Genotypic relatedness and phenotypic characterization of Staphylococcus schleiferi subspecies in clinical samples from dogs

Christine L. Cain, DVM; Daniel O. Morris, DVM, MPH; Kathleen O’Shea, BS; Shelley C. Rankin, PhD

Objective—To assess the degree of biological similarity (on the basis of genotype determined via pulsed-field gel electrophoresis [PFGE]) between isolates of 2 Staphylococcus schleiferi subspecies (S schleiferi subsp coagulans and S schleiferi subsp schleiferi) in clinical samples obtained from dogs.

Sample Population—161 S schleiferi isolates from 160 canine patients.

Procedures—A commercial microbiology identification system was used to identify each isolate as S schleiferi. Isolates underwent slide and tube coagulase testing and antimicrobial susceptibility testing. A mecA PCR assay and a latex agglutination test for penicillin-binding protein 2a (PBP2a) were also performed on each isolate. Clonal clusters with a similarity cutoff value of 80% were identified via PFGE.

Results—Of the 161 isolates, 61 (38%), 79 (49%), and 21 (13%) were obtained from cutaneous sites, ears, and other sites, respectively; 110 (68%) were coagulase negative, and 51 (32%) were coagulase positive. Among the coagulase-negative and coagulase-positive isolates, 65% (71/110) and 39% (20/51) were oxacillin resistant, respectively. All oxacillin-resistant isolates yielded positive results via mecA PCR assay and PBP2a latex agglutination testing. Via PFGE, 15 major clusters and 108 individual pulsed-field profiles were identified. Oxacillin-resistant and oxacillin-susceptible isolates clustered separately. Clonal clusters were heterogeneous and contained representatives of both subspecies.

Conclusions and Clinical Relevance—Coagulase-positive and coagulase-negative isolates were not genotypically distinct and may represent a single S schleiferi spp with variable coagulase production, rather than 2 biologically distinct subspecies. Further studies are needed to characterize clinical or epidemiological differences associated with infections with coagulase-positive and coagulase-negative S schleiferi in dogs. (Am J Vet Res 2011;72:96–102)

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Abbreviations

<table>
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<tr>
<th>ATCC</th>
<th>American Type Culture Collection</th>
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<tr>
<td>CC</td>
<td>Clonal cluster</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>PBP2a</td>
<td>Penicillin-binding protein 2a</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed-field gel electrophoresis</td>
</tr>
<tr>
<td>PFP</td>
<td>Pulsed-field profile</td>
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Staphylococci are gram-positive bacteria that are normally harbored as resident flora on the skin and mucosal surfaces of human and nonhuman animals. These organisms have the potential to be opportunistic pathogens and to result in infection.1 Staphylococcus schleiferi was identified as a coagulase-negative staphylococcus in 1988.2 Since that time, 2 subtypes of S schleiferi have been identified: S schleiferi subsp coagulans and S schleiferi subsp schleiferi. These 2 subtypes are classified as tube coagulase positive and tube coagulase negative (on the basis of results of a tube coagulase test), respectively. Staphylococcus schleiferi produces pseudocoagulases, resulting in false-positive tube coagulase reactions, and does not possess the true coagulase gene (coa) of Staphylococcus aureus.3 In 1990, Igimi et al4 identified S schleiferi subsp coagulans from the external auditory meatus of dogs with otitis externa. Staphylococcus schleiferi subsp coagulans is a pathogen of dogs; the organism causes skin and ear infections in that species.4,5 Coagulase-negative staphylococci have historically been regarded in human medicine as commensals with little pathogenic potential, but results of a recent study6 have emphasized their emerging importance as pathogens (especially as nosocomial pathogens). The pathogenic potential of S schleiferi subsp schleiferi has been of particular interest.6 This subspecies...
was first isolated in 1988 from a clinical sample collected from a human, and the first human case of _S. schleiferi_ endocarditis was reported in 1999. It is now classified as a normal component of human preaxillary flora, and most infections are presumed to be nosocomial in origin. Post-surgical wound infections most commonly result from procedures involving the axilla, such as pacemaker implantation. Until recently, _S. schleiferi_ subsp _coagulans_ had been associated only once with a human infection. In 2007, endocarditis caused by _S. schleiferi_ subsp _coagulans_ in an immunocompromised human was reported. This infection was presumed to be of nonhuman animal origin and constitutes the first documented evidence of possible zoonotic transmission of _S. schleiferi_ subsp _coagulans_.

Both _S. schleiferi_ subspecies have been isolated from veterinary patients, although the true incidence of _S. schleiferi_ infections is likely underestimated because of the misidentification of _S. schleiferi_ as _S. aureus_; both staphyloccocal species produce β-hemolysin and a heat-stable nuclease and share similar biochemical characteristics. Studies have been performed to examine the incidence of _S. schleiferi_ isolation from healthy dogs, dogs with pyoderma, and dogs with otitis. In 1 study, _S. schleiferi_ was isolated from cultures of canine skin samples but only from samples obtained from dogs with recurrent pyoderma, frequently while those animals were receiving antimicrobials, suggesting that _S. schleiferi_ is an opportunistic pathogen. _Staphylococcus schleiferi_ subsp _schleiferi_ has been isolated from the ears of dogs with otitis and also from the ears of dogs with no clinical signs of otitis, whereas _S. schleiferi_ subsp _coagulans_ was found in the ears of dogs that were only clinically affected by otitis externa. By contrast, in another study, _S. schleiferi_ subsp _coagulans_ was isolated from the ears of dogs with or without otitis externa. Therefore, the true reservoir of these subspecies remains in question.

A great deal of scientific and media attention has been directed at resistant staphylococci and, more specifically, at methicillin-resistant _S. aureus_. It is known that methicillin resistance in _S. aureus_ and its implication for human and nonhuman animal health. It is known that methicillin resistance in _S. aureus_ is mediated via acquisition of _PB2a_, which is encoded by the _mecA_ gene carried on the mobile staphyloccocal cassette chromosome _mec_ (SCCmec) element. Staphyloccocal isolates that have _mecA_-mediated methicillin resistance are considered to have in vivo resistance to all β-lactams, potentiated β-lactams, cephalexopins, and carbapenems. In 2004, Kania et al showed that methicillin-resistant isolates of _Staphylococcus intermedius_ and _S. schleiferi_ each carried the _mecA_ gene and expressed _PB2a_. Detection of the _PB2a_ antigen by use of latex agglutination or _mecA_ gene by use of PCR analysis provides definitive evidence of methicillin resistance in _Staphylococcus spp._

The genotypic similarity within a species can be investigated by use of PFGE. This technique is useful in epidemiological studies and has been used to explore the relatedness of bacterial strains that cause diseases. The purpose of the study reported here was to classify _S. schleiferi_ identified in clinical samples obtained from dogs into 2 subspecies ( _S. schleiferi_ subsp _coagulans_ and _S. schleiferi_ subsp _schleiferi_), to describe the phenotypic characteristics of the isolates (including oxacillin resistance and antimicrobial susceptibility), and to assess the degree of biological similarity (on the basis of genotype determined via PFGE) between the subspecies.

**Materials and Methods**

**Study population**—A convenience sample of 161 clinical isolates of _S. schleiferi_ derived from 160 dogs that were evaluated at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania was available for use in the study. Isolates were randomly obtained from clinical samples collected between January 2003 and December 2007 and represented 48% (161/334) of _S. schleiferi_ isolates during this time. Isolates were stored at −70°C in cryopreservation tubes. Body site source was noted for each isolate.

A commercial microbiology identification system was used to characterize organisms as _S. schleiferi_. Bio-type data were stored in the microbiology identification system. Susceptibility to antimicrobials was also determined by use of this panel, and results were interpreted by use of the Clinical Laboratory Standards Institute guidelines. Susceptibility of isolates to enrofloxacin and marbofloxacin was determined via Kirby-Bauer disk diffusion. In preparation for typing, all stored isolates were removed from the freezer, subcultured on sheep blood agar plates, and incubated at 35°C for 24 hours.

**Coagulase testing**—Coagulase production was determined by use of a tube coagulase test with rabbit plasma and EDTA to classify isolates as _S. schleiferi_ subsp _schleiferi_ or _S. schleiferi_ subsp _coagulans_. All isolates also underwent a slide test for clumping factor that was performed with rabbit plasma.

**mecA PCR assay and latex agglutination test for PB2a**—All _S. schleiferi_ isolates were tested for the presence of the _mecA_ gene by use of a PCR assay, as previously described. All _S. schleiferi_ isolates were also tested for the presence of PB2a via latex agglutination testing performed with a commercial test kit, according to the manufacturer’s instructions.

**PFGE**—Pulsed-field gel electrophoresis was performed on isolates, as previously described. All parts of the PFGE procedure were performed according to the CDC Pulse Net protocol for molecular typing of _S. aureus_. Following thawing and subculturing, 1 bacterial colony from each culture plate was inoculated into brain heart infusion broth to prepare a cell suspension and incubated at 35°C to 37°C for 18 to 24 hours. Cell suspensions were adjusted to appropriate turbidity and centrifuged, and then the supernatant was removed. Cell pellets were resuspended in Tris-EDTA buffer, equilibrated, and mixed with lysostaphin stock solution and 1.8% commercial agarose in Tris-EDTA buffer. The mixture was transferred to a plug mold, and gel plugs were allowed to solidify. The plugs were then removed and added to melt agarase buffer (6 mM Tris HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, and 0.5% sodium laurylsulfate).
define PFP clusters, as previously described. A similarity coefficient of 80% was selected to derive arithmetic mean values and based on Dice coefficients. A similarity coefficient of 80% was selected to define PFP clusters, as previously described.

Results

Most of the 161 S schleiferi isolates were obtained from skin or ear samples; 79 (49%) isolates were obtained from culture of samples from the external portion of ears or tympanic bullae, whereas 61 (38%) isolates were obtained from culture of samples from cutaneous sites. Twenty-one (13%) isolates were obtained from samples collected from body sites other than the skin or ears. These 21 isolates were obtained from samples of transtracheal or endotracheal washes (n = 9), urine or bladder mucosa (6 [4%]), conjunctiva (2 [1%]), pleural effusion (1 [< 1%]), CSF (1 [< 1%]), semen (1 [< 1%]), and peritoneal cavity fluid (1 [< 1%]). Of the 21 isolates from those other body sites, 12 (57%) were coagulase negative and 9 (43%) were coagulase positive.

Coagulase testing—Of the 161 isolates, 110 (68%) were coagulase negative and 51 (32%) were coagulase positive. Three of the 51 (6%) isolates that yielded positive results via the tube coagulase test were also positive for clumping factor as determined via slide coagulase testing with rabbit plasma. Only 1 of the 110 (0.9%) isolates that yielded negative results via tube coagulase testing was also positive for clumping factor as determined via slide coagulase testing with rabbit plasma.

Antimicrobial susceptibility testing—Results of the antimicrobial susceptibility testing revealed that 71 of the 110 (65%) coagulase-negative isolates and 20 of the 51 (39%) coagulase-positive isolates were oxacillin (methicillin) resistant (Table 1). Regardless of oxacillin susceptibility status, data indicated that trimethoprim-sulfamethoxazole was the most effective antimicrobial tested, followed (in descending order) by rifampin, chloramphenicol and tetracycline, and clindamycin. Oxacillin-resistant isolates had the poorest non-β-lactam susceptibility; < 50% of isolates were susceptible to marbofloxacin, enrofloxacin, or gentamicin.

meca PCR assays and latex agglutination tests for PBP2a—The 161 S schleiferi isolates underwent the meca PCR assay and PBP2a latex agglutination testing (Tables 2 and 3). For 91 (57%) isolates, the MIC for oxacillin was > 2 µg/mL, as determined by use of the commercial microbiology identification system. All 91 isolates were also positive for clumping factor as determined via slide coagulase testing with rabbit plasma. Only 1 of the 110 (0.9%) isolates that yielded negative results via tube coagulase testing was also positive for clumping factor as determined via slide coagulase testing with rabbit plasma.

Table 1—Results of antimicrobial susceptibility testing of 161 Staphylococcus schleiferi isolates derived from clinical samples obtained from 160 dogs indicating the MIC cutoffs of the various antimicrobials and the proportion of oxacillin-susceptible and oxacillin-resistant isolates that were susceptible to each antimicrobial.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC cutoff (µg/mL)</th>
<th>No. of oxacillin-susceptible isolates (%) of 70 isolates</th>
<th>No. of oxacillin-resistant isolates (%) of 91 isolates*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin</td>
<td>≤ 2</td>
<td>70 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate potassium</td>
<td>≤ 4; ≤ 2</td>
<td>70 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>≤ 8</td>
<td>66 (94)</td>
<td>81 (89)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤ 0.5</td>
<td>61 (87)</td>
<td>65 (71)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤ 0.5</td>
<td>57 (81)</td>
<td>61 (67)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤ 1</td>
<td>61 (87)</td>
<td>37 (41)</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>NA</td>
<td>60 (86)</td>
<td>23 (27)</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>NA</td>
<td>60 (86)</td>
<td>20 (23)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤ 4</td>
<td>67 (96)</td>
<td>81 (89)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≤ 2; ≤ 38</td>
<td>70 (100)</td>
<td>91 (100)</td>
</tr>
<tr>
<td>Rifampin</td>
<td>≤ 2</td>
<td>70 (100)</td>
<td>87 (96)</td>
</tr>
</tbody>
</table>

Susceptibility to enrofloxacin and marbofloxacin was determined via Kirby-Bauer disk diffusion.

*For enrofloxacin and marbofloxacin, number of oxacillin-resistant isolates tested was 85 and 87, respectively.

NA = Not applicable.

For enrofloxacin and marbofloxacin, number of oxacillin-resistant isolates tested was 85 and 87, respectively.

Table 2—Results of PCR analysis for the meca gene and latex agglutination testing for PBP2a production in 161 Staphylococcus schleiferi isolates derived from clinical samples obtained from 160 dogs and categorized on the basis of susceptibility or resistance to oxacillin.

<table>
<thead>
<tr>
<th>meca and PBP2a status</th>
<th>No. of oxacillin-susceptible isolates (%) of 70 isolates</th>
<th>No. of oxacillin-resistant isolates (%) of 91 isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>meca positive and PBP2a positive</td>
<td>4 (6)</td>
<td>91 (100)</td>
</tr>
<tr>
<td>meca negative and PBP2a negative</td>
<td>65 (93)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>meca positive and PBP2a negative</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>meca negative and PBP2a positive</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

β-lactam susceptibility; < 50% of isolates were susceptible to marbofloxacin, enrofloxacin, or gentamicin.

sarcosine) and incubated at 37°C for 4 hours. The plugs were rinsed with Tris-EDTA buffer, cut to the appropriate comb size, and incubated with Smal enzyme1 for DNA restriction. Plug pieces were placed into the wells of a 1% agarose running gel, and PFGE was performed. Electrophoresis conditions were as follows: 200 V (6 V/cm); temperature, 14°C; initial switch, 5 seconds; final switch, 40 seconds; and duration, 21 hours. Gels were stained with ethidium bromide, photographed with a commercial gel documentation system, and saved electronically for analysis.20 Staphylococcus aureus NCTC (National Collection of Type Cultures) 8325 was used as the reference standard. Computer software3 was used to identify percentage similarities on a dendrogram derived from the unweighted pair group method involving arithmetic mean values and based on Dice coefficients. A similarity coefficient of 80% was selected to define PFP clusters, as previously described.21
oxacillin-resistant isolates were positive for both the \textit{mecA} gene and PBP2a. The majority (93%) of oxacillin-susceptible isolates were negative for both the \textit{mecA} gene and PBP2a (Table 2). However, 4 oxacillin-susceptible isolates were positive for the \textit{mecA} gene and PBP2a. Seventy-three of the 110 (66%) coagulase-negative isolates and 22 of the 51 (43%) coagulase-positive isolates were positive for both the \textit{mecA} gene and PBP2a (Table 3). Thirty-seven of the 110 (34%) coagulase-negative isolates and 28 of the 51 (55%) coagulase-positive isolates were negative for both the \textit{mecA} gene and PBP2a.

PFGE—Among the 161 \textit{S. schleiferi} isolates, 108 distinct PFPs were identified via PFGE. A cutoff value of 80% was used to identify 13 CCs (data not shown). Four major CCs contained 80% (129/161) of the isolates. All CCs were heterogeneous and contained both coagulase-positive and coagulase-negative isolates, and coagulase status could not be predicted on the basis of genotype alone.

Table 3—Results of PCR analysis for the \textit{mecA} gene and latex agglutination testing for PBP2a production in 161 \textit{S. schleiferi} isolates derived from clinical samples obtained from 160 dogs and categorized on the basis of susceptibility or resistance to oxacillin and coagulase production.

<table>
<thead>
<tr>
<th>\textit{mecA} and PBP2a status</th>
<th>Oxacillin-susceptible isolates (n = 70)</th>
<th>Oxacillin-resistant isolates (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of coagulase-negative isolates (% of 39 isolates)</td>
<td>No. of coagulase-positive isolates (% of 31 isolates)</td>
</tr>
<tr>
<td>\textit{mecA} positive and PBP2a positive</td>
<td>2 (5)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>\textit{mecA} negative and PBP2a positive</td>
<td>37 (95)</td>
<td>28 (90)</td>
</tr>
<tr>
<td>\textit{mecA} positive and PBP2a negative</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>\textit{mecA} negative and PBP2a positive</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Figure 1—Results of PFGE after \textit{SmaI} restriction of 13 \textit{Staphylococcus schleiferi} isolates derived from clinical samples obtained from dogs and 2 ATCC \textit{S. schleiferi} type strains, each representative of a different CC. The predominant PFP (second column from right [SchSma.xxx]) and oxacillin (OX) susceptibility result for each of the 15 \textit{S. schleiferi} CCs (right-hand column [CCShxx]) are indicated. The scale on the left of the figure indicates the degree of relatedness among isolates. The groupings of vertical lines represent DNA fragments separated according to length and molecular weight. The column to the right of the vertical lines indicates the laboratory number for the representative isolates of each CC or the ATCC \textit{S. schleiferi} type strain designation (\textit{S. schleiferi} subspp. \textit{schleiferi} [ATCC 43808] and \textit{S. schleiferi} subspp. \textit{coagulans} [ATCC 49545]). The third column from the right indicates the MIC of OX (µg/mL) for each isolate.

Clonal cluster 1 contained 48 isolates, of which 38 (79%) were coagulase negative and 10 (21%) were coagulase positive. Forty-three (90%) isolates were oxacillin resistant, and 5 (10%) isolates were oxacillin susceptible. There were 14 PFPs, but 34 of the 48 (71%) isolates had a similar PFP (ie, PFP 62).

Clonal cluster 2 contained 43 isolates, of which 36 (84%) were coagulase negative and 7 (16%) were coagulase positive. Two isolates that yielded positive results via the tube coagulase test were also positive for clumping factor as determined via slide coagulase testing with rabbit plasma. Of the 43 isolates, 22 (51%) were oxacillin resistant and 21 (49%) were oxacillin susceptible. There were 27 PFPs; 8 (19%) isolates were grouped in PFP 42, and all were oxacillin susceptible. Six of 43 (14%) isolates were grouped in PFP 117, and 5 of these 6 isolates were oxacillin resistant.

Clonal cluster 3 contained 19 isolates, of which 11 (58%) were coagulase positive and 8 (42%) were coagulase negative. Three (16%) isolates were oxacillin
resistant, and 16 (84%) isolates were oxacillin susceptible. There were 8 PFPs, but 2 predominated; 7 isolates were grouped in PFP 1, and 6 were grouped in PFP 94.

Clonal cluster 4 contained 11 isolates, of which 7 (64%) were coagulase positive and 4 (36%) were coagulase negative. Four isolates were oxacillin resistant, and 7 isolates were oxacillin susceptible. There were 5 PFPs; the oxacillin-susceptible isolates were grouped in PFP 79.

A second dendrogram was constructed to compare the PFP of the predominant strain from each of the CCs (Figure 1). The dendrogram revealed that 2 clusters were formed that contained predominantly oxacillin-resistant isolates (MIC of oxacillin, > 2.00 μg/mL) and predominantly oxacillin-susceptible isolates (MIC of oxacillin, < 0.25 μg/mL). The dendrogram revealed that although ATCC 43808 (S schleiferi subsp schleiferi) was clustered with the oxacillin-susceptible isolates, ATCC 49545 (S schleiferi subsp coagulans) was not clustered with either group.

Discussion

In humans, S schleiferi has been associated mainly with infections of wounds, pacemaker implantation sites, and incision sites, although cases of endocarditis, brain empyema, and osteomyelitis have been reported. In dogs, S schleiferi has been reported most commonly in association with pyoderma and otitis externa. In the present study, most clinical samples were obtained from the skin and ears (external ear or tympanic bulla). In a smaller proportion of samples, S schleiferi was isolated from the respiratory and genitourinary tracts as well as from various other body sites (eg, conjunctiva, CSF; and pleural and peritoneal cavities). Although most serious and systemic infections in humans have been caused by S schleiferi subsp schleiferi rather than S schleiferi subsp coagulans, both subspecies were isolated from approximately equal proportions of the subset of samples obtained from body sites other than the skin and ears. An evaluation of the severity of these infections and determination of whether S schleiferi infections of noncutaneous or otic origin are more likely to develop in immunocompromised dogs or as a result of nosocomial transmission (as has been suggested in humans) are beyond the scope of our study. In contrast to a previous report by Jones et al, in which S schleiferi subsp coagulans was isolated more frequently than S schleiferi subsp schleiferi from 1,772 clinical samples obtained from dogs, most of the isolates from clinical samples in the present study were coagulase negative.

Slide coagulase testing for clumping factor detection is routinely performed to differentiate S schleiferi subsp schleiferi from other coagulase-negative Staphylococcus spp. Only coagulase-negative S schleiferi and Staphylococcus lugdunensis are reported to be positive for clumping factor. Similarly, results of slide coagulase testing may also be used to differentiate S schleiferi subsp coagulans from S aureus; coagulase-positive S schleiferi is typically negative for clumping factor, whereas S aureus is positive. In the present study, all isolates underwent slide coagulase testing for clumping factor but results were widely disparate from those expected. Six percent of coagulase-positive isolates were positive for clumping factor, whereas only 0.9% of coagulase-negative isolates were positive for clumping factor. It has been established that results of tube coagulase testing are generally more accurate than slide coagulase testing, with both false-negative and false-positive reactions to slide coagulase tests possible. False-negative reactions are estimated to occur in 5% to 10% of tests, and culture medium can affect test results; testing of colonies cultured on high-salt-content media can yield false-positive results, whereas tests performed on staphylococci obtained directly from blood culture broth media can yield false-negative results. However, the occurrence of false-negative reactions was unlikely to account for the fact that most isolates were negative for clumping factor in the present study. Previous reports of clumping factor production in S schleiferi subspecies are based on results of slide coagulase testing of only a few type strains and may not represent the majority of strains cultured from samples obtained from dogs. Therefore, clumping factor results should no longer be used in an algorithm to differentiate S schleiferi from other Staphylococcus spp.

Pulsed-field gel electrophoresis is a very useful technique for establishing biologic relatedness among strains within a bacterial species. Previous studies have revealed genotypic heterogeneity among strains of S intermedius that cause pyoderma in dogs and S schleiferi subsp schleiferi isolated from surgical site infections in humans and genotypic homogeneity among S schleiferi subsp coagulans from a small number of external ear samples obtained from dogs. Although relative homogeneity within S schleiferi was suggested, previous PFGE analysis of 28 oxacillin-resistant S schleiferi subsp coagulans from clinical isolates at our institution revealed a fair degree of genotypic heterogeneity. To the authors’ knowledge, no prior study has investigated the biologic similarity between S schleiferi subspecies by use of PFGE.

In the present study, results of PFGE indicated that isolates of S schleiferi that cause disease in dogs are genetically heterogeneous, as are isolates of Staphylococcus pseudintermedius. From the clinical samples that were investigated, 108 individual PFPs were identified and clustered into 15 CCs. When a second dendrogram was constructed to compare the PFPs of the predominant strain from each of the 15 CCs, the organisms clustered into 2 separate groups that contained predominantly oxacillin-resistant or oxacillin-susceptible isolates. This observation supports the hypothesis that methicillin-resistant S schleiferi strains are clonal, whereas methicillin-susceptible isolates are heterogeneous, as determined for methicillin-resistant S pseudintermedius strains on different continents. Within the 4 CCs that represented most of the isolates, some clusters contained mainly coagulase-negative or coagulase-positive isolates, but all clusters contained a mix of isolates by coagulase status. This finding suggests that there is a single S schleiferi sp with variable coagulase activity, rather than 2 biologically distinct subspecies. Staphylococcus schleiferi subsp coagulans ATCC strain 49545 clustered separately from
isolates obtained in the present study. The established type strain may not represent the majority of isolates cultured from samples obtained from dogs, although species misidentification is a less likely possibility. Sequencing of highly conserved genes such as 16S rDNA and sodA with sequence comparison to type strains of both S schleiferi subspecies would confirm species identification.36,37

Methicillin (oxacillin) resistance was first recognized in S aureus in the 1960s.38 Since then, widespread methicillin resistance has been detected in various staphylococcal species of human and veterinary medical importance, and the proportion of methicillin-resistant organisms appears to have increased over recent years.13 Methicillin resistance is particularly pervasive among S schleiferi isolates.5,12,13 Results of a previous study14 have supported a higher rate of methicillin resistance among coagulase-negative S schleiferi, compared with findings in coagulase-positive S schleiferi. Similarly, in the present study, S schleiferi subsp schleiferi was much more likely to be oxacillin resistant than S schleiferi subsp coagulans. In human medicine, S schleiferi subsp schleiferi is also generally considered to be more pathogenic than other coagulase-negative staphylococcal species,39 and this has been supported by data obtained from mice.40 The high rate of methicillin resistance in S schleiferi isolates from nonhuman animals raises the concern for potential transfer of the mecA gene to other staphylococci of human and veterinary medical importance.41 Staphylococcus schleiferi infection in dogs also appears to be associated with prior antimicrobial use, suggesting opportunistic invasion when normal staphylococcal flora is inhibited.42 Although evaluation of antimicrobial use was not within the scope of the present study, this is a possible area of future investigation.

Although the rate of oxacillin resistance among S schleiferi isolates in the present study was high, the isolates maintained an overall favorable antimicrobial susceptibility pattern. This finding is consistent with results of previous studies,12,13 which indicated that methicillin-resistant S schleiferi maintained the broadest antimicrobial susceptibility pattern, compared with the patterns for methicillin-resistant S intermedius and S aureus. Even among oxacillin-resistant isolates, susceptibility rates > 50% were maintained for trimethoprim-sulfamethoxazole, rifampin, chloramphenicol, tetracycline, clindamycin, and erythromycin. Oxacillin-resistant isolates did have poor susceptibility (< 50%) to fluoroquinolones. Although oxacillin-resistant S schleiferi may be more susceptible to fluoroquinolones than oxacillin-resistant S aureus,12 S schleiferi is more often fluoroquinolone resistant than S intermedius and that resistance is mediated by non-mecA mechanisms.43 For this reason, treatment of dogs with fluoroquinolones for pyoderma or otitis should be recommended only when indicated by results of culture and antimicrobial susceptibility testing.

The gold standard test for detection of oxacillin resistance in S aureus is the PBP2a latex agglutination test, the results of which have been correlated with oxacillin susceptibility patterns in small numbers of tested S schleiferi.49 All of the oxacillin-resistant isolates in our study were positive for the mecA gene and produced PBP2a as determined by latex agglutination testing. Most oxacillin-susceptible isolates were negative for both the mecA gene and PBP2a production. Smaller numbers of isolates that were phenotypically negative for oxacillin resistance were positive for the mecA gene, PBP2a production, or both. Possession of the mecA gene and production of PBP2a in S schleiferi do not confer oxacillin resistance. In a study by Kania et al.,44 a number of phenotypically oxacillin-susceptible S schleiferi isolates were also positive for mecA and PBP2a production.

In the present study, S schleiferi isolates derived from clinical samples obtained from dogs were, in general, genotypically heterogeneous as established by PFGE. However, on the basis of study findings, 2 biologically distinct subspecies by coagulase status could not be supported. Furthermore, very few coagulase-negative isolates were positive for clumping factor production, which indicates that this test should no longer be used for species differentiation. A high rate of oxacillin resistance was identified, and all oxacillin-resistant isolates were positive for the mecA gene and PBP2a production. Via PFGE, isolates clustered into 2 separate groups on the basis of predominant oxacillin susceptibility. Oxacillin-resistant isolates were more clonal, supporting acquisition of the mecA gene by and lateral dissemination of related S schleiferi clones.35 Staphylococcus schleiferi may represent a single bacterial species with variable coagulase production. Further study is needed to better characterize clinical and epidemiological differences in infections with coagulase-positive and coagulase-negative S schleiferi in dogs.

References

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