

Prevalence of methicillin-resistant *Staphylococcus* spp. in the conjunctival sac of healthy dogs

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Abstract

Objectives To determine the prevalence of selected coagulase-positive methicillin-resistant *Staphylococcus aureus* (MRS) in the conjunctival sac in a group of healthy dogs and to compare the prevalence of ocular MRS colonization with colonization of typically assessed body sites including the nasal cavity and rectum.

Animals studied 123 healthy dogs were used in the prevalence study: 40 dogs from a shelter and 83 privately owned dogs.

Procedures The sampling procedure included culturing three separate sites per subject in the following order: the lower conjunctival fornices, the nares, and rectum.

Results A low prevalence of 1.6% (2/123) of MRS was detected in healthy dogs.

Methicillin-resistant *Staphylococcus pseudintermedius* was isolated from two dogs, one from a conjunctival swab and the other from a rectal swab.

Conclusion The survey data indicate the ocular surface is a potential site of MRS colonization, although the prevalence was low in healthy dogs.

Key Words: conjunctiva, dog, eye, methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus pseudintermedius*, real-time PCR

INTRODUCTION

Staphylococci are common commensals that can be found at various body sites in healthy individuals, but some *Staphylococcus* spp, most notably coagulase-positive species such as *S. aureus* and *S. pseudintermedius*, are important opportunistic pathogens. An area of concern with staphylococci is their ability to acquire antimicrobial resistance, particularly methicillin resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen in human medicine.¹ Its prevalence and clinical significance in veterinary medicine has also been investigated in a variety of companion and food animals.^{2–7} While MRSA can cause infections, it is an opportunist and is more often found colonizing body sites of clinically healthy animals particularly the nares and intestinal tract.⁸ Methicillin resistance develops from the production of an altered penicillin-binding protein known as PBP2a. Detection of MRS by polymerase chain reaction (PCR) for the *mecA* gene coding for methicillin resistance is a well-established method.⁹

The close proximity of humans and animals creates the potential for the exposure of animals to MRSA. There has been extensive reporting of MRSA infection and

colonization with human epidemic MRSA clones in domestic animals since the late 1990s.^{10,11} In dogs and cats, however, the most prevalent and potentially more important pathogenic *Staphylococcus* species is *S. pseudintermedius*.¹²

Methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged and is a significant health concern in dogs, particularly with pyoderma, wound infections, and surgical site infections.¹³ Other species, including *S. schleiferi*, have been shown to cause infections of the skin and ear canals and may be methicillin resistant.¹⁴ As with MRSA, MRSP has more often been found colonizing healthy animals than sick animals, and these animals are presumably an important reservoir of MRSP transmission in the canine (and potentially human) population.

The conjunctiva harbors a complex microbiota that includes staphylococci. As with other staphylococcal colonization sites, methicillin-resistant staphylococci could be present and ocular colonization could be a risk factor for subsequent ocular infection or transmission to other individuals. A recent case report was the first to describe MRSA keratitis in a dog.¹⁵ Two cases of canine bilateral conjunctival MRS infections were documented at the Purdue University Veterinary Teaching Hospital (PUVTH)

and prompted the undertaking of a pilot study to determine the prevalence of ocular surface MRS in a group of healthy dogs. The specific objectives of this pilot study were 1) to determine the prevalence of selected coagulase-positive MRS on the conjunctiva in a group of healthy dogs and 2) to compare the prevalence of conjunctival MRS colonization with colonization of typically assessed body sites including the nasal cavity and rectum.

MATERIALS AND METHODS

Study animals

A total of 123 dogs were used in this study: 40 dogs from a shelter and 83 privately owned dogs, each from a different household. All dogs were considered healthy based on a history of no current major disease or illness. Exclusion criteria included serious systemic disease (such as but not limited to pneumonia, immune-mediated disease, renal failure, neoplastic disease, sepsis, diabetes mellitus and hyperadrenocorticism). Animals were additionally excluded if, at the time of sample collection, they had a known bacterial infection, were hospitalized in the PUVTH intensive care unit, or had dermatologic disease. Individuals with a history of ocular abnormalities or a history of having received ophthalmic medication, oral antimicrobials, or oral immunosuppressive medication in the previous 60 days were excluded from the study. Shelter dogs were excluded if receiving antimicrobials at the time of sample collection; however, history prior to entering the shelter was unknown. The protocol was approved by the Purdue University Animal Care and Use Committee and conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Owners provided consent before their animals were included in the study.

Sampling procedure

Three sites per subject were sampled in the following order: the lower conjunctival fornices, the nares, and rectum. A single rayon tipped swab was used for both right and left conjunctival fornices, and a separate swab was used for both nares. A third swab was used for the rectum. All samples were collected by one investigator (MM) wearing latex gloves, which were changed between patients. After collection, swabs were stored at 4 °C until processing. The maximum length of time samples were stored prior to processing was 2 weeks.

Laboratory methods

The protocol to identify selected resistant *Staphylococcus* spp was as follows: swabs were inoculated into 2 mL of enrichment broth containing 10 g/L tryptone T, 75 g/L sodium chloride, 10 g/L mannitol, and 2.5 g/L yeast extract, and incubated for 24 h at 35 °C. Aliquots of 100 µL were then inoculated onto both MRSA Chromogenic agar (BBL CHROMagar, Becton, Dickinson and

Co, Sparks, MD, USA) and mannitol salt agar with 2 g/mL oxacillin (MSA-OX) and incubated at 35 °C for 48 h. Suspect staphylococcal isolates were subcultured onto Columbia blood agar and incubated at 35 °C for 24 h. Isolates were identified as staphylococci based on colony morphology, Gram stain appearance, and a positive catalase reaction. A tube coagulase test (BBL™ Coagulase Plasma with EDTA; Becton, Dickinson and Co) was performed on all resistant staphylococci. Methicillin resistance was confirmed by demonstration of PBP2a antigen with a latex agglutination test (Oxoid Ltd., Basingstoke, Hampshire, UK). *Staphylococcus aureus* was identified by a latex agglutination test (Pastorex™Staph- Plus; Bio-Rad Laboratories, Redmond, WA, USA). Speciation of the remaining coagulase-positive-resistant isolates was performed using a multiplex-PCR assay.¹⁶ Isolates were typed by sequence analysis of the *mec*-associated direct repeat unit region (*dru* typing).¹⁷

Statistics

Summary statistics were reported as mean ± SD.

RESULTS

Study animals

One hundred and twenty-three dogs (50 neutered males, 49 spayed females, and 12 of each intact males and females) were included in the study. Breeds of dogs included 49 mixed breed and 74 pure-bred. A variety of body sizes and skull conformations were represented. The mean age of dogs was 47.5 ± 36.8 months.

Culture results

Methicillin-resistant *S. pseudintermedius* was isolated from 1.6% (2/123) dogs. Single swabs were positive for each dog, with MRSP isolated from the conjunctival sac of one dog and the rectum of another. Both dogs were privately owned. One isolate was classified as dt10 h strain type, while the other was dt9a strain type. No other methicillin-resistant coagulase-positive staphylococci were isolated.

DISCUSSION

The results of the prevalence study indicated that among healthy dogs in this study, MRS carriage was low. Two samples were MRSP positive, and no samples were MRSA positive. Of the two positive samples, only one was positive from the conjunctival sac. In a study by Guptill *et al.*¹⁸ at the PUVTH evaluating colonization of MRS in pet dogs, the prevalence was 16.6%. Both that study and the present study evaluated nasal and rectal swabs, and identical laboratory protocols and storage time guidelines were used. However, dogs in the study by Guptill *et al.*¹⁸ had a variety of medical conditions and were not excluded for receiving antibiotic or immunosuppressive drug therapy, which may have accounted for the lower prevalence

of MRS in the present study. In the present study, one dog was positive for MRSP from the rectum; however, the conjunctival and nasal culture results for this dog were negative. This dog had a history of visiting the PUVTH weekly and reportedly had uncharacterized staphylococcal infections that had been treated with antimicrobials in the past. No further information could be obtained on this dog. One other dog was MRSP positive from the conjunctiva but negative from the nares and rectum. Both dogs were clinically normal and, at last report, several months after sampling, had not developed a MRSP infection.

In this study, two different direct repeat units (*dru*) types were identified. In MRS spp, the *dru* region is useful in epidemiologic analysis of highly uniform epidemic strains. The two strains, dt10 h and dt9a, identified in this study are common types in North American dogs and are associated with sequence type 68.^{19,20} These clonal groups harbor resistance mechanisms for various antibiotics.²¹

Methicillin-resistant *S. pseudintermedius* is an important pathogen in the dog and is often resistant to multiple classes of antibiotics.²² Although MRSA is of concern because of the potential for transmission between humans and companion animals, MRSP is the more important pathogen for dogs' health due to the resistance to multiple antimicrobials, thus leaving fewer treatment options available.²³ Studies have reported MRSP colonization rates of 4.5–5% in healthy dogs, however, those studies were not able to determine risk factors.^{11,24} In a recent study,¹³ a significant association was found between prior administration of antimicrobials within 30 days and MRSP infection in dogs. Both of the MRSP-positive dogs of the present study had previously received antimicrobials; however, these were not given within 60 days of the sample collection. The duration of impact of antimicrobial therapy on MRSP colonization is not known.

In this study, all privately owned dogs were sampled first. Due to the low prevalence of MRS in these dogs, it was decided to sample dogs from a local shelter to see if animals from a different housing environment would have a higher prevalence of MRS. Although the same criteria restricting the use of oral and topical antibiotics or immunosuppressive drugs for 60 days prior to sampling could not be applied, we found no samples positive for MRS from the shelter dogs.

In order to batch samples, swabs were collected and stored for up to 2 weeks at 4 °C. This is unlikely to have affected the recovery rate in this study based on experiments from the laboratory in which the testing was performed (JS Weese, personal communication, 2013). In those unpublished studies, 2 weeks of storing swabs at 4 °C did not affect recovery of *Staphylococcus* spp. Likewise, in the study by Guptill *et al.*,¹⁸ swabs were stored up to 2 weeks before processing, and the recovery rate of *Staphylococcus* spp was much higher than in our study.

CONCLUSION

This study documents the ocular surface as a potential site of MRS colonization, although among healthy dogs in our study, the prevalence was very low.

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REFERENCES

1. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Reviews Microbiology* 2009; **7**: 629–641.
2. Abraham JL, Morris DO, Griffeth GC *et al.* Surveillance of healthy cats and cats with inflammatory skin disease for colonization of the skin by methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* ssp. *schleiferi*. *Veterinary Dermatology* 2007; **18**: 252–259.
3. Anderson ME, Weese JS. Evaluation of a real-time polymerase chain reaction assay for rapid identification of methicillin-resistant *Staphylococcus aureus* directly from nasal swabs in horses. *Veterinary Microbiology* 2007; **122**: 185–189.
4. Boost MV, O'Donoghue MM, James A. Prevalence of *Staphylococcus aureus* carriage among dogs and their owners. *Epidemiology and Infection* 2008; **136**: 953–964.
5. Cui S, Li J, Hu C *et al.* Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China. *Journal of Antimicrobial Chemotherapy* 2009; **64**: 680–683.
6. Devriese LA, Van Damme LR, Fameree L. Methicillin (cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. *Zentralblatt für Veterinärmedizin B. Journal of Veterinary Medicine. Series B* 1972; **19**: 598–605.
7. Lee JH. Occurrence of methicillin-resistant *Staphylococcus aureus* strains from cattle and chicken, and analyses of their *mecA*, *mecR1* and *mecI* genes. *Veterinary Microbiology* 2006; **114**: 155–159.
8. Weese JS. Methicillin-resistant *Staphylococcus aureus* in animals. *Institute for Laboratory Animal Research Journal* 2010; **51**: 233–244.
9. Strommenger B, Kettlitz C, Werner G *et al.* Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *Journal of Clinical Microbiology* 2003; **41**: 4089–4094.
10. Seguin JC, Walker RD, Caron JP *et al.* Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *Journal of Clinical Microbiology* 1999; **37**: 1459–1463.
11. Hanselman BA, Kruth SA, Rousseau J *et al.* Coagulase positive staphylococcal colonization of humans and their household pets. *Canadian Veterinary Journal* 2009; **50**: 954–958.
12. Bond R, Loeffler A. What's happened to *Staphylococcus intermedius*? Taxonomic revision and emergence of multi-drug resistance. *Journal of Small Animal Practice* 2012; **53**: 147–154.
13. Weese JS, Faires MC, Frank LA *et al.* Factors associated with methicillin-resistant versus methicillin-susceptible *Staphylococcus pseudintermedius* infection in dogs. *Journal of the American Veterinary Medical Association* 2012; **240**: 1450–1455.

14. Cain CL, Morris DO, Rankin SC. Clinical characterization of *Staphylococcus schleiferi* infections and identification of risk factors for acquisition of oxacillin-resistant strains in dogs: 225 cases (2003-2009). *Journal of the American Veterinary Medical Association* 2011; **239**: 1566-1573.
15. Tajima K, Sinjo A, Ito T *et al.* Methicillin-resistant *Staphylococcus aureus* keratitis in a dog. *Veterinary Ophthalmology* 2013; **16**: 240-243.
16. Sasaki T, Tsubakishita S, Tanaka Y *et al.* Multiplex-PCR method for species identification of coagulase-positive staphylococci. *Journal of Clinical Microbiology* 2010; **48**: 765-769.
17. Goering RV, Morrison D, Al-Doori Z *et al.* Usefulness of mec-associated direct repeat unit (dru) typing in the epidemiological analysis of highly clonal methicillin-resistant *Staphylococcus aureus* in Scotland. *Clinical Microbiology and Infection* 2008; **14**: 964-969.
18. Guptill LME, Dunham A, Weese JS. Methicillin-resistant coagulase positive *Staphylococcus* species colonization of pet dogs and cats [abstract]. 2nd ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary Public Health Implications, 2011.
19. Weese JSJR, Kadlec K, Guptil L *et al.* Direct repeat unit (dru) typing of methicillin-resistant *Staphylococcus pseudintermedius* from North America and Europe. International Society for Companion Animal Infectious Diseases Conference, San Francisco, CA, 2012.
20. Kadlec NM, Weese JS, Schwarz S. Distribution of dru types among methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs and cats. International Symposium on Staphylococci and Staphylococcal Infections, 2010.
21. Weese JS, Sweetman K, Edson H *et al.* Evaluation of minocycline susceptibility of methicillin-resistant *Staphylococcus pseudintermedius*. *Veterinary Microbiology* 2013; **162**: 968-971.
22. Perreten V, Kadlec K, Schwarz S *et al.* Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *Journal of Antimicrobial Chemotherapy* 2010; **65**: 1145-1154.
23. Beck KM, Waisglass SE, Dick HL *et al.* Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from skin and carriage sites of dogs after treatment of their methicillin-resistant or methicillin-sensitive staphylococcal pyoderma. *Veterinary Dermatology* 2012; **23**(369-375): e366-e367.
24. Morris DO, Boston RC, O'Shea K *et al.* The prevalence of carriage of methicillin-resistant staphylococci by veterinary dermatology practice staff and their respective pets. *Veterinary Dermatology* 2010; **21**: 400-407.