum spanning tree for 135 isolates based on the cgMLST of *S. aureus*. Colours correspond to the clonal complex. Each circle represents isolates with an allelic profile based on the sequence of 1861 core genome targets. Black numbers refer to the allelic differences between two isolates. Isolates with closely related genotypes were identified with a maximum of 24 allelic differences and are shaded. Each circle is marked with an alias ID and separated with "*" from the sequence type.

[29]. Since we could only detect one CC705 isolate, one might even speculate that CC3666 isolates have adopted the niche of CC705 isolates.

Due to the omnipresence of CC3666 in all provinces of Rwanda, this particular CC could emerge as an important clonal lineage associated with *S. aureus* mastitis in cattle. The same applies to *S. aureus* isolates belonging to CC3591. This clonal lineage was isolated only from bovine mastitis cases in Rwanda (Antok et al., [21]). All CC3591 isolates carried the bovine leukocidin *lukF-P83/lukM* genes, which were also observed in all analysed strains of this clonal lineage in the past (Antok et al., [21]). The *lukF-P83/lukM* genes are strongly associated with cytotoxicity to bovine neutrophils [32].

The Panton-Valentine leukocidin (PVL) genes were detected in six out of eight CC152 isolates. CC152 is a common human-associated clonal lineage from sub-Saharan Africa [33]. *S. aureus* isolates of *spa* type t355 and/or sequence type ST152 within CC152 were identified in previous studies on cows from Rwanda and Tan-

zania (Antok et al., [21,30]), but also across sub-Saharan Africa from humans [33]. We also observed the presence of the trimethoprim resistance gene *dfpG* in five PVL-positive isolates. As previously reported, in some CC152 isolates, *dfpG* is located in the PVL-encoding prophage ϕ Sa2 [33]. The existence of PVL and *sak/scn* genes also suggests a human origin.

Five isolates represented CC1. This CC1 is another human-associated clonal lineage with global distribution [22]. CC1 isolates are commonly found in livestock (including cases of bovine mastitis), companion animals, and wildlife [2,23]. Until present, CC1 bovine mastitis isolates have been observed in the Southern Province of Rwanda [21], whereas all five isolates of the present study were from the Northern Province.

The role of the three isolates belonging to new *spa* types t20974 and t21056 and the new ST7984 in the pathogenesis of bovine mastitis is currently uncertain. These were only isolates carrying the fosfomycin resistance gene *fosB*. The leukocidin *lukD/E* genes

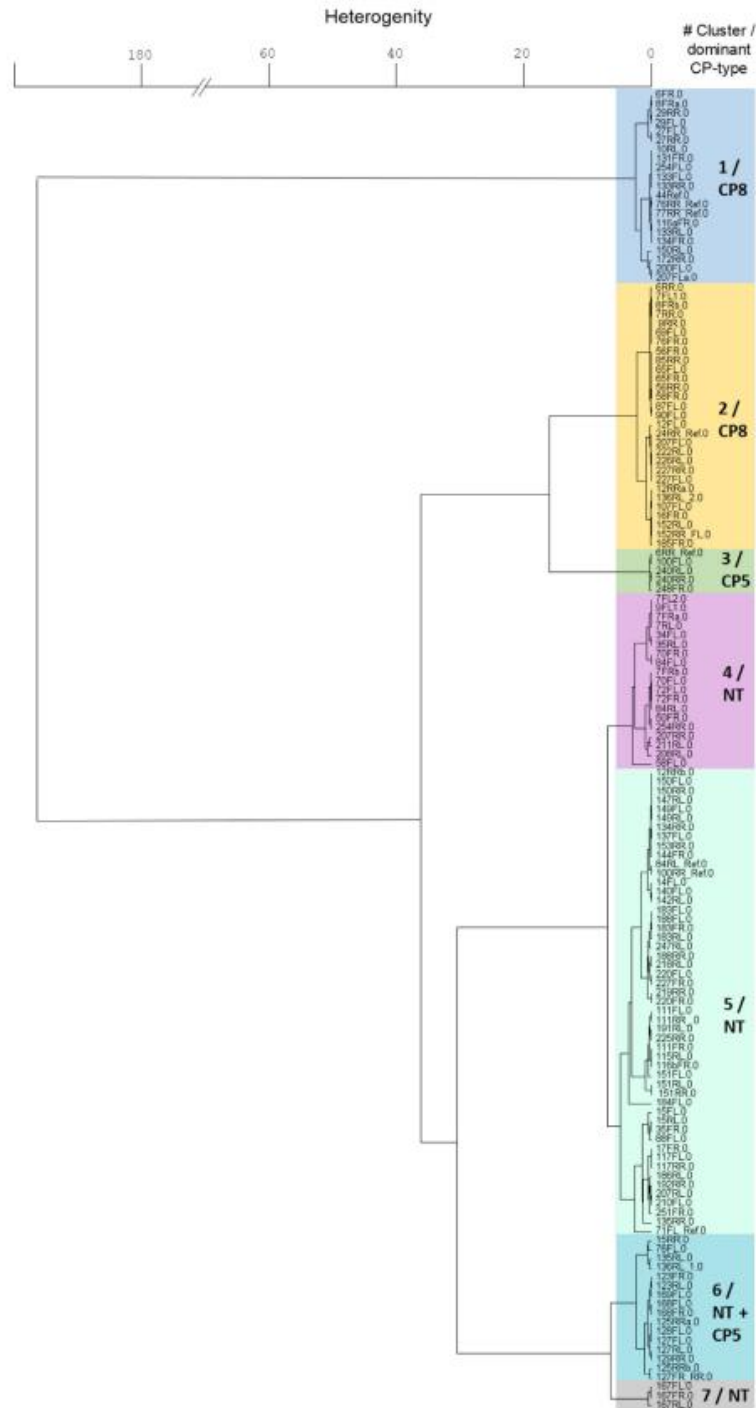


Fig. 3. FTIR spectroscopy-based dendrogram of *S. aureus* isolated from milk samples of cows with mastitis. Encapsulated: CP5 = serotype 5, CP8 = serotype 8; nonencapsulated: NT = none typeable.

were observed, but genes coding for staphylococcal enterotoxins could not be detected.

Finally, the two singletons belonging to CC130 and CC705 were observed for the first time in Rwanda in mastitis milk samples. CC130 is most likely of ovine origin with a subsequent spillover from humans into dairy cows [22]. Bovine CC130 is common in Europe and often harbours the *mecC* gene [22]. Both singletons carried *lukF-P83/lukM* genes. In addition, the worldwide common bovine CC705 was positive for the *egc* cluster, *sec* and *sel* genes, and *tst-1* (bovine variant), which may induce mammary inflammation [2].

5. Conclusion

In conclusion, a low diversity of bovine mastitis-associated *S. aureus* isolates was observed. More than 86.7% of all isolates belonged to CC97, CC3666 and CC3591. While bovine CC97 shows global dissemination, the data of the present study suggest that CC3666 and CC3591 either have emerged in Eastern Africa or, most likely, have been present there in the past but, due to paucity of data, remained unrepresented in the scientific literature. Whether these CCs will remain only in Eastern Africa or will become important global bovine lineages should be monitored, especially due to their virulence potential. The occurrence of human-associated CCs, like CC152, suggests interspecies transmission, which emphasizes the importance of hygiene measures and supports the need for a rigorous One-Health Surveillance program of the bovine–human interface. In addition, the presence of *S. aureus* isolates in milk is of public health concern in Rwanda, as *S. aureus*-positive tank and raw milk products could serve as vehicles for the bacterial transmission to humans.

Ethical approval: The study was discussed, 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, 003/2021/DRI 23.08.2021) in accordance with the Good Scientific Practice guidelines of the Rwandan national legislation.

Funding: This work was partially supported by “Veterinaer Projekt Ruanda – united vets friendship group.”

Competing interests: None to declare.

Acknowledgement: This work was partially supported by “Veterinaer Projekt Ruanda – united vets friendship group”. We are grateful to all participating farmers and the team of the NVVH in Musanze (Flora, Beatrice, Gaudiose, Grace, Josphride, Antoine, Jean Paul, Sylvie, Geraldine, Vital, Adelphine, Pierre, Aron, and Felicien). We would also like to express our thank to Anna Stöger, Tatjana Svoboda, and Michael Steinbrecher for their technical assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.01.017.

References

- Akers RM, Nickerson SC. Mastitis and its impact on structure and function in the ruminant mammary gland. *J Mammary Gland Biol Neoplasia* 2011;16:275–89. doi:10.1007/s10911-011-9231-3.
- Campos B, Pickering AC, Rocha LS, Aguilar AP, Fabres-Klein MH, de Oliveira Mendes TA, et al. Diversity and pathogenesis of *Staphylococcus aureus* from bovine mastitis: current understanding and future perspectives. *BMC Vet Res* 2022;18:115. doi:10.1186/s12917-022-03197-5.
- Sargeant JM, Leslie KE, Shirley JE, Pulkrabek BJ, Lim GH. Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. *J Dairy Sci* 2001;84:2018–24. doi:10.3168/jds.S0022-0302(01)74645-0.
- Ruegg PL. A 100-year review: mastitis detection, management, and prevention. *J Dairy Sci* 2017;100:10381–97. doi:10.3168/jds.2017-13023.
- Iraguha B, Hamudikuwanda H, Mushonga B. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *J S Afr Vet Assoc* 2015;86:1228. doi:10.4102/jsava.v86i1.1228.
- Gonçalves JL, Kamphuis C, Martins CMMR, Barreiro JR, Tomazi T, Gameiro AH, et al. Bovine subclinical mastitis reduces milk yield and economic return. *Livest Sci* 2018;210:25–32. doi:10.1016/j.livsci.2018.01.016.
- Manishimwe R, Nishimwe K, Ojok L. Assessment of antibiotic use in farm animals in Rwanda. *Trop Anim Health Prod* 2017;49:1101–6. doi:10.1007/s11250-017-1290-z.
- García SN, Mpatwenumugabo JPM, Ntampaka P, Nandi S, Cullor JS. A one health framework to advance food safety and security: an on-farm case study in the Rwandan dairy sector. *One Health* 2023;16:100531. doi:10.1016/j.onehlt.2023.100531.
- Mazimpaka E, Mbuza F, Michael T, Gatari EN, Bukonya EM, James O-A. Current status of cattle production system in Nyagatare District-Rwanda. *Trop Anim Health Prod* 2017;49:1645–56. doi:10.1007/s11250-017-1372-y.
- Kamana O, Ceuppens S, Jacxsens L, Kimonyo A, Uyttendaele M. Microbiological quality and safety assessment of the Rwandan milk and dairy chain. *J Food Prot* 2014;77:299–307. doi:10.4315/0362-028X.JFP-13-230.
- Mpatwenumugabo JP, Mukasafari MA, Ndahetuye JB, Wredle E, Båge R. A systematic literature review of milk consumption and associated bacterial zoonoses in East Africa. *J Appl Microbiol* 2023;134. doi:10.1093/jambio/ckad080.
- Antók F, Mayrhofer R, Marbach H, Masengesho JC, Keinprecht H, Nyirimbuga V, et al. Characterization of antibiotic and biocide resistance genes and virulence factors of *Staphylococcus* species associated with bovine mastitis in Rwanda. *Antibiotics (Basel)* 2019;9:1. doi:10.3390/antibiotics9010001.
- CLSI M100 performance standards for antimicrobial susceptibility testing. 32nd ed. Wayne, PA, USA: Clinical Laboratory Standard Institute; 2021.
- Loncaric I, Lepuschitz S, Ruppitsch W, Trstan A, Andreadis T, Bouchlis N, et al. Increased genetic diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from companion animals. *Vet Microbiol* 2019;235:118–26. doi:10.1016/j.vetmic.2019.05.013.
- Monecke S, Slickers P, Ehrlich R. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. *FEMS Immunol Med Microbiol* 2008;53:237–51. doi:10.1111/j.1574-695X.2008.00426.x.
- Argudin MA, Tenhagen B-A, Fetsch A, Sachsenröder J, Käsböhrer A, Schroeter A, et al. Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. *Appl Environ Microbiol* 2011;77:3052–60. doi:10.1128/AEM.02260-10.
- Seemann T. 2020. ABRicate.Github. Available online: <https://github.com/tseemann/abricate>, last accessed 26 August 2023.
- Grunert T, Wenning M, Barbagelata MS, Fricker M, Sordelli DO, Buzzola FR, et al. Rapid and reliable identification of *Staphylococcus aureus* capsular serotypes by means of artificial neural network-assisted Fourier transform infrared spectroscopy. *J Clin Microbiol* 2013;51:2261–6. doi:10.1128/JCM.00581-13.
- Jöhler S, Stephan R, Althaus D, Ehling-Schulz M, Grunert T. High-resolution subtyping of *Staphylococcus aureus* strains by means of Fourier-transform infrared spectroscopy. *Syst Appl Microbiol* 2016;39:189–94. doi:10.1016/j.syapm.2016.03.003.
- Watts A, Ke D, Wang Q, Pillay A, Nicholson-Weller A, Lee JC. *Staphylococcus aureus* strains that express serotype 5 or serotype 8 capsular polysaccharides differ in virulence. *Infect Immun* 2005;73:3502–11. doi:10.1128/IAI.73.6.3502-3511.2005.
- Ndahetuye JB, Leijon M, Båge R, Artursson K, Persson Y. Genetic characterization of *Staphylococcus aureus* from subclinical mastitis cases in dairy cows in Rwanda. *Front Vet Sci* 2021;8:751229. doi:10.3389/fvets.2021.751229.
- Yebra G, Harling-Lee JD, Lycett S, Aarestrup FM, Larsen G, Cavaco LM, et al. Multiclonal human origin and global expansion of an endemic bacterial pathogen of livestock. *Proc Natl Acad Sci U S A* 2022;119:e2211217119. doi:10.1073/pnas.2211217119.
- Matuszewska K, Murray GGR, Harrison EM, Holmes MA, Weinert LA. The evolutionary genomics of host specificity in *Staphylococcus aureus*. *Trends Microbiol* 2020;28:465–77. doi:10.1016/j.tim.2019.12.007.
- El-Ashker M, Gwida M, Monecke S, El-Gohary F, Ehrlich R, Elsayed M, et al. Antimicrobial resistance pattern and virulence profile of *S. aureus* isolated from household cattle and buffalo with mastitis in Egypt. *Vet Microbiol* 2020;240:108535. doi:10.1016/j.vetmic.2019.108535.
- Hoekstra J, Zomer AL, Rutten VPMG, Benedictus L, Stegeman A, Spaninks MP, et al. Genomic analysis of European bovine *Staphylococcus aureus* from clinical versus subclinical mastitis. *Sci Rep* 2020;10:18172. doi:10.1038/s41598-020-75179-2.
- Naushad S, Nobrega DB, Naqvi SA, Barkema HW, De Buck J. Genomic analysis of bovine *Staphylococcus aureus* isolates from milk to elucidate diversity and determine the distributions of antimicrobial and virulence genes and their association with mastitis. *mSystems* 2020;5. doi:10.1128/mSystems.00063-20.

- [27] Fitzgerald JR, Monday SR, Foster TJ, Bohach GA, Hartigan PJ, Meaney WJ, et al. Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. *J Bacteriol* 2001;183:63–70. doi:10.1128/JB.183.1.63-70.2001.
- [28] Thomas A, Chothe S, Byukusenge M, Mathews T, Pierre T, Kariyawasam S, et al. Prevalence and distribution of multilocus sequence types of *Staphylococcus aureus* isolated from bulk tank milk and cows with mastitis in Pennsylvania. *PLoS One* 2021;16:e0248528. doi:10.1371/journal.pone.0248528.
- [29] Bardiau M, Caplin J, Detilleux J, Graber H, Moroni P, Taminiau B, et al. Existence of two groups of *Staphylococcus aureus* strains isolated from bovine mastitis based on biofilm formation, intracellular survival, capsular profile and agr-typing. *Vet Microbiol* 2016;185:1–6. doi:10.1016/j.vetmic.2016.01.003.
- [30] Mzee T, Kumburu H, Kazimoto T, Leekitcharoenphon P, van Zwetselaar M, Masalu R, et al. Molecular characterization of *Staphylococcus aureus* isolated from raw milk and humans in eastern tanzania: genetic diversity and inter-host transmission. *Microorganisms* 2023;11:1505. doi:10.3390/microorganisms11061505.
- [31] Schlotter K, Ehricht R, Hotzel H, Monecke S, Pfeffer M, Donat K. Leukocidin genes lukF-P83 and lukM are associated with *Staphylococcus aureus* clonal complexes 151, 479 and 133 isolated from bovine udder infections in Thuringia, Germany. *Vet Res* 2012;43:42. doi:10.1186/1297-9716-43-42.
- [32] Vrieling M, Koymans KJ, Heesterbeek DAC, Aerts PC, Rutten VPMG, de Haas CJ, et al. Bovine *Staphylococcus aureus* secretes the leukocidin LukMF to kill migrating neutrophils through CCR1. *mBio* 2015;6:e00335. doi:10.1128/mBio.00335-15.
- [33] Baig S, Rhod Larsen A, Martins Simões P, Laurent F, Johannesen TB, Lilje B, et al. Evolution and population dynamics of clonal complex 152 community-associated methicillin-resistant *Staphylococcus aureus*. *mSphere* 2020;5. doi:10.1128/mSphere.00226-20.

3. Discussion

There are few studies from Rwanda describing the diversity of *S. aureus* in relation to mastitis in dairy cows and also few studies on antibiotic resistance in *S. aureus* in the country. The *S. aureus* isolates obtained here were characterized by phenotyping and genotyping methods. The present work was conducted to obtain results that extend or compare findings from previously conducted studies in Rwanda by examining samples from all provinces of Rwanda. A typically occurring CC in *S. aureus*-associated mastitis in cattle described worldwide in Algeria, Brazil, Europe, Japan and the USA is CC97 (26, 79–81). This was consistent with the results of our study, in which 37 % of isolates belonged to CC97, followed by CC3666 (33 %) and CC3591 (16 %). Nevertheless, CC97 also appears to be one of the predominant clonal complexes in apparently healthy cattle from which samples were taken in their studies (skin swab, nasal swab, milk) (82–84). But there are only a few studies on healthy cows and further investigations are necessary. CC97 is also occasionally described in humans (skin infection, pus, nasal carriage), especially when there is close contact between humans and cattle (26, 85), and this is also reflected in East Africa (86).

In the present study, 11 different *spa* types were found within CC97, with the most common *spa* type t9432, followed by t2421, t10103, and t3992, which is much more diverse than CC3666. Within this CC, two isolates were examined by WGS and could be assigned to ST97 and a new ST8320. The study by Antók et al. (87) describes the *spa* type t1236 in mastitis cows as predominant within ST97 and associated with CC97, while in the present study its occurrence is rather low. While the isolates examined by Antók et al. (87) originated from northern Rwanda and Kigali, the present study examined isolates from all provinces of Rwanda. Regarding the geographical distribution of the *spa* types, t1236 was also found in the present study, as in the previously mentioned study, in northern Rwanda, but also in the west. This could indicate a geographical distribution of the *spa* types but requires further investigation. It has been described that intramammary infections (IMI) can occur more frequently depending on the season, herd, and location, especially in spring and winter (15). This could also be reasons for the seasonal and geographical occurrence of certain *spa* types, depending on time and location of sampling. However, this is purely speculative and requires further investigation, especially since the northern isolates belonging to CC97 in the present study represent only a fraction of the samples collected from northern Rwanda, compared to Antók et al. (87). Similar to a study on the geographical and seasonal distribution of antibiotic patterns in africa, which found significant regional and seasonal differences (88), further

studies on *spa* types could also look like this, and investigations are necessary. Furthermore, the same *spa* types (t10103, t380, and t9432) are described by Antók et al. (87) as in the present study. *Spa* type t18853 was first described by Antók et al. in Rwanda (87) and was also found in the current study. In CC97 the resistance gene *blaZ* mediated resistance to penicillin was detected in most of the examined isolates and is often found in combination with *tet(K)*. This is also confirmed by previous studies from this region (73, 86, 87). The *blaZ* gene is a common cause of penicillin resistance in *S. aureus* infections worldwide. This can also be observed in cows with mastitis, including within CC97, where penicillin resistance is frequently found (51, 69, 82, 89). It has even been detected in apparently healthy cows and humans (82). CC3666 and CC3591, which were found in the present study, have so far only been described in Rwanda and partly also in neighboring countries, as for example the study by Antók et al shows with a more frequent occurrence of both CCs (87). A higher presence of CC3666 was also described in another study of cows with subclinical mastitis from Rwanda with around 17 % (73), as well as in some milk samples from cows from neighboring Tanzania (~13 %) (86). It appears that CC3666 is currently only present in these geographical areas and is restricted to livestock, particularly cattle but now also in goats (63). In the present study, all but five isolates assigned to CC3666 exhibit the *spa* type t18853, which was also detected by Antók et al (87). The remaining five isolates exhibit new *spa* types t20842 and t20844. Compared to CC97, CC3666 is far less diverse. ARGs were found in all isolates of this CC, either as the sole resistance gene *blaZ* or in combination with *tet(K)*, which are associated with penicillin and tetracycline resistance. These results are consistent with other studies from Rwanda (73, 86, 87). Furthermore, in the present study, the bovine superantigen *tsst-1* was found in all isolates except one commonly found in CC3666. This gene is widespread among mastitis isolates, as studies show (87, 90). All isolates from this CC were positive for the leukocidin gene *lukD* but negative for *lukE*. This is also reflected in a previous study from Rwanda (87). Another study describes the presence of sigma factor B (SigB) within CC3666, among others, which plays an important role as a stress regulator in *S. aureus* and may enable better survival in the bovine mammary gland in the case of SigB deficiency, but further studies are required (91).

All isolates from mastitis cows that could be assigned to CC3591 in the present study belonged to *spa* type t458, despite having different STs (ST7983, ST7110) and they were therefore less diverse. Isolates which belong to CC3591 have been so far only described in cows with mastitis in Rwanda (73, 87) and, like CC3666, it could evolve into a mastitis-specific CC in cows in this region. It can be observed that all CC3591 isolates in the present study contain the bovine

lukF-P83/lukM genes, which attacks bovine neutrophils and thus promotes the pathogenesis of mastitis (90). These genes have already been observed in previously analyzed strains from Rwanda (87).

Furthermore, CC152 and CC5477 are described as common in East Africa (86). While in this study isolates belonging to clonal complex 152 were found only sporadically (6 %) in mastitis milk from Rwanda, another study describes the occurrence in Rwanda as the most frequent CC with about 32 % (73). Like the current study, the study by Antók et al. also showed a rather low prevalence of CC152 in cows with mastitis (87). Further investigations are necessary, as the study by Ndahetuye et al. (73) examined only a fraction of the isolates compared to the current study. In Africa, there is an increasing tendency for human-derived *S. aureus* strains to belong to the human clonal complex 152 (92), and in recent years it has become one of the most important clonal lineages in human medicine (93). CC152 is described as a typically African complex in humans associated with *S. aureus* (94, 95). This CC exhibits higher resistance to trimethoprim, which could be due to a specific gene. It has been described that *dfrG* can occur independently of a genetic element or prophage, but it can also occur in the PVL-encoding prophage ϕ Sa2 (41). In this study, the trimethoprim resistance gene *dfrG* was also detected exclusively in isolates belonging to CC152 with the *spa* type t355. As other studies from East Africa also describe, this resistance gene is common in ST in CC152 (73, 86). According to the study by Mzee et al. (86), *dfrG* was also detected in CC121 with *spa* type t272 in humans without contact with animals. Another important finding in the present study is that Panton-Valentine leucocidin (PVL) genes were detected in six of the eight isolates that could be assigned to CC152. As mentioned above, CC152 is widely distributed among human isolates worldwide, but especially in Africa. CC152 is thought to have originated from a PVL-positive ancestor, which may explain the frequent association of PVL genes with CC152 (41). The emergence of cattle isolates in Africa that can be attributed to CC152 supports human-to-animal transmission (73, 82).

Other clonal complexes rarely observed in the present study were CC1, CC130 and CC705. The CC1 found in this study (4 %) has been detected in other studies primarily in human isolates worldwide (80, 93, 96). However, some other studies also describe a worldwide occurrence in cattle (38, 49, 81, 89). In Africa, CC1 is primarily detected in human isolates (92, 93), but has also been detected in dwarf goats in Nigeria (47) and cattle in Mozambique (97). According to Boss et al. (80), CC130 and CC705 originate from a common ruminant lineage, which in turn probably originated from a human ancestor. While CC705 has adapted to cattle, CC130 is considered to be adapted to sheep (80). However, Shepherd et al. (98) and Campos

et al. (81) contradict this statement, as CC130 has also been found to occur in cattle (81, 98) or also appears to be common in wild animals such as lagomorphs and hedgehogs as further studies show (99, 100). In the present study, this clone lineage was detected among 1 % of the isolates. CC705 occurs sporadically in bovine isolates worldwide (e.g. China, Germany, Italy, Switzerland) (81), which is also reflected in this study with 1 %.

In comparison, according to a study by Boss et al. (80), 80 % of mastitis cases in Europe are attributable to only six different clonal complexes (CC8, CC71, CC97, CC133, CC479, CC705) with CC8, CC705 and CC97 being the largest occurring complexes. The remaining 20 % are to be considered as isolated cases and negligible (80). A more recent study by Nemati et al. (101) also describes similar results with a frequent occurrence of CC8, CC705 and CC97 in Europe. Furthermore, CC151 and CC398 are described as quite common in Europe (89).

The occurrence of typical European clonal lines in Africa, such as CC705, could have developed from the import of European cattle breeds to Africa during various projects. This is purely conjectural and has not yet been investigated in detail.

As already mentioned in the introduction, CC8 is one of the typical human clonal complexes. As one of the most frequent clonal complexes in mastitis cattle in Europe, CC8 is described as having originated from a human CC8 ancestor. This also supports a human to animal transmission (80). This bovine-adapted CC8 (CC8bov) is frequently found in Alpine regions in Austria, for example. It has been detected primarily in cattle kept by different farms in communally used areas, where transmission from cow to cow occurs, causing the spread of CC8bov. This makes monitoring necessary to minimize spread among animals and ultimately to significantly reduce transmission from animals to humans (102). CC8 has also been described as a typical of humans in Africa and has occasionally been described in goats of that continent (47).

In the present study, bovine mastitis-associated *S. aureus* isolates from all five provinces of Rwanda were examined. In addition to new STs and *spa* types found, the results demonstrate a high resistance and pathogenicity due to various virulence factors, similar to previous studies. However, there are still few studies on mastitis in dairy cows from East Africa, especially Rwanda, and further investigations are necessary. As a pathogen containing mobile genetic elements, it is highly adaptable and, if inadequately monitored, can spread across species and become a medical or economic problem, especially in developing countries.

4. Summary

Staphylococcus aureus is a predominant mastitis pathogen in cows worldwide. Subclinical to clinical and acute to chronic forms are possible and directly impact the welfare and milk yield of animals as well as the quality of the milk. Frequent, too rapid and often prophylactic use of antibiotics, poorer veterinary care, especially in rural areas, and the connection with a lack of hygiene measures, for example during milking, favor the occurrence of mastitis in dairy cows. Frequent consequences are the development of resistance, chronic mastitis, a decline in performance, and a negative impact on human health. A previously conducted study by Antók et al. (2019) focused on the characterization of *Staphylococcus* species limited to the northern province of Rwanda and Kigali. In general, little information is available on *Staphylococcus aureus*-associated mastitis in cattle from sub-Saharan Africa. The aim of the current study is to investigate the diversity of *Staphylococcus aureus* from cows with mastitis at the national level of Rwanda, i.e. all five provinces.

A total of 1080 milk samples were collected from different farms across Rwanda. One hundred and thirty-five *Staphylococcus aureus* isolates were obtained and characterized by antimicrobial susceptibility testing and *spa* typing. In addition, the presence of the trimethoprim resistance gene *dfpG* was tested using PCR. Seven clonal complexes were detected, which are CC97, CC3666, CC3591, CC152, CC1, CC130 and CC705. While CC97 was frequently found globally in both mastitis-affected and healthy cows, CC3666 and CC3591 were found exclusively in Rwanda and partly also in neighboring countries. As a complex typically found in humans in Africa, CC152 is becoming increasingly important in these countries, especially since it is also frequently found in bovine isolates. Most of the isolated strains were assigned to CC97, followed by CC3666 and CC3591. Nineteen different *spa* types were detected. Within CC97, the most common *spa* type was t9432. Within CC3666, t18853 was identified as the most common *spa* type, while CC3591 harboured t458. Strains belonging to CC152 presented the *spa* type t355 and had the *dfpG* resistance gene.

In the present study, isolates associated with bovine mastitis were investigated in all five provinces of Rwanda and the findings support previous studies. Pathogenic *Staphylococcus* spp. strains in dairy cows able to cause mastitis has far-reaching consequences for the entire dairy industry in Rwanda, including the population, and plays a crucial role in One Health. An understanding of this disease, the antibiotic use and hygiene practices must be improved, and long-term success can be achieved with appropriate medical care and monitoring strategies.

5. Zusammenfassung

Staphylococcus aureus ist ein weltweit vorherrschender Mastitiserreger bei Kühen. Subklinische bis klinische über akute bis chronische Ausprägungsformen sind möglich und haben einen direkten Einfluss auf das Wohlergehen und die Milchleistung von Tieren sowie auf die Qualität der Milch. Eine häufige, zu schnelle, oft prophylaktische Verwendung von Antibiotika, schlechtere tierärztliche Versorgung besonders in ländlichen Gegenden und der Zusammenhang mit mangelnden Hygienemaßnahmen beispielsweise beim Melkvorgang begünstigen das Auftreten von Mastitiden bei Milchkühen. Häufige Folgen sind Resistenzbildung, chronische Mastitiden, Leistungsrückgang aber auch negativer Impact auf die Gesundheit des Menschen sind zu verzeichnen. Eine bereits zuvor durchgeführte Studie von Antok et al. (2019) beschäftigte sich mit der Charakterisierung von *Staphylococcus*-Arten auf die nördliche Provinz Ruandas und Kigali beschränkt. Generell sind nur wenige Informationen *Staphylococcus aureus* assoziierter Mastitis bei Rindern aus Subsahara-Afrika vorhanden. Ziel der aktuellen Studie ist es, die Diversität von *Staphylococcus aureus* von Kühen mit Mastitis auf nationaler Ebene Ruandas, das heißt aller fünf Provinzen zu untersuchen.

Insgesamt wurden 1080 Milchproben aus verschiedenen Betrieben in Ruanda entnommen. Es wurden 135 *Staphylococcus aureus*-Isolate gewonnen und durch Prüfung der Empfindlichkeit gegenüber antimikrobiellen Mitteln und *spa*-Typisierung charakterisiert. Darüber hinaus wurde das Vorhandensein des Trimethoprim-Resistenzgens *dfrG* mittels PCR getestet.

Es konnten sieben klonale Komplexe nachgewiesen werden, welche CC97, CC3666, CC3591, CC152, CC1, CC130 und CC705 sind. Während CC97 weltweit häufig sowohl bei mastitiskranken als auch bei gesunden Kühen gefunden wurde, wurden CC3666 und CC3591 ausschließlich in Ruanda und teilweise auch in den Nachbarländern nachgewiesen. Als typisch für Menschen in Afrika vorkommender Komplex gewinnt CC152 in diesen Ländern zunehmend an Bedeutung, insbesondere da er auch häufig in Rinderisolaten vorkommt. Die Mehrheit der untersuchten Stämme sind CC97 zuzuordnen, gefolgt von CC3666 und CC3591. Neunzehn verschiedene *spa*-Typen wurden nachgewiesen. Innerhalb des CC97 ist der häufigste *spa*-Typ t9432. Innerhalb des CC3666 wurde t18853 als häufigster *spa*-Typ identifiziert, während CC3591 ausschließlich t458 beinhaltet. Dem CC152 zugehörige Stämme beinhalten alle den *spa*-Typ t355 und weisen das *dfrG* Resistenzgen auf.

Im Rahmen dieser Studie wurden in allen fünf Provinzen Ruandas Isolate untersucht, die mit Rindermastitis in Verbindung stehen und die Erkenntnisse stützen bisherige Studien. Der

Nachweis von pathogenen *Staphylokokkus* Stämmen in Milchkühen, welche Mastitis verursachen können, hat weitreichende Folgen für die gesamte Milchwirtschaft in Ruanda einschließlich der Bevölkerung und spielt im Rahmen von One Health eine entscheidende Bedeutung. Ein Verständnis für Krankheit, Antibiotikaeinsatz und Hygiene muss geschaffen werden und mit geeigneter medizinischer Versorgung sowie Überwachungsstrategien können langfristige Erfolge erzielt werden.

6. List of abbreviations

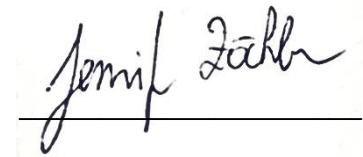
AMR:	antimicrobial resistance
ARG:	antibiotic resistance gene
CC:	clonal complex
CoNS:	coagulase-negative <i>Staphylococci</i> spp.
CoPS:	coagulase-positive <i>Staphylococci</i> spp.
E.:	<i>Escherichia</i>
EPS:	extracellular polymere substance
HGT:	horizontal gene transfer
IMI:	intramammary infection
In:	integron
MLST:	multi-locus sequence typing
MRSA:	methicillin-resistant <i>Staphylococcus aureus</i>
NAS:	non- <i>aureus</i> staphylococci
NDS:	National Dairy Strategy
PVL:	Panton-Valentine leucocidin
RDDP:	Rwanda Dairy Development Project
S.:	<i>Staphylococcus</i>
Sc.:	<i>Streptococcus</i>
SCC <i>mec</i> :	staphylococcal chromosomal cassette <i>mec</i>
SigB:	sigma-factor-B
ST:	sequence type
Tn:	transposon
TSST-1:	toxic shock syndrom toxin-1
WGS:	whole-genome sequencing

7. Eigenständigkeitserklärung

Hiermit erkläre ich, dass die entscheidenden Arbeiten selbst durchgeführt wurden und keine andere als die gelistete Literatur verwendet wurde. Die zur Beurteilung bereitgestellte Diplomarbeit wurde eigenständig verfasst. Die Diplomarbeit wurde an keiner anderen Stelle eingereicht oder veröffentlicht, außer der Einreichung und Veröffentlichung des Papers, welches Teil der Diplomarbeit ist.

Rabenstein/Pielach, 14.10.2025

Ort, Datum

A handwritten signature in black ink, reading "Jennifer Zöchbauer", written over a horizontal line.

Jennifer Zöchbauer

8. References

1. Vries A de, Kaylegian KE, Dahl GE. MILK Symposium review: Improving the productivity, quality, and safety of milk in Rwanda and Nepal. *Journal of Dairy Science* 2020; 103(11):9758–73. Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S0022030220307815>.
2. Adesogan AT, Havelaar AH, McKune SL, Eilittä M, Dahl GE. Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters. *Global Food Security* 2020; 25:100325. doi: 10.1016/j.gfs.2019.100325.
3. Mazimpaka E, Mbuza F, Michael T, Gatari EN, Bukonya EM, James O-A. Current status of cattle production system in Nyagatare District-Rwanda. *Trop Anim Health Prod* 2017; 49(8):1645–56. Verfügbar unter: <https://link.springer.com/article/10.1007/s11250-017-1372-y>.
4. MINAGRI - Ministry of Agriculture and animal Resources. ANNUAL REPORT 2022/23 2023 [Stand: 13.04.2024]. Verfügbar unter: <https://www.minagri.gov.rw/index.php?eID=dumpFile&t=f&f=87019&token=4b614ef2bca23247b616cacb693bbdcd909e9e33>.
5. Arcese R. IFAD. Rwanda Dairy Development Project: Detailed Design Report. 2016.
6. Kibibi V, Nkechi IE. Analysis of Daily Production and Development Status in Rwanda. *International Journal of Engineering Research & Technology (IJERT)* 2021.
7. Hahirwa JG, Karinganire C. Exploring the success and challenges of the Girinka¹ programme and the need for social work involvement: Southern Province, Rwanda. *RWJour* 2017; 4(1):121. Verfügbar unter: <https://www.ajol.info/index.php/rj/article/view/163306>.
8. Military ON, Mbonyinkebe D, Lebailly P. Impact of social protection programs on economic resilience of poor households in eastern province of Rwanda 2013 [Stand: 17.04.2024]. Verfügbar unter: http://www2.agrosym.rs.ba/agrosym/agrosym_2013/documents/6rd/rd18.pdf.
9. MINISTRY OF AGRICULTURE AND ANIMAL RESOURCES (MINAGRI). National Dairy Strategy; 2013 [Stand: 17.04.2024].
10. Lukuyu BA, Kitalyi A, Franzel S, Duncan A and Baltenweck I. Constraints and options to enhancing production of high quality feeds in dairy production in Kenya, Uganda and Rwanda [ICRAF Working Paper no. 95] 2009 [Stand: 13.04.2024]. Verfügbar unter: <https://cgspace.cgiar.org/server/api/core/bitstreams/6b8b7484-079d-4de4-bf5d-7acd5da1d14d/content>.
11. Claire D`A H, Cyprian E, Jules M, Mupenzi M, Felix N, Paul Ampon W. Livestock Farming and Management: The Case of Meat Production and Processing in Rwanda. *Asian J. of Animal Sciences* 2017; 11(2):96–107. doi: 10.3923/ajas.2017.96.107.
12. Kim J, Hanotte O, Mwai OA, Dessie T, Bashir S, Diallo B et al. The genome landscape of indigenous African cattle. *Genome Biol* 2017; 18(1):34 [Stand: 26.04.2024]. Verfügbar unter: <https://link.springer.com/content/pdf/10.1186/s13059-017-1153-y.pdf>.

13. Wurzinger M, Ndumu D, Baumung R, Drucker A, Okeyo AM, Semambo DK et al. Comparison of production systems and selection criteria of Ankole cattle by breeders in Burundi, Rwanda, Tanzania and Uganda. *Trop Anim Health Prod* 2006; 38(7-8):571–81.
Verfügbar unter: <https://link.springer.com/article/10.1007/s11250-006-4426-0>.
14. Argent J, Augsburg B, Rasul I. Livestock asset transfers with and without training: Evidence from Rwanda. *Journal of Economic Behavior & Organization* 2014; 108:19-39. doi: 10.1016/j.jebo.2014.07.008.
15. Sadoon AS. Clinical and subclinical mastitis in buffaloe in Mosul area, Iraq. *IJVS* 2021; 36(1):177-86.
Verfügbar unter: https://www.vetmedmosul.com/article_169998_13578.html.
16. Karimuribo ED, Fitzpatrick JL, Bell CE, Swai ES, Kambarage DM, Ogden NH et al. Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: risk, intervention and knowledge transfer. *Preventive Veterinary Medicine* 2006; 74(1):84-98. doi: 10.1016/j.prevetmed.2006.01.009.
17. Ashraf A, Imran M. Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of bovine mastitis. *Animal Health Research Reviews* 2020; 21(1):36–49.
Verfügbar unter: <https://www.cambridge.org/core/journals/animal-health-research-reviews/article/causes-types-etiological-agents-prevalence-diagnosis-treatment-prevention-effects-on-human-health-and-future-aspects-of-bovine-mastitis/A0F2F51966BD0776B87E405A6E3A54A0>.
18. Iraguha B, Hamudikuwanda H, Mushonga B. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *J S Afr Vet Assoc* 2015; 86(1):1228. doi: 10.4102/jsava.v86i1.1228.
19. Ndahetuye JB, Persson Y, Nyman A-K, Tukei M, Ongol MP, Båge R. Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda. *Trop Anim Health Prod* 2019; 51(7):2037–44. doi: 10.1007/s11250-019-01905-2.
20. Kamana O, Jacxsens L, Kimonyo A, Uyttendaele M. A survey on hygienic practices and their impact on the microbiological quality and safety in the Rwandan milk and dairy chain. *Int J of Dairy Tech* 2017; 70(1):52–67.
Verfügbar unter: <https://onlinelibrary.wiley.com/doi/10.1111/1471-0307.12322>.
21. Sinha MK, Thombare NN, Mondal B. Subclinical mastitis in dairy animals: incidence, economics, and predisposing factors. *ScientificWorldJournal* 2014; 2014:523984.
Verfügbar unter: <https://www.hindawi.com/journals/tswj/2014/523984/>.
22. Heikkilä A-M, Liski E, Pyörälä S, Taponen S. Pathogen-specific production losses in bovine mastitis. *Journal of Dairy Science* 2018; 101(10):9493–504.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S0022030218307525>.
23. Tesfaye GY, Regassa FG, Kelay B. Milk yield and associated economic losses in quarters with subclinical mastitis due to *Staphylococcus aureus* in Ethiopian crossbred dairy cows. *Trop Anim Health Prod* 2010; 42(5):925–31.
Verfügbar unter: <https://link.springer.com/article/10.1007/s11250-009-9509-2>.

24. Clegg J, Soldaini E, McLoughlin RM, Rittenhouse S, Bagnoli F, Phogat S. Staphylococcus aureus Vaccine Research and Development: The Past, Present and Future, Including Novel Therapeutic Strategies. *Front. Immunol.* 2021; 12:705360.
Verfügbar unter: <https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.705360/full>.
25. Löffler B, Hussain M, Grundmeier M, Brück M, Holzinger D, Varga G et al. Staphylococcus aureus panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLOS Pathogens* 2010; 6(1):e1000715.
Verfügbar unter: <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1000715>.
26. Akkou M, Bouchiat C, Antri K, Bes M, Tristan A, Dauwalder O et al. New host shift from human to cows within Staphylococcus aureus involved in bovine mastitis and nasal carriage of animal's caretakers. *Veterinary Microbiology* 2018; 223:173–80. doi: 10.1016/j.vetmic.2018.08.003.
27. Bystroń J, Podkowik M, Piasecki T, Wieliczko A, Molenda J, Bania J. Genotypes and enterotoxin gene content of *S. aureus* isolates from poultry. *Veterinary Microbiology* 2010; 144(3-4):498–501. doi: 10.1016/j.vetmic.2010.01.029.
28. Nemeghaire S, Argudín MA, Feßler AT, Hauschild T, Schwarz S, Butaye P. The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. *Veterinary Microbiology* 2014; 171(3-4):342–56. doi: 10.1016/j.vetmic.2014.02.005.
29. Kayser FH, Böttinger EC, Zinkernagel RM, Haller O, Eckert J, Deplazes P. *Medizinische Mikrobiologie: Taschenlehrbuch*. 12. Auflage. Stuttgart: Thieme Verlag; 2010.
30. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev* 2014; 27(4):870–926. doi: 10.1128/CMR.00109-13.
31. Foster T. *Staphylococcus* [Chapter 12]. In: Foster T, Hrsg. *Medical Microbiology*. 4th edition: University of Texas Medical Branch at Galveston; 1996
Verfügbar unter: <https://www.ncbi.nlm.nih.gov/books/NBK8448/?report=reader>.
32. Shehab El-Din EMR, El-Sokkary MMA, Bassiouny MR, Hassan R. Epidemiology of Neonatal Sepsis and Implicated Pathogens: A Study from Egypt. *BioMed Research International* 2015; 2015:509484 [Stand: 25.05.2024].
Verfügbar unter: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4471255/pdf/BMRI2015-509484.pdf>.
33. Sukur H, Esendal OM. Presence and antimicrobial resistance of coagulase-negative staphylococci isolated from animals in a Veterinary Teaching Hospital in Cyprus. *Vet. Med.* 2020; 65(5):191–8 [Stand: 20.06.2024].
Verfügbar unter: <https://www.agriculturejournals.cz/pdfs/vet/2020/05/01.pdf>.
34. Aires-de-Sousa M. Methicillin-resistant *Staphylococcus aureus* among animals: current overview. *Clin Microbiol Infect* 2017; 23(6):373–80.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S1198743X16305572>.

35. Guinane CM, Ben Zakour NL, Tormo-Mas MA, Weinert LA, Lowder BV, Cartwright RA et al. Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. *Genome Biol Evol* 2010; 2:454–66.
Verfügbar unter: <https://academic.oup.com/gbe/article/doi/10.1093/gbe/evq031/573716>.
36. Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A et al. Determinants of *Staphylococcus aureus* nasal carriage. *Clin Diagn Lab Immunol* 2001; 8(6):1064–9. doi: 10.1128/CDLI.8.6.1064-1069.2001.
37. Nagase N, Sasaki A, Yamashita K, Shimizu A, Wakita Y, Kitai S et al. Isolation and Species Distribution of *Staphylococci* from Animal and Human Skin. *J. Vet. Med. Sci.*; 2002(64(3)):245–50.
38. Dendani Chadi Z, Dib L, Zeroual F, Benakhla A. Usefulness of molecular typing methods for epidemiological and evolutionary studies of *Staphylococcus aureus* isolated from bovine intramammary infections. *Saudi Journal of Biological Sciences* 2022; 29(8):103338.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S1319562X22002546>.
39. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; 38(3):1008–15. doi: 10.1128/JCM.38.3.1008-1015.2000.
40. Pennone V, Prieto M, Álvarez-Ordóñez A, Cobo-Díaz JF. Antimicrobial Resistance Genes Analysis of Publicly Available *Staphylococcus aureus* Genomes. *Antibiotics* 2022; 11(11):1632.
Verfügbar unter: <https://www.mdpi.com/2079-6382/11/11/1632>.
41. Baig S, Rhod Larsen A, Martins Simões P, Laurent F, Johannesen TB, Lilje B et al. Evolution and Population Dynamics of Clonal Complex 152 Community-Associated Methicillin-Resistant *Staphylococcus aureus*. *mSphere* 2020; 5(4). doi: 10.1128/mSphere.00226-20.
42. McCarthy AJ, Lindsay JA, Loeffler A. Are all methicillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. *Vet Dermatol* 2012; 23(4):267-75, e53-4 [Stand: 17.05.2025].
Verfügbar unter: <https://onlinelibrary.wiley.com/doi/pdfdirect/10.1111/j.1365-3164.2012.01072.x?download=true>.
43. Lindsay JA, Moore CE, Day NP, Peacock SJ, Witney AA, Stabler RA et al. Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. *J Bacteriol* 2006; 188(2):669–76. doi: 10.1128/JB.188.2.669-676.2006.
44. McCarthy AJ, Witney AA, Lindsay JA. *Staphylococcus aureus* temperate bacteriophage: carriage and horizontal gene transfer is lineage associated. *Front. Cell. Infect. Microbiol.* 2012; 2:6.
Verfügbar unter: <https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2012.00006/full>.
45. Matuszewska M, Murray GGR, Harrison EM, Holmes MA, Weinert LA. The Evolutionary Genomics of Host Specificity in *Staphylococcus aureus*. *Trends in Microbiology* 2020; 28(6):465–77.
Verfügbar unter: [https://www.cell.com/trends/microbiology/fulltext/S0966-842X\(19\)30322-1](https://www.cell.com/trends/microbiology/fulltext/S0966-842X(19)30322-1).

46. Romanò A, Gazzola A, Bianchini V, Cortimiglia C, Maisano AM, Cremonesi P et al. Staphylococcus aureus From Goats Are Genetically Heterogeneous and Distinct to Bovine Ones. *Front. Vet. Sci.* 2020; 7:628. doi: 10.3389/fvets.2020.00628.
47. Shittu AO, Taiwo FF, Froböse NJ, Schwartbeck B, Niemann S, Mellmann A et al. Genomic analysis of Staphylococcus aureus from the West African Dwarf (WAD) goat in Nigeria. *Antimicrob Resist Infect Control* 2021; 10(1):122.
Verfügbar unter: <https://link.springer.com/article/10.1186/s13756-021-00987-8>.
48. Porrero MC, Hasman H, Vela AI, Fernández-Garayzábal JF, Domínguez L, Aarestrup FM. Clonal diversity of Staphylococcus aureus originating from the small ruminants goats and sheep. *Veterinary Microbiology* 2012; 156(1-2):157–61.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S037811351100558X>.
49. Cvetnić L, Samardžija M, Duvnjak S, Habrun B, Cvetnić M, Jaki Tkalec V et al. Multi Locus Sequence Typing and spa Typing of Staphylococcus Aureus Isolated from the Milk of Cows with Subclinical Mastitis in Croatia. *Microorganisms* 2021; 9(4). doi: 10.3390/microorganisms9040725.
50. Patel K, Godden SM, Royster EE, Crooker BA, Johnson TJ, Smith EA et al. Prevalence, antibiotic resistance, virulence and genetic diversity of Staphylococcus aureus isolated from bulk tank milk samples of U.S. dairy herds. *BMC genomics* 2021; 22(1):367.
Verfügbar unter: <https://pubmed.ncbi.nlm.nih.gov/34016049/>.
51. Schmidt T, Kock MM, Ehlers MM. Molecular Characterization of Staphylococcus aureus Isolated from Bovine Mastitis and Close Human Contacts in South African Dairy Herds: Genetic Diversity and Inter-Species Host Transmission. *Front Microbiol* 2017; 8:511. doi: 10.3389/fmicb.2017.00511.
52. Monecke S, Ruppelt A, Wendlandt S, Schwarz S, Slickers P, Ehricht R et al. Genotyping of Staphylococcus aureus isolates from diseased poultry. *Veterinary Microbiology* 2013; 162(2-4):806–12.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S0378113512005573>.
53. Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA et al. Recent human-to-poultry host jump, adaptation, and pandemic spread of Staphylococcus aureus. *Proc Natl Acad Sci U S A* 2009; 106(46):19545–50.
Verfügbar unter: <https://www.pnas.org/doi/full/10.1073/pnas.0909285106>.
54. Silva V, Correia S, Rocha J, Manaia CM, Silva A, García-Díez J et al. Antimicrobial Resistance and Clonal Lineages of Staphylococcus aureus from Cattle, Their Handlers, and Their Surroundings: A Cross-Sectional Study from the One Health Perspective. *Microorganisms* 2022; 10(5):941.
Verfügbar unter: <https://www.mdpi.com/2076-2607/10/5/941>.
55. Sung JM-L, Lloyd DH, Lindsay JA. Staphylococcus aureus host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. *Microbiology (Reading)* 2008; 154(Pt 7):1949–59.
Verfügbar unter: <https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.2007/015289-0?crawler=true>.

56. Strommenger B, Braulke C, Heuck D, Schmidt C, Pasemann B, Nübel U et al. spa Typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. *J Clin Microbiol* 2008; 46(2):574–81. doi: 10.1128/JCM.01599-07.
57. Fox LK, Gay JM. Contagious mastitis. *Vet Clin North Am Food Anim Pract* 1993; 9(3):475–87. doi: 10.1016/s0749-0720(15)30615-0.
58. Rasmussen G, Monecke S, Ehricht R, Söderquist B. Prevalence of clonal complexes and virulence genes among commensal and invasive *Staphylococcus aureus* isolates in Sweden. *PLOS ONE* 2013; 8(10):e77477.
Verfügbar unter: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0077477>.
59. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLOS ONE* 2011; 6(4):e17936.
Verfügbar unter: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0017936>.
60. Haag AF, Fitzgerald JR, Penadés JR. *Staphylococcus aureus* in Animals. *Microbiol Spectr* 2019; 7(3). doi: 10.1128/microbiolspec.GPP3-0060-2019.
61. Otto M. *Staphylococcus aureus* toxins. *Curr Opin Microbiol* 2014; 17:32–7 [Stand: 31.07.2024]. Verfügbar unter: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3942668/pdf/nihms543594.pdf>.
62. Pérez VKC, Da Costa GM, Guimarães AS, Heinemann MB, Lage AP, Dorneles EMS. Relationship between virulence factors and antimicrobial resistance in *Staphylococcus aureus* from bovine mastitis. *Journal of Global Antimicrobial Resistance* 2020; 22:792–802.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S2213716520301557>.
63. Loncaric I, Keinprecht H, Irmaso E, Cabal-Rosel A, Stessl B, Ntakirutimana C et al. Diversity of *Staphylococcus aureus* isolated from nares of ruminants. *J. Appl. Microbiol.* 2024; 135(1). Verfügbar unter: <https://academic.oup.com/jambio/article/135/1/ixad304/7504751?login=true>.
64. Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial Biofilm and its Role in the Pathogenesis of Disease. *Antibiotics* 2020; 9(2):59.
Verfügbar unter: <https://www.mdpi.com/2079-6382/9/2/59>.
65. Idrees M, Sawant S, Karodia N, Rahman A. *Staphylococcus aureus* Biofilm: Morphology, Genetics, Pathogenesis and Treatment Strategies. *International Journal of Environmental Research and Public Health* 2021; 18(14):7602.
Verfügbar unter: <https://www.mdpi.com/1660-4601/18/14/7602#B24-ijerph-18-07602>.
66. Otto M. *Staphylococcal* Biofilms. *Microbiol Spectr* 2018; 6(4). doi: 10.1128/microbiolspec.GPP3-0023-2018.
67. Mushayija JP, Nikwigize S, Karangwa C, Manishimwe R, Habimana R, Rutayisire E. Knowledge, Attitudes and Practices on Antibiotics Use among Cattle Keepers in Nyagatare District, Rwanda. *Austin Journal of Public Health and Epidemiology* 2021.
Verfügbar unter: <https://austinpublishinggroup.com/public-health-epidemiology/fulltext/ajphe-v8-id1111.php>.

68. Manishimwe R, Nishimwe K, Ojok L. Assessment of antibiotic use in farm animals in Rwanda. *Trop Anim Health Prod* 2017; 49(6):1101–6.
Verfügbar unter: <https://link.springer.com/article/10.1007/s11250-017-1290-z>.
69. Oliveira RP de, Da Silva JG, Aragão BB, Carvalho RG de, Juliano MA, Frazzon J et al. Diversity and emergence of multi-resistant *Staphylococcus* spp. isolated from subclinical mastitis in cows in of the state of Piauí, Brazil. *Brazilian journal of microbiology* : [publication of the Brazilian Society for Microbiology] 2022; 53(4):2215–22.
Verfügbar unter: <https://pubmed.ncbi.nlm.nih.gov/36074251/>.
70. Jamali H, Radmehr B, Ismail S. Short communication: prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Journal of Dairy Science* 2014; 97(4):2226–30.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S0022030214001246>.
71. Molineri AI, Camussone C, Zbrun MV, Suárez Archilla G, Cristiani M, Neder V et al. Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine mastitis: Systematic review and meta-analysis. *Preventive Veterinary Medicine* 2021; 188:105261.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S0167587721000052>.
72. Manishimwe R, Ndayisenga B, Habimana R, Mwikarago IE, Habiyaremye T, Shyaka A et al. Consumption Trends in Antibiotics for Veterinary Use in Rwanda: A Retrospective Study between 2019 and 2021 2022.
Verfügbar unter: <https://www.researchsquare.com/article/rs-1807218/v1>.
73. Ndahetuye JB, Leijon M, Båge R, Artursson K, Persson Y. Genetic Characterization of *Staphylococcus aureus* From Subclinical Mastitis Cases in Dairy Cows in Rwanda. *Front Vet Sci* 2021; 8:751229. doi: 10.3389/fvets.2021.751229.
74. Dantas G, Morten O. A. Sommer, Oluwasegun RD, Church GM. Bacteria Subsisting on Antibiotics. *American Association for the Advancement of Science* 04.04.2008 [Stand: 18.09.2024].
Verfügbar unter: <https://www.science.org/doi/10.1126/science.1155157>.
75. Jian Z, Zeng L, Xu T, Sun S, Yan S, Yang L et al. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *Journal of Basic Microbiology* 2021; 61(12):1049–70.
Verfügbar unter: <https://onlinelibrary.wiley.com/doi/10.1002/jobm.202100201>.
76. DocCheck Mb. Resistenzgen - DocCheck Flexikon: DocCheck Community GmbH; 2024 [Stand: 18.09.2024].
Verfügbar unter: <https://flexikon.doccheck.com/de/Resistenzgen>.
77. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, Marusza W. Molecular Mechanisms of Drug Resistance in *Staphylococcus aureus*. *International Journal of Molecular Sciences* 2022; 23(15):8088.
Verfügbar unter: <https://www.mdpi.com/1422-0067/23/15/8088>.
78. Schwarz S, Feßler AT, Loncaric I, Wu C, Kadlec K, Wang Y et al. Antimicrobial Resistance among *Staphylococci* of Animal Origin. *Microbiol Spectr* 2018; 6(4):10.1128/microbiolspec.arba-0010-2017.
Verfügbar unter: <https://pmc.ncbi.nlm.nih.gov/articles/PMC11633598/>.

79. Rabello RF, Moreira BM, Lopes RMM, Teixeira LM, Riley LW, Castro ACD. Multilocus sequence typing of *Staphylococcus aureus* isolates recovered from cows with mastitis in Brazilian dairy herds. *Journal of Medical Microbiology* 2007; 56(Pt 11):1505–11.
Verfügbar unter: <https://www.microbiologyresearch.org/content/journal/jmm/10.1099/jmm.0.47357-0>.
80. Boss R, Cosandey A, Luini M, Artursson K, Bardiau M, Breitenwieser F et al. Bovine *Staphylococcus aureus*: Subtyping, evolution, and zoonotic transfer. *Journal of Dairy Science* 2016; 99(1):515–28.
Verfügbar unter: <https://www-sciencedirect-com.ezproxy.vetmeduni.ac.at/science/article/pii/S0022030215008346>.
81. Campos B, Pickering AC, Rocha LS, Aguilar AP, Fabres-Klein MH, Oliveira Mendes TA de et al. Diversity and pathogenesis of *Staphylococcus aureus* from bovine mastitis: current understanding and future perspectives. *BMC Vet Res* 2022; 18(1):115.
Verfügbar unter: <https://link.springer.com/article/10.1186/S12917-022-03197-5>.
82. El-Ashker M, Monecke S, Gwida M, Saad T, El-Gohary A, Mohamed A et al. Molecular characterisation of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* clones isolated from healthy dairy animals and their caretakers in Egypt. *Veterinary Microbiology* 2022; 267:109374.
Verfügbar unter: <https://www.sciencedirect.com/science/article/pii/S037811352200044X>.
83. Conceição T, Lencastre H de, Aires-de-Sousa M. Healthy Bovines as Reservoirs of Major Pathogenic Lineages of *Staphylococcus aureus* in Portugal. *Microb Drug Resist* 2017; 23(7):845–51. doi: 10.1089/mdr.2017.0074.
84. Kümmel J, Stessl B, Gonano M, Walcher G, Bereuter O, Fricker M et al. *Staphylococcus aureus* Entrance into the Dairy Chain: Tracking *S. aureus* from Dairy Cow to Cheese. *Front. Microbiol.* 2016; 7:1603.
Verfügbar unter: <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2016.01603/full#F1>.
85. Rodrigues RA, Pizauro LJJ, Varani AdM, Almeida CC de, Silva SR, Cardozo MV et al. Comparative genomics study of *Staphylococcus aureus* isolated from cattle and humans reveals virulence patterns exclusively associated with bovine clinical mastitis strains. *Front Microbiol* 2022; 13:1033675.
Verfügbar unter: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.1033675/full>.
86. Mzee T, Kumburu H, Kazimoto T, Leekitcharoenphon P, van Zwetselaar M, Masalu R et al. Molecular Characterization of *Staphylococcus aureus* Isolated from Raw Milk and Humans in Eastern Tanzania: Genetic Diversity and Inter-Host Transmission. *Microorganisms* 2023; 11(6):1505.
Verfügbar unter: <https://www.mdpi.com/2076-2607/11/6/1505>.
87. Antók FI, Mayrhofer R, Marbach H, Masengesho JC, Keinprecht H, Nyirimbuga V et al. Characterization of Antibiotic and Biocide Resistance Genes and Virulence Factors of *Staphylococcus* Species Associated with Bovine Mastitis in Rwanda. *Antibiotics (Basel)* 2019; 9(1). doi: 10.3390/antibiotics9010001.

88. Karzis J, Petzer I-M, Donkin EF, Naidoo V, Etter EMC. Climatic and regional antibiotic resistance patterns of *Staphylococcus aureus* in South African dairy herds. *The Onderstepoort Journal of Veterinary Research* 2019; 86(1):e1-e9.
Verfügbar unter: <https://pmc.ncbi.nlm.nih.gov/articles/PMC6676993/>.
89. Hoekstra J, Zomer AL, Rutten, Victor P. M. G., Benedictus L, Stegeman A, Spaninks MP et al. Genomic analysis of European bovine *Staphylococcus aureus* from clinical versus subclinical mastitis. *Sci Rep* 2020; 10(1):18172.
Verfügbar unter: <https://www.nature.com/articles/s41598-020-75179-2>.
90. Schlotter K, Ehricht R, Hotzel H, Monecke S, Pfeffer M, Donat K. Leukocidin genes lukF-P83 and lukM are associated with *Taphylococcus aureus* clonal complexes 151, 479 and 133 isolated from bovine udder infections in Thuringia, Germany. *Vet Res* 2012; 43(1):42.
Verfügbar unter: <https://veterinaryresearch.biomedcentral.com/articles/10.1186/1297-9716-43-42>.
91. Walzl A, Marbach H, Belikova D, Vogl C, Ehling-Schulz M, Heilbronner S et al. Prevalence of the SigB-Deficient Phenotype among Clinical *Staphylococcus aureus* Isolates Linked to Bovine Mastitis. *Antibiotics* 2023; 12(4):699.
Verfügbar unter: <https://www.mdpi.com/2079-6382/12/4/699>.
92. Lawal OU, Ayobami O, Abouelfetouh A, Mourabit N, Kaba M, Egyir B et al. A 6-Year Update on the Diversity of Methicillin-Resistant *Staphylococcus aureus* Clones in Africa: A Systematic Review. *Front. Microbiol.* 2022; 13:860436. doi: 10.3389/fmicb.2022.860436.
93. Garrine M, Costa SS, Messa A, Massora S, Vubil D, Ácacio S et al. Antimicrobial resistance and clonality of *Staphylococcus aureus* causing bacteraemia in children admitted to the Manhiça District Hospital, Mozambique, over two decades. *Front. Microbiol.* 2023; 14:1208131. doi: 10.3389/fmicb.2023.1208131.
94. Ruffing U, Alabi A, Kazimoto T, Vubil DC, Akulenko R, Abdulla S et al. Community-Associated *Staphylococcus aureus* from Sub-Saharan Africa and Germany: A Cross-Sectional Geographic Correlation Study. *Sci Rep* 2017; 7(1):154.
Verfügbar unter: <https://www.nature.com/articles/s41598-017-00214-8>.
95. Obanda BA, Cook EAJ, Fèvre EM, Bebora L, Ogara W, Wang S-H et al. Characteristics of *Staphylococcus aureus* Isolated from Patients in Busia County Referral Hospital, Kenya. *Pathogens* 2022; 11(12) [Stand: 27.06.2025].
Verfügbar unter: https://d1wqtxts1xzle7.cloudfront.net/102057563/pdf-libre.pdf?1683708207=&response-content-disposition=inline%3B+filename%3DCharacteristics_of_Staphylococcus_aureus.pdf&Expires=1751036211&Signature=JYwmi7XWn-8u8MHMHTLb8oSqGzcZtnrJEh~7Djb40ca54LpzvlglaJM5CwznaSyJOG7C3acH8OsIs9njpfKm0RXH3DNIv6YpJUqiVPtfc79o2RugT~0G-yhjeyMg7IxRqT421Wjv7oEPHUXvU-ElmCkyNyDy9TZ3YxERABqA2M3xG6uJh5Xhj2Txh8OC-WIrZ4BbHv4fz1j5edvxcREI-P2gl~2MizYWkhkGISdW1mmbHYMq28i3VQN2rXFCGE90LozHeODu9NBemtA3KBBWXtz4WwUTYI2rf0w-krSrDkG6k1DfHwzBu8SdoPvKYMg9mOR9VXFuPir5B~cJk3~-wA__&Key-Pair-Id=APKAJLOHF5GGSLRBV4ZA.

96. Holmes MA, Zadoks RN. Methicillin resistant *S. aureus* in human and bovine mastitis. *J Mammary Gland Biol Neoplasia* 2011; 16(4):373–82.
Verfügbar unter: <https://link.springer.com/article/10.1007/s10911-011-9237-x>.
97. Nhatsave N, Garrine M, Messa A, Massinga AJ, Cossa A, Vaz R et al. Molecular Characterization of *Staphylococcus aureus* Isolated from Raw Milk Samples of Dairy Cows in Manhica District, Southern Mozambique. *Microorganisms* 2021; 9(8):1684.
Verfügbar unter: <https://www.mdpi.com/2076-2607/9/8/1684>.
98. Shephard MA, Fleming VM, Connor TR, Corander J, Feil EJ, Fraser C et al. Historical zoonoses and other changes in host tropism of *Staphylococcus aureus*, identified by phylogenetic analysis of a population dataset. *PLOS ONE* 2013; 8(5):e62369.
Verfügbar unter: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0062369#pone-0062369-g001>.
99. Moreno-Grúa E, Pérez-Fuentes S, Viana D, Cardells J, Lizana V, Aguiló J et al. Marked Presence of Methicillin-Resistant *Staphylococcus aureus* in Wild Lagomorphs in Valencia, Spain. *Animals* 2020; 10(7):1109.
Verfügbar unter: <https://www.mdpi.com/2076-2615/10/7/1109>.
100. Abdullahi IN, Fernández-Fernández R, Juárez-Fernández G, Martínez-Álvarez S, Eguizábal P, Zarazaga M et al. Wild Animals Are Reservoirs and Sentinels of *Staphylococcus aureus* and MRSA Clones: A Problem with "One Health" Concern. *Antibiotics* 2021; 10(12):1556.
Verfügbar unter: <https://www.mdpi.com/2079-6382/10/12/1556#app1-antibiotics-10-01556>.
101. Nemati G, Romanó A, Wahl F, Berger T, Rojo LV, Graber HU. Bovine *Staphylococcus aureus*: a European study of contagiousness and antimicrobial resistance. *Front. Vet. Sci.* 2023; 10:1154550.
Verfügbar unter: <https://www.frontiersin.org/journals/veterinary-science/articles/10.3389/fvets.2023.1154550/full>.
102. Ramezanigardaloud N, Loncaric I, Mikuni-Mester P, Alinaghi M, Ehling-Schulz M, Khol JL et al. Prevalence and Diversity of *Staphylococcus aureus* in Bulk Tank Milk from Community-Based Alpine Dairy Pastures in Tyrol, Austria. *Animals* 2025; 15(14):2153.
Verfügbar unter: <https://www.mdpi.com/2076-2615/15/14/2153#B4-animals-15-02153>.