# *Staphylococcus delphini* and Methicillin-Resistant *Staphylococcus pseudintermedius* in Horses at a Veterinary Teaching Hospital

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## Introduction

*Staphylococcus aureus* is a well known pathogen in horses, yet the role of other coagulase-positive staphylococcal species is unclear. Non-*aureus* staphylococcal species, including *Staphylococcus pseudintermedius* and *Staphylococcus delphini,* are important pathogens in some species, can be multidrug resistant and could be a concern in horses.

## Materials and Methods

- Methicillin-resistant or unusual staphylococci isolated at the Ontario Veterinary College (OVC) by the Animal Health Laboratory (AHL) routinely undergo further characterization. During 2011, six staphylococci isolates from horses that were not methicillin-resistant *S. aureus* (MRSA) were tested.
- Isolates were identified through Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF), *S. pseudintermedius* or *S. delphini* PCR, and *sod*A sequence analysis.
- Isolates were further characterized, as indicated, by *dru* typing, PFGE, *mecA* PCR, *mecA* homologue ($mecA_{LGA251}$) PCR, penicillin-binding protein 2a (PBP2a) latex agglutination test (LAT), and broth microdilution and/or disc diffusion.
- A search of AHL's database was performed to determine the frequency of *S. pseudintermedius* and *S. delphini* isolation and identification for all equine submissions (OVC and private practice) from January 2011-August 2012.

## Results

Six isolates were evaluated; 2 were identified as methicillin-resistant *S. pseudintermedius* (MRSP) and 4 as *S. delphini* (Table 1).

Methicillin-resistant *S. pseudintermedius*

- MRSP-1 was classified as dt11a, a predominant MRSP clone in dogs; MRSP-2 could not be *dru* typed.
- A high level of resistance was noted, including to beta-lactams, chloramphenicol, clindamycin, erythromycin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole (TMS); Table 2.
- The *mecA* PCR, $mecA_{LGA251}$ PCR, and PBP2a LAT for MRSP-2 were negative.

*Staphylococcus delphini*

- *S. delphini* isolates were classified as group A [n=1; typical hosts: Mustelidae (i.e. mink, ferret, badger)] and group B (n=3); Table 1. All were initially identified biochemically as *S. pseudintermedius.*
- Three of the isolates were incidental findings following *Streptococcus equi* surveillance testing.
- A low level of resistance was identified, with one isolate (SD-4) resistant to erythromycin (Table 2).

**Table 1: Isolates Identified as MRSP and *S. delphini***

| Isolate | Species Identification | Signalment | History | Sample Location | Comments | Identification Methods |
|---|---|---|---|---|---|---|
| MRSP-1 | MRSP, dt11a | 1-yr filly | Sinusitis | Frontal sinus (surgery) | Mixed infection | A |
| MRSP-2 | MRSP* | 16-yr mare | Urolith | Urine | | A, $mecA_{LGA251}$ PCR , disc diffusion |
| SD-1 | *S. delphini* (group B) | 8-yr mare | Chronic otitis externa | Ear canal (swab) | Mixed infection | B |
| SD-2 | | 5-yr mare | *Streptococcus equi* surveillance | Nasopharyngeal wash | | B |
| SD-3 | *S. delphini* (group A) | 5-yr mare | *Streptococcus equi* surveillance | Nasopharyngeal wash | | B |
| SD-4 | *S. delphini* (group B) | 4-yr mare | *Streptococcus equi* surveillance | Nasopharyngeal wash | | B |

A: MALDI-TOF, MRSP PCR, *dru* typing, *mecA* PCR, PBP2a LAT, broth microdilution
B: MALDI-TOF, *S. delphini* PCR, *sodA* sequence analysis, broth microdilution
*Unable to *dru* type

**Table 2: Resistance Patterns for MRSP and *S. delphini* Isolates[1]**

| Isolate | Ampicillin | Chloramphenicol | Ciprofloxacin | Clindamycin | Erythromycin | Gentamicin | Levofloxacin | Linezolid | Moxifloxacin | Nitrofurantoin | Oxacillin | Penicillin | Rifampin | Tetracycline | TMS | Vancomycin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MRSP-1 | R | R | R | R | R | R | R | S | R | S | R | R | S | R | R | S |
| MRSP-2 | R[2] | R | R | R | R | R | R | S | R | S | R | R | S | R | R | S |
| SD-1 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| SD-2 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| SD-3 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| SD-4 | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S |

[1] Result obtained with broth microdilution method, unless noted
[2] Result obtained with disc diffusion method

- Two of the *S. delphini* isolates (SD-1, SD-2) were possibly related by PFGE, with a 4 band difference (Figure). The remaining isolates were unrelated to each other and the 2 related isolates.

AHL Database search

- Eight additional *S. pseudintermedius* cases were identified; 6 (75%) of which were MRSP. Five were from infections, one horse was colonized and inadequate data were available to classify the other two. One additional *S. delphini* isolate was identified.

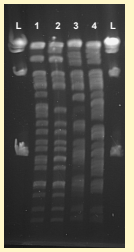


**Figure: PFGE gel image.**
Lane (isolate): 1 (SD-1), 2 (SD-2), 3 (SD-3), 4 (SD-4); L=ladder

## Discussion

- MRSP is an important emerging pathogen in dogs and cats, yet has been rarely identified in horses. Although not available for these cases, obtaining a history of domestic animal contact would be useful, as between-species transmission may have occurred.
- The roles of these bacteria in disease in horses is unclear but given their ability to cause opportunistic infections in other species and limited reports of *S. pseudintermedius* infections in horses, they should not be dismissed.
- We were unable to document the presence of *mec* for one of the MRSP isolates. It is possible *mec* was lost during manipulation or storage of the isolate; alternatively, a novel *mec* element may be involved.
- While rarely reported, MRSP might be overlooked in horses. Misidentification as *S. aureus* is possible if laboratories assume coagulase positive staphylococci from horses are *S. aureus*. Since typical phenotypic methods to detect methicillin-resistance in *S. aureus* are ineffective for *S. pseudintermedius*, MRSP can be misidentified as methicillin-susceptible *S. pseudintermedius* or *S. aureus*. Given the rapid expansion of this pathogen in dogs and its highly resistant nature, ongoing surveillance is indicated.
- *S. delphini* has rarely been identified in horses, but it may be misidentified with conventional methods. Although in these cases colonization/contamination appeared most likely, these findings suggest this opportunist can be found in horses and might be pathogenic in certain situations.
- The use of additional identification methods (e.g., MALDI-TOF, *Staphylococcus* species-specific PCR) is important to differentiate *S. delphini* and *S. pseudintermedius* and will be critical in ongoing surveillance for the emergence of these species in horses.