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DNA microarray-based characterization and antimicrobial resistance phenotypes of clinical MRSA strains from animal hosts

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Conflict of Interest

The authors declare no conflicts of interest.

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of severe infections in humans and animals worldwide. Studies elucidating the population structure, staphylococcal cassette chromosome *mec* types, resistance phenotypes, and virulence gene profiles of animal-associated MRSA are needed to understand spread and transmission.

Objectives: The objective of this study was to determine 1) clonal complexes and *spa* types, 2) resistance phenotypes, and 3) virulence/resistance gene profiles of MRSA isolated from animals in Switzerland.

Methods: We analyzed 31 presumptive MRSA isolates collected from clinical infections in horses, dogs, cattle, sheep, and pigs, which had tested positive in the Staphaurex Latex Agglutination Test. The isolates were characterized by *spa* typing and DNA microarray profiling. In addition, we performed antimicrobial susceptibility testing using the VITEK 2 Compact system.

Results: Characterization of the 31 presumptive MRSA isolates revealed 3 methicillin-resistant *Staphylococcus pseudintermedius* isolates, which were able to grow on MRSA2 Brilliance agar. Of the 28 MRSA isolates, the majority was assigned to CC398 (86%), but CC8 (11%) and CC1 (4%) were also detected. The predominant *spa* type was t011 (n = 23), followed by t009 (n = 2), t034 (n = 1), t008 (n = 1), and t127 (n = 1).

Conclusions: The results of this study extend the current body of knowledge on the population structure, resistance phenotypes, and virulence and resistance gene profiles of MRSA from livestock and companion animals.

Keywords: MRSA; MRSP

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a global health concern in humans and animals [1]. MRSA colonization and infection were first reported in animals in 1972 in asymptomatic dogs in Nigeria and a case of bovine mastitis in Belgium [2,3]. Staphylococcal resistance to beta lactam antibiotics is, in the majority of cases, either mediated by enzymatic inactivation through beta lactamase encoded by *blaZ* or by target site replacement through alternate penicillin-binding proteins encoded by *mecA* or *mecC* [4]. The

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mec genes are located on the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*), for which at least 13 major types and various subtypes have been reported [4].

Host specificity, or lack thereof, in different clonal lineages has been of particular interest in view of the transmission of MRSA from livestock and companion animals to humans [5,6]. Different staphylococcal lineages, including the most common clonal complex (CC) among livestock-associated MRSA (LA-MRSA) CC398, have been detected in various animal species, as well as in humans [7,8]. Transmission of MRSA from livestock to humans occurs through direct contact, environmental contamination, or handling and consumption of contaminated meat or milk [7]. In recent years, CC398 has emerged as the predominant MRSA lineage in pigs, cattle, and horses in Europe, as well as in many regions worldwide [7,8]. Acquisition of MRSA in companion animals was suggested to primarily represent a reverse zoonosis, with colonized owners transmitting the MRSA strains to their pets [7].

The European Food Safety Authority recently stressed that additional data on MRSA from animal origins is crucial to identify the spread and evolution of zoonotically acquired MRSA in humans [9]. Therefore, in this study, we aimed to determine 1) CCs and *spa* types, 2) resistance phenotypes, and 3) virulence/resistance gene profiles of clinical MRSA isolated from livestock and companion animals.

MATERIALS AND METHODS

Bacterial isolates

All isolates identified as presumptive MRSA by a routine laboratory diagnostic service between 2013 and 2016, and tested positive in a Staphaurex Plus Latex Agglutination Test (Thermo Fisher Scientific, Switzerland), were included in the study. The results revealed 31 presumptive MRSA isolates from horses, dogs, pigs, cattle, and a sheep. Eighteen isolates originated from clinical cases of infections in horses (abscesses: n = 15, joint infections: n = 2, sepsis: n = 1). Five isolates originated from abscesses (n = 3) or wound infections (n = 2) in dogs. Five isolates were from pigs (sepsis: n = 2, abscess: n = 1, joint infection: n = 1, nasal secretion: n = 1), 2 from clinical cases of mastitis in cattle, and one from an abscess in a sheep. The presumptive MRSA isolates were streaked from cryogenic stocks on Columbia sheep blood agar (Thermo Fisher Scientific) and incubated overnight at 37°C. Subsequently, isolates were checked for purity, streaked on Brilliance MRSA2 agar (Thermo Fisher Scientific), and incubated overnight at 37°C. Tests for acetoin production and urease activity were performed to differentiate *Staphylococcus pseudintermedius* from *S. aureus* [10].

Penicillin-binding protein latex agglutination test

Detection of the penicillin-binding protein PBP2 α was performed using the Oxoid PBP2' Latex Agglutination Test Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Determination of minimum inhibitory concentration (MIC) of antimicrobial agents

Antimicrobial susceptibility testing (microdilution method) was conducted with the automated VITEK 2 Compact system using the AST-GP69 susceptibility testing card (bioMérieux, France) according to the manufacturer's instructions. The VITEK 2 ceftiofur screen test on the AST-GP69 card and the oxacillin MIC was used to identify methicillin-resistant staphylococci [11,12]. The advanced expert system of the VITEK 2 Compact system

provided MIC and susceptibility interpretations, which were automatically edited in some phenotypes [13]. Interpretation of results was performed per the guidelines of the Clinical and Laboratory Standards Institute [11].

DNA extraction, DNA microarray profiling, and SplitsTree analysis

DNA microarray analysis was performed using a Staphytype genotyping kit 2.0 and the ArrayMate reader and software (Alere Technologies GmbH, Germany) and following the manufacturer's instructions. Chromosomal DNA of all isolates was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany). The DNA microarray can be used to determine the presence or absence of 334 target sequences, corresponding to 171 distinct resistance and virulence genes and their allelic variants. It also allows for SCC*mec* typing, as well as the assignment of strains to CCs based on comparison of the hybridization profile of the analyzed strain to those of well-characterized reference strains [14]. To visualize the degree of similarity of the DNA microarray gene profiles, SplitsTree4 software (<http://www.splittree.org/>) was used as previously described [15].

spa typing

All isolates were *spa* typed as described elsewhere [16]. Briefly, the polymorphic X region of the *spa* gene was amplified by polymerase chain reaction (PCR). Amplicons were purified using the GenElute PCR Purification Kit (Sigma-Aldrich, USA). Sequencing was outsourced with the obtained sequences subsequently assigned to *spa* types using the *spa*-server (<http://www.spaserver.ridom.de/>).

RESULTS

Out of the 31 presumptive MRSA isolates, 3 canine isolates did not produce acetoin, tested urease positive, and were discarded as “wrong species” in the DNA microarray. Species identification using the identification card for Gram-positive bacteria (VITEK 2 GP ID card) revealed that all 3 isolates were representative of *S. pseudintermedius*. These methicillin-resistant *S. pseudintermedius* (MRSP) isolates were positive in the plasma coagulase test, and yielded positive results in the PBP2 α latex agglutination test and the Staphaurex Latex Agglutination Test. All 3 MRSP isolates harbored various resistance genes, including *blaZ*/I/R and *mecA*/I/R (**Supplementary Table 1**). While all *S. pseudintermedius* yielded negative results in the ceftiofloxacin-screen test, all were classified as resistant to oxacillin.

All 28 of the tested *S. aureus* strains were resistant to penicillin, oxacillin, and tetracycline, and except for strain MRSA-18, all strains also yielded positive results in the ceftiofloxacin-screen test (**Table 1**). The MIC₅₀/MIC₉₀ values indicate the MIC at which $\geq 50\%$ and $\geq 90\%$ of the MRSA strains tested in our study would be inhibited (**Table 1, Supplementary Tables 2 and 3**). Resistance to trimethoprim/sulfamethoxazole (75%), gentamicin (61% resistant, 14% intermediate), enrofloxacin (32%), and marbofloxacin (32%) was frequently detected among the tested MRSA strains. In contrast, resistance to erythromycin, nitrofurantoin, and clindamycin was relatively rare (4% each), and all MRSA strains were susceptible to vancomycin, rifampicin, and chloramphenicol. While no clinical breakpoints were available for fusidic acid and mupirocin, all isolates yielded MICs of $\leq 0.5 \mu\text{g/mL}$ and $\leq 2 \mu\text{g/mL}$, respectively, representing the lowest concentrations tested. There were also no clinical breakpoints available for ampicillin/sulbactam, imipenem, or kanamycin, which yielded heterogeneous results when used for susceptibility testing of the MRSA isolates.

Table 1. Overview of resistance phenotypes and MIC₅₀/MIC₉₀ values determined for the 28 methicillin-resistant *Staphylococcus aureus* isolates tested in this study

Antimicrobial agent	Susceptible (%)	Intermediate (%)	Resistant (%)	MIC ₅₀	MIC ₉₀	Clinical breakpoints applied*
Penicillin	0	-	100	≥ 0.5	≥ 0.5	Human clinical breakpoint
Ampicillin/sulbactam (2/1) [†]	-	-	-	8	≥ 32	-
Oxacillin	0	-	100	≥ 4	≥ 4	Human clinical breakpoint
Imipenem	-	-	-	≤ 1	≤ 1	-
Vancomycin	100	0	0	≤ 0.5	1	Human clinical breakpoint
Erythromycin	96	0	4	≤ 0.25	0.5	Human clinical breakpoint
Fusidic acid	-	-	-	≤ 0.5	≤ 0.5	-
Mupirocin	-	-	-	≤ 2	≤ 2	-
Rifampicin	100	0	0	≤ 0.5	≤ 0.5	Human clinical breakpoint
Kanamycin	-	-	-	≥ 64	≥ 64	-
Gentamicin	25	14	61	≥ 16	≥ 16	Human clinical breakpoint
Enrofloxacin	68	0	32	≤ 0.5	≥ 4	Veterinary clinical breakpoint (dogs)
Marbofloxacin	68	0	32	≤ 0.5	≥ 4	Veterinary clinical breakpoint (dogs)
Tetracycline	0	0	100	≥ 16	≥ 16	Human clinical breakpoint
Nitrofurantoin	96	0	4	≤ 16	32	Human clinical breakpoint
Chloramphenicol	100	0	0	≤ 4	8	Human clinical breakpoint
Clindamycin	96	0	4	≤ 0.25	≤ 0.25	Veterinary clinical breakpoint (dogs)
Trimethoprim/sulfamethoxazole (1/19) [†]	25	-	75	≥ 16	≥ 16	Human clinical breakpoint

This overview of resistance phenotypes and MIC₅₀/MIC₉₀ values is based on human or veterinary clinical breakpoints. For the MIC distribution, see

Supplementary Table 2.

MIC, minimum inhibitory concentration.

*Due to a lack of available veterinary clinical breakpoints for all host species, classification as susceptible/intermediate/resistant is based on human clinical breakpoints or veterinary clinical breakpoints determined for dogs [11]; [†]MIC values given represent the MIC values for ampicillin and trimethoprim, respectively.

All isolates exhibited unique DNA microarray hybridization profiles and therefore represented different strains (**Table 2**). An overview of the similarities among the DNA microarray hybridization patterns is provided using SplitsTree results (**Fig. 1**). Of the *S. aureus* strains, a substantial majority was assigned to CC398 (86%), followed by CC8 (11%), and CC1 (4%). The predominant *spa* type was t011 (n = 23), which was detected in isolates from horses, pigs, cattle, sheep, and a dog. In addition, 2 isolates were designated as t009 (horse), and one isolate each as t034 (cattle), t127 (dog), and t008 (horse). All genomic characterization results, as well as all resistance phenotypes, are available as **Supplementary Tables 1 and 2**, respectively.

DISCUSSION

Although the DNA microarray approach used in this study allows screening for the presence of 334 target sequences, corresponding to 171 distinct resistance and virulence genes and their allelic variants, it does not allow for conclusions on antimicrobial resistance phenotype [14]. Therefore, we also determined MICs of various antimicrobial agents of interest. We also included typing by PCR analysis and sequencing of the polymorphic X region of the *spa* gene, as microarray analysis only allows for reliable prediction of CCs, not *spa* types [14].

Of the clinical MRSA strains tested in this study, the majority was assigned to CC398 and *spa* type t011, with one CC398 strain being assigned to t034. The *spa* types t011 and t034 are the predominant *spa* types in CC398 isolates in Switzerland and Europe [17-19]. MRSA assigned to these *spa* types have also been detected in nasal swabs of Swiss veterinarians with occupational contact to livestock [17]. *S. aureus* of CC398 likely originated in humans, and the host jump to livestock led to the acquisition of methicillin and tetracycline resistance, while at the same time reducing the capacity for colonization and transmission in humans [19]. Consistent with our results, LA-MRSA of CC398 has been reported to characteristically

MRSA from different animal hosts

Table 2. Overview of typing and characterization results

CC	spa type	Host	SCCmec	Resistance genes*	Resistance phenotype†	Virulence genes*
CC398 (n = 24)	t011 (n = 23)	Horse (n = 15)	IV	<i>mecA, blaZ, aacA-aphD, tetM, sdrM</i>	n = 6: PEN, OXA, FOX, TET, SXT, GEN	-
					n = 3: PEN, OXA, FOX, TET, SXT, (GEN)	-
					n = 3: PEN, OXA, FOX, TET, SXT, GEN, EFX, MAR	-
					n = 1: PEN, OXA, FOX, TET, SXT, GEN, EFX, MAR, NIT	-
					n = 1: PEN, OXA, FOX, TET, SXT, GEN, EFX, MAR	-
					n = 1: PEN, OXA, TET, SXT	-
		Pig (n = 5)	V (n = 3)	<i>mecA, blaZ, tetK/M, sdrM</i>	PEN, OXA, FOX, TET	-
			IV (n = 2)	<i>mecA, blaZ, aacA-aphD, tetM, sdrM</i>	PEN, OXA, FOX, TET	-
		Cattle (n = 1)	IV	<i>mecA, blaZ, aacA-aphD, sdrM</i>	PEN, OXA, FOX, TET, SXT, (GEN)	-
					PEN, OXA, FOX, TET, SXT, GEN, EFX, MAR	-
Dog (n = 1)	IV	<i>mecA, blaZ, aacA-aphD, tetM, sdrM</i>	PEN, OXA, FOX, TET, SXT, GEN, EFX, MAR	-		
			PEN, OXA, FOX, TET	-		
CC8 (n = 3)	t009 (n = 2)	Horse	Atypical	<i>mecA, blaZ, aacA-aphD, aphA3, sat, tetM, sdrM</i>	PEN, OXA, FOX, TET, SXT, GEN	<i>seb, seq</i>
					PEN, OXA, TET, SXT, GEN, EFX, MAR	<i>seb, sek, seq</i>
					PEN, OXA, FOX, TET, GEN, EFX, MAR	<i>sek, seq, sak, chp, scn</i>
	t034 (n = 1)	Cattle	V	<i>mecA, blaZ, ermA, tetK/M, sdrM</i>	PEN, OXA, FOX, TET	-
CC1 (n = 1)	t127 (n = 1)	Dog	IV	<i>mecA, blaZ, tetK, sdrM</i>	PEN, OXA, FOX, TET	<i>seh</i>

CC, spa type, and SCCmec type were determined for the MRSA isolates (n = 28) included in this study. The table also provides information on resistance phenotypes, and it lists resistance and virulence genes detected by DNA microarray.

CC, clonal complex; SCCmec, staphylococcal cassette chromosome mec; MRSA, methicillin-resistant *Staphylococcus aureus*; PEN, penicillin; OXA, oxacillin; FOX, ceftioxin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; ERY, erythromycin; MAR, marbofloxacin; EFX, enrofloxacin; CLI, clindamycin; VAN, vancomycin; NIT, nitrofurantoin; CHL, chloramphenicol; RIF, rifampicin.

*The presence of selected resistance and virulence genes determined by DNA microarray is stated. For a full report see **Supplementary Table 1**. None of the isolates harbored *mecC, pvl* encoding Panton-Valentine leukocidin, or genes encoding exfoliative or toxic shock syndrome toxins; †Due to lack of available clinical breakpoints for all host species, classification as intermediate (antimicrobial indicated in brackets) or resistant is based on human clinical breakpoints or veterinary clinical breakpoints determined for dogs, which may not apply to the host species from which the MRSA was isolated.

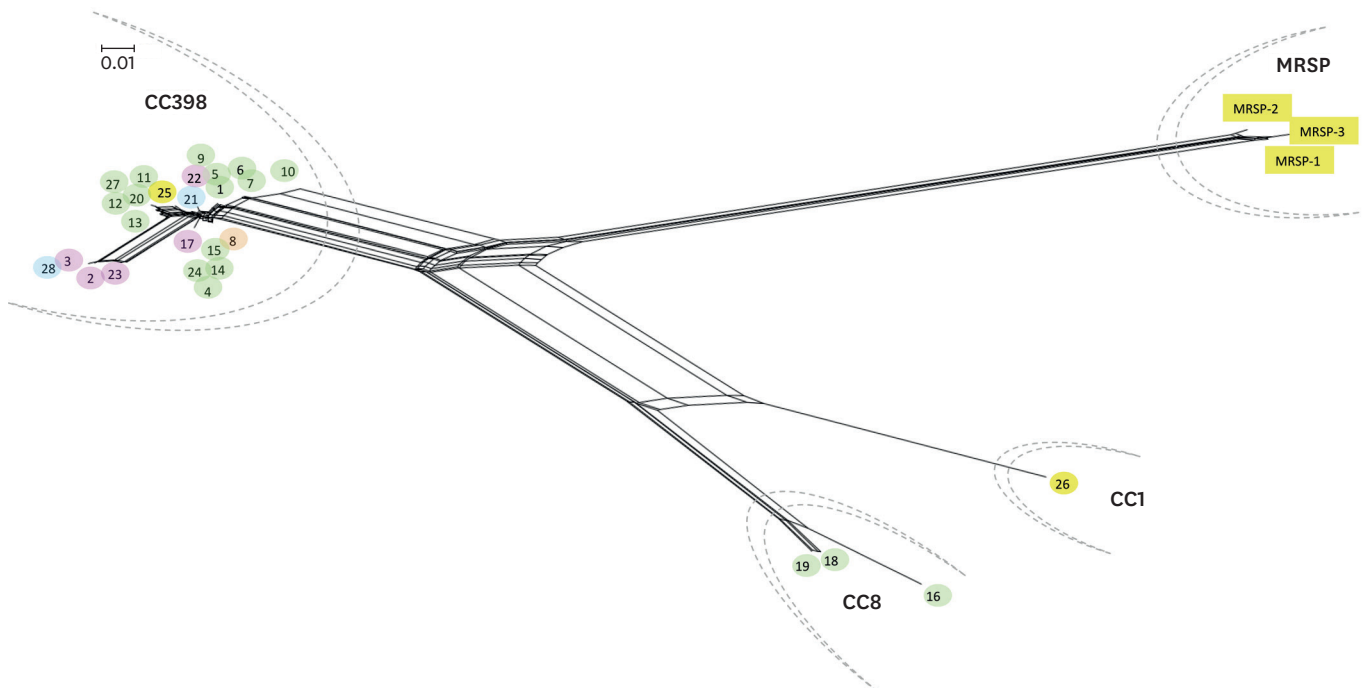


Fig. 1. SplitsTree depicting the similarity between the virulence and resistance gene profiles of the investigated strain collection, comprising 28 MRSA strains (numbers only) and 3 MRSP strains. Host species are indicated by color codes: 18 strains originated from horses (green), 5 from dogs (yellow), 5 from pigs, 2 from cows (pink), and one from a sheep (orange).

MRSA, methicillin-resistant *Staphylococcus aureus*; MRSP, methicillin-resistant *Staphylococcus pseudintermedius*; CC, clonal complex.

lack various virulence factors, including genes coding for enterotoxins, toxic shock syndrome toxin, and leukocidins [19-22]. Our data, as well as the results in previous studies, suggest that CC398 is replacing equine-adapted MRSA lineages as the predominant CC detected in horses [5,7,19,23,24]. The only other CC detected among the equine MRSA strains in this study was CC8, with one isolate assigned to t008 and 2 isolates assigned to t009. The *spa* type t008 has been previously linked to *pvl*-positive strains in calves in Switzerland and pigs in Cuba, as well as to community-associated MRSA strain USA300, which causes severe infections in humans [25]. While the t008 strain detected in this study did not harbor *pvl*, we detected genes of the immune evasion gene cluster encoding staphylokinase (*sak*), the chemotaxis inhibitory protein (*chp*), and the complement inhibitor (*scn*). These genes are associated with the integrase group 3 phages present in MRSA CC398 strains of human origin but are usually absent in *S. aureus* from pigs or cattle [5]. The *spa* type t009 has been reported in MRSA, causing equine infections in Germany [23]. Both equine CC8/t009 strains detected in this study are closely related to the strain known as Hannover EMRSA or UK-EMRSA-10, an MRSA strain of CC8/ST254 with an atypical SCC*mec* that has been sporadically detected in humans [26].

Apart from CC398 and CC8, only one other CC (CC1) was detected. The single MRSA strain assigned to CC1 was from a dog and belonged to t127, a *spa* type previously detected in equine, bovine, and porcine MRSA, as well as in MRSA isolated from veterinarians [23,27-29]. The MRSA CC1 strain in the present study was closely related to MRSA AK-1, a strain frequently identified among community-associated and hospital-associated MRSA in New Zealand [30]. None of the canine strains in our study was assigned to CC22, which had been detected in 82% of the MRSA strains isolated from domestic cats and dogs in a previous Swiss study [31].

All MRSA isolates tested in our study were not only resistant to penicillin and oxacillin, but also tetracycline. Consistent with our findings, LA-MRSA were reported to characteristically harbor not only *mecA*, which confers resistance to beta lactams, but also the tetracycline resistance determinant *tetM*, either alone or in combination with *tetK* and/or *tetL* [21,32]. Various other genes conferring resistance to antimicrobial agents were also detected in this study, including genes involved in resistance to macrolide/lincosamide/streptogramin B (*ermA*, *msrA*, *mphC*), aminoglycosides (*aacA-aphD*, *aphA3*), streptothricins (*sat*), and trimethoprim (*dhfrSI*). These results are consistent with those in a previous report on the occurrence of resistance genes in MRSA from an animal origin [4].

The VITEK system used for antimicrobial susceptibility testing in this study is highly popular in routine diagnostic testing. However, it should be noted that, for technical reasons, not every result included in a VITEK report is based on testing of the respective concentration of the antimicrobial agent, but rather on inferring results from data points of higher/lower concentrations. Therefore, MIC values generated using this system need to be interpreted with caution. In addition, a lack of suitable clinical breakpoints for many host species impedes MIC interpretation and the classification of strains as susceptible, intermediate, or resistant [33]. We used veterinary clinical breakpoints determined for dogs for MIC interpretation in canine, equine, porcine, bovine, and ovine MRSA to determine susceptibility to enrofloxacin, marbofloxacin, and clindamycin. Human clinical breakpoints were used for penicillin, oxacillin, vancomycin, erythromycin, rifampicin, gentamicin, tetracycline, nitrofurantoin, chloramphenicol, and trimethoprim/sulfamethoxazole. No breakpoints were available for ampicillin/sulbactam (2/1), imipenem, fusidic acid, mupirocin, and kanamycin. Additional veterinary clinical breakpoints are urgently needed to allow for accurate susceptibility classification of veterinary isolates and to improve therapeutic recommendations.

It has been suggested that human infections caused by methicillin-susceptible *S. pseudintermedius* are likely underreported due to misidentification as MRSA based on false-positive results in the PBP2 α latex agglutination test combined with coagulase positivity and beta hemolysis [34]. Our results indicate that differentiation between MRSA and MRSP originating from animals is a challenge to routine diagnostic services. All MRSP isolates in our study tested positive for plasma coagulase, Staphaurex Latex Agglutination, and PBP2 α , and exhibited phenotypes on MRSA2 Brilliance agar that were practically indistinguishable from those of MRSA isolates. While MRSP is more likely than MRSA to test negative on cefoxitin screening, cefoxitin-negative MRSA results frequently occur [35,36], and one such incidence was detected in this study. Routine diagnostic testing of presumptive MRSA strains for acetoin production and urease activity could lower the rate of MRSP being misclassified as MRSA. Dogs are frequent carriers and natural hosts of the opportunistic pathogen *S. pseudintermedius*, which can cause canine pyoderma, ear and wound infections, as well as urinary tract infections [37]. MRSP is gaining attention as an emerging nosocomial pathogen in dogs and is linked to surgical wound infections, joint prosthesis infection, and fatal cases of necrotizing fasciitis and septicemia [37]. Clear diagnostic differentiation of MRSP from MRSA is not only crucial in preventing spread of the organisms and selecting suitable therapeutic interventions but also in improving the understanding of MRSP-associated diseases.

Strains of CC398/t011 were predominant among the collection of clinical MRSA strains from animal sources tested in this study. MRSA of CC398/t011 were detected in equine, porcine, bovine, ovine, and canine hosts, stressing the wide dissemination of this characteristically LA-MRSA lineage in both livestock and companion animals. Nonetheless, consistent with previous publications [21,32,38,39], the CC398 strains characterized in this study harbored very few virulence factors such as genes coding for enterotoxins, toxic shock syndrome toxin, and leukocidins. While this study focused on MRSA isolated from diseased animals in Switzerland, MRSA with similar genomic traits and phenotypic resistance patterns have also been detected in asymptomatic animal carriers, suggesting that isolates colonizing these animals serve as reservoirs for infection. A Swiss study by Huber et al. [17] that included 800 pigs, 300 calves, 400 cattle, and 100 chicken carcasses at slaughter as well as 142 bovine mastitis milk samples, detected MRSA among 10 pigs, 3 calves, one bull, and 2 mastitis milk samples. The following combinations of sequence, *spa*, and SCC*mec* types were detected: ST398-t034-V in the pigs, ST1-t127-IV in the bull, and ST398-t011-V in the bovine mastitis milk samples. A study by Overesch et al. [18], in which nasal cavities of pigs were sampled, revealed that between 2.0% and 5.9% of the animals were MRSA carriers in 2009, and the following combinations of sequence, *spa*, and SCC*mec* types were detected: ST398-t034-V, ST49-t208-V, ST398-t011-V, ST398-t1451-V, and ST1-t2279-IV. A study by Kittl et al. [40] published in 2020 revealed a marked increase in MRSA prevalence among pigs compared to 2% in 2009, with 44% being MRSA carriers in 2017 as well as a moderate increase in calves (8.1% in 2017).

We included in this study all isolates identified as presumptive MRSA by a routine laboratory diagnostic service between 2013 and 2016, isolates that had tested positive in the Staphaurex Plus Latex Agglutination Test. A remarkably high share of the MRSA isolates originated from infections in horses, mostly specified as abscesses, but more rarely also in association with joint infections and sepsis. This pattern is consistent with a recent Swiss study identifying horses as a risk factor for LA-MRSA carriage in veterinarians [40].

In conclusion, the presented results extend the current body of knowledge on the population structure, resistance phenotypes, and virulence and resistance gene profiles of MRSA isolates

from diseased livestock and companion animals. The predominant CC detected was the livestock-associated CC398 (86%), but CC8 (11%) and CC1 (4%) were also observed. The predominant *spa* type was t011 (n = 23), followed by t009 (n = 2), t034 (n = 1), t008 (n = 1), and t127 (n = 1). The same CCs were described in veterinarians and farmers in Switzerland, further underlining the importance of MRSA in animals as a potential source of MRSA nasal carriage and infection in humans.

The high rate of MRSP misclassified as MRSA detected in this study emphasizes the need for diagnostic approaches that differentiate between MRSA and MRSP. Screening for MRSA using chromogenic media such as MRSA2 Brilliance agar should be complemented with acetoin production and urease testing to avoid misclassification of MRSP as MRSA. Such screening is particularly relevant for samples from dogs, which are a natural host of MRSP.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Genomic characteristics. Comprehensive overview of all DNA microarray hybridization profiles, indicating *spa* typing and clonal complex assignment, as well as sample type for each isolate (<http://doi.org/10.17632/r9kpz942sh.1>)

[Click here to view](#)

Supplementary Table 2

Resistance phenotype overview: overview of MICs determined (<http://doi.org/10.17632/r9kpz942sh.1>)

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Supplementary Table 3

Resistance phenotype of each isolate, including detailed MIC results for each isolate (<http://doi.org/10.17632/r9kpz942sh.1>)

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