Prevalence and whole genome-based phylogenetic, virulence and antibiotic resistance characteristics of nasal *Staphylococcus aureus* in healthy Swiss horses

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Prävalenz und genombasierte Phylogenie, Virulenz und Antibiotikaresistenz von *Staphylococcus aureus* aus der Nase von gesunden Schweizer Pferden

Zwischen Januar 2020 und August 2020 wurden von 100 gesunden Pferden in der Schweiz Nasenabstriche gesammelt. Die Proben wurden von Pferden aus 40 verschiedenen Ställen in 12 verschiedenen Kantonen entnommen und sowohl auf Methicillin-resistente (MRSA), als auch auf Methicillin-sensitive *S. aureus* (MSSA) mit Hilfe von selektiven Agarplatten untersucht. Die Antibiotika-Empfindlichkeit der Staphylokokken wurde durch Messung der minimalen Hemmkonzentration (MHK) bestimmt. Weiter wurden Resistenz – und Virulenzgene, sowie auch phylogenetische Eigenschaften mittels Genom-Sequenzierung untersucht. Zehn Pferde waren positiv für *S. aureus* (10 %, KI: 95 %, 0,0552-0,1744), und vier davon waren MRSA (4 %, CI: 95 %, CI: 1,5 % – 9 %). MRSA Stämme wurden in drei verschiedenen Ställen in der gleichen Region eines Kantons nachgewiesen, während MSSA-Stämme in fünf verschiedenen Kantonen und fünf verschiedenen Ställen nachgewiesen wurden. Alle MRSA-Isolate waren genetisch verwandt (ST398-t011-IVa), während die MSSA unterschiedlich waren (ST1-t127/t398/t1508, ST816-t1294, ST133-t1403, ST30-t012). MRSA Stämme zeigten Resistenzen gegen Penicillin (*blaZ*), Cefoxitin (*mecA*), Trimethoprim (*dfrK*), Gentamicin, Kanamycin (*aac(6')-Ie – aph(2'')-Ia*) und Tetracyclin (*tet*(M)). MSSA Stämme wiesen entweder keine Resistenz auf oder nur eine Resistenz gegen eines der getesteten Antibiotika wie Penicillin (*blaZ*) und Erythromycin (*erm*(T)). Virulenzgene waren bei MSSA häufiger zu finden als bei MRSA. Diese Studie liefert einen ersten Einblick in die Prävalenz und die Genotypen von *S. aureus* bei gesunden Schweizer Pferden und deckt eine mögliche Quelle von Stämmen auf, die sowohl bei Pferden als auch beim Menschen zu Infektionen führen können.

Schlüsselwörter: Antibiotika, Genotypisierung, Pferd, Resistenz, Tiere, Virulenz-Faktoren

Summary

A total of 100 nasal swabs were collected from healthy horses in Switzerland between January 2020 and August 2020. The samples were taken from horses at 40 different stables in 12 different cantons and screened for both methicillin-resistant (MRSA) and methicillin-susceptible *S. aureus* (MSSA) using selective agar plates. *S. aureus* were tested for antibiotic susceptibility by measurement of the minimal inhibitory concentration (MIC) and for virulence factors, antibiotic resistance genes and phylogenetic characteristics using whole genome sequence analysis. Ten horses were found to be positive (10 %, CI: 95 %, 0,0552 – 0,1744) for *S. aureus*, and four of them harboured MRSA (4 %, CI: 95 %, CI: 1,5 % – 9 %). The MRSA were detected in horses from three different stables in the same region of one canton and MSSA were detected in horses from five different cantons. All the MRSA isolates were genetically related (ST398-t011-IVa), while the MSSA were diverse (ST1-t127/t398/t1508, ST816-t1294, ST133-t1403, ST30-t012). MRSA showed resistance to penicillin (*blaZ*), cefoxitin (*mecA*), trimethoprim (*dfrK*), gentamicin, kanamycin (*aac(6')-Ie – aph(2'')-Ia*), and tetracycline (*tet*(M)). MSSA were resistant to either none or one of the antibiotics tested like penicillin (*blaZ*) and erythromycin (*erm*(T)). Virulence genes were more abundant in MSSA than in MRSA. This study provides first insight into the prevalence and type of *S. aureus* in healthy Swiss horses and reveals a source of strains, which may cause infections in both horses and humans.

Keywords: Animals, antibiotic, equine, genotyping, resistance, virulence factors

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[§]Equal contribution

Introduction

Staphylococcus aureus is part of the normal skin and nasal flora of many healthy humans and can also colonize a variety of animals including livestock, companion animals, wildlife, and horses.[2,19,20,26] It is also recognized as one of the most important opportunistic pathogens in humans and animals.[49] In humans, it can cause different types of infections ranging from minor skin infections to a wide range of more serious infections like pneumonia, meningitis, endocarditis, osteomyelitis, toxic shock syndrome, bacteraemia, as well as food poisoning.[57] In animals, *S. aureus* has also been associated with a wide range of infections predominantly affecting skin and soft tissues such as bovine mastitis, necrosis in chicken, pyoderma, otitis, urinary tract infections in companion animals, joint infections, equine pastern dermatitis, pneumonia, sinusitis, omphalitis, osteomyelitis, tenosynovitis, metritis, mastitis and bacteraemia in horses.[8,23,30,65] Infections with *S. aureus* most frequently occur after surgery and use of indwelling medical devices in companion animals and horses.[51,65,68]

S. aureus can possess an array of different virulence factors enabling host innate immune system evasion and enhancing pathogenicity like adhesins, cytotoxins, and superantigens (enterotoxins and toxic shock syndrome toxin-1).[10,55] Some *S. aureus* strains have become very difficult to treat with antibiotics due to chromosomal mutations and acquisition of mobile genetic elements containing antibiotic resistance genes.[21,35] Methicillin-resistant *S. aureus* (MRSA) is of particular concern, it is resistant to all beta-lactam antibiotics, except the anti-MRSA cephalosporins, as well as to the majority of other classes of antibiotics.[48] MRSA are characterized by the presence of a methicillin resistance gene *mec* gene encoding an alternative penicillin binding protein (PBP2a), located on staphylococcal cassette chromosome *mec* (SCC*mec*) elements.[35] Specific MRSA genotypes have spread in different environments and are identified as healthcare-acquired (HA-MRSA), community-acquired (CA-MRSA), or livestock-acquired MRSA (LA-MRSA).[35,53] LA-MRSA emerged in livestock in the 2000's and a particular genotype belonging to sequence type (ST) ST398 has spread mainly in pigs, but also in cattle, horses and companion animals.[25,27] It may also be transmitted to people who come into close contact with animals. Farmers, veterinarians, animal health-care personnel, care-takers and owners are most likely to become colonized and in rare cases develop infections with LA-MRSA.[9,12,13,52] On the other hand, animals may also acquire *S. aureus* from humans.[25]

In horses, the nose appears to be the most common site for *S. aureus* colonization.[64] Most horses show a transient colonization with MRSA. The bacteria are normally eliminated within weeks after colonization, however some of them remain colonized for prolonged periods of time.[45,64] Horses frequently acquire MRSA through hospitalization, where they become either colonized or develop infections, mostly after having undergone surgery.[63] Both hospitalized horses and horse clinic personnel may represent a reservoir of both methicillin-susceptible *S. aureus* (MSSA) and MRSA. Transmission may occur in both directions.[14,28,54] Recently, MRSA from horses belonging to ST398 *spa* type t011 (ST398-t011) were found to harbour the novel leukocidin LukPQ, which is associated with a specific staphylococcal complement inhibitor (SCIN), named eqSCIN, located on a prophage ΦSaeq1.[15,33] This eqSCIN was shown to have a broader activity than the common human-specific SCIN of *S. aureus*, blocking innate immune responses in humans, horses, and pigs, thus facilitating immune evasion and dissemination among different hosts.[15] In Swiss equine clinic settings, MRSA were found to belong mainly to ST398-t011, harbouring the SCC*mec* IVa (ST398-t011-IVa), while MSSA belonged to ST1-t2863, ST1-t127 and ST1660-t3043.[51] However, the nasal carriage prevalence and type of MRSA and MSSA in the healthy Swiss horse population is unknown, prompting us to screen healthy horses without hospitalization in the 6 months prior to analysis for the presence of *S. aureus*. The isolates were tested for antibiotic susceptibility by measurement of the MIC and for virulence factors, antibiotic resistance genes and phylogenetic characteristics using whole genome sequence analysis.

Materials and Method

Study population and data collection

A total of 100 horses from 40 different stables in 12 cantons (Figure 1), where the horse population is the largest in Switzerland (>2500 horses) (Aargau, Bern, Basel-Landschaft, Fribourg, Jura, Luzern, Solothurn, St. Gallen, Thurgau, Ticino, Vaud, Zurich, with the exception of the canton Valais and Graubünden, where no horses were tested),[1] were sampled between January 2020 and August 2020. The maximum of horses tested per stable was four. The horse owners were contacted by phone or via social media. In February 2021, the horses that tested positive for MRSA underwent a second screening. The health status of every horse was assessed by clinical examination before the samples were taken. The clinical examination included recording of heart rate, respiratory rate, rectal temperature, palpation of submandibular lymph nodes, and inspecting mucous membranes and skin for signs of infectious diseases. Only horses without any signs of health disorder were enrolled in the study (Table 1). Information about the horses was collected by means of a questionnaire including age, breed, species, sex, history of previous medical disorders, antibiotic treatment in the preceding two weeks, and hospitalization in the preceding six months

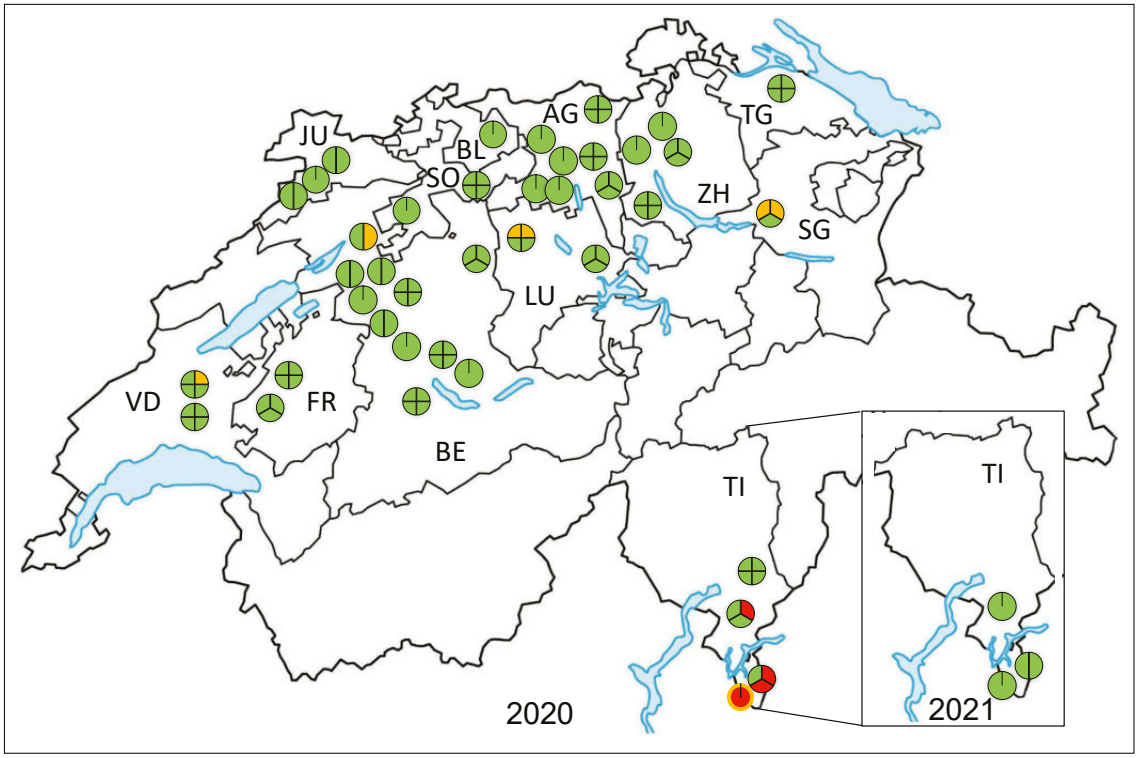

Figure 1: Geographical distribution of the 100 healthy horses screened for nasal carriage of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) in Switzerland in 2020. The window indicates the carriage status in 2021 of the four horses which were MRSA-positive in 2020. The geographical location of stables and number of horse screened per stables are indicated by coloured pie charts with each black radius line of the circle corresponding to one horse. Green pie pieces represent horses with no *S. aureus*, red pie represent horses with MRSA, and orange pie pieces represent horses with MSSA. The orange/red circle indicates one horse which harboured both one MRSA and one MSSA. Number of horses and stables tested in the different cantons: AG, Aargau (15 horses, 7 stables); BE, Bern (26 horses, 11 stables); BL, Basel-Landschaft (1 horse, 1 stable); FR, Fribourg (7 horses, 2 stables); JU, Jura (5 horses, 3 stables); LU, Luzern (7 horses, 2 stables); SO, Solothurn (5 horses, 2 stables); SG, St. Gallen (3 horses, 1 stable); TG, Thurgau (3 horses, 1 stable); TI, Ticino (11 horses, 4 stables); VD, Vaud (8 horses, 2 stables); ZH, Zurich (9 horses, 4 stables). Number of MSSA detected in Vaud (n=1), Bern (n=1), Luzern (n=2), St.Gallen (n=2) and Ticino (n=1). Number of MRSA detected in Ticino (n=4).

(Table 1). The period of two weeks for the antibiotic treatment and six months for hospitalization were arbitrarily chosen. The short period for no antibiotic treatments should insure absence of antimicrobial selective pressure and maximize reliability of owner information. The considerably longer period of six months for hospitalization reflects the much higher risk of colonization with MRSA in hospitalized horses[66] and owners can be expected to reliably recall events like a hospitalization. The study was approved by the veterinary ethical committees of all concerned cantons in Switzerland (VD3297+) and written consent was given by all owners. After the clinical examination, the samples were taken from one nostril of the horses. A swab (Puritan Opti-Swab, Puritan Medical Products, Gulford, ME, US) was inserted at least 10 cm into the nares and further rubbed on the ventral nasal passage against the mucosa for a few seconds. The swabs were transported within a transporter fluid (SwabAX liquid amies from Axonlab) at room temperature to the Institute of Veterinary Bacteriology in Bern where they were processed directly.

Sample processing and identification of *S. aureus*

The swabs were incubated in 5 ml Mueller-Hinton broth (Becton, Dickinson and Company) containing 6,5 % NaCl at 37 °C overnight under agitation. One loopful of the incubated broth was streaked onto plates of chromogenic SaSelect™ agar (Bio-Rad, Hercules, CA, US) for the selection of *S. aureus*, and of BBL™ CHROMagar™ MRSA II (Becton and Dickinson Company, Franklin Lakes, NJ, US) for the selection of MRSA. The two plates were incubated at 37°C overnight. Resulting colonies displaying pink to orange colours on the SaSelect™ agar and pink to purple colours on the BBL™ CHROMagar™ MRSA II were selected, and identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics GmbH, Bremen, Germany). After identification, the *S. aureus* colonies were purified on BBL™ Trypticase™ Soy Agar plates containing 5 % sheep blood (TSA-S, Becton and Dickinson Company, Franklin Lakes, NJ, US), and identification was confirmed

Table 1: Epidemiological and medical information of the 100 horses tested for *Staphylococcus aureus* carriage.

Horse	Stable	MSSA/MRSA carriage	Age [year]	Breed	Sex	Temperature [°C]	Heart rate [min^{-1}]	Respiratory rate [min^{-1}]	Underlying disease (history and clinical signs)	Hospitalisation (in the last 6 months)	Administration of antibiotics (in the last 2 weeks)
H1	S1	MSSA	10	warmblood	f	37,5	20	12	None noted	No	No
H2	S1		10	warmblood	f	37,2	36	8	None noted	No	No
H3	S1		n.a.	warmblood	m	37	40	16	None noted	No	No
H4	S1		11	warmblood	f	37	32	12	None noted	No	No
H5	S2	MRSA	21	warmblood	g	n.d.	n.d.	n.d.	None noted	No	No
H6	S2	MRSA	12	mixed breed	f	n.d.	n.d.	n.d.	None noted	No	No
H7	S2		12	warmblood	g	n.d.	n.d.	n.d.	None noted	No	No
H8	S3		19	light draught horse	f	n.d.	n.d.	n.d.	None noted	No	No
H9	S3	MRSA	11	light draught horse	f	n.d.	n.d.	n.d.	None noted	No	No
H10	S3		4	light draught horse	f	n.d.	n.d.	n.d.	None noted	No	No
H11	S4		11	warmblood	f	37,7	36	16	None noted	No	No
H12	S4		15	warmblood	f	37,6	32	12	None noted	No	No
H13	S4		5	warmblood	f	36,5	28	18	None noted	No	No
H14	S5		12	mixed breed	f	37,1	20	36	None noted	No	No
H15	S6		5	western horse	g	37,1	40	12	None noted	No	No
H16	S7	MSSA	7	pony	g	37,2	40	24	None noted	No	No
H17	S7		10	light draught horse	g	36,8	40	20	None noted	No	No
H18	S7	MSSA	27	light draught horse	g	37,9	36	28	None noted	No	No
H19	S8		13	trotter	m	37,5	16	12	None noted	No	No
H20	S9		9	light draught horse	f	38	28	16	None noted	No	No
H21	S9		14	light draught horse	g	37,6	28	12	None noted	No	No
H22	S9		6	light draught horse	f	38	32	12	None noted	No	No
H23	S10		8	light draught horse	f	37,2	40	12	None noted	No	No
H24	S11		12	light draught horse	g	36,8	24	16	None noted	No	No
H25	S11		9	mixed breed	g	37,8	32	16	None noted	No	No
H26	S11	MSSA	22	pony	g	37,5	36	20	None noted	No	No
H27	S11	MSSA	15	pony	g	37,9	52	20	None noted	No	No
H28	S12		13	iberian horse	g	37,4	28	12	None noted	No	No
H29	S12		17	warmblood	g	37,5	28	16	None noted	No	No
H30	S12		11	light draught horse	f	37,8	32	12	None noted	No	No
H31	S13		15	warmblood	g	37,4	32	12	None noted	No	No
H32	S13		19	western horse	f	37,5	40	12	None noted	No	No
H33	S13		7	western horse	f	36,4	36	12	None noted	No	No
H34	S14		9	heavy draught horse	g	37,6	28	12	None noted	No	No
H35	S13		18	light draught horse	g	37,6	40	12	None noted	No	No
H36	S15		8	warmblood	g	37,6	28	12	None noted	No	No
H37	S15		20	warmblood	g	37,5	24	12	None noted	No	No
H38	S16		17	warmblood	f	35,2	24	12	None noted	No	No
H39	S16		3	warmblood	g	37,2	36	12	None noted	No	No
H40	S17		14	warmblood	f	37,7	36	16	None noted	No	No
H41	S17		19	pony	g	38,1	48	20	None noted	No	No
H42	S17		9	pony	f	37,9	44	16	None noted	No	No
H43	S18	MRSA/MSSA	9	iberian horse	g	n.d.	n.d.	n.d.	None noted	No	No
H44	S19		24	arabian	g	36,4	36	12	None noted	No	No
H45	S19		10	arabian	f	36,5	24	12	None noted	No	No
H46	S20		10	not specified	f	38	40	12	None noted	No	No
H47	S20		10	warmblood	g	38	40	12	None noted	No	No
H48	S20		14	pony	g	37,4	36	12	None noted	No	No
H49	S20		14	pony	g	37,9	32	16	None noted	No	No
H50	S21		6	pony	f	37,4	28	16	None noted	No	No
H51	S22		13	warmblood	f	37,4	36	12	None noted	No	No

Horse	Stable	MSSA/MRSA carriage	Age [year]	Breed	Sex	Temperature [°C]	Heart rate [min^{-1}]	Respiratory rate [min^{-1}]	Underlying disease (history and clinical signs)	Hospitalisation (in the last 6 months)	Administration of antibiotics (in the last 2 weeks)
H52	S23	MSSA	17	warmblood	g	38	28	12	None noted	No	No
H53	S23		11	warmblood	g	37,7	32	12	None noted	No	No
H54	S24		8	warmblood	f	38	40	16	None noted	No	No
H55	S24		14	warmblood	g	37,4	36	12	None noted	No	No
H56	S24		22	light draught horse	f	37,4	36	12	None noted	No	No
H57	S24		14	warmblood	g	37,9	36	12	None noted	No	No
H58	S25		24	warmblood	g	n.d.	n.d.	n.d.	None noted	No	No
H59	S25		17	warmblood	f	n.d.	n.d.	n.d.	None noted	No	No
H60	S25		18	warmblood	g	n.d.	n.d.	n.d.	None noted	No	No
H61	S25		15	warmblood	f	n.d.	n.d.	n.d.	None noted	No	No
H62	S26		13	warmblood	m	37,8	24	12	None noted	No	No
H63	S26		16	light draught horse	g	37,5	32	16	None noted	No	No
H64	S26		15	pony	g	37,5	28	12	None noted	No	No
H65	S26		11	iberian horse	g	37,2	36	12	None noted	No	No
H66	S27		19	warmblood	g	37,8	28	12	None noted	No	No
H67	S27		6	warmblood	g	37,2	28	12	None noted	No	No
H68	S28		17	light draught horse	f	37,9	36	16	None noted	No	No
H69	S29		5	warmblood	g	n.d.	n.d.	n.d.	None noted	No	No
H70	S29		17	warmblood	f	n.d.	n.d.	n.d.	None noted	No	No
H71	S29		31	warmblood	f	n.d.	n.d.	n.d.	None noted	No	No
H72	S29		11	warmblood	g	n.d.	n.d.	n.d.	None noted	No	No
H73	S30		11	warmblood	f	37,6	32	12	None noted	No	No
H74	S30		9	warmblood	f	37,7	28	16	None noted	No	No
H75	S30		10	light draught horse	f	37,5	28	12	None noted	No	No
H76	S30		11	pony	g	37,7	36	16	None noted	No	No
H77	S31		7	warmblood	f	37,6	32	16	None noted	No	No
H78	S32		15	warmblood	f	38	28	16	None noted	No	No
H79	S32		16	warmblood	g	36,9	36	20	None noted	No	No
H80	S32		6	warmblood	f	37,7	32	16	None noted	No	No
H81	S32		20	warmblood	f	36,7	44	32	None noted	No	No
H82	S33		19	warmblood	g	36,8	32	8	None noted	No	No
H83	S33		10	warmblood	g	37,7	40	12	None noted	No	No
H84	S34		20	warmblood	f	37,9	24	12	None noted	No	No
H85	S35		20	light draught horse	g	37,7	28	16	None noted	No	No
H86	S35		9	warmblood	f	37,5	28	12	None noted	No	No
H87	S36		11	arabian	g	37,6	28	12	None noted	No	No
H88	S36		17	pony	g	37,5	32	16	None noted	No	No
H89	S36		20	pony	f	37,5	28	16	None noted	No	No
H90	S37		6	warmblood	g	37,7	28	16	None noted	No	No
H91	S37		14	pony	g	37,9	24	20	None noted	No	No
H92	S38		6	pony	g	37,8	32	16	None noted	No	No
H93	S38		19	warmblood	g	37,3	24	12	None noted	No	No
H94	S38		8	not specified	f	37,6	28	16	None noted	No	No
H95	S38		27	iberian horse	m	37,2	28	16	None noted	No	No
H96	S39		4	light draught horse	g	37,9	32	20	None noted	No	No
H97	S39		24	pony	f	37,7	28	16	None noted	No	No
H98	S39		4	light draught horse	f	37,5	32	16	None noted	No	No
H99	S39		12	light draught horse	g	37,4	32	12	None noted	No	No
H100	S40		9	pony	f	38,1	40	16	None noted	No	No

MSSA, MRSA, methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*. f, female; m, male; g, geldings. n.d., not determined. n.a., not available.

by MALDI-TOF MS after 18h of incubation at 37 °C. The *S. aureus* cultures were frozen at – 80 °C in glycerol stocks.

Minimal inhibitory concentration (MIC)

Minimal inhibitory concentration (MIC) of 19 antibiotics was determined by broth microdilution method using Sensititre EUST plates (Thermo Fisher Scientific) and following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[56] These plates were chosen to apply the same procedures as those described in Directive 2003/99/EC for a harmonized antimicrobial resistance monitoring in zoonotic and indicator bacteria in animals in the European Union and Switzerland.[17,18] The MIC were interpreted using the EUCAST resistance breakpoints for *S. aureus*, except for kanamycin and sulfamethoxazole for which the breakpoints from of the Clinical and Laboratory Standards Institute (CLSI) were used: gentamicin (>1 µg/ml), kanamycin (≥64 µg/ml), penicillin (>0,125 µg/ml), cefoxitin (>4 µg/ml), trimethoprim (>4 µg/ml), tetracycline (>2 µg/ml), erythromycin (>2 µg/ml), clindamycin (>0,5 µg/ml), rifampicin (>0,5 µg/ml), fusidic acid (>1 µg/ml), chloramphenicol (>8 µg/ml), tiamulin (>2 µg/ml), synercid (>2 µg/ml), vancomycin (>2 µg/ml), linezolid (>4 µg/ml) and sulfamethoxazole (≥512 µg/ml).[11,56] For streptomycin for which no resistance breakpoint exists, a breakpoint of >32 µg/ml was tentatively used. Inducible clindamycin resistance was determined by disk diffusion using *D*-test and broth dilution by the addition of 1µg/ml of erythromycin in the Mueller-Hinton broth used for MIC determination following CLSI recommendations.[11]

Whole genome sequencing (WGS)

WGS libraries were prepared from DNA extracted from colonies grown on TSA-SB agar plates overnight at 37°C using the Nextera™ DNA Flex microbial colony extraction protocol (Illumina Inc., San Diego, CA, US). Prior to library preparation, the DNA was purified using the AMPure XP magnetic beads (Beckman Coulter, Brea, CA). Libraries were prepared with the Nextera DNA Flex Library Prep Kit following the manufacturer's instructions (Illumina), and sequenced (2x150 bp paired-end) on an Illumina MiSeq platform at the Next Generation Sequencing Platform, Institute of Genetics, Vetsuisse Faculty, University of Bern. The Illumina reads of the MSSA and MRSA strains from healthy horses were deposited into the NCBI database under BioProject PRJNA692738 with SRA experiment acc. no. SRX9863707 (JIH106N, isolate name: horse106_nose), SRX9863708 (JIH110N, isolate name: horse110_nose_MRSA), SRX9863709 (JIH111N, isolate name: horse111_nose_MRSA), SRX9863712 (JIH114N, isolate name: horse114_nose_MRSA), SRX9863713 (JIH121N, isolate name: horse121_nose), SRX9863715 (JIH123N, isolate name: horse123_nose), SRX9863719 (JIH131N, isolate name: horse131_nose), SRX9863720 (JIH132N, isolate name: horse132_nose), SRX9863722 (strain JIH148Na, isolate name: horse148_nose), SRX9863723

Table 2: Genetic characteristics and antibiotic resistance profile of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from healthy horses in Switzerland.

Strains and origin					Genotype			Antibiotic resistance phenotype and genotype (resistance breakpoints (R) and MIC in mg/L)											
								GEN	KAN	GEN/KAN	PEN		FOX		TMP		TET		ERY	
Strain	MSSA/ MRSA	Horse	Stable	Canton	ST	*spa*	*SCC-mec*	R>1	R≥64		R>0,125		R>4		R>4		R>2		R>2	
JIH106N	MSSA	H1	S1	VD	1	t127		≤1	≤4		≤0,12		4		≤2		≤0,5		≤0,25	
JIH121N	MSSA	H16	S7	SG	1	t398		≤1	≤4		≤0,12		4		≤2		≤0,5		≤0,25	
JIH131N	MSSA	H26	S11	LU	816	t1294		≤1	≤4		≤0,12		4		≤2		≤0,5		≤0,25	
JIH148Na	MSSA	H43	S18	TI	133	t1403		≤1	≤4		≤0,12		4		≤2		≤0,5		≤0,25	
JIH123N	MSSA	H18	S7	SG	398	t4030		≤1	≤4		≤0,12		4		≤2		≤0,5		>8	*erm*(T)
JIH132N	MSSA	H27	S11	LU	30	t012		≤1	≤4		2	*blaZ*	4		≤2		≤0,5		0,5	
JIH157N	MSSA	H52	S23	BE	1	t1508		≤1	≤4		>2	*blaZ*	4		≤2		≤0,5		≤0,25	
JIH111N	MRSA	H5	S2	TI	398	t011	IVa	8	>64	*aac(6')-Ie-aph(2'')-Ia*	>2	*blaZ*	>16	*mecA*	>32	*dfrK*	>16	*tet*(M)	≤0,25	
JIH110N	MRSA	H6	S2	TI	398	t011	IVa	4	>64	*aac(6')-Ie-aph(2'')-Ia*	>2	*blaZ*	16	*mecA*	>32	*dfrK*	>16	*tet*(M)	≤0,25	
JIH114N	MRSA	H9	S3	TI	398	t011	IVa	8	64	*aac(6')-Ie-aph(2'')-Ia*	>2	*blaZ*	16	*mecA*	>32	*dfrK*	>16	*tet*(M)	≤0,25	
JIH148Nb	MRSA	H43	S18	TI	398	t011	IVa	8	>64	*aac(6')-Ie-aph(2'')-Ia*	>2	*blaZ*	16	*mecA*	>32	*dfrK*	>16	*tet*(M)	≤0,25	

Cantons: BE, Bern; LU, Luzern; SG, Sankt Gallen; TI, Ticino; VD, Vaud. MIC, minimum inhibitory concentration; ST, Sequence type; SCC*mec*, *Staphylococcus* Cassette Chromosome *mec*; GEN, gentamicin; KAN, kanamycin; PEN, penicillin; FOX, cefoxitin; TMP, trimethoprim; TET, tetracycline; ERY, erythromycin. Antibiotic resistance genes and their functions: *aac(6')-Ie – aph(2'')-Ia*, aminoglycoside acetyltransferase and phosphotransferase tandem genes; *blaZ*, β-lactamase gene; *dfrK*, acquired dihydrofolate reductase; *erm*(T), macrolide, lincosamide and streptogramin B 23S rRNA methylase gene (inducible clindamycin resistance); *mecA*, methicillin-resistance gene encoding PBP2a for resistance to β-lactam-antibiotics; *tet*(M), ribosome protection tetracycline resistance gene. The MIC breakpoints for *S. aureus* were those recommended from the European Committee on Antimicrobial Susceptibility Testing,[56] except for kanamycin for which breakpoint was used from the Clinical and Laboratory Standard Institute.[11]

(JIH148Nb, isolate name: horse148_nose_MRSA), and SRX9863724 (JIH157N, isolate name: horse157_nose). WGS Illumina reads of clinical MRSA strains (17KM2889, 17KM0012, 17KM0743), isolated from infection sites of horses in 2017,[32] were obtained from the NCBI database (SRA experiment acc. no. SRX6491197, SRX6491199, SRX6491200 within BioProject PRJNA556204) and used for comparative genomic analysis. The reads of the MRSA strains JIH110N, JIH111N, JIH114N, JIH148Nb, 17KM2889, 17KM0012, 17KM0743 were also deposited into the Swiss Pathogen Surveillance Platform (SPSP) (https://spsp.ch/).

WGS-based genetic characterization

The WGS raw reads were processed using the software Seqsphere+ (version 7, Ridom GmbH, Münster, Germany) which uses the tools trimmomatic for quality filtering, trimming, and removing adapters from the reads, and velvet for read assembly. SeqSphere+ was also used for multilocus sequence typing (MLST), *spa* typing, and detection of the antibiotic resistance genes using AMRFinder and virulence factors using the Virulence Factor Database (VFDB). The presence of *scn-eq* and *lukPQ* (not yet present in Seqsphere+) was determined by read mapping the illumina reads against the reference sequence containing the genes (GenBank accession number LT671578) using Geneious Prime® 2021.1.1 (Biomatters Ltd.). Point mutations associated with resistance mechanisms were identified using ResFinder and default parameters and the SCC*mec* type was determined using SCCmecFinder, both tools located at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). A core genome multilocus sequence typing (cgMLST) scheme was created with 1,861 queried target genes, as previously described.[36] All genes, which were present in all isolates, were defined as the core genome and included for further analysis. A phylogenetic minimum spanning tree was constructed based on core genome multilocus sequence typing (cgMLST) analysis of 1,861 genes using Seqsphere+. The tree was visualized using iTOL.[37]

Statistical method

Prevalence and CI 95 % were determined using *VassarStats*-calculator. (http://vassarstats.net/prop1.html)

Results

Study population

The horses were 45 mares, 4 stallions and 51 geldings with an average age of 13 years (range 3 to 31 years). The breed of 98 horses was known and consisted as warmbloods (n=47), light draft horses (n=20), pony breeds (n=16), iberian horses (n=4), arabians (n=3), western horses (n=3), mixed breeds (n=3), heavy draft horses (n=1) and trotter (n=1). Based on the clinical examination and the information from the questionnaire, all 100 horses had no underlying diseases, had not been in a horse clinic in the last 6 month and did not receive any antibiotic treatment in the last two weeks, and therefore met the inclusion criteria (Table 1).

Geographical distribution of the study population

The 100 healthy horses screened for nasal carriage of *S. aureus* during this study were from 12 different Swiss cantons and 40 different stables (Figure 1). The number of horses and stables which were investigated in each canton was as follows: 26 horses from 11 stables in the canton Bern, 15 horses from 7 stables in the canton Aargau, 11 horses from 4 stables in the canton Ticino, 9 horses from 4 different stables in the canton Zurich, 8 horses from 2 stables in the canton Vaud, 7 horses from 2 stables in the canton Fribourg, 7 horses from 2 stables in the canton Luzern, 5 horses from 3 stables in the canton Jura, 5 horses from 2 stables in the canton Solothurn, 3 horses from 1 stable in the canton St. Gallen, 3 horses from 1 stable in the canton Thurgau, and 1 horse in the canton Basel-Landschaft (Figure 1).

Prevalence and geographical distribution of *S. aureus* positive horses

Ten horses were found to carry *S. aureus* in their nasal cavities, consisting of three horses with MRSA, six with MSSA, and one with both MSSA and MRSA (Figure 1) (Table 2). This represents an overall prevalence of *S. aureus* in the healthy horse population of Switzerland of 10 % (95 %, CI: 5 % – 17 %), and a prevalence of MRSA of 4 % (95 %, CI: 1,5 % – 9 %). The four MRSA strains were detected in four different horses from three stables in the Canton Ticino. The seven MSSA were detected in seven different horses from five different stables situated in the canton Bern (one horse), Luzern (two horses of one stable), St. Gallen (two horses of one stable), Vaud (one horse) and Ticino (one horse which also carried MRSA) (Figures 1 and 2). All the horses harbouring MRSA in 2020 were tested negative for MRSA one year later (Figure 1).

Antimicrobial resistance, virulence factors and phylogenetic analysis of *S. aureus*

The *S. aureus* isolates of each horse were genetically diverse, except for the four MRSA, which clustered into the same branch of the cgMLST phylogenetic tree (Figure 2). All MRSA carried a SCC*mec* type IVa cassette (*mecA*/Δ*mecR1*/IS*1272*/*ccrB2*/*ccrA2*) and belonged to ST398-t011-IVa. One MSSA also belonged to ST398, but differed from the MRSA by a different *spa* type (t4030) and gathered into a different branch of the cgMLST tree. The other six MSSA strains formed different branches of the cgMLST tree and belonged to different

lineages including three different sublineages of ST1 (ST1-t127, ST1-t398, ST1-t1508), and 3 unique lineages of ST816-t1294, ST133-t1403, and ST30-t012 (Figure 2). The four MRSA ST398-t011-IVa also diverged from clinical MRSA ST398-t011-IVa strains, which were chosen as representative of the MRSA lineages causing infections in a veterinary medical teaching hospital (ISME equine clinic, University of Bern) in Switzerland (Figure 2). The four MRSA strains harboured several antibiotic resistance genes and were resistant to penicillin, cefoxitin, trimethoprim, tetracycline, gentamicin and kanamycin (Table 2).

The seven MSSA strains were either susceptible to all tested antibiotics or exhibited resistance to only one of the tested antibiotics such as to penicillin (strains JIH132N and JIH 157N), and erythromycin (JIH123N). Strain JIH123N contained an *erm*(T) gene conferring resistance to erythromycin and inducible resistance to clindamycin (Table 2). The strain expressed an MIC to clindamycin of < 0,5 µg/ml, which increased to 8 µg/ml when induced with erythromycin. Two MSSA strains (JIH132N and JIH148Na) harboured the fosfomycin resistance gene *fosB*, but the resistance phenotype was not measured.

Several virulence genes were detected among the different *S. aureus* strains (Figure 2). All *S. aureus* contained genes associated with host innate immune system evasion (*adsA*, *aur*, *sbi*), adhesins (*ebp*, *ica*), hemolysins (*hld*, *hlgA/B/C*), leukocidins (*lukAB*, also known as *lukHG*, as well as *lukED* and *lukPQ*), lipase (*lip*), heme-iron uptake (*srtB*), whereas enterotoxins (*sea*, *seh*, *sell*), toxic shock syndrome toxin-1 (*tsst-1*), β-hemolysin (sphingomyelinase) (*hlb*), pore-forming toxins (*hly/hla*), complete capsule operon (*cap8*), host innate immune system evasion (*chp*, *map*, *scn*, *scn-eq*, *sdrE*), adhesins (*clfA/B*, *fnbA*), co-factors associated with coagulase and immune system evasion (*coa*, *vWbp*, *sak*), type VII secretion system (T7SS, also known as ESAT-6) (virulence and im-

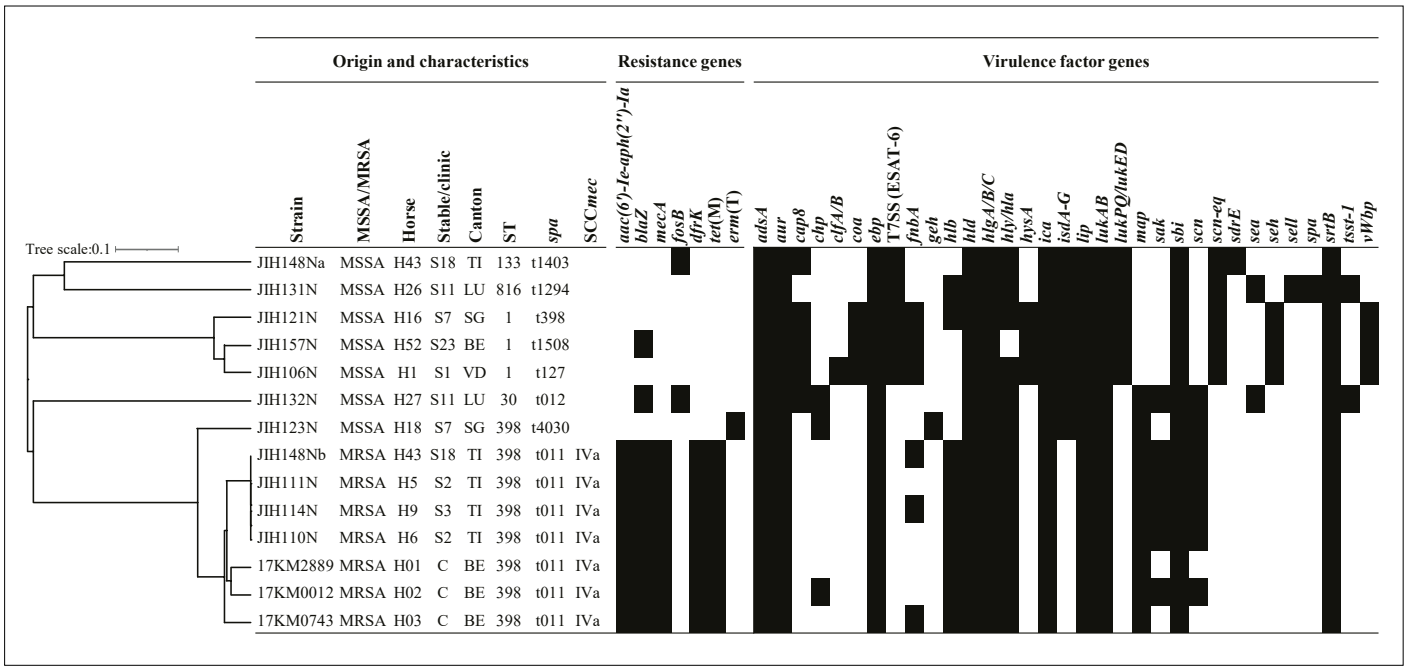

Figure 2: Antibiotic resistance genes and virulence factors of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) isolates from healthy horses in Switzerland. Cantons: BE, Bern; LU, Luzern; SG, Sankt Gallen; TI, Ticino; VD, Vaud. MIC, minimum inhibitory concentration; ST, Sequence type; SCC*mec*, *Staphylococcus* Cassette Chromosome *mec*. Antibiotic resistance genes and their functions: *aac(6')-Ie – aph(2')-Ia*, aminoglycoside acetyltransferase and phosphotransferase tandem genes; *blaZ*, β-lactamase gene; *dfrK*, acquired dihydrofolate reductase; *erm*(T), macrolide, lincosamide and streptogramin B 23S rRNA methylase gene (inducible clindamycin resistance); *fosB*, fosfomycin-modifying enzyme gene; *mecA*, methicillin-resistance gene encoding PBP2a for resistance to β-lactam-antibiotics; *tet*(M), ribosome protection tetracycline resistance gene. Virulence genes and their functions: *adsA*, adenosine synthase A; *aur*, aureolysin; *cap8* (*cap8A-cap8P*), capsular polysaccharide production; *chp*, chemotaxis inhibitory protein; *clfA/B*, clumping factor A and B (fibrinogen-binding protein); *coa*, coagulase; *ebp*, elastin-binding protein; T7SS, type VII secretion system (also known as ESAT-6 (early secreted antigenic target of 6 kDa) secretion system); *fnbA*, fibronectin-binding protein A; *geh*, glycerol ester hydrolase (lipase); *hlb*, β-hemolysin (sphingomyelinase); *hld*, δ-toxin (phenol soluble modulin); *hlgA/B/C*, pore-forming toxins; *hly*/*hla*, α-toxin (pore-forming toxin); *hysA*, staphylococcal hyaluronidase; *ica*, adhesion gene cluster; *isdA-G*, iron-responsive surface determinant gene locus (heme-iron uptake into cells); *lip*, triacylglycerol lipase; *lukAB*, leukocidin AB; *lukED*, leukotoxin (pore-forming toxin); *lukPQ*, equine associated leukocidin; *map*, major histocompatibility complex (MHC) class II analog protein; *sak*, staphylokinase; *sbi*, second immunoglobulin-binding protein; *scn*, human-associated staphylococcal complement inhibitor; *scn-eq*, equine-associated staphylococcal complement inhibitor; *sdrE*, surface protein serine–aspartate repeat protein E; *sea*, *seh*, *sell*, staphylococcal enterotoxins; *spa*, immunoglobulin G-binding protein A; *srtB*, sortase B (regulation of heme-iron uptake into cells); *tsst-1*, toxic shock syndrome toxin-1; *vWbp*, von Willebrand factor binding protein.

mune evasion), lipase (*geh*), hyaluronidase (*hysA*), heme-iron uptake into cells (*isdA-G*), were present in individual strains (Figure 2). Virulence factors like the enterotoxins Se, toxic shock syndrome toxin-1 Tsst-1, leukocidins LukED and LukPQ, T7SS, heme-iron uptake IsdA-G, coagulases Coa and vWbp as well as eqSCIN were only present in MSSA strains. On the other hand, only the MRSA and MSSA ST398 as well as MSSA ST30 contained SCIN and the major histocompatibility complex (MHC) class II analog protein Map, and except for ST30, also the staphylokinase Sak.

Discussion

This is the first study to provide an overview of the distribution and prevalence of *S. aureus* in healthy horses from different cantons of Switzerland. Although samples were not obtained from horses of all 26 Swiss cantons, the data give a general information on *S. aureus* carriage in cantons of Switzerland, where horse population is the largest.[1] Ten percent of the horses harboured *S. aureus* in the nasal cavities, with 4 % being MRSA. Of note, the MRSA prevalence was the results of four horses located in different stables in one specific region of Switzerland in the canton of Ticino. These MRSA were genetically related indicating local dissemination and an outbreak situation at the time of screening. The origin and dissemination vectors of these MRSA between horses of different stables remained unknown. The horses had no history or clinical signs of infectious disease, no antibiotics had been administered in the past two weeks, and no contact to a veterinary clinic in the past six months, where the risk of MRSA acquisition is higher.[51,59] Additionally, deeper genomic analysis of these MRSA ST398-t011-IVa using cgMLST showed that they were distinct from some of those isolated from infection sites of hospitalized horses in Switzerland (Figure 2).[32] The four horses had no history of hospitalization or of travelling to another country. They were occasionally ridden together, where MRSA transmission may have occurred. On occasion, these horses were also ridden by the same persons, and three of these riders had the same veterinarian. Humans may therefore also have played a role in the transmission, but they were not screened for MRSA carriage during this study. Nevertheless, the four MRSA-positive horses were tested negative for MRSA one year later and did not receive any antimicrobial treatments between the two sampling times. It is known that most of the adult horses harbouring MRSA show a transient colonization.[42,45,61] The bacteria are normally eliminated within weeks, but for some reason, a small percentage of horses continue to be colonized for months and perhaps persistently,[45,64] which was not the case for the four horses in Ticino.

Although the MRSA prevalence in horses in Switzerland in 2020 was likely due to a small outbreak in one region, this study provided insights into MRSA carriage in healthy horses in Switzerland. As observed in other countries, the MRSA carriage prevalence in the healthy horse community tends to be usually lower (<0,53 % to 4 %) [6,7,28,31,58] compared to the reported prevalence in horses at hospital admission and in hospitalized horses, which ranges between 1,9 % to 16,4 %.[42,50,51,60,61,67] Similarly to our study, regional dissemination has already been shown to be responsible for increase in prevalence, but such prevalence would decrease when considered on a nationwide scale.[7,24,64] In order to avoid these possible biases, future prevalence studies should be based on a nationwide sampling strategy representative of the horse population in each cantons. Furthermore, the presence of genetically related MRSA in horses from one single region of Switzerland indicated that local MRSA outbreaks may also occur outside of horse clinics, where MRSA acquisition and colonization is known to be a risk for both horses and personnel.[3,51,61] In Switzerland, a pilot study conducted in 2010 showed that only 2,2 % of the horses newly admitted to the veterinary medical teaching hospital (ISME equine clinic, University of Bern) were MRSA carriers,[51] but since then no routine screening has been performed neither at the clinic level nor at the national level within an official surveillance program.

The identified MRSA were all of MRSA ST398-t011-IVa, which is the predominant lineage reported in horses in many countries associated with both carriage and infections.[42] Use of WGS analysis allows to discriminate between different sublineages of MRSA ST398-t011, which is very useful for comparative analyses of strains from different origins and for tracking strains in outbreaks.[32,40] For instance, cgMLST showed that MRSA ST398-t011 strains from infection sites of horses hospitalized in Switzerland diverged from those isolated from the horses in Ticino, indicating an unrelated origin. Similarly, the three MSSA strains belonging to ST1 could be distinguished from each other by different *spa* types and by cgMLST, further emphasizing the need and advantage of WGS for strain typing and molecular epidemiology. Among them, ST1-t127 seems to have a promiscuous colonisation potential as it was found in different animal species and humans,[5,16,22,41,46,47] with a particular affinity to horses where it was found to be the predominant lineage of MSSA in horses in the USA and Denmark, and in horses with equine pastern dermatitis in Switzerland.[28,30,39] Furthermore, it had been previously identified in infection sites of horses in Switzerland.[51] The other MSSA lineages ST816-t1294 as well as ST30 and ST133 were also identified among the MSSA strains from the horses in the USA and Spain, but at lower proportions.[39,43] Unlike MRSA, which were

resistant to several classes of antibiotics, the MSSA were either susceptible to all tested antibiotics or resistant to only one of them. However, virulence factors were more abundant among the MSSA strains. The absence of important virulence factors like superantigens and leukocidins is a known characteristic of LA-MRSA ST398 and is due to the lack of specific pathogenicity islands as well as phage-related chromosomal islands carrying such virulence genes.[4,29,38] However, the reason for the paucity of phage-related virulence factors in LA-MRSA ST398 is still not clear. [34] Superantigens such as phage-related enterotoxins and toxic shock syndrome toxin-1,[44] as well as genes associated with pore-forming toxins, capsule, coagulase, adhesins, hyaluronidase, heme-iron uptake, T7SS and eqSCIN associated immune evasion were only detected among MSSA. While the classic SCIN of *S. aureus* is specific to human hosts, the eqSCIN represents an animal-adapted SCIN variant which blocks the innate immune responses in a broad range of hosts including horses, humans, and pigs.[15] The presence of eqSCIN in *S. aureus* ST1, ST816 and ST133 may explain their particular affinity to horses,[39,43] as well as the potential to colonize humans. Although eqSCIN was originally identified in MRSA ST398-t011 from horses in different countries,[15,62] it was not present in the MRSA ST398-t011 from the nasal cavities of the Swiss horses. On the other hand, they carried the human-specific SCIN. The absence of a broader immune evasion system in the MRSA ST398-t011 from Ticino may be one of the reasons why these strains were unable to establish themselves and persist in this horse community. Overall, the presence of different virulence factors among both MRSA and MSSA from horses underlines their different pathogenicity and zoonotic potential. In this regards, WGS-based comparative analysis and online platforms like the Swiss Pathogen Surveillance Platform (SPSP) (https://spsp.ch/) that enable access to whole genome sequences of human and animal pathogens and their associated clinical and epidemiological metadata play a key role in modern molecular epidemiology and outbreak investigations.

Conclusion

This study showed that the prevalence of *S. aureus* in the nasal cavities of horses is relatively low, and the presence of MRSA may be associated with sporadic local dissemination. Although, healthy horses of Switzerland do not represent a large reservoir of *S. aureus*, the strains analysed here were found to carry a variety of virulence genes, which may affect both horses and humans. Nasal colonization may represent a source of strains with the potential to develop different types of infections, including skin and postsurgical disorders in equine clinics.[30,67] They can also be transferred to humans with close contact with horses with clinic personnel being at a particular risk.[13,51] Further surveillance and investigations of *S. aureus* isolates of both human and animal origin should be performed using WGS allowing genomic comparison and rapid identification of new emerging strains, which are more adapted to zoonotic dissemination and to cause infections.

Acknowledgments

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Prévalence, phylogénie et caractéristiques des virulences et résistances aux antibiotiques de *Staphylococcus aureus* provenant du nez de chevaux sains en Suisse

Au total, 100 écouvillons nasaux ont été prélevés sur des chevaux sains en Suisse entre janvier 2020 et août 2020. Les échantillons ont été prélevés sur des chevaux de 40 écuries différentes dans 12 cantons différents et ont été soumis à un dépistage de *S. aureus* résistant à la méthicilline (MRSA) et de *S. aureus* sensible à la méthicilline (MSSA) à l'aide de plaques de gélose sélectives. Les *S. aureus* ont été testés pour leur sensibilité aux antibiotiques en mesurant la concentration minimale inhibitrice (CMI) et pour les facteurs de virulence, les gènes de résistance aux antibiotiques et les caractéristiques phylogénétiques en analysant la séquence du génome entier. Dix chevaux se sont révélés positifs (10 %, IC: 95 %, 0,0552 – 0,1744) pour *S. aureus*, et quatre d'entre eux étaient porteurs de MRSA (4 %, IC: 95 %, IC: 1,5 % – 9 %). Les MRSA ont été détectés chez des chevaux provenant de trois écuries différentes de la même région d'un canton et les MSSA ont été détectés chez des chevaux provenant de cinq cantons différents. Tous les isolats de MRSA étaient génétiquement apparentés (ST398-t011-IVa), tandis que les MSSA étaient divers (ST1-t127/t398/t1508, ST816-t1294, ST133-t1403, ST30-t012). Les MRSA étaient résistants à la pénicilline (*blaZ*), à la céfoxitine (*mecA*), au triméthoprime (*dfrK*), à la gentamicine, à la kanamycine (*aac(6')-Ie – aph(2")-Ia*) et à la tétracycline (*tet*(M)). Les MSSA étaient résistants à aucun ou à un des antibiotiques testés soit à la pénicilline (*blaZ*) ou à l'érythromycine (*erm*(T)). Les gènes de virulence étaient plus abondants chez les MSSA que chez les MRSA. Cette étude donne, pour la première fois, un aperçu de la prévalence et du type de *S. aureus* chez les chevaux suisses en bonne santé et révèle la présence de souches susceptibles de provoquer des infections chez les chevaux et les humains.

Mots clés: Animaux, antibiotique, équin, facteurs de virulence, génotypage, résistance

Prevalenza, filogenomica e caratteristiche di virulenza e antibioresistenza di *Staphylococcus aureus* dal naso di cavalli svizzeri sani

Un totale di 100 tamponi nasali sono stati raccolti da cavalli sani in Svizzera tra gennaio 2020 e agosto 2020. I campioni sono stati prelevati da cavalli in 40 diverse scuderie in 12 diversi cantoni e sono stati analizzati sia per lo *S. aureus* resistente alla meticillina (MRSA) che per lo *S. aureus* sensibile alla meticillina (MSSA) utilizzando piastre di agar selettivi. Gli *S. aureus* sono stati testati per la suscettibilità agli antibiotici mediante la misurazione della MIC (concentrazione minima inibente), per i fattori di virulenza, i geni di resistenza agli antibiotici e le caratteristiche filogenetiche mediante l'analisi della sequenza dell'intero genoma. Dieci cavalli sono risultati positivi per *S. aureus* (10 %, CI: 95 %, 0,0552 – 0,1744) quattro dei quali sono stati identificati come MRSA (4 %, CI: 95 %, CI: 1,5 % – 9 %). Gli MSSA sono stati isolati in cavalli di cinque diversi cantoni e gli MRSA in cavalli di tre diverse stalle nella stessa regione di un solo cantone. Tutti gli isolati di MRSA erano geneticamente correlati (ST398-t011-IVa) mentre gli MSSA erano diversi (ST1-t127/t398/t1508, ST816-t1294, ST133-t1403, ST30-t012). Gli MRSA hanno mostrato resistenza alla penicillina (*blaZ*), alla cefoxitina (*mecA*), al trimetoprim (*dfrK*), alla gentamicina, alla kanamicina (*aac(6')-Ie – aph(2")-Ia*) e alla tetraciclina (*tet*(M)). Gli MSSA non hanno mostrato alcuna resistenza oppure solo a uno degli antibiotici testati come la penicillina (*blaZ*) e l'eritromicina (*erm*(T)). I geni di virulenza erano più abbondanti in MSSA che in MRSA. Questo studio ha fornito una prima visione della prevalenza e del tipo di *S. aureus* nella popolazione di cavalli svizzeri sani e ha rivelato una potenziale fonte di ceppi che possono causare infezioni sia nei cavalli che nell'uomo.

Parole chiave: Animali, antibiotici, equini, fattori di virulenza, tipizzazione genetica, resistenza

Prevalence and whole genome-based phylogenetic, virulence and antibiotic resistance characteristics of nasal *Staphylococcus aureus* in healthy Swiss horses

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Literaturnachweis

[1] Ackermann C, von Niederhäusern R: Kennzahlen der Schweizer Pferdebranche: Stand 2019. Herausgeber: Agroscope, Schweizer Nationalgestüt SNG, Avenches, 2020: 1–36.

[2] Aires-de-Sousa M: Methicillin-resistant *Staphylococcus aureus* among animals: current overview. *Clin Microbiol Infect* 2017; 23(6): 373–380.

[3] Albert E, Biksi I, Német Z, Csuka E, Kelemen B, Morvay F, et al.: Outbreaks of a methicillin-resistant *Staphylococcus aureus* clone ST398-t011 in a Hungarian equine clinic: Emergence of rifampicin and chloramphenicol resistance after treatment with these antibiotics. *Microb Drug Resist* 2019; 25(8): 1219–1226.

[4] Argudín MA, Tenhagen BA, Fetsch A, Sachsenröder J, Käsbohrer A, Schroeter A, et al.: Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. *Appl Environ Microbiol* 2011; 77(9): 3052–3060.

[5] Aspiroz C, Lozano C, Vindel A, Lasarte JJ, Zarazaga M, Torres C: Skin lesion caused by ST398 and ST1 MRSA, Spain. *Emerg Infect Dis* 2010; 16(1): 157–159.

[6] Axon JE, Carrick JB, Barton MD, Collins NM, Russell CM, Kiehne J, et al.: Methicillin-resistant *Staphylococcus aureus* in a population of horses in Australia. *Aust Vet J* 2011; 89(6): 221–225.

[7] Boyle AG, Rankin SC, Duffee LA, Morris D: Prevalence of methicillin-resistant *Staphylococcus aureus* from equine nasopharyngeal and guttural pouch wash samples. *J Vet Intern Med* 2017; 31(5): 1551–1555.

[8] 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(1): 115.

[9] Chen C, Wu F: Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) colonisation and infection among livestock workers and veterinarians: a systematic review and meta-analysis. *Occup Environ Med* 2021; 78: 530–540.

[10] Cheung GYC, Bae JS, Otto M: Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence* 2021; 12(1): 547–569.

[11] Clinical and Laboratory Standards Institute CLSI: *Performance Standards for Antimicrobial Susceptibility Testing.* 31st ed CLSI supplement M100 Clinical and Laboratory Standards Institute, USA, 2021.

[12] Crespo-Piazuelo D, Lawlor PG: Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) prevalence in humans in close contact with animals and measures to reduce on-farm colonisation. *Ir Vet J* 2021; 74(1): 21.

[13] Cuny C, Abdelbary MMH, Kock R, Layer F, Scheidemann W, Werner G, et al.: Methicillin-resistant *Staphylococcus aureus* from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans. *One Health* 2016; 2: 11–17.

[14] Cuny C, Witte W: MRSA in equine hospitals and its significance for infections in humans. *Vet Microbiol* 2017; 200: 59–64.

[15] de Jong NWM, Vrieling M, Garcia BL, Koop G, Brettmann M, Aerts PC, et al.: Identification of a staphylococcal complement inhibitor with broad host specificity in equid *Staphylococcus aureus* strains. *J Biol Chem* 2018; 293(12): 4468–4477.

[16] Earls MR, Kinnevey PM, Brennan GI, Lazaris A, Skally M, O'Connell B, et al.: The recent emergence in hospitals of multidrug-resistant community-associated sequence type 1 and *spa* type t127 methicillin-resistant *Staphylococcus aureus* investigated by whole-genome sequencing: Implications for screening. *PLoS One* 2017; 12(4): e0175542.

[17] European Food Safety Authority, Aerts M, Battisti A, Hendriksen R, Kempf I, Teale C, et al.: Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. *EFSA J* 2019; 17(6): e05709.

[18] Federal Office of Public Health and Federal Food Safety and Veterinary Office: *Swiss Antibiotic Resistance Report 2020. Usage of Antibiotics and Occurrence of Antibiotic Resistance in Switzerland*. FOPH publication number: 2020-OEG-64 11.2020 2020; 2020.

[19] Fitzgerald JR: Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. *rends Microbiol* 2012; 20(4): 192–198.

[20] Fluit AC: Livestock-associated *Staphylococcus aureus*. *Clin Microbiol Infect* 2012; 18(8): 735–744.

[21] Foster TJ: Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. *FEMS Microbiol Rev* 2017; 41(3): 430–449.

[22] Franco A, Hasman H, Iurescia M, Lorenzetti R, Stegger M, Pantosti A, et al.: Molecular characterization of *spa* type t127, sequence type 1 methicillin-resistant *Staphylococcus aureus* from pigs. *J Antimicrob Chemother* 2011; 66(6): 1231–1235.

[23] Gosbell I: Methicillin-resistant *Staphylococcus aureus* in veterinary practice. *Aust Vet J* 2011; 89(5): 148–151.

[24] Guérin F, Fines-Guyon M, Meignen P, Delente G, Fondrinier C, Bourdon N, et al.: Nationwide molecular epidemiology of methicillin-resistant *Staphylococcus aureus* responsible for horse infections in France. *BMC Microbiol* 2017; 17(1): 104.

[25] Haenni M, Châtre P, Dupieux-Chabert C, Métayer V, Bes M, Madec JY, et al.: Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in horses, cats, and dogs over a 5-year period in France. *Front Microbiol* 2017; 8: 2493.

[26] Heaton CJ, Gerbig GR, Sensius LD, Patel V, Smith TC: *Staphylococcus aureus* epidemiology in wildlife: a systematic review. *Antibiotics (Basel)* 2020; 9(2): 89.

[27] Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, et al.: Community-acquired MRSA and pig-farming. *Ann Clin Microbiol Antimicrob* 2006; 5: 26.

[28] Islam MZ, Espinosa-Gongora C, Damborg P, Sieber RN, Munk R, Husted L, et al.: Horses in Denmark are a reservoir of diverse clones of methicillin-resistant and -susceptible *Staphylococcus aureus*. *Front Microbiol* 2017; 8: 543.

[29] Jamrozy DM, Fielder MD, Butaye P, Coldham NG: Comparative genotypic and phenotypic characterisation of methicillin-resistant *Staphylococcus aureus* ST398 isolated from animals and humans. *PLoS One* 2012; 7(7): e40458.

[30] Kaiser-Thom S, Gerber V, Collaud A, Hurni J, Perreten V: Prevalence and WGS-based characteristics of *Staphylococcus aureus* in the nasal mucosa and pastern of horses with equine pastern dermatitis. *BMC Vet Res* 2022; 18(1): 79.

[31] Kaspar U, von Lutzau K, Schlattmann A, Rosler U, Kock R, Becker K: Zoonotic multidrug-resistant microorganisms among non-hospitalized horses from Germany. *One Health* 2019; 7: 100091.

[32] Kittl S, Brodard I, Heim D, Andina-Pfister P, Overesch G: Methicillin-resistant *Staphylococcus aureus* Strains in swiss pigs and their relation to isolates from farmers and veterinarians. *Appl Environ Microbiol* 2020; 86(5): e01865–01819.

[33] Koop G, Vrieling M, Storisteanu DM, Lok LS, Monie T, van Wigcheren G, et al.: Identification of LukPQ, a novel, equid-adapted leukocidin of *Staphylococcus aureus*. *Sci Rep* 2017; 7: 40660.

[34] Kraushaar B, Hammerl JA, Kienöl M, Heinig ML, Sperling N, Dinh Thanh M, et al.: Acquisition of virulence factors in livestock-associated MRSA: Lysogenic conversion of CC398 strains by virulence gene-containing phages. *Sci Rep* 2017; 7(1): 2004.

[35] Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, et al.: Methicillin-resistant *Staphylococcus aureus*. *Nat Rev Dis Primers* 2018; 4: 18033.

[36] Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A: Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J Clin Microbiol* 2014; 52(7): 2365–2370.

[37] Letunic I, Bork P: Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 2019; 47(W1): W256-W259.

[38] Lienen T, Schnitt A, Hammerl JA, Maurischat S, Tenhagen BA: Genomic distinctions of LA-MRSA ST398 on dairy farms from different German federal states with a low risk of severe human infections. *Front Microbiol* 2020; 11: 575321.

[39] Little SV, Hillhouse AE, Lawhon SD, Bryan LK: Analysis of virulence and antimicrobial resistance gene carriage in *Staphylococcus aureus* infections in equids using whole-genome sequencing. *mSphere* 2021; 6(4): e0019620.

[40] 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: 118–126.

[41] Lozano C, Aspiroz C, Lasarte JJ, Gómez-Sanz E, Zarazaga M, Torres C: Dynamic of nasal colonization by methicillin-resistant *Staphylococcus aureus* ST398 and ST1 after mupirocin treatment in a family in close contact with pigs. *Comp Immunol Microbiol Infect Dis* 2011; 34(1): e1–7.

[42] Maddox TW, Clegg PD, Williams NJ, Pinchbeck GL: Antimicrobial resistance in bacteria from horses: Epidemiology of antimicrobial resistance. *Equine Vet J* 2015; 47(6): 756–765.

[43] Mama OM, Gómez P, Ruiz-Ripa L, Gómez-Sanz E, Zarazaga M, Torres C: Antimicrobial resistance, virulence, and genetic lineages of staphylococci from horses destined for human consumption: High detection of *S. aureus* isolates of lineage ST1640 and those carrying the *lukPQ* gene. *Animals (Basel)* 2019; 9(11): 900.

[44] Novick RP, Christie GE, Penadés JR: The phage-related chromosomal islands of Gram-positive bacteria. *Nat Rev Microbiol* 2010; 8(8): 541–551.

[45] Peterson AE, Davis MF, Awantang G, Limbago B, Fosheim GE, Silbergeld EK: Correlation between animal nasal carriage and environmental methicillin-resistant *Staphylococcus aureus* isolates at U.S. horse and cattle farms. *Vet Microbiol* 2012; 160(3–4): 539–543.

[46] Pilla R, Castiglioni V, Gelain ME, Scanziani E, Lorenzi V, Anjum M, et al.: Long-term study of MRSA ST1, t127 mastitis in a dairy cow. *Vet Rec* 2012; 170(12): 312.

[47] Porrero MC, Mentaberre G, Sánchez S, Fernández-Llario P, Gómez-Barrero S, Navarro-Gonzalez N, et al.: Methicillin resistant *Staphylococcus aureus* (MRSA) carriage in different free-living wild animal species in Spain. *Vet J* 2013; 198(1): 127–130.

[48] Purrello SM, Garau J, Giamarellos E, Mazzei T, Pea F, Soriano A, et al.: Methicillin-resistant *Staphylococcus aureus* infections: A review of the currently available treatment options. *J Glob Antimicrob Resist* 2016; 7: 178–186.

[49] Raineri EJM, Altulea D, van Dijl JM: Staphylococcal trafficking and infection-from 'nose to gut' and back. *FEMS Microbiol Rev* 2022; 46(1): fuab041.

[50] Roudaud M, Allano M, Fairbrother JH, Sauvé F: A retrospective study on methicillin-resistant *Staphylococcus* spp. isolated from horses admitted to a Canadian veterinary teaching hospital between 2008 and 2018. *Can Vet J* 2020; 61(11): 1197–1202.

[51] Sieber S, Gerber V, Jandova V, Rossano A, Evison JM, Perreten V: Evolution of multidrug-resistant *Staphylococcus aureus* infections in horses and colonized personnel in an equine clinic between 2005 and 2010. *Microb Drug Resist* 2011; 17(3): 471–478.

[52] Smith TC, Wardyn SE: Human Infections with *Staphylococcus aureus* CC398. *Curr Environ Health Rep* 2015; 2(1): 41–51.

[53] Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al.: Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. *Int J Antimicrob Agents* 2012; 39(4): 273–282.

[54] Steinman A, Masarwa S, Tirosh-Levy S, Gleser D, Kelmer G, Adler A, et al.: Methicillin-resistant *Staphylococcus aureus spa* type t002 outbreak in horses and staff at a veterinary teaching hospital after its presumed introduction by a veterinarian. *J Clin Microbiol* 2015; 53(9): 2827–2831.

[55] Tam K, Torres VJ: *Staphylococcus aureus* secreted toxins and extracellular enzymes. *Microbiol Spectr* 2019; 7(2): GPP3–0039–2018.

[56] The European Committee on Antimicrobial Susceptibility Testing EUCAST: *Breakpoint tables for interpretation of MICs and zone diameters 2021*; Version 11.0, 2021. http://www.eucast.org.

[57] Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr.: *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 2015; 28(3): 603–661.

[58] Van den Eede A, Martens A, Feryn I, Vanderhaeghen W, Lipinska U, Gasthuys F, et al.: Low MRSA prevalence in horses at farm level. *BMC Vet Res* 2012; 8: 213.

[59] Van den Eede A, Martens A, Flore K, Denis O, Gasthuys F, Haesebrouck F, et al.: MRSA carriage in the equine community: an investigation of horse-caretaker couples. *Vet Microbiol* 2013; 163(3–4): 313–318.

[60] Van den Eede A, Martens A, Lipinska U, Struelens M, Deplano A, Denis O, et al.: High occurrence of methicillin-resistant *Staphylococcus aureus* ST398 in equine nasal samples. *Vet Microbiol* 2009; 133(1–2): 138–144.

[61] van Duijkeren E, Moleman M, Sloet van Oldruitenborgh-Oosterbaan MM, Multem J, Troelstra A, Fluit AC, et al.: Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. *Vet Microbiol* 2010; 141(1–2): 96–102.

[62] Walther B, Klein KS, Barton AK, Semmler T, Huber C, Merle R, et al.: Equine methicillin-resistant sequence type 398 *Staphylococcus aureus* (MRSA) harbor mobile genetic elements promoting host adaptation. *Front Microbiol* 2018; 9: 2516.

[63] Waqar N, Amin Q, Munir T, Ikram MS, Shahzad N, Mirza A, et al.: A cross-sectional study of methicillin-resistant *Staphylococcus aureus* at the equine-human interface. *Trop Anim Health Prod* 2019; 51(7): 1927–1933.

[64] Weese JS: Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel. *Vet Clin North Am Equine Pract* 2004; 20(3): 601–613.

[65] Weese JS: Methicillin-resistant *Staphylococcus aureus* in animals. *ILAR J* 2010; 51(3): 233–244.

[66] Weese JS, Lefebvre SL: Risk factors for methicillin-resistant *Staphylococcus aureus* colonization in horses admitted to a veterinary teaching hospital. *Can Vet J* 2007; 48(9): 921–926.

[67] Weese JS, Rousseau J, Willey BM, Archambault M, McGeer A, Low DE: Methicillin-resistant *Staphylococcus aureus* in horses at a veterinary teaching hospital: frequency, characterization, and association with clinical disease. *J Vet Intern Med* 2006; 20(1): 182–186.

[68] Wipf JR, Perreten V: Methicillin-resistant *Staphylococcus aureus* isolated from dogs and cats in Switzerland. *Schweiz Arch Tierheilkd* 2016; 158(6): 443–450.

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