# Whole genome sequencing of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* isolated from 4 horses in a veterinary teaching hospital and its ambulatory service

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## Abstract

Genomic characterization was conducted on 2 methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from 2 horses hospitalized during an overlapping period of time and 2 methicillin-sensitive *S. aureus* (MSSA) strains isolated from 2 distinct horses. Phylogenetic proximity was traced and the genotypic and phenotypic characteristics of the antimicrobial resistance of the strains were compared.

Whole genome sequencing of MRSA strains for this report was similar but differed from whole genome sequencing of MSSA strains. The MRSA strains were closely related, belonging to sequence type (ST) 612, *spa* type t1257, and SCC*mec* type IVd2B. The MSSA strains were also closely related, belonging to ST1660, *spa* type t3043, and having no detectable staphylococcal cassette chromosome *mec* elements. All MSRA and MSSA strains were Panton-Valentine leukocidin negative. There were discrepancies in the genotypic analysis and the antimicrobial susceptibility testing (phenotypic analysis) of MRSA strains for rifampin, trimethoprim-sulfamethoxazole, gentamicin, amikacin, and enrofloxacin.

## *Résumé*

*La caractérisation génomique a été effectuée sur deux souches de* Staphylococcus aureus *résistantes à la méticilline (SARM) isolées de deux chevaux hospitalisés sur une période de chevauchement, et de deux* S. aureus *sensibles à la méticilline (SASM) isolés de deux chevaux distincts. Leur proximité phylogénétique a été retracée. Les caractéristiques génotypiques et phénotypiques de la résistance aux antimicrobiens de ces souches ont été comparées.*

*Le séquençage complet du génome des souches de SARM pour ce rapport était similaire, mais différent du séquençage complet du génome des souches de SASM. Les souches de SARM étaient étroitement apparentées, appartenant à la séquence type (ST) 612, au* spa *type t1257 et au SCC*mec *type IVd2B. Les souches MSSA étaient étroitement apparentées appartenant au ST1660,* spa *type t3043 et aucun élément de la cassette contenant le gène* mec *n'a été détecté. Toutes les souches MSRA et MSSA étaient négatives pour la leucocidine Panton-Valentine. Il y avait des divergences entre l'analyse génotypique et les tests de sensibilité aux antimicrobiens (phénotype) des souches de SARM pour la rifampicine, le triméthoprime-sulfaméthoxazole, la gentamicine, l'amikacine et l'enrofloxacine.*

*(Traduit par les auteurs)*

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are a serious concern in equine medicine. Infections involving MRSA in horses have a clinical impact, represent a risk for nosocomial diseases and have the potential to be transmitted to humans (1–5). Unfortunately, there is a limited number of antimicrobials approved for use in horses in Canada to address such important infectious diseases.

To the authors' knowledge, whole genome sequencing (WGS) of Canadian equine MRSA has not been described. The WGS of 2 MRSA strains isolated from 2 horses hospitalized at the veterinary teaching hospital (VTH) of the Faculty of Veterinary Medicine of the Université de Montréal, during an overlapping period is described. Two equine methicillin-sensitive *S. aureus* (MSSA) strains were described, to compare MRSA and MSSA genomic characteristics. The genotypic and phenotypic characteristics for antimicrobial resistance of these *S. aureus* strains were compared and their phylogenetic relatedness was assessed.

Two frozen MRSA strains collected from 2 horses hospitalized at the VTH over the same period in 2017 (71 d overlapping period) were selected. The horses shared the same facilities and were kept in separate stalls with the same hospital staff handling them. The first MRSA sample (MRSA 1) was taken from the left hind limb fetlock wound of an 8-year-old Quarter Horse mare hospitalized at the VTH for recurrent lymphangitis. Prior to admission, this horse was treated with intravenous (IV) penicillin (day −35 to day −27), IV gentamicin (day −35 to day −32), IV enrofloxacin (day −32 to

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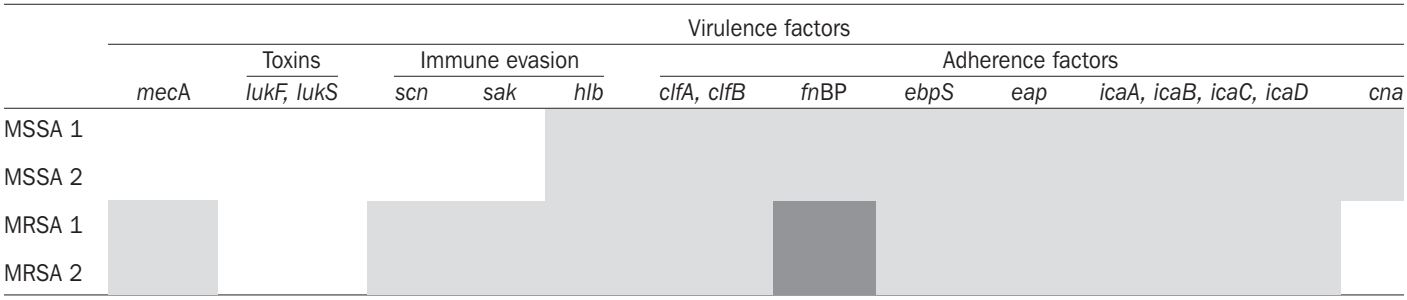
**Table I. Reference strains.**

| Host specificity | Isolation site | Collection date | Sample type | Isolation source |
|---|---|---|---|---|
| *Equus caballus* | Germany | 2016 | Pure culture | Ocular surface swab |
| *Equus caballus* | Germany | 2016 | Pure culture | Ocular surface swab |
| *Equus caballus* | Germany | 2014 | Nasal swab | N/A |
| *Equus caballus* | Germany | 2014 | Fecal sample | N/A |
| *Equus caballus* | Germany | 2014 | Nasal swab | N/A |
| *Equus caballus* | Germany | 2016 | Pure culture | Ocular surface swab |
| *Equus caballus* | Germany | 2015 | Nasal swab | N/A |
| *Sus scrofa domesticus* | Germany | 2012 | Cell culture | N/A |
| *Homo sapiens* | United Kingdom | 2003 | N/A | Blood |
| *Homo sapiens* | Australia | 2009 | N/A | Vet Hospital |
| Not Available (N/A) | USA | N/A | N/A | N/A |
| *Homo sapiens* | Saudi Arabia | 2017 | N/A | N/A |
| *Equus caballus* | Japan | 2018 | N/A | N/A |
| *Homo sapiens* | Jordan | 2007 | N/A | Wound |

**Table II. Equine methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) genomic characteristics identified in 4 strains.**




*luk* — leucocidin; *scn* — staphylococcal complement inhibitor; *sak* — staphylokinase; *hlb* — beta-hemolysin; *clf* — clumping factor; *fn*BP — fibronectin-binding protein; *ebpS* — elastin-binding protein; *eap* — extracellular adherence protein; *ica* — intercellular adhesion protein; *cna* — collagen adhesin.

gene absent

gene identified

non-conclusive result

day −19), and oral chloramphenicol (day −28 to day −12) based on bacterial culture results (MRSA and *Enterobacter cloacae*) and antimicrobial susceptibility testing (AST). Upon admission (day 0), a sample was taken for a bacterial culture and AST. While the results of the bacterial culture and AST were pending, the horse received 4 d of IV enrofloxacin and oral chloramphenicol (day 0 to day 3). Methicillin-resistant *S. aureus, Proteus vulgaris, Pseudomonas aeruginosa,* and *Streptococcus equi* spp. *zooepidemicus* were identified on the bacterial culture. Based on the results of the AST, the clinical findings, and a second bacterial culture performed 26 d following admission (day 25), IV ceftiofur and oral chloramphenicol were administered for 30 d (day 4 to day 33) and 69 d (day 0 to day 68), respectively.

The second MRSA sample (MRSA 2) was taken from a chronic wound between the right tuber coxae and ischium of a 25-year-old Hanoverian mare. Before her admission, trimethoprim-sulfamethoxazole (TMS) (unknown route of administration) was administered for 7 d (day −18 to day −12), followed by intramuscular penicillin and enrofloxacin (unknown route of administration) for 7 d (day −11 to day −5), and trimethoprim-sulfadiazine (unknown route of administration) and enrofloxacin (unknown route of administration) for 5 d (day −4 to day 0), due to treatment failures (i.e., antimicrobial treatments were not based on a bacterial culture and AST results). Upon admission, a sample was submitted to the bacteriology laboratory for bacterial culture, but no growth was observed. A second sample was obtained for bacterial culture 14 d after being admitted (day 13), which revealed a pure culture of MRSA. During the hospitalization, the horse was treated with oral enrofloxacin and metronidazole for 20 d (day 0 to day 19), followed by oral chloramphenicol for 10 d (day 20 to day 30).

Two frozen MSSA strains (MSSA 1 and 2) sampled in 2016 were also sequenced. Methicillin-sensitive *S. aureus* 1 was from a skin

**Table III. Genotypic and phenotypic characteristics of antimicrobial resistance in equine methicillin-resistant *S. aureus* (MRSA) (*n* = 2) and methicillin-sensitive *S. aureus* (MSSA) (*n* = 2). Italicized words represent discrepancies between the genotypic and phenotypic patterns of antimicrobial resistance.**

| | MSSA 1 | MSSA 2 | MRSA 1 | MRSA 2 |
|---|---|---|---|---|
| *Genotypic analyses* | Absence of resistance genes | Absence of resistance genes | P: β-lactam antibiotics<br>N: phenicols<br>N: *quinolones*<br>P: tetracyclines<br>P: macrolides<br>P: *aminoglycosides*<br>N: *rifampin*<br>N: *trimethoprim*<br>N: *sulphonamid* | P: β-lactam antibiotics<br>N: phenicols<br>N: *quinolones*<br>P: tetracyclines<br>P: macrolides<br>P: *aminoglycosides*<br>N: *rifampin*<br>N: *trimethoprim*<br>N: *sulphonamid* |
| *Phenotypic analyses (AST)* | Absence of antibiotic resistance | Absence of antibiotic resistance | R: β-lactam antibiotics<br>S: chloramphenicol<br>I: *enrofloxacin*<br>R: tetracycline<br>R: erythromycin<br>S: *amikacin*<br>R: gentamicin<br>R: *rifampin*<br>R: *TMS* | R: β-lactam antibiotics<br>S: chloramphenicol<br>I: *enrofloxacin*<br>R: tetracycline<br>R: erythromycin<br>S: *amikacin*<br>I: *gentamicin*<br>R: *rifampin*<br>R: *TMS* |

AST — antimicrobial susceptibility testing; P — positive for the gene; N — negative for the gene; S — susceptible; I — intermediate; R — resistant.

sample of a horse hospitalized at the VTH for colic and presenting papules. Methicillin-sensitive *S. aureus* 2 was isolated from a nasal swab sampled by the ambulatory practice of the VTH.

All specimens were obtained using sterile cotton swabs (BBL CultureSwab; Becton Dickinson Canada, Mississauga, Ontario) with Stuart Transport Medium (Becton Dickinson Canada).

The samples were cultured aerobically. A single colony subculture was frozen at −80°C in tryptic soy broth (CM0129) (Oxoid Ltd, Basingstoke, England) with 15% glycerol. The *S. aureus* clone was inoculated on Difco™ Columbia blood agar base (279240) with 5% sheep blood (Becton Dickinson and Company, Sparks, Maryland, USA) and incubated at 35°C ± 2°C with 5% carbon dioxide. Bacteria were identified with conventional biochemical tests for MSSA 1 and the matrix-assisted laser desorption ionization time-of-flight mass spectrometry method was used for MSSA 2 and both MRSA. The same clone was used for sequencing.

All AST was repeated in September 2019 using BBL™ Mueller Hinton II agar (Becton Dickinson and Company, Sparks, Maryland, USA) for the Kirby-Bauer disk diffusion method, according to the most recent guidelines published by the Clinical Laboratory Standards Institute (CLSI). If CLSI criteria were not available for horses or for bacteria isolated from other animals, human criteria were used for the interpretation. Clinical Laboratory Standards Institute criteria specific to horses were only available for ceftiofur and enrofloxacin. All samples were tested for the following antimicrobials: amikacin, ampicillin, cefoxitin, ceftiofur, chloramphenicol, enrofloxacin, erythromycin, gentamicin, penicillin G, rifampin, tetracycline, and TMS. Strains phenotypically resistant to cefoxitin were considered MRSA.

Bacterial DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, Maryland, USA), as described in the company's protocol. DNA was quantified using the Qubit dsDNA High Sensitivity Assay Kit (ThermoFisher Scientific, Waltham, Maine, USA) and a Qubit Fluorometer (ThermoFisher Scientific). The next-generation sequencing libraries were synthesized with the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, California, USA), as described in the Nextera XT protocol. The quality of the library was assessed using the Agilent High Sensitivity DNA Kit (Agilent, Santa Clara, California, USA) in a Bioanalyzer (Agilent). Libraries were sequenced in a v3 600-cycle cartridge using a MiSeq instrument and PhiX Control at around 1% (Illumina).

Reads were trimmed by the MiSeq system together with the CLC Genomics Workbench software (CLC-GW) (version 12.0.3; Qiagen, Redwood City, California, USA). *De novo* analysis was performed using the *De Novo* Assemble Metagenome application in the CLC-GW and confirmed by SPAdes Genome Assembler in BaseSpace (Illumina). Contigs were used to prepare the phylogenetic trees and the Bacterial Analysis Pipeline GoSeqIt ApS. All genome contig sequences for the 4 cases described in this paper are available in GenBank with the BioSample accession number SAMN15351203-6 for strains FMV17-17660 (MRSA 1), FMV17-17156 (MRSA 2), FMV16-5587 (MSSA 1), and FMV16-7294 (MSSA 2).

The genotypic characterizations of isolates [sequence type (ST), *spa* typing schemes, staphylococcal cassette chromosome *mec* (SCC*mec*) type, Panton-Valentine leukocidin (PVL), leucocidin-F and -S subunits *(lukF, lukS)*, staphylococcal complement inhibitor *(scn)*, staphylokinase *(sak)*, clumping factor A and B *(clfA, clfB)*, fibronectin binding protein (*fn*BP), collagen adhesin *(cna)*, elastin-binding protein

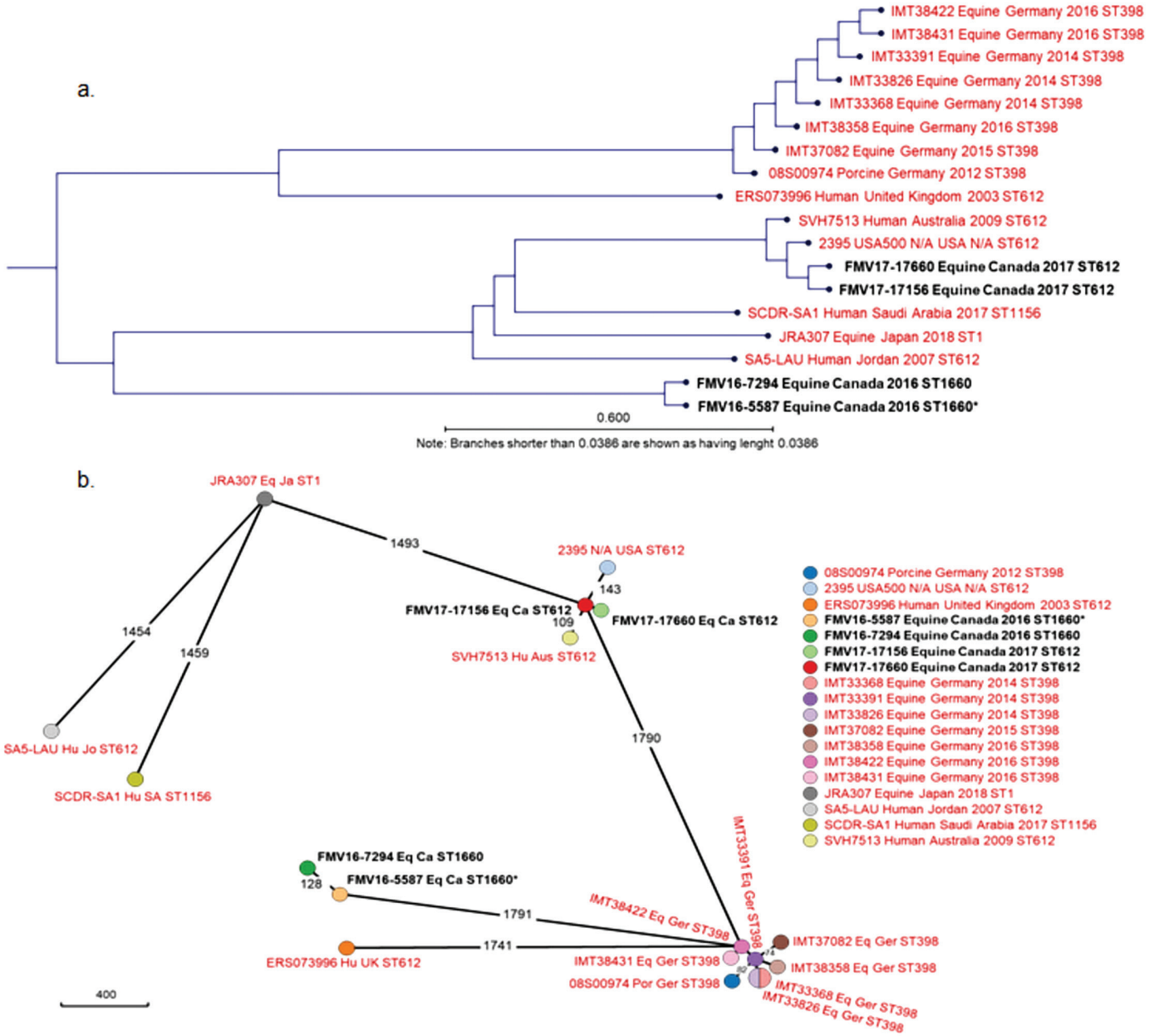



**Figure 1. a — Neighbor joining phylogenetic tree of FMV17-17660 (MRSA 1), FMV17-17156 (MRSA 2), FMV16-5587 (MSSA 1), FMV16-7294 (MSSA 2), and whole sequenced *S. aureus* genomes of different species. The more genetic characteristics shared between strains, the closer they are in the phylogenetic tree. Each isolate is listed as strain name, species, country of origin, and sequence type (ST). The scale bar indicates the percentage of difference in the nucleotide sequence based on the average nucleotide identity. * Represents the nearest ST. N/A — Not available. b — Phylogenetic analysis was performed by using the core genome multilocus sequence typing (cgMLST) approach based on 1865 cgMLST loci. BacWGSTdb 2.0 was used to construct and visualize the minimal spanning tree generated based on the cgMLST allelic profiles, which supports manipulations of both tree layout and the user specified metadata attributes. The lines connecting the circles indicate the clonal relationship between different isolates and the numbers on the connecting lines illustrate the numbers of allelic differences. Numbers below 70 are not shown on the Figure. The scale bar represents a pairwise allelic difference of 400 cgMLST loci (13,14).**

EbpS *(ebpS)*, extracellular adherence protein *(eap)*, intercellular adhesion protein icaA *(icaA)*, intercellular adhesion protein icaB *(icaB)*, intercellular adhesion protein icaC *(icaC)*, intercellular adhesion protein icaD *(icaD)*, and phospholipase C *(hlb)*] were acquired by performing genomic comparisons with other complete *S. aureus* genomes in the Center for Genomic Epidemiology public database (https://cge.cbs.dtu.dk/services/) (6,7).

The online version of the ResFinder 3.2 tool (https://cge.cbs.dtu.dk/services/ResFinder/database.php) was used to identify the antimicrobial resistance genes, the single-end and paired-end reads, and the FastQ file, as previously described (8). The classes of antibiotics of interest reported were phenicol, β-lactam antibiotics, quinolone, tetracycline, trimethoprim, macrolide, aminoglycoside, sulfonamide, and rifampin (9–11).

**Table IV. Genetic comparisons between the reported *S. aureus* strains and the Canadian epidemic methicillin-resistant *S. aureus*-5 (CMRSA-5) (15).**

| | MSSA 1 | MSSA 2 | MRSA 1 | MRSA 2 | CMRSA-5 |
|---|---|---|---|---|---|
| ST | 1660 | 1660 | 612 | 612 | 8 |
| Spa type | t3043 | unknown | t1257 | t1257 | t064 |
| SCC*mec* | Negative | Negative | IVd2B | IVd2B | IV |
| PVL | Negative | Negative | Negative | Negative | Negative |

MSSA — methicillin-sensitive *S. aureus;* ST — sequence type; SCC*mec* — staphylococcal cassette chromosome *mec;* PVL — Panton-Valentine leukocidin.

For the phylogenetic analysis, the whole genome sequenced strains were compared to others available on GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (Table I). A neighbor-joining phylogenetic tree with whole genome sequences was created using Comparison (beta) 1.0 based on the average nucleotide identity calculation from the CLC Genomics Workbench (Qiagen) Whole Genome Alignment 20.0 plugin. Phylogenetic analysis using the core genome multilocus sequence typing (cgMLST) approach based on the 1865 cgMLST loci in the scheme for *S. aureus* was performed using BacWGSTdb 2.0 (http://bacdb.cn/BacWGSTdb/).

The genotypic characteristics of the *S. aureus* strains are summarized in Table II. The ST obtained from MRSA 1 and 2 was similar (ST612). Both MRSA strains harbored the SCC*mec* type IVd2B. Methicillin-sensitive *S. aureus* 1 and 2 displayed the same ST (ST1660) and did not harbor the SCC*mec* gene. The *spa* types identified for both MRSA strains and for MSSA 1 were type t1257 and t3043, respectively; the *spa* type for MSSA 2 was unidentified.

The same genetic patterns for virulence factors and antimicrobial resistance were identified on both MRSA strains. Methicillin-resistant *S. aureus* 1 and 2 were positive for *mecA*, *scn*, *sak*, *clfA*, *clfB*, *ebpS*, *eap*, *icaA*, *icaB*, *icaC*, *icaD*, and *hlb* genes. It was inconclusive for the *fn*BP gene. Both MSSA strains were positive for *clfA*, *clfB*, *fn*BP, *cna*, *ebpS*, *eap*, *icaA*, *icaB*, *icaC*, *icaD*, and *hlb* genes. All *S. aureus* were PVL negative.

The identified antimicrobial resistance genes were in accordance with the AST results, except for amikacin, rifampin, TMS, and enrofloxacin for MRSA 1 and 2. The results are presented in Table III.

The genes identified for each *S. aureus* and their pattern of branching in the phylogenetic tree suggests that the MRSA and MSSA strains of this report are distinct. The phylogenetic trees based on the whole genome (Figure 1) and based on the core genome indicate that both MRSA strains are closely related. These 2 different ways of comparing the strains are 2 alignment methods currently used in the literature (12–14). Whole genome alignment compares full length genomes and plasmid sequences, while core genome alignment compares the set of genes shared by all strains in a species (i.e., 1865 cgMLST loci for *S. aureus*) that mostly encode functions related to basic cellular biology (12–14). The MRSA strains are not exactly on the same branch, but they belong to a common subcluster and harbor similar genes. Indeed, they harbored the same ST, *spa* type, and SCC*mec*, and were negative for PVL. Moreover, the genes associated with virulence factors and antimicrobial resistance were similar.

The genotypic characteristics of Canadian equine MRSA (Canadian epidemic MRSA-5) are partially reported (Table IV) (15,16). Canadian epidemic MRSA-5 was reported as ST8 and *spa* type t064, which differs from the reported MRSA strains (ST612 and *spa* type t1257). Canadian epidemic MRSA-5 and both reported MRSA strains were SCC*mec* type IV and PVL negative. These results add new information regarding the epidemiological data relating to the circulating equine MRSA strains in Canada.

Interestingly, the MRSA strains in this report share some common genotypic characteristics with the ST612 Australian strain [SVH7513(horse)AUS] (17). Worldwide, the ST612 strain has only been isolated in horses in Australia (17). As for SVH7513(horse)AUS, the MRSA strains reported here also share some similar genotypic characteristics with the human MRSA USA500 strains previously reported in North America. However, a whole genome comparison between the reported MRSA strains and the ST612 Australian and human MRSA USA 500 strains would be required to clearly identify the homology between these MRSA strains. The MRSA USA500 strain (ST8; *spa* types t064, t008, or t211; SCC*mec* IV; PVL negative) is responsible for both community- and hospital-associated infections, and is considered to be a significant infectious agent (4,18,19).

We report the staphylococcal genes identified, but their expression was not studied. Therefore, the clinical relevance of the virulence factors identified is unknown. All *S. aureus* strains in this report harbor genes responsible for adhesion, colonization, and invasion, as well as the ability to form a biofilm. They have the potential to evade the immune system by interfering with the complement cascade, neutrophil function, and diapedesis. They also harbor genes responsible for cytolytic toxins (4,20). The reported MRSA strains harbor genes responsible for the inhibition of the complement system and the degradation of immunoglobulin opsonins *(sak, scn)* (4). These characteristics may influence the severity of the disease if they are expressed.

The results of the genotypic and phenotypic antibiotic resistance profiles for each MRSA strain show discrepancies. This could be explained by a consensus sequence for the genotypic antibiotic resistance analyses, but this was not the methodology used in this report. Another explanation could be antibiotic resistances carried by mechanisms such as genetic mutations (rifampin, TMS, and enrofloxacin) or unexpressed genes of resistance (amikacin), which were not studied in this report (21). A limitation to the AST results is the absence of horse-specific cutoff values for the interpretation of the antimicrobial resistance of all antimicrobials tested. Although it is unlikely that the discrepancies between the genotypic and phenotypic antibiotic resistance profiles are a consequence of the absence of horse-specific cutoff values for all tested antimicrobials, this might have influenced the interpretation of the AST.

The use of multiple classes of antibiotics for a prolonged period in these horses is likely to be one of the major contributing factors to the pattern of resistance of the MRSA strains described in this report (22).

Considering the phylogenetic relatedness of MRSA 1 and 2, an indirect transmission from one horse to the other cannot be excluded (common shared areas, common hospital handlers), despite the application of standard infection control measures.

In conclusion, complete genome sequencing tools are useful in hospital settings to provide epidemiological information and may

assist veterinary hospitals in improving biosecurity and antimicrobial stewardship (23,24).

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## References

1. Cuny C, Abdelbary MM, Köck R, 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 2015;2:11–17.
2. Maddox TW, Clegg PD, Diggle PJ, et al. Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 1: Prevalence of antimicrobial-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*. Equine Vet J 2012;44:289–296.
3. Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus:* Molecular characterization, evolution, and epidemiology. Clin Microbiol Rev 2018;31:e00020–18.
4. Foster TJ, Geoghegan JA. *Staphylococcus aureus*. In: Tang YW, Liu D, Schwartzman J, Sussman M, Poxton I, eds. Molecular Medical Microbiology. 2nd ed. London: Elsevier, 2015:655–674.
5. Cuny C, Witte W. MRSA in equine hospitals and its significance for infections in humans. Vet Microbiol 2017;200:59–64.
6. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 2012;50:1355–1361.
7. Bartels MD, Petersen A, Worning P, et al. Comparing whole-genome sequencing with Sanger sequencing for spa typing of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2014;52:4305–4308.
8. Kleinheinz KA, Joensen KG, Larsen MV. Applying the ResFinder and VirulenceFinder web-services for easy identification of acquired antibiotic resistance and *E. coli* virulence genes in bacteriophage and prophage nucleotide sequences. Bacteriophage 2014;4:e27943.
9. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCC*mec*): Guidelines for reporting novel SCC*mec* elements. Antimicrob Agents Chemother 2009;53:4961–4967.
10. Kondo Y, Ito T, Ma XX, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: Rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother 2007;51:264–274.
11. Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–2644.
12. Bosi E, Monk JM, Aziz RK, Fondi M, Nizet V, Palsson BØ. Comparative genome-scale modelling of *Staphylococcus aureus* strains identifies strain-specific metabolic capabilities linked to pathogenicity. Proc Natl Acad Sci USA 2016;113:E3801–E3809.
13. Feng Y, Zou S, Chen H, Yu Y, Ruan Z. BacWGSTdb 2.0: A one-stop repository for bacterial whole-genome sequence typing and source tracking. Nucleic Acids Res 2021;49:D644–D650.
14. Ruan Z, Yu Y, Feng Y. The global dissemination of bacterial infections necessitates the study of reverse genomic epidemiology. Brief Bioinform 2020;21:741–750.
15. 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;20:182–186.
16. Weese JS, Archambault M, Willey BM, et al. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000–2002. Emerg Infect Dis 2005;11:430–435.
17. Murphy RJT, Ramsay JP, Lee YT, et al. Multiple introductions of methicillin-resistant *Staphylococcus aureus* ST612 into Western Australia associated both with human and equine reservoirs. Int J Antimicrob Agents 2019;54:681–685.
18. Frisch MB, Castillo-Ramírez S, Petit RA 3rd, et al. Invasive methicillin-resistant *Staphylococcus aureus* USA500 strains from the U.S. Emerging Infections Program constitute three geographically distinct lineages. mSphere 2018;3:e00571–17.
19. Boyle-Vavra S, Li X, Alam MT, et al. USA300 and USA500 clonal lineages of *Staphylococcus aureus* do not produce a capsular polysaccharide due to conserved mutations in the *cap5* locus. mBio 2015;6:e02585–14.
20. Downer R, Roche F, Park PW, Mecham RP, Foster TJ. The elastin-binding protein of *Staphylococcus aureus* (EbpS) is expressed at the cell surface as an integral membrane protein and not as a cell wall-associated protein. J Biol Chem 2002;277:243–250.
21. Boerlin P, White DG. Antimicrobial resistance and its epidemiology. In: Giguère S, Prescott JF, Dowling PM, eds. Antimicrobial Therapy in Veterinary Medicine. 5th ed. Ames, Iowa: Wiley-Blackwell, 2013:21–40.
22. Weese JS, Lefebvre SL. Risk factors for methicillin-resistant *Staphylococcus aureus* colonization in horses admitted to a veterinary teaching hospital. Can Vet J 2007;48:921–926.
23. Morris DO, Loeffler A, Davis MF, Guardabassi L, Weese JS. Recommendations for approaches to meticillin-resistant staphylococcal infections of small animals: Diagnosis, therapeutic considerations and preventative measures. Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Vet Dermatol 2017;28:304–e69.
24. Quainoo S, Coolen JPM, van Hijum SAFT, et al. Whole-genome sequencing of bacterial pathogens: The future of nosocomial outbreak analysis. Clin Microbiol Rev 2017;30:1015–1063.