Incidence of Inducible Clindamycin Resistance in *Staphylococcus pseudintermedius* from Dogs

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Clindamycin is increasingly used to treat canine pyoderma. Eight of 608 *Staphylococcus pseudintermedius* isolates were positive for inducible clindamycin resistance by double-disk diffusion testing and PCR detection of *ermB*. *Staphylococcus pseudintermedius* isolates that are erythromycin resistant but clindamycin susceptible by *in vitro* antimicrobial susceptibility testing should be tested for inducible clindamycin resistance.

Increased prevalence of methicillin resistance and multidrug resistance in *Staphylococcus pseudintermedius* has resulted in greater use of clindamycin to treat canine pyoderma because of its perceived clinical efficacy and good distribution into the skin (1, 2). Clindamycin is a lincosamide that reversibly binds to the bacterial 50S ribosomal subunit, thereby inhibiting protein synthesis (3). In some cases, staphylococci may appear to be susceptible to clindamycin when tested *in vitro*, but the infected patient may fail to respond to therapy despite being treated with what seems to be an appropriate drug concentration for an appropriate duration. Lincosamides bind to the same or closely related binding sites in the bacterial ribosome as macrolides such as erythromycin. Resistance to macrolide, lincosamide, and streptogramin B antibiotics (MLS phenotype) can occur through acquisition of a methylase enzyme that removes a methyl group from an adenine residue in the 23S rRNA component of the 50S subunit of the ribosome (4–6). Removal of this methyl group alters the site to which the antimicrobial drug binds, altering its efficacy. An active efflux pump encoded by the *msrA* gene also confers resistance to macrolides and streptogramin antibiotics but not lincosamides such as clindamycin (MS phenotype) (6).

Approximately 40 *erm* genes that encode methylases have been reported in different bacterial genera, with *ermA*, *ermB*, and *ermC* the genes most commonly found among staphylococci (7). In *Staphylococcus aureus*, *ermA* and *ermC* confer erythromycin resistance in 94 to 98% of isolates (8). In *S. pseudintermedius*, *ermB* is responsible primarily for MLS resistance, but its expression can be constitutive or inducible (9). Detailed descriptions of the mechanisms of *erm* gene expression and mutations leading to constitutive MLS resistance have been previously published (10, 11). Mutation in the macrolide-inducible DNA sequence preceding *ermB* genes can alter resistance from inducible to constitutive (10). These mutations occur at a rate of about one in every 2×10^6 replications (11, 12). Infections in which bacteria are present and dividing in purulent material in numbers greater than this are common, which means that these mutations readily occur, resulting in constitutive MLS resistance, and strains carrying the mutation will dominate within the bacterial population at the site of infection, particularly in the presence of antimicrobial selection pressure (10). Therefore, if bacteria carrying inducible MLS resistance are present in an infection, mutations may result in constitutive MLS resistance leading to treatment failure. Routine antimicrobial susceptibility testing can detect constitutive MLS resistance but fails to detect inducible resistance (13). Inducible

clindamycin resistance should be suspected in isolates that are erythromycin resistant but clindamycin susceptible upon *in vitro* antimicrobial susceptibility testing. In this study, we evaluated the frequency of inducible clindamycin resistance in *S. pseudintermedius* from patients presented to the Texas A&M Veterinary Medical Teaching Hospital (VMTH) by using double-disk diffusion testing (D-test) for inducible clindamycin resistance and the presence of *ermB* by PCR.

A total of 608 canine *Staphylococcus pseudintermedius* isolates collected from the VMTH between 2007 and 2012 were screened for inducible clindamycin resistance. At the time of initial collection, all isolates were presumptively identified as *S. pseudintermedius* based on Gram stain, colony color, polymyxin B susceptibility, production of coagulase and catalase, and ability to grow on salt-mannitol agar. All isolates were tested for antimicrobial susceptibility using commercially available systems (Vitek [bioMérieux, Durham, NC] or TREK Sensititre [TREK Diagnostics, Cleveland, OH]) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for MIC testing (14). Isolates were screened to identify those that were intermediate or resistant to erythromycin and susceptible or intermediate to clindamycin. Those meeting the criteria were further tested for the presence of a positive D-test according to the CLSI guidelines (15). Quality control strains for antimicrobial susceptibility testing included *S. aureus* ATCC 43300, ATCC 25923, and ATCC 29213. Quality control strains for the D-test included *S. aureus* BAA-977 and BAA-976. All quality control strains were obtained from the American Type Culture Collection (Manassas, VA). Eight isolates met the screening criteria and underwent further testing. All eight were susceptible for clindamycin on the MIC panel; seven were erythromycin resistant and one was intermediate to erythromycin. One isolate exhibited intermediate resistance to clindamycin; however, it was susceptible to erythromycin and was not tested further. Species identification of the eight isolates was confirmed by PCR using primers and methods previously described (16). Bacterial DNA was purified for the *ermB* PCR using a DNeasy blood and tissue kit

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TABLE 1 Inducible clindamycin resistance, erythromycin resistance, and oxacillin resistance in *Staphylococcus pseudintermedius*

Isolate no.	MIC (µg/ml) (interpretation)[a]			D-test result	PCR test for *ermB*
	Clindamycin	Erythromycin	Oxacillin		
11-001	≤0.5 (S)	≥8 (R)	≥8 (R)	Positive	Positive
11-025	≤0.5 (S)	≥8 (R)	≥8 (R)	Positive	Positive
11-033	≤0.5 (S)	1 (I)	≥8 (R)	Positive	Positive
11-064	≤0.5 (S)	≥8 (R)	≥8 (R)	Positive	Positive
12-012	≤0.5 (S)	≥8 (R)	≤0.25 (S)	Positive	Positive
17-016	≤0.5 (S)	≥8 (R)	2 (R)	Positive	Positive
18-007	≤0.5 (S)	≥8 (R)	≥8 (R)	Positive	Positive
24-014	≤0.5 (S)	≥8 (R)	≤0.25 (S)	Positive	Positive

[a] MIC for clindamycin, erythromycin, and oxacillin. Breakpoints for antimicrobials were from CLSI VET01-A4 (15). Abbreviations for interpretations are as follows: R, resistant to antimicrobial; S, susceptible to antimicrobial; I, intermediate susceptibility to antimicrobial. Guidelines for D-test performance and interpretation were from CLSI M02-A11 (15).

(Qiagen, Germantown, MD) according to the manufacturer's instructions for Gram-positive bacteria. PCR amplification of a 639-bp product specific for *ermB* was performed using primers (5) and methods (17) previously described, with an alteration in the annealing temperature to 46°C followed by 1% gel electrophoresis. All primers and PCR reagents were purchased from Sigma-Genosys, Houston, TX, and TaKaRa Bio Company, Otsu, Shiga, Japan, respectively. The resultant PCR product was confirmed as *ermB* by sequencing at the DNA Core Laboratory at the Texas A&M University College of Veterinary Medicine.

The isolates in this study came from eight dogs that presented to the VMTH between February 2008 and April 2010. Two isolates were collected from each of the following sites: infected tibial plateau leveling osteotomy (TPLO) implants, skin lesions, and the urinary tract (one from an infection and one from a bladder stone). One of the skin lesion isolates came from a dog with generalized demodicosis and deep pyoderma, and the second was collected from the prescrubbed surgical site for a torn cranial cruciate ligament repair. The remaining two isolates came from a blood culture and postsurgical lavage of the peritoneum following exploratory abdominal surgery.

Upon presentation to the VMTH, six of the eight dogs had received prior antibiotic therapy with one or more antimicrobial drugs within 6 weeks of entering the hospital. Five of these dogs were receiving antimicrobial therapy at the time of culture. One dog received erythromycin, and another received clindamycin. In this study, all isolates considered resistant or intermediate to erythromycin but susceptible to clindamycin *in vitro* tested positive for inducible clindamycin resistance by D-test and the presence of *ermB* associated with MLS resistance (Table 1 and Fig. 1). Two of the isolates were methicillin susceptible (25%), while the remaining six were methicillin resistant (75%).

S. pseudintermedius is the most common bacterial agent isolated from canine pyoderma and surgical and nonsurgical wound infections (18, 19). Of the eight dogs that provided isolates evaluated in this study, four had skin lesions or TPLO implant-related surgical infections. Treatment of staphylococcal infection at these sites in dogs typically involves therapy with β-lactam antibiotics, such as penicillins and cephalosporins. With increased prevalence of methicillin resistance, alternatives to β-lactam antibiotics have been sought (1, 20). In addition to being resistant to β-lactam antibiotics, methicillin-resistant *S. pseudintermedius* (MRSP) strains are increasingly resistant to other antibiotics. A recent multicenter study in Europe and North America showed that MRSP isolates are

commonly resistant to virtually all classes of antibiotics approved for use in dogs (19, 21). Six of the isolates in this study were MRSP strains, while two were methicillin susceptible. In methicillin-resistant *Staphylococcus* isolates from North America collected from 2006 to 2008, 17.7% (11/62) of *S. aureus* isolates carried inducible clindamycin resistance compared to 0% (0/46) of *S. pseudintermedius* isolates (22). In MRSP isolates from Europe and North America collected from 2004 to 2009, 1.9% (2/103) of isolates were positive for *ermB* and displayed inducible resistance to clindamycin (9). In the study described here, the differences in inducible clindamycin resistance could be attributed to either rapid changes in antimicrobial resistance patterns or geographic differences in the occurrence of inducible resistance.

Increased methicillin resistance and inducible clindamycin resistance in *S. pseudintermedius* has significant implications for ca-
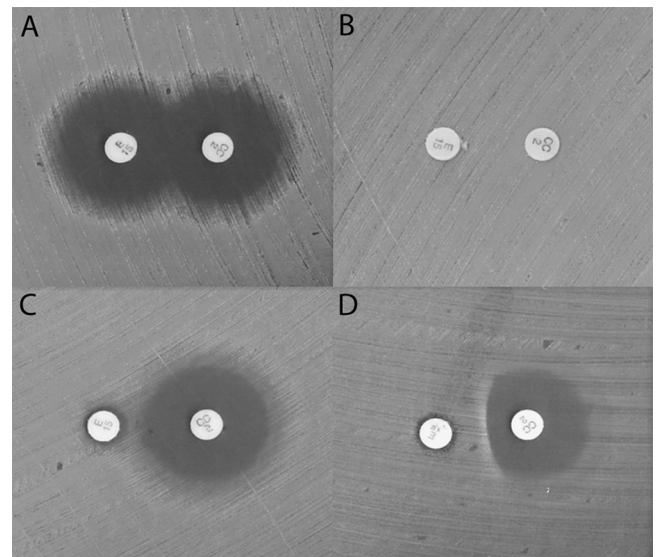

FIG 1 Disk diffusion testing for inducible clindamycin resistance of *Staphylococcus pseudintermedius* isolates. The disk labeled E15 contained 15 µg of erythromycin, and the disk labeled CC2 contained 2 µg of clindamycin. The disks are spaced 15 mm apart. (A) *Staphylococcus aureus* ATCC 25923, erythromycin and clindamycin susceptible, negative D-test; (B) *S. pseudintermedius* clinical isolate 11-012, erythromycin and clindamycin resistant, negative D-test; (C) *S. aureus* BAA-976, erythromycin resistant, clindamycin susceptible, negative D-test; (D) *S. aureus* BAA-977, erythromycin resistant, inducible clindamycin resistant, positive D-test.

nine and human health. While *S. pseudintermedius* infection in humans is relatively uncommon, zoonotic transmission of *S. pseudintermedius* to the owner of an infected pet or veterinary staff is a potential threat (18, 23). Recent studies have demonstrated that 5.3% of veterinary dermatologists and their technical staff carry MRSP and that owners of dogs with deep pyoderma can carry *S. pseudintermedius* strains identical to those carried by their infected pets (18, 24). Additionally, there is the potential for transmission of antimicrobial resistance genes from canine isolates of *S. pseudintermedius* to human isolates of *S. aureus* (25, 26).

For empirical, systemic treatment of canine pyoderma, amoxicillin-clavulanate and first-generation cephalosporins are the most common first-line drugs selected (2). With increased occurrence of antimicrobial resistance, clindamycin is recommended as an appropriate, alternative choice due to its favorable safety profile, clinical efficacy, and distribution into the skin (1, 2). Infections refractory to empirical therapy should be cultured and isolated bacteria tested for antimicrobial susceptibility. *S. pseudintermedius* isolates that are resistant to macrolides such as erythromycin but susceptible to clindamycin should be tested for the presence of inducible clindamycin resistance either by D-test or genetic testing. While the occurrence of such isolates is relatively low (1.32% [8/608] in our study), failure to detect these isolates can result in treatment failures in infected patients and associated increased patient morbidity and expense for clients. In two of the cases presented here, clindamycin was used for antibiotic therapy, resulting in treatment failure. Clinicians must recognize the potential for inducible clindamycin resistance and be able to recognize the potentially predictive pattern on antimicrobial susceptibility results. Performing the D-test is not a standard practice in all microbiology laboratories. The laboratory should be asked to perform this test whenever an *S. pseudintermedius* isolate is reported as susceptible (or intermediate) to clindamycin while resistant (or intermediate) to erythromycin with *in vitro* antimicrobial susceptibility tests (27).

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REFERENCES

1. **Frank LA, Loeffler A.** 2012. Meticillin-resistant *Staphylococcus pseudintermedius*: clinical challenge and treatment options. Vet. Dermatol. **23:** 283–291.
2. **Guardabassi L, Houser GA, Frank LA, Papich MG.** 2008. Guidelines for antimicrobial use in dogs and cats, p 183–206. *In* Guardabassi L, Jensen LB, Kruse H (ed), Guide to antimicrobial use in animals. Blackwell Publishing, Ltd., Ames, IA.
3. **Rich M, Deighton L, Roberts L.** 2005. Clindamycin-resistance in methicillin-resistant *Staphylococcus aureus* isolated from animals. Vet. Microbiol. **111:** 237–240.
4. **Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH.** 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. J. Clin. Microbiol. **41:** 4740–4744.
5. **Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:** 2562–2566.
6. **Fernandes CJ, O'Sullivan MV, Cai Y, Kong F, Zeng X, Gilbert GL, Kotsiou G.** 2007. Agar dilution method for detection of inducible clindamycin resistance in *Staphylococcus* spp. J. Clin. Microbiol. **45:** 4018–4020.
7. **Le Bouter A, Leclercq R, Cattoir V.** 2011. Molecular basis of resistance to macrolides, lincosamides and streptogramins in *Staphylococcus saprophyticus* clinical isolates. Int. J. Antimicrob. Agents **37:** 118–123.
8. **Khan SA, Nawaz MS, Khan AA, Cerniglia CE.** 1999. Simultaneous detection of erythromycin-resistant methylase genes *ermA* and *ermC* from *Staphylococcus* spp. by multiplex-PCR. Mol. Cell. Probes **13:** 381–387.
9. **Perreten V, Kadlec K, Schwarz S, Gronlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L.** 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J. Antimicrob. Chemother. **65:** 1145–1154.
10. **Woods CR.** 2009. Macrolide-inducible resistance to clindamycin and the D-test. Pediatr. Infect. Dis. J. **28:** 1115–1118.
11. **Leclercq R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. **34:** 482–492.
12. **Daurel C, Huet C, Dhalluin A, Bes M, Etienne J, Leclercq R.** 2008. Differences in potential for selection of clindamycin-resistant mutants between inducible *erm*(A) and *erm*(C) *Staphylococcus aureus* genes. J. Clin. Microbiol. **46:** 546–550.
13. **Jorgensen JH, Crawford SA, McElmeel ML, Fiebelkorn KR.** 2004. Detection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing. J. Clin. Microbiol. **42:** 1800–1802.
14. **Clinical and Laboratory Standards Institute.** 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard VET01-A4— 4th ed. CLSI, Wayne, PA.
15. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial disk susceptibility tests; approved standard M02-A11—11 ed, vol 32. CLSI, Wayne, PA.
16. **Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K.** 2010. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J. Clin. Microbiol. **48:** 765–769.
17. **Sutcliffe J, Tait-Kamradt A, Wondrack L.** 1996. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob. Agents Chemother. **40:** 1817–1824.
18. **Morris DO, Boston RC, O'Shea K, Rankin SC.** 2010. The prevalence of carriage of meticillin-resistant staphylococci by veterinary dermatology practice staff and their respective pets. Vet. Dermatol. **21:** 400–407.
19. **Moodley A, Stegger M, Ben Zakour NL, Fitzgerald JR, Guardabassi L.** 2009. Tandem repeat sequence analysis of staphylococcal protein A (*spa*) gene in methicillin-resistant *Staphylococcus pseudintermedius*. Vet. Microbiol. **135:** 320–326.
20. **Papich MG.** 2012. Selection of antibiotics for meticillin-resistant *Staphylococcus pseudintermedius*: time to revisit some old drugs? Vet. Dermatol. **23:** 352–360.
21. **Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach GF, Kreienbrock L, Schwarz S, Simon D, Nolte I.** 2011. Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. Vet. Microbiol. **150:** 191–197.
22. **Faires MC, Gard S, Aucoin D, Weese JS.** 2009. Inducible clindamycin-resistance in methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs and cats. Vet. Microbiol. **139:** 419–420.
23. **Vincze S, Paasch A, Walther B, Ruscher C, Lubke-Becker A, Wieler LH, Kohn B.** 2010. Multidrug-and methicillin resistant *Staphylococcus pseudintermedius* as a cause of canine pyoderma: a case report. Berl. Münch. Tierärztl. Wochenschr. **123:** 353–358.

24. **Guardabassi L, Loeber ME, Jacobson A.** 2004. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. Vet. Microbiol. **98:**23–27.

25. **Ryffel C, Tesch W, Birch-Machin I, Reynolds PE, Barberis-Maino L, Kayser FH, Berger-Bachi B.** 1990. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Gene **94:**137–138.

26. **Wisplinghoff H, Rosato AE, Enright MC, Noto M, Craig W, Archer GL.** 2003. Related clones containing SCC*mec* type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. Antimicrob. Agents Chemother. **47:**3574–3579.

27. **Rubin JE, Ball KR, Chirino-Trejo M.** 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. Can. Vet. J. **52:**153–157.