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Prevalence of Methicillin-Resistant *Staphylococcus aureus* from Equine Nasopharyngeal and Guttural Pouch Wash Samples

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as a cause of nosocomial infections in both human and veterinary medicine. Studies that examine the nasopharynx and guttural pouches of the horse as carriage sites for MRSA have not been reported.

Hypothesis/Objective: MRSA colonizes the nasopharynx and guttural pouch of horses. To determine the prevalence of MRSA in equine nasopharyngeal wash (NPW) and guttural pouch lavage (GPL) samples in a field population of horses.

Samples: One hundred seventy-eight samples (123 NPW and 55 GPL) from 108 horses.

Methods: Prospective study. Samples were collected from a convenience population of clinically ill horses with suspected *Streptococcus equi* subsp. *equi* (*S. equi*) infection, horses convalescing from a known *S. equi* infection, and asymptomatic horses undergoing *S. equi* screening. Samples were submitted for *S. aureus* aerobic bacterial culture with mannitol salt broth and two selective agars (cefoxitin CHROMagar as the PBP2a inducer and mannitol salt agar with oxacillin). Biochemical identification of *Staphylococcus* species and pulsed-field gel electrophoresis (PFGE), to determine clonal relationships between isolates, were performed.

Results: Methicillin-resistant *Staphylococcus* (MRS) was isolated from the nasopharynx of 7/108 (4%) horses. Three horses had MRSA (2.7%), and 4 had MR-*Staphylococcus pseudintermedius* (MRSP). MRSA was isolated from horses on the same farm. PFGE revealed the 3 MRSA as USA 500 strains.

Conclusions and Clinical Importance: Sampling the nasopharynx and guttural pouch of community-based horses revealed a similarly low prevalence rate of MRSA as other studies sampling the nares of community-based horses. More study is required to determine the need for sampling multiple anatomic sites when screening horses for MRSA.

Key words: Community; Horse; MRSA.

Staphylococcus aureus is a Gram-positive organism frequently associated with infection of the skin and mucous membranes. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multiple drug-resistant organism that is a pathogen of humans and animals. There might be a zoonotic risk from horses with active infection or carrier status,[1–3] there is evidence of human to equid transmission.[4] Community-acquired MRSA strains have been identified in nonhospitalized humans and equid populations that have no typical risk factors for MRSA acquisition.[5] As the same MRSA strain has been found in both community-acquired and nosocomial infections in equine hospitals, defining the prevalence of MRSA in a general field population might help to identify the true level of risk for cross-transmission among horses and between horses and people.

Studies in North America, Europe, Australia, Asia, and the Middle East to determined the prevalence of MRSA in both hospitalized and nonhospitalized horses.[1,4–9] Nasal carriage rates of MRSA in horses in the community ranged from 0 to 4.7% in North America, although targeted surveillance of farms with horses known to carry MRSA revealed a prevalence as high as 12%.[5] MRSA nasal carriage in the general equine population of the United Kingdom ranged from 0 to 0.6%.[10] Prevalence of MRSA carriage in hospitalized horses ranged from 2.7% in Canada[11] to 12% in the United Kingdom.[12] The previously reported prevalence might under-represent true carriage as all samples were

Abbreviations:

GPL	guttural pouch lavage
MR	methicillin resistant
MRSA	methicillin-resistant *Staphylococcus aureus*
MRS	methicillin-resistant *Staphylococcus*
MRSP	methicillin-resistant *Staphylococcus pseudintermedius*
MSA	mannitol salt agar
MSB	mannitol salt broth
MSSS	methicillin-susceptible *Staphylococcus* species
NPW	nasopharyngeal wash
PBP2a	penicillin binding protein
PFGE	pulsed-field gel electrophoresis
S. equi	*Streptococcus equi* subsp. *equi*

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acquired from the nasal passage of horses. Many human hospitals use a combination of sites which include the nares, throat, and perineum for routine screening and emphasize the importance of sampling the throat.[13–17] In a study that examined colonization of humans with concurrent MRSA infection, the throat was the only positive site in 17% of patients and 33% of their close human contacts.[16] It was also found that colonization persisted for extended periods of time in the throat, which might be the reason that treated patients recolonize their nares.[16] To our knowledge, studies which sample the nasopharynx or guttural pouches of the horse for MRSA have not been reported. The objective of the study reported here was to determine the prevalence of MRS in equine nasopharyngeal and guttural pouch wash samples, in order to address the hypothesis that MRS colonizes the nasopharynx and guttural pouches of horses.

Material and Methods

Samples

Nasopharyngeal wash (NPW) and guttural pouch lavage (GPL) samples were collected from a convenience population of clinically ill horses with suspected *Streptococcus equi* subsp. *equi* (*S. equi*) infection, horses convalescing from a known *S. equi* infection, and asymptomatic horses undergoing *S. equi* screening. Antibiotic treatment status was not available. Lavage samples were submitted to the University of Pennsylvania's New Bolton Center Clinical Microbiology Laboratory for bacterial culture. More than one sample originated from some horses due to the nature of diagnostic testing protocols for strangles. The experimental protocol was approved by the University of Pennsylvania's Institutional Animal Care and Use Committee.

Testing

Aerobic culture and bacterial species identification for streptococcal species and real-time polymerase chain reaction (PCR) for *S. equi* were performed as previously described.[18] To detect MRSA, aerobic culture was performed by incubating 100 μL of wash fluid sample for a minimum of 18 hour at 35°C in mannitol salt broth[a] (MSB) to select for the presence of *Staphylococcus*. A 10-μL loop was used to subculture samples on both CHROMagar-MRSA[b] and mannitol salt agar (MSA) with 4 μg/mL of oxacillin[c], and these plates were incubated overnight at 35°C. Presumptive MRSA isolates (mauve colonies on CHROMagar-MRSA plates and yellow colonies on MSA plates) were tested for the production of catalase. Catalase positive isolates were then tested for penicillin binding protein (PBP2a) to identify methicillin-resistant (MR) staphylococci via rapid slide latex agglutination kit (Oxoid PBP2' Latex Test). Species were identified on a Sensititre ARIS @x[d] with the GPID panel, and antimicrobial susceptibility was determined with a veterinary companion animal equine MIC panel (COMPAN1F). Methicillin-resistant *Staphylococcus* (MRS) isolates were stored in Microbank® cryopreservation tubes.[e] Macrorestriction pulsed-field gel electrophoresis (PFGE) was performed on MRSA isolates as previously described.[19] Clonal relatedness was determined from the dendrogram with a cutoff of 80%.[19]

Results

One hundred and seventy-eight samples (123 NPW from 78 different horses and 55 GPL from 38 different horses) from 108 different horses were tested for MRS. The median age of subjects was 6 years (range 0.2–27, IQR 3–10 years). Breed information was available for 105 horses and was distributed as follows: 32/105 (30%) Standardbreds, 19/105 (18%) Thoroughbreds, 17/105 (16%) Warmbloods, 14/105 (13%) pony breeds, 11/105 (10%) Quarter Horses, and 12/105 (12%) other breeds. For animals where sex was recorded, the majority 52/103 (50%) were female, 13/103 (13%) were male, and 38/103 (37%) were geldings. Horses were sampled from 34 farms; 78 (73%) of the horses came from 5 farms (32, 26, 9, 7, and 4 horses, respectively), and 29/107 (27%) were from individual farms. One hundred and seventy-seven samples had the horse's strangles clinical status identified on the laboratory submission form: 24 different horses were classified as clinical, 38 different horses were classified as convalescent, and 58 different horses were classified as asymptomatic at the time of sampling. Of the 108 horses from which samples were obtained, 62 (57%) had a single sample tested, 27 (25%) had two samples tested, and 15 (14%) had 3 samples tested. Three horses had 4 samples tested, and 1 horse had 6 samples tested. Five horses had more than one sample type (NPW and GPL).

Seven presumptive MRS were isolated from equine nasopharyngeal washes from 108 different horses (4%). None were isolated from guttural pouch washed. All 7 were PBP2a positive and were identified as MRSA (4/108, 3.7%) and MR-*Staphylococcus pseudintermedius* (MRSP) (4/108, 3.7%). MRSA was found in nasopharyngeal samples from 3 different horses asymptomatic for strangles: twice from different samples 7 days apart from a 4-month-old Standardbred filly, once from her 10-year-old Standardbred dam, and once from a 2-year-old Standardbred filly; all resided on the same breeding farm. Of the 32 horses sampled on this farm, these three resulted in an on-farm MRSA prevalence of 9%. Results of the PGFE comparison are found in Figure 1; all four samples were strain USA 500. Thirty-four samples were positive for *S. equi* (31 positive on direct PCR and 13 positive on bacterial culture), none concurrently with MRS.

Discussion

This study demonstrated a low community prevalence (2.7%) of MRSA in equine nasopharyngeal and guttural pouch wash samples. When data from one farm are excluded, the prevalence of MRSA (in 76 horses) was 0. This is similar to previous reports of nasal carriage rates in low risk community equine populations. For example, MRSA was not isolated from the nares of 600 healthy Japanese Thoroughbred racehorses,[4] from 300 community horses in Canada,[20] or from 206 community horses in Israel.[9] The cluster of positive horses from one farm in the present study (9% of 32 horses) is similar to the 12% prevalence found in a study that targeted high-risk populations in Canada and New York state. Horses were at higher risk for MRSA if they resided on farms larger than 20 horses.[5] In the present study, the MRSA-positive farm housed over 100 horses.

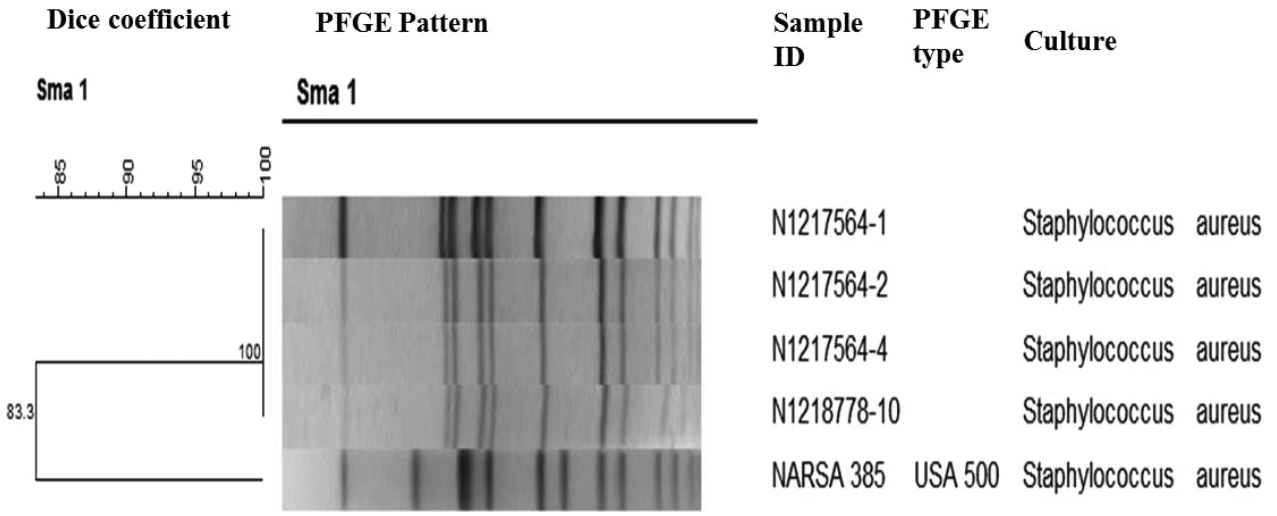

Fig 1. Genetic characteristics of the 4 MRSA isolates from community-based equine nasopharyngeal washes compared to a standard known USA 500 clone. Clonal relatedness was determined from the dendrogram with a cutoff of 80%.[19] PGFE: pulsed-field gel electrophoresis.

MRSA was found twice in the nasopharynx of the same foal, 7 days apart, demonstrating at least temporary colonization. As the antibiotic treatment status was not recorded on the laboratory submission, we do not know if this played a role in our low recovery of *Staphylococcus*.

In both humans and small animals, the nares are the most common site used to screen for the presence of MRSA. A combination of multiple locations is recommended, including the throat, axilla, groin, and perineum in humans[13] and the mouth, ears, axilla, and perineum in dogs[21] for optimal screening for MRSA. Multiple samplings have only been studied once in horses (combined perianal and nares sampling at the farm level)[22] and yielded low MRSA prevalence (1 positive nares sample of 373 samples from 189 horses), and this is not commonly implemented in practice. Foti et al.[23] sampled the conjunctiva in donkeys and identified MRSA in 4% (2/46). A study in 2013 found the nasal vestibulum was the preferred sampling location compared to the reflection of the nasal diverticulum (alar fold) and the ventral meatus.[24] We were unable to compare the ability to recover MRSA from the throat to that of the nares within the same horse or the same population of horses because no samples from nares were tested.

MRSA was not isolated from any guttural pouch samples. This might be a reflection of the very low prevalence in our population and smaller number of guttural pouch samples obtained, rather than the lack of MRSA in this anatomic location. *Staphylococcus* has been isolated from the guttural pouch but these isolates were not identified to species level.[25–27] Clinical infections of MRSA in the guttural pouch and other areas of the upper respiratory tract of the horses are rare.[25]

MRSP was also isolated from four nasopharynx samples from four different horses. There is one other previous report of MRSP found in the nares of a hospitalized horse in Turkey[28] and one in the nares of a healthy harness-racing horse in Italy.[29] MRSP was not found in the nares of 300 community-based horses in Canada.[20] MRSP was found at a prevalence of 0.1% in 4,710 German equine clinical specimens.[30] The clinical importance of this organism has not yet been determined.[31,32] Although in a recent study, MRSP was found to have the highest binding affinity for equine corneocytes *in vitro* when compared to methicillin-susceptible *Staphylococcal species* (MSSS) and MRSA.[33] No methicillin-resistant coagulase negative *Staphylococcus* was detected due to this methodology and was not the focus of this study.

The results of the PFGE showed that the 4 isolates were identical and belonged to the USA 500 clone (Fig 1). USA 500 was a common cause of both nosocomial and community-onset infections in people in the late 1990s and is now most frequently isolated from horses (both nosocomial and community-acquired infections) and people who work with horses.[7,8,34] Infections with USA 500 have been identified in horses and their caregivers.[3,7,8]

With samples that were submitted for the purpose of *S. equi* detection enabled us to obtain nasopharyngeal and guttural pouch samples and examine the throat as a potential site for MRSA screening with no unnecessary testing of the horses. It did limit this population to animals living on farms with known or suspected strangles outbreaks resulting in clustering. Only a subset of animals was considered completely healthy (asymptomatic), whereas others were convalescing from a *S. equi* infection or were clinical for active *S. equi* infection. Only a small portion of samples (34) were positive for *S. equi* on either culture or PCR. Two different culture methods to isolate MRSA were used to obtain the most sensitive and specific MRSA recovery rate possible. All 4 MRSA samples were positive on both methods. Although inhibition of staphylococcal species by

streptococcal species in human nasal passages[35] and human breast milk[36] occurs, there is no information available on the potential for *S. equi* and MRSA to compete for the same niche in the equine pharynx and guttural pouch. Group C streptococcal species do not produce detectable levels of H_2O_2 which is one mechanism of action for bactericidal activity against *S. aureus*.[35] Due to the nature of strangles outbreak testing, a large percentage of the horses sampled came from a small percentage of the farms in the study, and some samples were repeated from the same horses.

Sampling the nasopharynx and guttural pouch of community-based horses revealed a low prevalence rate of MRSA similar to other studies that sampled the nares of community-based horses. More work should be performed to study the potential for increased recovery when screening horses for MRSA by multiple anatomic sites as has been shown useful in human and small animal medicine in both community and hospital-based populations.

Footnotes

[a] Mannitol Salt Broth, Northeast Laboratory, Waterville, ME
[b] CHROMagar-MRSA, Remel, Overland Park, KS
[c] Mannitol salt agar, Remel, Overland Park, KS
[d] Sensititre, Trek Diagnostics, Cleveland, OH
[e] Microbank® cryopreservation tubes, Prolab Diagnostics, Richmond, ON, CA

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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