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Equine Infectious Disease

Prevalence and Risk Factors of *Staphylococcus aureus* Nasal Colonization in Horses Admitted to a Veterinary Teaching Hospital

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Keywords: antimicrobial resistance | epidemiology | equine | infection control | MRSA | nosocomial

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections, including in veterinary settings.

Hypothesis/Objectives: To investigate the prevalence, risk factors for *Staphylococcus aureus* (SA) and MRSA colonization, and the duration of MRSA colonization.

Animals: Elective cases admitted to the Veterinary Teaching Hospital were recruited (228 horses).

Methods: A cross-sectional study was conducted over 3 years. Nasal swabs were collected at admission and cultured for SA. Methicillin-resistant isolates were identified using matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) technology, oxacillin minimal inhibitory concentration (MIC), and PCR testing. Horses colonized with MRSA were resampled until two negative cultures were obtained. Stabling management, activity, and medical history were obtained from owners and medical files. Multivariable logistic regressions were used to model associations between risk factors and colonization.

Results: The prevalence of SA and of MRSA nasal carriage was 17.5% (95% CI: 12.4–22.7) and 6.2% (95% CI: 2.9–9.4), respectively. Of the 10 horses colonized by MRSA and monitored over time, only one tested positive after 3 months. More than 10 horses on the premises (OR 6.0 – 95% CI 1.1–64.2), previous hospitalization (OR 6.0 – 95% CI 1.0–35.2), and year of admission (2022 vs. 2020–2021; OR 9.0 – 95% CI 1.7–92.2) were associated with MRSA nasal carriage.

Conclusions and Clinical Importance: The prevalence of MRSA nasal colonization is of concern; however, the carriage seems transitory. Apart from the medical risk factors, the importance of social interactions in MRSA transmission needs to be elucidated in horses.

Abbreviations: AAVLD, American Association of Veterinary Laboratory Diagnosticians; CDVUM, Centre de diagnostic vétérinaire de l'Université de Montréal; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; CoPS, coagulase-positive *Staphylococcus*; DAG, directed acyclic graph; FVM, Faculty of Veterinary Medicine; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; OR, odd ratio; PVL, Panton-Valentine leukocidin; SA, *Staphylococcus aureus*; TMS, trimethoprim/sulfamethoxazole; VTH, Veterinary Teaching Hospital; XDR, extensively drug-resistant.

1 | Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern in human and veterinary medicine. According to a global annual mortality study, the age-standardized mortality rate for *Staphylococcus aureus* (SA) was 14.6 deaths per 100 000 persons, causing an estimated 1.1 million of lives lost in 2019 [1]. This placed SA as the second cause for deaths associated with bacterial infections in individuals older than 15 years, after *Mycobacterium tuberculosis* [1, 2]. This opportunistic pathogen is found mostly in the host nasal mucosa and occasionally on the skin. In humans, nasal colonization significantly increases the risk of infection [3, 4]. SA has several intrinsic virulence factors and mobile genetic elements that support the antimicrobial resistance dissemination. SA and MRSA cause various types of infections in horses, from mild skin and soft tissue infection to fatal septicemia [5, 6]. In veterinary hospitals, MRSA is also a frequent nosocomial pathogen [7]. Studies assessing similarities between human and horse MRSA concluded on a potential risk of zoonotic transmission [8–11].

The prevalence of MRSA colonization in horses varies greatly by the geographic location, the characteristics of the sample, and the sampling. When sampled immediately after admission in veterinary hospitals, the prevalence for horses in Canada and in Europe ranged from 2% to 10% [12–17]. Only a few studies investigated the risk factors of colonization in horses, and they mostly evaluated aspects related to the medical history of the patient [17, 18].

A retrospective study showed a significant increase in the proportion of methicillin-resistant *Staphylococcus* spp. isolates among horses admitted to the Veterinary Teaching Hospital (VTH) of the Faculty of Veterinary Medicine (FVM) of the Université de Montréal between 2008 and 2018 [19]. This increase prompted various questions regarding the risk factors and the management of nosocomial infections in hospital settings. Furthermore, there is a scarcity of recent information on MRSA epidemiological characteristics and prevalence in horses in Canada.

This study aimed to estimate the prevalence and risk factors of nasal SA and MRSA in horses upon admission to the VTH of the Université de Montréal. Secondary objectives included examining MRSA presence on the skin and determining the duration of nasal carriage. We hypothesized that nasal SA colonization is common among admitted horses, while nasal MRSA colonization is rare. Additionally, we proposed that the horse's lifestyle, activity type, and medical history are risk factors for colonization. Finally, we suspected that nasal carriers likely harbor MRSA on their skin and that carriage is short term.

2 | Materials and Methods

2.1 | Study Design and Group

A cross-sectional study was conducted on horses electively presented at the VTH of the FVM of the Université de Montréal (Saint-Hyacinthe, Québec, Canada). The horses were enrolled in the study after obtaining an informed consent from their owners. They were given the opportunity to provide a second consent to participate in the longitudinal follow-up study for MRSA nasal colonization. At the beginning of each week, the list of scheduled patients for the following week was extracted from the hospital database (VetView Version 2.1.14.1, Athens, GA, USA). Owners were contacted by email or by phone approximately 1 week before their appointment at the VTH. All horses were included, and each horse was eligible only once during the study. However, the horses admitted for emergency care were excluded due to the challenges of obtaining consent before admission.

A sample size of 450 horses was targeted to estimate the prevalence of MRSA nasal colonization with a precision of 2% at a 95% confidence level, given an expected prevalence of 5% (https://epitools.ausvet.com.au/oneproportion). During the first 2 weeks, 10 horses were randomly selected from the list and their owners contacted. After evaluation of the participation rate over these first weeks, all owners on the list were subsequently contacted.

2.2 | Data Collection

To investigate the risk factors, an online survey was developed and administered to participating owners (SurveyMonkey Inc., San Mateo, California, USA; fr.surveymonkey.com). A fact sheet explaining MRSA in horses and offering recommendations for colonized horses was provided to owners during recruitment. The questions were developed after reviewing the scientific literature related to the biology of *Staphylococcus* spp. and the risk factors for colonization, in both human and equine medicine. It was also inspired by other similar epidemiological studies [20, 21]. The questionnaire was pre-tested by experts in veterinary dermatology, epidemiology, equine medicine, and a horse owner. It took approximately 20 min to complete. If needed, information was completed through phone calls or consultation of medical records after consent. The questionnaire included questions on signalment (age, sex, breed), medical history (date and reason of presentation, admission service, previous hospitalization in the previous 6 months, previous antimicrobial treatment in the previous 6 months), barn management (type of housing, number of other horses in the stable, presence of other animal species on the farm, number of people involved in horse care), and type of activity (including transportation, frequency, and type of events; Data S1). All questions related to the medical history, type of housing, and type of activity pertained to the period covering the previous 6 months.

2.3 | Sample Collection

Each horse was swabbed in the first 2 h upon admission at the VTH to reduce the risk of SA colonization acquired at the hospital. The sampling was performed by an equine veterinarian or veterinary technician. The nasal swabbing technique was standardized based on the previous literature to enhance the sensitivity [22]. Briefly, while wearing clean non-sterile gloves, the sampler inserted a rayon-tipped swab (BBL CultureSwab, COPAN Diagnostics, Murrieta, CA, USA) approximately 10 cm in both nostrils, successively. It was gently rubbed against the

mucosa of the ventral meatus, and the nasal vestibulum as the swab was withdrawn. Another swab was used to sample a combination of four cutaneous sites: forehead, left neck, withers, and perineum. At each site, a 10-cm^2 area of skin was rubbed 10 times, except for the perineum where the swab tip was rotated just below the anus. Both swabs were immediately inserted in a Stuart's transport medium separately and kept refrigerated until submission to the bacteriology laboratory (within a maximum of 24 h).

To estimate the duration of colonization, the MRSA-positive horses were resampled (only the nostrils) until two consecutive negative samples were obtained. This longitudinal follow-up was conducted at standardized intervals: 14, 30, 90, and 180 days after admission. The sampling was performed using the same procedure by the first author or by the referring veterinarian. In the latter case, the standardized sampling protocol and the required material (swabs, gloves) were sent to the referring veterinarian.

This study protocol was approved by the Animal Ethics and Care Committee of the Université de Montréal (19-Rech-2038).

2.4 | Isolation and Identification of SA and MRSA

The microbiological analyses were performed by the AAVLD-accredited bacteriology laboratory at the Centre de diagnostic vétérinaire de l'Université de Montréal (CDVUM, Saint-Hyacinthe, Canada). The nasal swabs were placed in a selective enrichment broth for Gram-positive bacteria, consisting of a BHI broth (Brain–Heart Infusion, BD Bacto Brain Heart Infusion 237 500, Becton Dickinson, Franklin Lakes, US) supplemented with 1% colistin (Sigma C1511-10MU, MilliporeSigma Canada Ltd., Oakville, Canada) and 1% nalixidic acid (Sigma N4382, Sigma-Aldrich, Saint Louis, US). The broth was incubated at 35°C ± 2°C, aerobically, for 16–24 h. The cutaneous swabs were stored at 5°C ± 2°C and were inoculated in the same manner within 48 h, only if MRSA was isolated from the nasal sample. After incubation and with a sterile swab, the BHI broth was inoculated on a MRSA selective agar (MRSA*Select* II, Bio-Rad, Clinical Diagnostics Division, Bio-Rad Laboratories, Montreal, Canada) and a Columbia agar (CBA) with 5% sheep blood (Difco Columbia blood agar base, Becton Dickinson and Company, Sparks, Maryland, USA). The CBA was used to detect the presence or absence of SA. Both plates were incubated at 35°C ± 2°C for 16–24 h under aerobic conditions. The exposure of MRSA*Select* II to light reduces the sensitivity of the method; thus, the exposure was minimized (≤ 8 h) before and during incubation as recommended. After incubation, the MRSA appeared as pink colonies and the non-MRSAs were inhibited or appeared as white or colorless colonies. The identification of SA was confirmed by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) technology. A horse was considered colonized by methicillin-susceptible *Staphylococcus aureus* (MSSA) when there was no growth on the chromogenic agar plate, but colonies on the CBA. The identified colonies were inoculated onto CBA plates, and up to three isolates per plate were kept at −80°C ± 15°C. Nasal MRSA absence/presence was reported to the owner and to the VTH's medical team if owners' consent was obtained.

2.5 | Antimicrobial Susceptibility Testing of MRSA Isolates

The methicillin resistance was confirmed by oxacillin MIC and PCR testing. Antimicrobial susceptibility (including oxacillin) was assessed by microdilution in Müller-Hinton broth using a Sensititre plate (Sensititre Vet Equine EQUIN1F Plate, Thermo Fisher Scientific, Basingstoke, UK) at the Animal Health Laboratory (AHL, https://www.uoguelph.ca/ahl, University of Guelph, Canada). Twenty different antimicrobials were tested: amikacin, azithromycin, ceftiofur, chloramphenicol, enrofloxacin, oxacillin +2% NaCl, penicillin, ticarcillin, ampicillin, clarithromycin, gentamicin, ticarcillin/clavulanic acid, cefazolin, ceftazidime, erythromycin, trimethoprim/sulfamethoxazole (TMS), doxycycline, imipenem, rifampin, and tetracyclin. The MRSA was classified as susceptible, intermediate, or resistant in accordance with the CLSI VET01S and M100 guidelines [23]. For oxacillin, a MIC ≤ 2 μg/mL corresponds to a susceptible isolate, whereas a MIC ≥ 4 μg/mL is a MRSA. A multidrug-resistant (MDR) bacteria was defined as an isolate non-susceptible to at least one agent in three or more antimicrobial categories. An extensively drug-resistant (XDR) bacteria is an isolate non-susceptible to at least one agent in all but two or fewer antimicrobial categories [24].

A multiplex PCR targeting the *mecA* and *mecC* genes was used to confirm the identification of the suspected MRSA, with primers and conditions as previously described, respectively, by Zhang et al. and Cuny et al. [25, 26].

2.6 | Statistical Analysis

All data were compiled into an Excel spreadsheet. SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) was used for all data curation and analyses. Descriptive statistics were used to describe the study sample, the microbiological results of the longitudinal study, and the antimicrobial susceptibility profiles. When a group of horses from the same premise was admitted on the same day, only one horse of the group was randomly selected and included in the prevalence and risk factor analyses to avoid potential clustering effects.

2.6.1 | Prevalence Estimation

We estimated the prevalence of SA and MRSA nasal colonization with 95% confidence intervals (CIs).

2.6.2 | Risk Factors

Two outcomes were studied: SA and MRSA nasal colonization. The potential risk factors from the questionnaire were organized into 20 categorical variables. Explanatory variables were categorized into two (number of horses on the premise) or three groups (age, frequency of transportation, number of in-contact persons). The type of activity was categorized as “Competition/Professional” (including racing, western or classic riding, carriage driving, shows), “Pleasure” (including any types of riding activities at a pleasure level with no competition/professional

activity reported), "Groundwork" (including groundwork and young horses involved in pre-training activities), and "Rest" (including retired horses due to age or medical reason and broodmares). The reason of presentation was divided into "Healthy," "Non-healthy," and "Skin and soft tissue (SST) affections." "Healthy" horses referred to electively admitted horses for a theriogenology follow-up, elective surgeries (castration, osteochondritis dissecans treated by arthroscopy), or the follow-up of a resolved medical condition. "Non-healthy" horses referred to horses admitted for any type of medical or surgical issue, excluding SST affections. The variable "Shared material" referred to material used commonly for different horses, as grooming materials, bridles, saddles, saddle pads, clippers, and blankets. The variable "Contacts with other horses" was defined as a direct contact between horses, as nose-to-nose contacts. Categories showing a few observations were combined for categorical variables to improve model convergence. For example, for the variable "Year of admission," the horses admitted in 2020 and 2021 were grouped into one category, because of the low number of horses admitted in 2020. The resulting 20 categorized variables are presented in Table 1.

A directed acyclic graph (DAG; Figure 1) was drawn with all variables to identify potential confounders and intermediate variables, based on the information obtained from the questionnaire, available literature, and biological knowledge, including one variable (i.e., MRSA-colonized stablemate) that was not evaluated in this study. Initially, the association between each variable and MRSA or SA status was tested using univariable logistic regressions. Exact tests were employed when at least one cell of the contingency table had a small expected count (≤ 5). Variables with $p \leq 0.20$ (likelihood ratio test, univariable analyses) that were directly associated with MRSA or SA according to the DAG and their potential confounders were considered for inclusion in the multivariable logistic regression analysis. These retained risk factors underwent further screening for pair-wise correlations using chi-square tests. In case of two significantly associated variables, only one variable was retained based on higher biological relevance if possible or smaller P-value otherwise. Selected variables were included in a full logistic regression model. A manual backward elimination approach ($p > 0.05$) was used for the final model selection. During variable removal, if at least one coefficient of the remaining variables changed by more than 30%, the eliminated variable was considered a confounder and kept into the final model; otherwise, it was removed. Odds ratios (ORs) with 95% confidence intervals were used to present the final results. The goodness-of-fit of the final model was evaluated with the Hosmer–Lemeshow test.

3 | Results

3.1 | Description of the Study Group

The recruitment period covered 50 non-consecutive weeks from March 2020 to May 2022. The sampling was performed during 2 weeks in March 2020 (nine horses) before being interrupted due to the COVID-19 pandemic, then during 28 weeks in 2021 (from January to May and from September to November; 123 horses), and during 20 weeks in 2022 (from January to May, 96 horses). Of 494 eligible horses, we obtained owner consents for 299 horses and sampled 228 horses (Figure 2).

The 228 horses came from 188 different stables. Horses originated mainly from Quebec (222 horses) and a few from Ontario (6 horses). In 14 occasions, two to four horses from the same stable were transported to the VTH at the same date. The study sample was mostly composed of geldings and mares. The median age was 8 years (mean 8.9 years), ranging from 8 months to 32 years. Overall, nasal colonization by SA was detected in 42 horses: 29 horses from 23 different stables had an MSSA isolate. The 13 MRSA-colonized horses came from 13 different stables, and no one of them came from the same stables as the MSSA-colonized horses.

3.2 | Prevalence and Risk Factor Analysis

The statistical analyses were based on a sample of 211 horses after the random selection of one horse per group transported from the same stable at the same date. The estimated prevalence of SA and MRSA nasal colonization was 17.5% (95% CI: 12.4–22.7; 37/211) and 6.2% (95% CI: 2.9–9.4; 13/211) at the time of admission, respectively.

After univariable analyses (Table 1) and based on potential confounders from the DAG, the following variables were kept for the multivariable analysis for the SA colonization outcome: season and year of admission, age, previous hospitalization, and medical status. The age and medical status were highly associated ($X^2 = 57$, $p < 0.01$): 83% of the horses under 2 years of age were presented for elective purposes, whereas 83% of the horses over 10 years old were in the "Non-healthy" group. The age was kept in the model based on the P-value criterion. After backward selection, the final model for SA colonization outcome included the age (as a potential confounder), the year of admission and a previous hospitalization in the previous 6 months. Being admitted in 2022 and a previous hospitalization in the previous 6 months were significantly associated with nasal SA colonization (Table 2).

For the MRSA colonization outcome, a previous hospitalization, presence of pets on site, number of horses on site, year of admission, and transportation were considered for the multivariable analysis. Transportation and previous hospitalization were highly correlated: 100% of hospitalized horses were previously transported. A previous hospitalization was kept in the model based on P-values and biological relevance. After backward selection, the final model for MRSA colonization outcome included a previous hospitalization in the previous 6 months, number of horses on site, and year of admission. A horse that has been hospitalized in the past 6 months, was admitted in 2022, or was housed in a stable with more than 10 horses had higher odds of being colonized by MRSA (Table 2). No lack of fit was detected according to the Hosmer–Lemeshow test ($p = 0.95$ and $p = 0.71$, respectively, for SA and MRSA final models).

3.3 | Skin Colonization and Longitudinal Follow-Up

Of the 13 nasally MRSA-colonized horses at admission, no MRSA was detected on the skin. Ten horses were available for the longitudinal follow-up (Figure 3). Only two horses tested positive on multiple occasions: one horse tested positive up to 90 days after

TABLE 1 | Description of potential risk factors considered for nasal MRSA and SA colonization in horses admitted to a Veterinary Teaching Hospital ($n = 211$ horses) including P-values from univariable logistic regressions.

		SA colonization			MRSA colonization		
Characteristics	**Number of horses**	***N***	**%**	***p***	***N***	**%**	***p***
Sex				0.70[a]			NA
Female	82	12	14.6		5	6.1	
Male	28	5	17.9		0		
Gelding	101	20	19.8		8	7.9	
Age (years)				**0.05**			0.67[a]
0-2	30	9	30		3	10.0	
> 2–10	102	20	19.6		6	5.9	
> 10	79	8	10.1		4	5.1	
Breed				NA			NA
Quarter Horses and related[b]	92	17	18.5		8	8.7	
Canadian	26	6	23.1		0		
Standardbred	8	1	12.5		0		
Thoroughbred	10	1	10		0		
Riding horses[c]	40	8	20		4	10.0	
Draught horses[d]	7	2	28.6		0		
Spanish breeds[e]	9	1	11.1		0		
Ponies	12	0	0		0		
Others[f]	7	1	12.5		1	14.3	
Type of activity[g]				0.23[a]			0.91[a]
Competition	92	12	13		5	5.4	
Groundwork	20	5	25		1	5	
Pleasure	63	15	23.8		5	7.9	
Rest	25	3	12		1	4	
Type of housing[g]				0.87			0.75[a]
Stable	69	13	18.8		4	5.8	
Outdoor	48	10	20.8		2	4.2	
Combined	76	13	17.1		6	7.9	
Service of admission				0.45[a]			0.71[a]
Orthopedic	57	7	12.3		2	3.5	
Surgery	74	20	23		6	8.1	
Internal medicine	55	9	16.4		4	7.3	
Others	25	4	16		1	4	
Season of admission				0.11[a]			0.22[a]
Spring	68	17	25		7	10.3	
Fall	41	4	9.8		1	2.4	

(Continues)

TABLE 1 | (Continued)

Characteristics	Number of horses	SA colonization			MRSA colonization		
		N	%	*p*	*N*	%	*p*
Winter	102	16	15.7		5	4.9	
Year of admission				**< 0.01**			**< 0.01**[a]
2020–2021	121	13	10.7		2	1.7	
2022	90	24	26.7		11	12.2	
Medical status				0.18[a]			0.74[a]
Healthy	66	14	21.2		4	6.1	
Non-Healthy	128	18	14.1		7	5.5	
SST affections[h]	17	5	29.4		2	11.8	
Previous hospitalization in the previous 6 months				**0.01**			0.09[a]
Yes	29	10	34.5		4	13.8	
No	182	27	14.8		9	5	
Previous antimicrobial treatment in the previous 3 months				0.27			0.70[a]
Yes	36	9	25		3	8.3	
No	160	27	16.9		9	5.6	
Transportation frequency in the previous 6 months[g]				0.78[a]			1[a]
None	86	15	17.4		5	5.8	
1–4 transports	84	15	17.9		5	6	
≥ 5	25	3	12		2	8	
Transportation contacts in the previous 6 months[g]				0.86			0.12[a]
Never transported	86	15	17.4		5	5.81	
Alone	53	10	18.9		6	11.32	
With other horses	55	8	14.6		1	1.8	
Previous event[i] in the previous 6 months[g]				0.21[a]			0.49[a]
Yes	33	3	9.1		1	3.0	
No	161	32	19.9		11	6.8	
Direct contact with horses on the premises[g]				0.80[a]			NA
Yes	170	31	18.2		12	7.1	
No	28	4	14.3		0	0	
Number of horses housed on the farm[g]				0.83			0.07[a]
0–10	89	15	16.9		2	2.3	
≥ 11	111	20	18		10	9.0	
Number of persons in contact[g,j]				1[a]			0.86[a]
1 person	22	4	18.2		1	4.6	
2–5	156	28	18.2		9	5.8	
≥ 6	22	4	18.2		2	9.1	
Shared material[g]				0.23			0.20

(Continues)

TABLE 1 | (Continued)

Characteristics	Number of horses	SA colonization			MRSA colonization		
		N	%	*p*	*N*	%	*p*
Yes	131	21	16.0		6	4.6	
No	64	14	21.9		6	9.4	
Pets on the farm[g]				0.48			0.19[a]
Presence	147	28	19.1		11	7.5	
Absence	54	8	14.8		1	1.9	
Food animal[k] on the farm[g]				0.53			0.52[a]
Presence	53	8	15.1		2	3.8	
Absence	148	28	18.9		10	6.8	

Note: N, number of horses colonized by SA or MRSA; %, percentage of horses with nasal SA or MRSA colonization among all horses in the category from the corresponding row; NA, a univariate logistic regression could not be performed due to the low count of horses in some categories.
[a]Indicates an exact logistic regression.
[b]Quarter Horses and related: Appaloosa, Appendix, Paint, Quarter Horse.
[c]Riding horses: Arabian, Belgian Warmblood, Dutch Warmblood, Friesian, Hanovrian, Holsteiner, KWPN, Morgan, Oldenbourg, Selle Français, Selle Luxembourgeois, Trakehner, Westphalian.
[d]Draught horses: Clydesdale, Gypsy, Percheron.
[e]Spanish breeds: Andalou, Lusitanian, Pure Race Espagnole.
[f]Others: crossed breeds.
[g]Between 10 and 18 horses had missing values for these variables.
[h]SST affections: skin and subcutaneous tissue affections.
[i]Previous event: any type of equestrian events (show, competition, etc.).
[j]Number of persons in contact: number of persons in direct contact regularly with the horse in the previous 6 months (anyone who has ridden, trained, and/or groomed the horse regularly).
[k]Food animals: cattle, small ruminants, pigs, and poultry.

admission and then could not be followed up thereafter, while the other horse tested positive at 14 days and 1 month after admission but was negative thereafter. Eight horses were negative at the first resampling (14 days after admission).

3.4 | Antimicrobial Resistance

All MRSA isolates (18) were PCR-positive for the *mecA* gene and PCR-negative for *mecC*. The 18 MRSA isolates were submitted for MIC testing: 13 were isolated upon admission, and five from the longitudinal follow-up. The susceptibility testing results are summarized in Table 3. All isolates were resistant to oxacillin and therefore considered resistant to all β-lactams that were tested: ampicillin, cefazolin, ceftazidime, ceftiofur, imipenem, penicillin, ticarcillin, and ticarcillin/clavulanic acid (results are not included in Table 3). All isolates were susceptible to chloramphenicol. All isolates were resistant to multiple antimicrobials (enrofloxacin, gentamicin, rifampin, tetracycline, and the combination of trimethoprim–sulfamethoxazole) and met the definition of MDR bacteria. Moreover, two of them were XDR. The susceptibility patterns were the same over time for the two horses who had multiple MRSA-positive cultures.

4 | Discussion

In this study, 17.5% of horses (95% CI: 12.4–22.7) admitted to the VTH were colonized by SA and 6.2% (95% CI: 2.9–9.4) harbored MRSA. The SA nasal prevalence estimated in this study is higher than previously reported in healthy horses. Indeed, the SA nasal prevalence in 497 healthy horses in Atlantic Canada and in 100 horses in Switzerland were 7.9% (no MRSA-colonized horses) and 10% (95% CI: 5%–17%), respectively [27]. The prevalence of MRSA colonization in horses enrolled in this study is comparable to the 2%–10% previously reported in equine hospitals [12–14, 17, 28].

When interpreting the results, the performance of the sampling method has to be considered. In one study, the nasal vestibulum was found to be more sensitive compared to the ventral meatus and the diverticulum for detecting MRSA-colonized horses, which justified the localization and technique chosen in this study [22]. Moreover, transmission by handlers before sampling had to be prevented to exclude nosocomial infections. In this study, horses were sampled shortly after admission, with a maximum time frame of 2 h, to minimize this risk. This protocol reduced the number of eligible horses, as potential participants were excluded if samples could not be collected within the specified time frame.

We identified important risk factors associated with colonization by SA and MRSA in this equine sample. In a similar sample of horses admitted to a VTH in Ontario, risk factors for being colonized by MRSA included a previous colonization, a previously identified colonized stablemate in the barn, administration of antibiotics in the previous 30 days, and being admitted to the neonatal intensive care unit and to a service other than the surgical department [17, 18]. Surprisingly, our study did not identify previous administration of antimicrobials as a significant risk factor. This variable, as well as healthcare exposure (hospital stay, surgery, medical/surgical

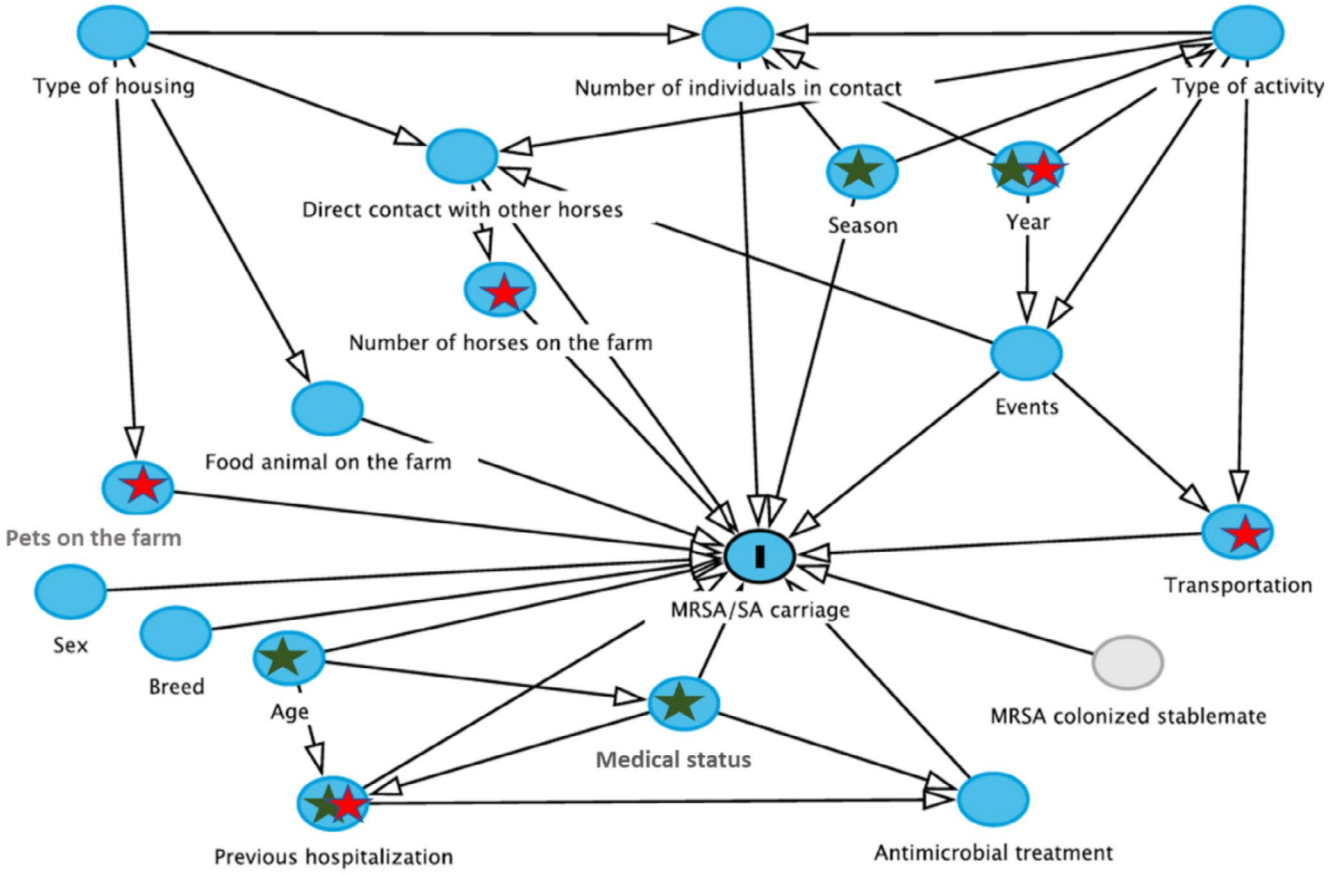

FIGURE 1 | Directed acyclic graphic illustrating the relationships between variables involved in the MRSA and SA nasal colonization in horses. Blue circles: Studied variables. Gray circle: Not studied variable. Red and green stars: Variables kept in the multivariable analysis for the MRSA and SA colonization outcomes, respectively.

devices, etc.), was identified as a significant risk factor in humans [29, 30]. In this study, none of the MRSA-colonized horses had received antimicrobials in the last month prior to admission and only three horses in the previous 3 months. The sample size in this subgroup being low, it may have prevented us from detecting an association. Being hospitalized in the previous 6 months represented a significant risk of colonization in our study sample. This finding suggests that the hospital environment may represent a MRSA reservoir and exposes, in turn, new patients and staff to MRSA [28]. Interestingly, in a recent prospective study in Italy [31], the antimicrobial treatment rate in the previous year constituted a risk factor for methicillin-resistant staphylococci nasal carriage at the barn level but not at the individual level. It should be noted that this epidemiological study was performed on horses in the community, excluding those that received antimicrobials in the previous 2 months.

Being housed in a stable with more than 10 horses was a significant risk factor for MRSA colonization. That is in accordance with a previous study conducted on 972 farm horses in North America, in which the authors concluded that horses living on a farm with more than 20 horses were associated with a higher risk of MRSA colonization [32]. In humans, activities in group or in crowded places that involve skin-to-skin contact and shared equipment or supplies increase the risk of MRSA colonization or infection [33, 34]. The COVID-19 pandemic significantly affected all forms of social interaction, including equine events, activities, and movements. In 2022, the overall activities in Quebec normalized compared with the previous 2 years. Thus, we suspected that horses had more contact opportunities with other horses and people in 2022 compared to those in 2020–2021 period. This could explain the increased odds of being colonized by SA or MRSA in 2022.

In humans, longitudinal studies identified two to three carriage patterns of SA or MRSA, which were classified as persistent, intermittent, and non-carriers [3]. The risk of infection varies depending on the type of carriers: "persistent" carriers have higher SA bacterial loads and a higher risk of acquiring SA infection [4]. One study described the spontaneous mid/long-term nasal MRSA carriage in horses, during which a small group of nine horses diagnosed with an MRSA-infected wound were followed up [35]. The horses were tested six to seven times for 12–24 months. At least five sites were sampled each time, including both nostrils and the previously infected site. The carriage time was defined by the authors as the period between the first positive sample of the infected site and the second negative sample, from two consecutive ones, at any site. It ranged from 55 to 711 days, with a mean of 143 days. No distinction can be made between a persistent carriage and a repeated colonization in this study, because no data were available regarding phenotypic or

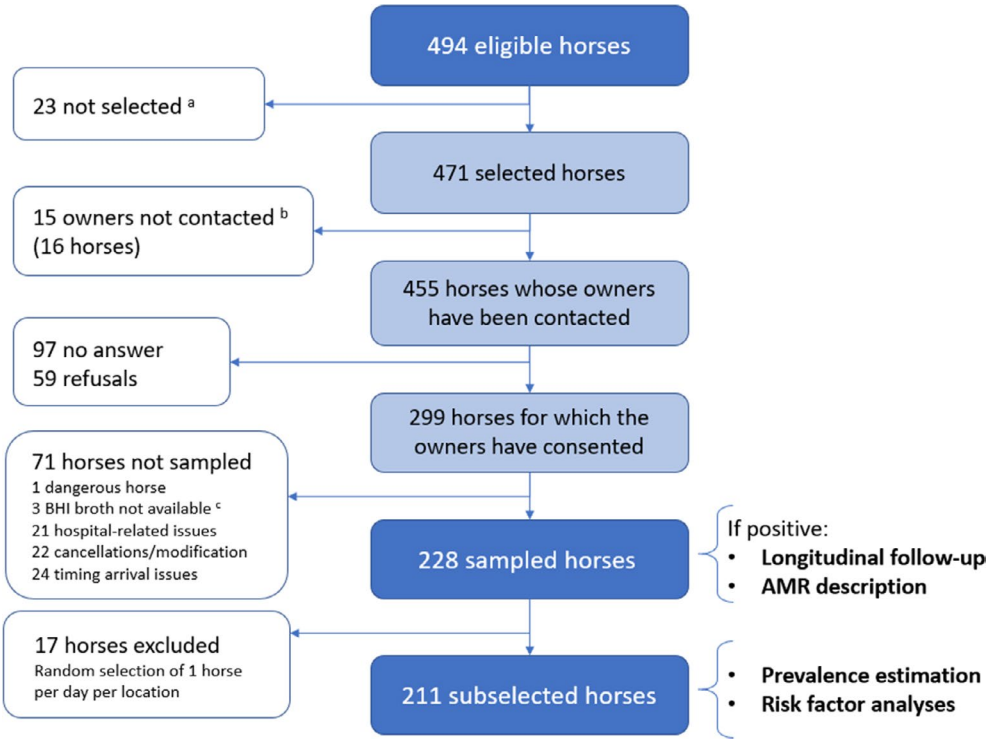

FIGURE 2 | Flow chart of enrolled horses in the study. (a) The recruitment strategy was modified after the first 2 weeks. Initially, the horses were randomly selected from the weekly appointment list. Regarding the consent rate, all scheduled horses on the list were eligible for sampling each week, after the first 2 weeks. (b) Fifteen owners were not contacted due to various technical issues (missing or erroneous contact details, time-related issues, etc.). (c) In one occasion, the BHI broths were not available due to supply issues.

genotypic characteristics of isolates. Weese et al. [32] reported the longitudinal colonization of MRSA-colonized horses stabled in two previously identified farms. Targeted interventions were specifically implemented after the initial samplings, as infection control measures and occasionally antimicrobial treatment. The incidence of colonized horses declined significantly in the first 30–50 days. Two on 8 horses and approximately 5 on 29 horses were colonized persistently, until around 120 days. Our study suggests a transient carriage in positive horses. Most of them (8 out of 10 horses with an available follow-up) were negative 14 days following their admission, without any specific intervention. This rapid and spontaneous loss of colonization warrants additional exploration to elucidate the pattern of MRSA persistence in equine sample. Considering that the antimicrobial susceptibility patterns remained the same over time for the two other horses, we can hypothesize that a prolonged colonization was likely. To deepen our understanding on the MRSA strains in our equine sample and the changes over time for the same horse, the genomic characterization of the MRSA and MSSA strains isolated in this study is currently under investigation. According to a previous report on two MRSA strains isolated from horses previously hospitalized at the VTH in 2017 [36], both belonged to the sequence type 612 (not previously reported in horses in North America) and harbored an *spa* type t1257, a SCC*mec* IVd2B, and a negative *pvl* (Panton-Valentine Leukocidin) gene. Those differed from the ones previously reported in horses in Canada, the Canadian epidemic MRSA-5 [17].

None of the nasally MRSA-colonized horses tested positive for MRSA on the skin. We selected cutaneous areas frequently touched by human hands and by the noses of horses, during individual and mutual grooming, or that are known to be colonized by MRSA in other species [4, 37, 38]. These findings suggest reduced transmission occasions between horses or with handlers. There are conflicting conclusions on this topic in the literature. The concurrent presence of MRSA on the skin and in the nasal passages in horses was described in various horse populations [38, 39]. In 51 horses presented with acute or chronic wounds in an equine hospital in the United Kingdom, *Staphylococcus* spp. was the most frequent genus isolated from healthy skin samples [40]. In contrast to this, and similar to our findings, Adams et al. reported no coagulase-positive *Staphylococcus* isolates from the skin of 20 healthy horses admitted to a VTH for routine elective surgery [41]. Another study on healthy horses did not report a predominance of *Staphylococcus* genus in the cutaneous microbiota [42]. As described, the four cutaneous sites were pooled in this study to decrease the analysis costs and laboratory workload. The performance of this pooling technique is not described in horses. In humans, pooling three samples presented a similar sensitivity for the detection of MRSA colonization compared to testing individual swabs [43]. Finally, it should be noted that as the skin samples were only processed if horses were colonized in the nostril, the number of positive horses in our study may have been reduced. Few horses can harbor MRSA isolates on the skin and not in the nostril (2/12 horses) [38].

The antimicrobial susceptibility patterns of the MRSA isolates are of concern. All isolates of this study were MDR and two were XDR. The MRSA strains are not intrinsically more virulent than MSSA strains [44]. However, an infection caused by MRSA, especially if MDR, will be more challenging to treat.

This is one of the reasons why MRSA represents an ongoing nosocomial threat. All isolates in our study were resistant to enrofloxacin, gentamicin, rifampin, tetracyclines, and TMS. This raises the question of whether the previous exposure to the hospital environment may have played a role in the observed multidrug resistance of the isolates, as observed in humans. A high proportion of MDR isolates among MRSA in horses has been occasionally reported: 93.4% of the 132 isolates from horses hospitalized at the surgery or medicine departments of a Belgian VTH were multi-resistant (to β-lactam, tetracycline, trimethoprim, and gentamicin) [22]. In Québec, the resistance patterns of frequently isolated bacteria in horses presented at the VTH of the Université de Montréal between 2007 and 2013 were reported, including coagulase-positive

TABLE 2 | Odds ratios from two final multivariable logistic regressions modeling SA and MRSA nasal colonization in horses admitted to a Veterinary Teaching Hospital.

			Positive horses		Odds ratios	
Characteristics		**Number of horses**	**Number**	**%**	**Estimate**	**95% CI**
Outcome: SA colonization ($n = 211$ horses)						
Hospitalization in the previous 6 months	**Yes**	**29**	**10**	**34.5**	**3.5**	**1.4–8.7**
	No	182	27	14.8	Ref	
Year of admission	**2022**	**90**	**24**	**26.7**	**3.3**	**1.5–7.1**
	2020–21	121	13	10.7	Ref	
Outcome: MRSA colonization ($n = 200$ horses)[a]						
Hospitalization in the previous 6 months	**Yes**	**28**	**4**	**14.3**	**6.0**	**1.0–35.2**
	No	172	8	4.7	Ref	
Year of admission	**2022**	**87**	**10**	**11.5**	**9.0**	**1.7–92.2**
	2020–21	113	2	1.8	Ref	
Number of horses on site	**≥11**	**111**	**10**	**9**	**6.0**	**1.1–64.2**
	0–10	89	2	2.3	Ref	

Abbreviations: CI, confidence interval; Ref, reference value.
[a]Indicates an exact logistic regression.

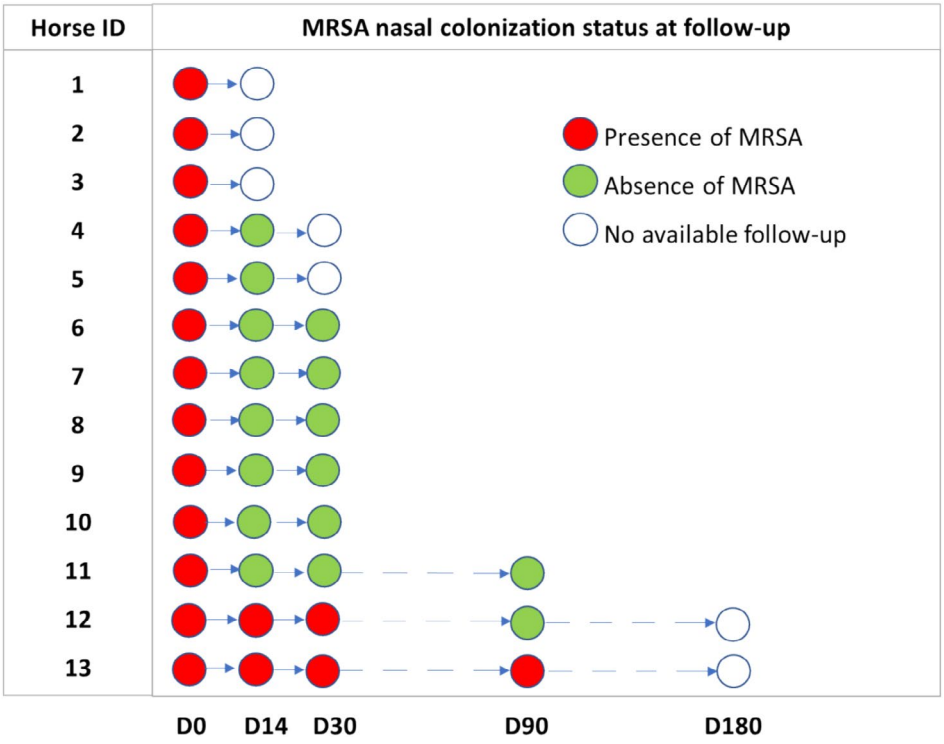

FIGURE 3 | Longitudinal follow-up of the 13 MRSA-colonized horses. D0: Admission.

TABLE 3 | Minimum inhibitory concentrations for the 18 MRSA isolated from the nasal cavities of horses at admission to a Veterinary Teaching Hospital and during the longitudinal sampling.

	Minimum inhibitory concentrations (μg/mL)												
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	Number of susceptible (%)
AMI							17	1					17 (94%)
AZI					16			2					16 (89%)
CHL								18					18 (100%)
CLA					16				2				16 (89%)
DOX								18					0 (0%)
ENRO						10	8						0 (0%)
ERY				16					2				16 (89%)
GEN								1	17				0 (0%)
OXA+								18					0 (0%)
RIF								18					0 (0%)
TET									18				0 (0%)
SXT								18					0 (0%)

Note: The numbers of isolates in each MIC category by an antimicrobial agent are presented in each row. White areas represent the concentrations of antimicrobials tested by the broth microdilution method. Numbers in gray areas have an MIC superior to the last concentration tested. Numbers in the first white area starting from the left have an MIC inferior or equal to the corresponding concentration. Black lines represent thresholds used to define susceptible and resistant breakpoints. Number of susceptible (%) is the number and percentage of susceptible isolates.
Abbreviations: AMI, amikacin, AZI, azithromycin, CHL, chloramphenicol, CLA, clarithromycin, DOX, doxycycline, ENRO, enrofloxacin, ERY, erythromycin, GEN, gentamicin, OXA+, oxacillin +2% NaCl, RIF, rifampin, TET, tetracycline, SXT, trimethoprim/sulfamethoxazole.

Staphylococcus (CoPS), but not MRSA specifically [45]. In this study, 15% of 26 CoPS were resistant to cefoxitin. Among the 42 CoPS isolates, 60% or more were susceptible for all antimicrobials tested (amikacin, ampicillin, ceftiofur, chloramphenicol, enrofloxacin, erythromycin, gentamicin, penicillin, tetracycline, and TMS).

This study presents some limitations. The final sample size was inferior to the targeted size. This affected the accuracy of the estimated prevalence. Regarding the risk factor analysis, the small sample size can lead to a type II error and the high number of tested variables to a type I error. The causes are multiple, for instance: (i) difficulties in reaching owners on time; (ii) sampling of horses could not be performed upon admission; (iii) some owners preferred not to add any procedures during their horse's hospitalization; (iv) some others expressed concerns about the potential negative impact on their horse's management at the VTH or at the barn if their horse was colonized by MRSA; and (v) some information from the questionnaire was missing or incomplete. All of these choices negatively affected the final number of horses recruited but improved the strength of the findings. A non-differential recall bias could affect the results of our questionnaire for some exposure variables, such as previous antimicrobial treatment in the previous 6 months, which would lead to underestimating the strength of the relationship. If associations are attenuated, risk factors could be missed. Finally, the external validity regarding prevalence may be limited to our VTH equine sample. However, the information related to the risk factors and carriage pattern of MRSA in horses regarding the absence on the skin and nasal colonization over time may apply to other similar horse populations.

In conclusion, this study underscores the notable prevalence of nasal MRSA colonization in a VTH horse sample, with all isolates demonstrating multidrug resistance. The carriage was predominantly transient. Significant risk factors for MRSA colonization included increased contact opportunities and previous exposure to hospital environments. Understanding the prevalence and risk factors for colonization is crucial for guiding infection prevention and control programs in veterinary hospitals. Overall, the goal is to reduce risks of MRSA and MDR bacterial exposure and nosocomial infections in both horses and handlers. Tailored preventive measures, such as heightened barrier precautions (e.g., gloves, dedicated gowns, and materials) for horses with prior hospital exposure, could be recommended.

Acknowledgments

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Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Approved by the Animal Ethics and Care Committee of the Université de Montréal, number 19-Rech-2038. Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.