Methicillin resistance of staphylococci isolated from the skin of dogs with pyoderma

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Objective—To determine the methicillin-resistant profile of staphylococcal isolates from the skin of dogs with pyoderma.

Animals—90 dogs with pyoderma.

Procedure—Staphylococci isolated from dogs with pyoderma were tested for susceptibility to methicillin by use of a standard disk diffusion test with oxacillin disks. The DNA extracted from the isolates was tested for the *mecA* gene that encodes the penicillin-binding protein 2a (PBP2a) by use of a polymerase chain reaction (PCR) assay. The expression of PBP2a was determined with a commercial latex agglutination assay. Species of staphylococcal isolates were identified by use of morphologic, biochemical, and enzymatic tests.

Results—Most of the isolated staphylococci were methicillin-susceptible, coagulase-positive *Staphylococcus intermedius* isolates. Whereas only 2 of 57 *S intermedius* isolates were resistant to methicillin, approximately half of the isolates had the *mecA* gene and produced PBP2a. *Staphylococcus schleiferi* was the second most common isolate. Widespread resistance to methicillin was found among *S schleiferi* isolates. More coagulase-negative *S schleiferi* isolates were identified with *mecA* gene-mediated resistance to methicillin, compared with coagulase-positive *S schleiferi* isolates.

Conclusions and Clinical Relevance—The latex agglutination assay for the detection of PBP2a expression coupled with the PCR assay for the *mecA* gene may provide new information about emerging antimicrobial resistance among staphylococcal isolates. (*Am J Vet Res* 2004;65:1265–1268)

Staphylococcus intermedius is the most common etiologic agent of pyoderma in dogs.[1] Coagulase-positive staphylococcal species (species in which > 90% of isolates are positive) are often considered as primary pathogens or as being more virulent than coagulase-negative *Staphylococcus* spp (species in which < 10% of isolates are positive), although a direct role for coagulase in disease has yet to be found. Coagulase-negative *Staphylococcus* spp have also been associated with infections in people[2] and dogs.[3] Of special interest are the coagulase-variable species *S schleiferi* (*S schleiferi* subsp *coagulans* and *S schleiferi* subsp *schleiferi*) that infect humans[4,5] and appear to be an emerging pathogen in veterinary medicine.[3,6,7] Because coagulase-negative staphylococci often have multiple antimicrobial resistances,[2] inclusion of these apparently more opportunistic *Staphylococcus* spp in the study of methicillin resistance is warranted.

Determination of antimicrobial susceptibility is crucial to the selection of antimicrobials that are effective therapeutic agents for the treatment of pyoderma. *Staphylococcus intermedius* is often penicillin resistant as a result of chromosomally encoded β-lactamase production.[8,9] Thus, first-generation cephalosporins are usually considered the antimicrobials of choice when treating *S intermedius* infections and are preferable over third-generation antimicrobials to prevent the selection of antimicrobial-resistant organisms. Most *S intermedius* isolates from dogs and S *schleiferi* isolates from wounds and other opportunistic infections of humans[4,10] are susceptible to methicillin. In a recent study[3] of dogs with pyoderma, methicillin-resistant *S schleiferi* isolates were identified. Cross-resistance between methicillin and other β-lactam agents is well established for **methicillin-resistant *Staphylococcus aureus* (MRSA)** organisms; however, it is not clear whether other *Staphylococcus* spp have similar cross-resistance.[11] Multiple resistances to other antimicrobial drug classes also commonly develop in MRSA organisms. It would be desirable to know whether similar antimicrobial-resistant correlations exist for *Staphylococcus* spp isolated from dogs with pyoderma.

The disk diffusion susceptibility test method is commonly used on *Staphylococcus* isolates. Oxacillin disks are used for in vitro testing because of the greater stability of disks and test results that correlate better with the methicillin resistance of *S aureus* isolates from humans. Disk diffusion testing with cefoxitin may provide even better in vitro antimicrobial-resistant correlations for MRSA organisms.[12] Test conditions and choice of breakpoint for oxacillin resistance have a major effect on the detection of methicillin resistance in *mecA* gene-positive MRSA organisms. Optimal conditions for determining methicillin resistance may vary with species and have not been well established for most coagulase-negative species or coagulase-positive species other than *S aureus*. It is reasonable to assume that heterologous, multiple, and cross-resistance properties may also be of clinical relevance in these species. Genetic characterization of methicillin-resistant genes in these species will be important for determining the likelihood of conversion to the methicillin-resistant phenotype.[13]

Alternative tests for determining methicillin resistance of staphylococci include the **polymerase chain reaction (PCR)** assay for the detection of the *mecA*

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gene, which encodes the **penicillin-binding protein 2a (PBP2a)**, and a latex particle agglutination test that detects the PBP2a antigen. The PCR assay provides genetic information but has the potential problem of producing false-positive results regarding methicillin resistance as a result of mutations that result in inactive PBP2a. In addition, regulatory genes can suppress *mecA* gene expression.[14] The latex particle agglutination test provides direct evidence of gene expression; however, it does not indicate whether the PBP2a is functional. In addition, this test has been validated only for *S aureus*. Therefore, in the study reported here, all 3 available tests were used to determine the methicillin-resistant profiles of staphylococcal isolates obtained from the skin of dogs with pyoderma.

Materials and Methods

Bacterial isolates—Ninety staphylococcal isolates from dogs with pyoderma submitted to the bacteriology laboratory at the University of Tennessee between April 1999 and December 2001 were evaluated in this study. Included were isolates obtained from dogs through veterinary services at the university hospital and also isolates from referral submissions. Isolates obtained from dogs through the university hospital included those previously reported in a study[3] on the isolation of *S schleiferi* from the skin of dogs with pyoderma.

Staphylococcal isolates from the skin of dogs with pyoderma were characterized by use of conventional biochemical tests. These included the tube coagulase test[15]; hemolysis patterns; and acid production from maltose, trehalose, and lactose.[15] Additional biochemical characterization was performed by use of a commercial kit according to the instructions of the manufacturer.[a] The use of this test for the identification of *S schleiferi* was validated in a previous study.[3] Antimicrobial susceptibility tests were performed via the standard disk diffusion method[15] by use of commercially available Mueller-Hinton agar plates and antimicrobial disks.[b] For *S schleiferi* and coagulase-negative species, interpretive breakpoints recommended by the National Committee for Clinical Laboratory Standards guideline for isolates from humans were used.[16]

Latex particle agglutination test—The PBP2a was detected with a latex particle agglutination test[c] that has been validated by the manufacturer for the determination of methicillin resistance of *S aureus*. The test was performed according to the instructions of the manufacturer. Briefly, an extract was prepared from fresh subcultures of bacteria. The extract was incubated with anti-PBP2a monoclonal antibody-sensitized latex particles and control particles for 3 minutes with rocking. Isolates were scored as having a positive reaction if particle agglutination resulted in clearing of the latex suspension. Agglutination without clearing was scored as a weak positive reaction. Isolates that failed to produce PBP2a were subcultured on induction media containing 1 mg of oxacillin/L and incubated overnight. Organisms that grew in the presence of the antimicrobial were retested, and these data were used in the final results.

PCR assay—The *mecA* gene was detected by use of a PCR assay with primers M1 and M2, as described by Vannuffel et al.[17] These primers yield a 310-bp product corresponding to bases 885 to 1,194, of the *mecA* gene of *S aureus*.[18] Staphylococci grown on blood agar plates were subcultured to Luria-Bertani broth media and grown overnight at 37°C with shaking at 220 rpm. Bacteria were harvested by centrifugation of 3 mL of culture, and DNA was extracted with a commercial kit according to the instructions of the manufacturer.[d] Amplification mixtures consisted of 5 µL of DNA, 5 pmol of each primer, and 25 µL of Taq premix,[e] thereby providing 1.25 units of *Taq* polymerase in a final concentration of 10mM Tris-HCl, 50mM KCl, and 1.5mM $MgCl_2$. The cycling parameters included an initial step of 95°C for 90 seconds; followed by 30 cycles of 94°C for 60 seconds, 50°C for 30 seconds, and 72°C for 60 seconds; and a final extension for 10 minutes at 72°C. The PCR products were loaded (10 µL/sample) on 2% agarose gels that contained ethidium bromide and viewed on a UV transilluminator.

Results

A total of 23 out of 90 (26%) staphylococcal isolates obtained from dogs with pyoderma were resistant to methicillin (**Table 1**). Most (17/23) of the resistant organisms were *S schleiferi* isolates. Other resistant staphylococci included *S epidermidis* (n = 3), *S intermedius* (2), and *S warneri* (1) isolates. All *S schleiferi* and *S intermedius* isolates that were resistant to methicillin on the basis of disk diffusion test results contained the *mecA* gene and expressed PBP2a. All 3 *S epidermidis* isolates contained the *mecA* gene but did not produce detectable PBP2a. The methicillin-resistant *S warneri* organism was negative for both the *mecA* gene and PBP2a.

Among the 8 methicillin-susceptible *S schleiferi* isolates, 4 were positive for expression of the PBP2a and 4 contained the *mecA* gene. Most (55/57) of the *S intermedius* isolates were susceptible to methicillin. Within this group of organisms, however, 25 were PBP2a positive and 22 were *mecA* gene positive. The methicillin-susceptible *S epidermidis* (n = 1), *S warneri* (2), and *S simulans* (1) isolates were all negative for the *mecA* gene and PBP2a.

Table 1—Methicillin-resistant characteristics of *Staphylococcus* spp isolated from the skin of dogs with pyoderma.

	Methicillin resistance				Methicillin susceptibility			
	PBP2a		*MecA*		PBP2a		*MecA*	
Bacterial species	**Pos (No.)**	**Neg (No.)**	**Pos (No.)**	**Neg (No.)**	**Pos (No.)**	**Neg (No.)**	**Pos (No.)**	**Neg (No.)**
S schleiferi subsp *coagulans*	5	0	5	0	4	3	3	4
S schleiferi subsp *schleiferi*	12	0	12	0	0	1	1	0
S intermedius	2	0	2	0	25	30	22	33
S epidermidis	0	3	3	0	0	1	0	1
S warneri	0	1	0	1	0	2	0	2
S simulans	0	0	0	0	0	1	0	1
Total	**19**	**4**	**22**	**1**	**29**	**38**	**26**	**41**

PBP2a = Penicillin-binding protein that was detected by use of a latex particle agglutination test. *MecA* = Gene encoding PBP2a that was detected by use of a polymerase chain reaction assay. Pos = Number of isolates with a positive reaction. Neg = Number of isolates with a negative reaction.

Within the methicillin-susceptible *S intermedius* isolates, 10 of 25 (40%) PBP2a test results were graded as weak positive reactions. Only 3 other isolates tested produced weak PBP2a-positive reactions. Among all of the isolates, 6 were coagulase positive and methicillin resistant, 17 were coagulase negative and methicillin resistant, 52 were coagulase positive and methicillin susceptible, and 15 were coagulase negative and methicillin susceptible.

Discussion

Most of the isolates in our study were *S intermedius*. *Staphylococcus intermedius* has consistently been reported as an important organism associated with pyoderma in dogs.[8] It has rarely been resistant to methicillin.[9,19] In a study of 25 methicillin-resistant staphylococci isolated from dogs, Gortel et al[19] found only 1 *S intermedius* isolate, an observation consistent with our results. It is interesting to mention, however, that in our study, 2 methicillin-resistant *S intermedius* isolates each contained the *mecA* gene and produced PBP2a. Furthermore, a large proportion of the methicillin-susceptible *S intermedius* isolates was positive for the *mecA* gene and produced PBP2a.

Staphylococcus schleiferi was the second most common isolate. Similar to *S intermedius,* a large proportion of the methicillin-susceptible *S schleiferi* isolates expressed PBP2a and had positive test results for the *mecA* gene.

Because the *mecA* gene PCR primers used in our study do not amplify the entire *mecA* gene, it is possible that some methicillin-susceptible organisms produced an inactive PBP2a from a truncated *mecA* gene. Susceptible isolates that have positive PCR assay results for the *mecA* gene could contain a full-length copy of the *mecA* gene but fail to express PBP2a as a result of a mutation in the open reading frame or promoter. Another possibility is that they have a full-length functional gene that is transcriptionally downregulated. *Staphylococcus aureus* expression of the *mecA* gene can be repressed by the *mecI* and *mecRI* genes.[14] In fact, results of a study[20] on *S aureus* indicate that repression of the *mecA* gene may play an important role in the survival of the gene in a new host.[20] Mapping the gene structure surrounding the *mecA* gene in *S intermedius* and *S schleiferi* may shed light on the mechanism by which it is regulated in these organisms. Production of low amounts of PBP2a by many of the methicillin-susceptible *S intermedius* isolates, resulting in a weak agglutination reaction, supports the hypothesis of a decrease in gene expression. A low degree of PBP2a expression and positive results on the PCR assay could also be explained by the phenomenon of heterogeneous resistance, as reviewed by Chambers.[21]

The detection of methicillin-resistant staphylococcal organisms in dogs with pyoderma is contrary to historical trends.[9,22] The antimicrobial history was unknown for many of the dogs from which the isolates of our study were obtained. Specimens from routine pyodermas are seldom submitted for bacterial culture[1]; hence, change of resistance among skin-colonizing flora is not documented. Specimens for bacterial culture are more often obtained from dogs with recurrent infections or dogs that failed to respond to conventional antimicrobial treatment,[1] as was true for isolates used in our study. Therefore, it is likely that the antimicrobial-resistant profiles of isolates from dogs with recurrent pyoderma are outcomes of heavy antimicrobial usage and not typical of those found in healthier dogs or dogs with their first episode of pyoderma.

For *S aureus*, it has been experimentally found that organisms that previously did not have the *mecA* gene mobile element selected against its expression.[20] *Staphylococcus aureus* organisms that have the element removed, however, readily express the *mecA* gene when it is reinserted. Expression of the *mecA* gene in *S schleiferi* and *S intermedius* isolates may represent an evolutionary step that could be driven by the routine use of antimicrobials to treat bacterial infections in dogs.

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[a]BBL crystal gram-positive ID kit, Becton, Dickinson & Co, Franklin Lakes, NJ.
[b]BBL Mueller Hinton II agar plates and Sensi-Discs, Becton, Dickinson & Co, Franklin Lakes, NJ.
[c]Oxoid PBP2' latex agglutination test, Oxoid Inc, Ogdensburg, NY.
[d]Easy-DNA, protocol #3, Invitrogen Corp, Carlsbad, Calif.
[e]Premix Taq, TaKaRa Shuzo Co Ltd, Otsu, Shiga, Japan.

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