

# Isolation of *Staphylococcus schleiferi* from healthy dogs and dogs with otitis, pyoderma, or both

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**Objective**—To determine the frequency of isolation and susceptibility patterns of *Staphylococcus schleiferi* from healthy dogs and dogs with otitis, pyoderma, or both that had or had not received antimicrobial treatment.

**Design**—Prospective study.

**Animals**—50 dogs.

**Procedure**—Dogs were allocated to 1 of 4 groups: healthy dogs (n = 13), dogs without otitis but with pyoderma (10), dogs with otitis but without pyoderma (11), and dogs with otitis and pyoderma (16). Bacteriologic culture of ear swab specimens was performed in all dogs. Bacteriologic culture of skin swab specimens was also performed in dogs with concurrent pyoderma. Isolates were identified as *S schleiferi* subsp *schleiferi* or *S schleiferi* subsp *coagulans* on the basis of growth and biochemical characteristics.

**Results**—*S schleiferi* was not isolated from any dogs with pyoderma only. *Staphylococcus schleiferi* subsp *schleiferi* was isolated from the ears of 2 healthy dogs, and the skin and ears of 2 dogs and the skin of 1 dog with otitis and pyoderma. *Staphylococcus schleiferi* subsp *coagulans* was isolated from the ears of 3 dogs with otitis only, and the ears of 6 dogs and the skin of 2 dogs with otitis and pyoderma. One of the *S schleiferi* subsp *schleiferi* isolates from ears, 2 of the *S schleiferi* subsp *coagulans* isolates from ears, and 1 of the *S schleiferi* subsp *coagulans* isolates from the skin were resistant to methicillin. One methicillin-resistant isolate from the ears and 1 from the skin were also resistant to fluoroquinolones.

**Conclusions and Clinical Relevance**—*S schleiferi* subsp *schleiferi* was detected in healthy dogs and dogs with otitis and pyoderma. Methicillin-resistant and -susceptible *S schleiferi* subsp *schleiferi* and *S schleiferi* subsp *coagulans* were detected as the predominant organisms in dogs with otitis. (*J Am Vet Med Assoc* 2005;227:928–931)

In the early 1990s, *Staphylococcus schleiferi*, an organism with variable coagulase activity, was recognized as a

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human as well as a veterinary pathogen. Two subspecies were initially identified: a coagulase-negative subspecies, *S schleiferi* subsp *schleiferi*, was isolated from humans in 1988<sup>1</sup> and a coagulase-positive subspecies, *S schleiferi* subsp *coagulans*, was isolated from the external auditory meatus of dogs with otitis externa in 1990.<sup>2</sup> In humans, both subspecies have been associated with wound infections,<sup>3,4</sup> endocarditis,<sup>5,6</sup> osteomyelitis,<sup>5,7</sup> bacteremia,<sup>5</sup> urinary tract infections,<sup>8</sup> and meningitis.<sup>9</sup> In dogs, *S schleiferi* subsp *coagulans* has been associated with pyoderma<sup>10-12</sup> and otitis externa.<sup>2</sup> Holm et al<sup>12</sup> reported that *S schleiferi* subsp *coagulans* was isolated more frequently from dogs with recurrent pyoderma but was also associated with the first episodes of pyoderma; however, the importance of this finding and antimicrobial susceptibility patterns of this subspecies were not addressed. In a recent study,<sup>11</sup> *S schleiferi* subsp *schleiferi* and *S schleiferi* subsp *coagulans* were isolated from dogs with pyoderma. That study<sup>11</sup> was the first to associate *S schleiferi* subsp *schleiferi* with pyoderma in dogs and identify methicillin-resistant variants; 11 of 15 *S schleiferi* isolates were methicillin-resistant.

*Staphylococcus schleiferi* subsp *coagulans* was first identified in 1988; however, this species has only recently gained attention in human and veterinary medicine. Before 1988, the most recent reclassification of *Staphylococcus* species isolated from domestic animals occurred in 1976, when *Staphylococcus intermedius* was differentiated from *Staphylococcus aureus*.<sup>13</sup> As a result, *S schleiferi* may be underreported by automated staphylococcal identification systems because the coagulase-positive subspecies, *S schleiferi* subsp *coagulans*, is phenotypically similar to *S aureus*<sup>4,5</sup> and therefore similar to *S intermedius*. Moreover, in human medicine, coagulase-negative staphylococci (CoNS) have historically been considered nonpathogenic<sup>14</sup> and all staphylococcal organisms other than *S aureus* were reported simply as CoNS.<sup>15</sup> On the basis of this information, we expect that the coagulase-negative subspecies, *S schleiferi* subsp *schleiferi*, has also been underreported.

With the exception of pyoderma, otitis is one of the most common dermatologic conditions treated in dogs. Organisms frequently isolated from otitis in dogs include *S intermedius* and *Pseudomonas aeruginosa*.<sup>16,17</sup> *Staphylococcus schleiferi* subsp *coagulans* has been associated with otitis externa in dogs but is not frequently reported.<sup>2</sup> Difficulty associated with differentiating *S intermedius* from *S schleiferi* could contribute to the underdiagnosis of *S schleiferi* in ears and may be a problem in animals as it is in humans because *S schleiferi* is phylogenetically and phenotypically similar to *S intermedius*.<sup>2</sup>

Frank et al<sup>11</sup> reported that *S schleiferi* isolated from skin is frequently resistant to methicillin and less often to

the fluoroquinolones, antimicrobial classes that are commonly used for the treatment of otitis and pyoderma in dogs. Many animals with otitis have pyoderma concurrently.<sup>18</sup> Because of frequent exposure to various antimicrobials used to treat pyoderma, *S schleiferi* isolates from ears of dogs have the potential to develop resistance. In 1 study,<sup>2</sup> only *S schleiferi* subsp *coagulans* was identified and susceptibility patterns were not examined. Frank et al<sup>11</sup> reported that resistant *S schleiferi* isolates were identified only from cases of recurrent pyoderma in dogs and were obtained most frequently when antimicrobials were being administered PO. In addition, when these same strains were subjected to further testing, the mechanism of resistance was identified as the **penicillin-binding protein 2a (PBP2a)**, which is encoded by the *mecA* gene.<sup>19</sup> The presence of a functional PBP2a renders the organism resistant to methicillin and, consequently, the entire  $\beta$ -lactam class of antimicrobials. Additionally, many methicillin-susceptible *S intermedius* isolates identified by Kania et al<sup>19</sup> also produced PBP2a, which may not have been functional.<sup>19</sup> However, with exposure of these isolates to antimicrobials and selection pressure, upregulation and expression of the protein and, consequently, methicillin resistance may occur. Therefore, it is important to determine how often *S schleiferi* is isolated from ears of dogs and whether patterns of resistance similar to those detected in *S schleiferi* isolated from dogs with pyoderma exist. The purpose of the study reported here was to determine the frequency of isolation and susceptibility patterns of *S schleiferi* from healthy dogs and dogs with otitis, pyoderma, or both that had or had not received antimicrobial treatment.

## Materials and Methods

**Dogs**—Fifty client-owned dogs > 1 year old were enrolled in the study with the informed consent of the owners. The protocol was reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee. Dogs were allocated to 1 of 4 groups: healthy dogs (n = 13), dogs without otitis but with pyoderma (10), dogs with otitis but without pyoderma (11), and dogs with otitis and pyoderma (16). Dogs were chosen for the study on the basis of otoscopic and cytologic examination findings. Cytologic examination of dry swab specimens from the junction of the horizontal and vertical canals of both ears for yeast and bacteria and otoscopic examinations were performed for all dogs. To be included in one of the groups without otitis, results of otoscopic examination and cytologic examination of ear swab specimens must have been consistent with that of clinically normal ears. In all dogs, a dry swab specimen<sup>a</sup> from at least 1 ear was submitted for bacteriologic culture. For dogs in which results of cytologic examination varied between ears (eg, different proportions of cocci and rods), dry swab specimens<sup>a</sup> from both ears were submitted for bacteriologic culture. In dogs with concurrent pyoderma, a dry swab specimen<sup>a</sup> from a closed papule or pustule was obtained from the skin and submitted for bacteriologic culture to determine whether the ear and skin pathogens were similar. Any previous antimicrobial treatments administered topically or PO were recorded, regardless of the condition requiring treatment. Infections were defined as recurrent if the dog had lesions consistent with active disease, such as papules, pustules, crusts, otic discharge, signs of otic pain, or otic inflammation, and had been treated previously for otitis or pyoderma.

**Identification of *S schleiferi***—Standard methods for bacteriologic culture were used; all staphylococcal species isolated, including *S schleiferi* subsp *coagulans* and *S schleiferi* subsp *schleiferi*, were recorded. Organisms obtained via bac-

teriologic culture were initially identified as staphylococci on the basis of growth and colony characteristics on primary plating medium. Colonies considered typical of *S schleiferi* were opaque, off-white,  $\geq 1$  mm in diameter after 24 hours of incubation, and usually surrounded by a double zone of hemolysis on blood agar medium. All visually distinct colony types were selected for identification. Colonies were identified biochemically as *S schleiferi* subsp *coagulans* on the basis of positive coagulase and Voges-Proskauer test results and negative maltose, trehalose, and lactose fermentation test results. Isolates with results matching those expected for *S schleiferi* subsp *coagulans*, except for negative coagulase test results, were identified as *S schleiferi* subsp *schleiferi* by use of a commercial biochemical identification system.<sup>b</sup>

**Methicillin susceptibility determination**—Oxacillin was used as the isoxazolyl penicillin class representative for indirectly judging methicillin susceptibility. Oxacillin susceptibility testing was performed according to NCCLS guidelines by use of the disk diffusion method or an automated broth microdilution susceptibility testing system.<sup>c</sup> The interpretive breakpoints used were those established for human isolates of coagulase-negative *Staphylococcus* spp ( $\leq 0.25$   $\mu\text{g/mL}$  for the broth microdilution method<sup>20</sup> and  $\geq 18$  mm for the disk diffusion method<sup>21</sup>). These breakpoints were used instead of the breakpoints listed for all *Staphylococcus* spp isolated from animals ( $\leq 2$   $\mu\text{g/mL}$  for the broth microdilution method and  $\geq 13$  mm for the disk diffusion method<sup>22</sup>) because we believed that the use of these recommended standards for animal isolates might erroneously result in resistant isolates being classified as susceptible.

## Results

Organisms other than *Staphylococcus* spp were isolated from 4 of the 13 healthy dogs; a *Staphylococcus* similar to *S intermedius* that was not *S aureus* or *S schleiferi* and not further identified was isolated from 1 dog, *S intermedius* was isolated from 1 dog, and CoNS was isolated from 6 dogs. One of the CoNS isolates was resistant to methicillin, and *S schleiferi* subsp *schleiferi* was isolated from 2 of those 6 dogs in addition to the CoNS (Table 1). All isolates were susceptible to fluoroquinolones. Antimicrobials had not been administered topically or PO in any of those dogs during the 12 months prior to study initiation. An additional dog was originally included in this group; however, after further questioning, the owner indicated that multiple antimicrobials had been administered PO for recurrent urinary tract disease. A methicillin-resistant *S schleiferi* subsp *schleiferi* and a methicillin-resistant CoNS were isolated from the ears of this dog. Both isolates were sensitive to fluoroquinolones. This dog was not included in the final data analysis.

No growth was detected on bacteriologic culture of ear swab specimens in 6 of the 10 dogs without otitis but with pyoderma; *S intermedius* was identified on bacteriologic culture of skin swab specimens. *Staphylococcus intermedius* was identified on bacteriologic culture of both ear and skin swab specimens from the remaining 4 dogs. All isolates from this group were sensitive to fluoroquinolones and methicillin.

No growth was detected on bacteriologic culture of ear swab specimens in 1 of 11 dogs with otitis but without pyoderma. *Staphylococcus intermedius* was isolated from 7 of the remaining 10 dogs, *S schleiferi* subsp *coagulans* was isolated from 2 dogs, and methicillin-resistant *S schleiferi* subsp *coagulans* was isolated from 1 dog. Eight of 11 dogs in this group had recurrent otitis. Of those 8

Table 1—*Staphylococcus* spp isolated from bacteriologic culture of ear swab specimens from healthy dogs (n = 13), dogs without otitis but with pyoderma (10), dogs with otitis but without pyoderma (11), and dogs with otitis and pyoderma (16).

Group	<i>Staphylococcus</i> spp	No. of dogs with first episode of infection	No. of dogs with recurrent infection
Dogs without otitis or pyoderma	<i>S intermedius</i> (n = 1 dog)	NA	NA
	CoNS (5)	NA	NA
	CoNS (MR; 1)	NA	NA
	<i>S schleiferi</i> subsp <i>schleiferi</i> (2)	NA	NA
	<i>S schleiferi</i> subsp <i>schleiferi</i> (MR; 0)	NA	NA
	Other (5)	NA	NA
Dogs with pyoderma only	<i>S intermedius</i>	1	3
	No growth	6	0
Dogs with otitis only	<i>S intermedius</i>	2	5
	<i>S schleiferi</i> subsp <i>coagulans</i>	1	1
	<i>S schleiferi</i> subsp <i>coagulans</i> (MR)	0	1
	No growth	0	1
Dogs with otitis and pyoderma	<i>S intermedius</i>	3	2
	CoNS	0	1
	<i>S schleiferi</i> subsp <i>schleiferi</i>	0	2
	<i>S schleiferi</i> subsp <i>schleiferi</i> (MR)	0	1
	<i>S schleiferi</i> subsp <i>coagulans</i>	0	5
	<i>S schleiferi</i> subsp <i>coagulans</i> (MR)	0	1
	Other	0	1
No growth	1	0	

More than 1 *Staphylococcus* spp was isolated from some dogs.  
NA = Not applicable. CoNS = Coagulase-negative staphylococci. MR = Methicillin resistant.

dogs, no growth was detected on bacteriologic culture of ear swab specimens in 1 dog, *S intermedius* was isolated from 5 dogs, *S schleiferi* subsp *coagulans* was isolated from 1 dog, and methicillin-resistant *S schleiferi* subsp *coagulans* was isolated from the remaining dog. All isolates from this group were sensitive to fluoroquinolones.

*Staphylococcus* spp were isolated from the ears of all but 1 of the 16 dogs with otitis and pyoderma; no growth was detected on bacteriologic culture of ear swab specimens from 1 dog. Of the *Staphylococcus* spp isolated, a CoNS was isolated from the ears and a methicillin- and fluoroquinolone-resistant CoNS was isolated from the skin of 1 dog. *Staphylococcus intermedius* was detected on bacteriologic culture of ear swab specimens from 5 of the 16 dogs. In those 5 dogs, *S intermedius* was also detected on bacteriologic culture of skin swab specimens. One of the *S intermedius* isolates from the skin was resistant to methicillin. None of these isolates was resistant to fluoroquinolones.

*Staphylococcus schleiferi* subsp *coagulans* was detected on bacteriologic culture of ear swab specimens in 5 of the 16 dogs; in those 5 dogs, *S schleiferi* subsp *coagulans* as well as *S intermedius* were detected on bacteriologic culture of skin specimens from 1 dog. Only *S intermedius* was detected on bacteriologic culture of skin swab specimens from the remaining 4 dogs. None of those isolates was resistant to methicillin or the fluoroquinolones.

Methicillin-resistant *S schleiferi* subsp *coagulans* was isolated from the ears of 1 of the 16 dogs; this isolate was sensitive to fluoroquinolones. A *Staphylococcus* sp was not detected on bacteriologic culture of skin swab specimens.

Methicillin- and fluoroquinolone-sensitive *S schleiferi* subsp *schleiferi* was isolated from the ears and skin of 1 of the 16 dogs. In the remaining dog, both a methicillin-resistant and methicillin-sensitive *S schleiferi*

subsp *schleiferi* were isolated from the ears and a methicillin-resistant *S schleiferi* subsp *coagulans* was isolated from the skin. All isolates from this dog were also resistant to fluoroquinolones.

## Discussion

Most of the isolates in our study were obtained from dogs with otitis and were identified as *S intermedius*. This is in agreement with results of other studies,<sup>17,23</sup> which have consistently reported *S intermedius* in association with otitis. However, in contrast to results of 1 study<sup>24</sup> in which *S aureus* was the most frequently isolated coagulase-positive species in dogs, *S aureus* was not isolated from any dog in our study. Results of that study<sup>24</sup> also indicate that CoNS are the most common of all staphylococcus species isolated, which is not in agreement with results of our study.

*Staphylococcus schleiferi* was the most prevalent species detected on bacteriologic culture of ear swab specimens from dogs with both otitis and pyoderma; *S schleiferi* was isolated 9 times, whereas *S intermedius* was isolated 5 times. Most dogs in this group with both otitis and pyoderma had recurrent infections, which is consistent with results of a previous study.<sup>11</sup> This finding was expected because most dogs had been treated with antimicrobials administered PO, as topical otic formulations, or both. Although it is not presently known exactly how antimicrobial treatment affects all cutaneous pathogenic *Staphylococcus* populations in dogs with pyoderma or otitis, it is reasonable to conclude that initial elimination of the more numerous and sensitive *Staphylococcus* spp would permit expression and expansion of the remaining potentially methicillin-resistant, slow-growing subpopulations of *S schleiferi*. In addition, animals with bacterial infections, such as otitis and pyoderma, often have an underlying disease process, such as an adverse

reaction to food or atopic dermatitis.<sup>18,25,26</sup> Such conditions may encourage colonization of the skin with *S intermedium*, but this situation may permit an increase in other pathogenic bacteria as well, such as *S schleiferi*.<sup>27</sup> Furthermore, the population of pathogenic bacteria colonizing the mucosal surfaces increases significantly in dogs with pyoderma.<sup>28</sup>

To the authors' knowledge, our study is the first to report both subspecies of *S schleiferi*, *S schleiferi* subsp *coagulans* and *S schleiferi* subsp *schleiferi*, in association with otitis. Only *S schleiferi* subsp *coagulans* has been previously reported in association with otitis.<sup>2</sup> Additionally, to the authors' knowledge, our study is the first to report isolation of *S schleiferi* subsp *schleiferi* from healthy dogs. *Staphylococcus schleiferi* as a normal inhabitant of the skin of carnivores has been alluded to but poorly documented. Thus, results of the study reported here confirm that this may in fact be true.

In our study, recurrent infections were most frequently associated with methicillin resistance. In a previous study,<sup>11</sup> *Staphylococcus schleiferi* subsp *coagulans* was not found to be methicillin resistant when associated with pyoderma; however, this varies from our findings. In our study, 2 of 9 isolates of *S schleiferi* subsp *coagulans* from ears of dogs were methicillin resistant. Similar to a previous study,<sup>11</sup> 1 of the 3 isolates of *S schleiferi* subsp *schleiferi* from ears of dogs was methicillin resistant.

When evaluating results of bacteriologic cultures of ear and skin swab specimens in these dogs, the same organism was not typically isolated from the ears and the skin when both were sampled. Of the dogs in which *S schleiferi* was isolated from the ears (8 dogs, 9 isolates), *S schleiferi* was also isolated from the skin in only 3 of those dogs. In one of those dogs, both the ear and skin isolates were reported as resistant to both fluoroquinolones and methicillin. The remaining isolates were sensitive to both classes of antimicrobials.

Results of the study reported here indicated that *S schleiferi* subsp *schleiferi* could be detected in ears of healthy dogs as well as dogs with otitis, whereas *S schleiferi* subsp *coagulans* was detected only in dogs with otitis.

- BBL CultureSwab Collection & Transport System, Becton, Dickinson & Co, Sparks, Md.
- BBL Crystal Gram-positive ID System/GP, Becton Dickinson Microbiology Systems, Cockeysville, Md.
- Vitek GPS-207 card, BioMerieux, St Louis, Mo.

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