Title: Incidence of inducible clindamycin resistance in *Staphylococcus pseudintermedius* from dogs

Running title: Inducible clindamycin resistance in *S. pseudintermedius*

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ABSTRACT

Clindamycin is increasingly used to treat canine pyoderma. Eight of 608 *Staphylococcus pseudintermedius* isolates were positive for inducible clindamycin resistance by D-test 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.

Keywords

*Staphylococcus pseudintermedius*

Inducible *ermB* resistance

Clindamycin

Erythromycin

Increased prevalence of methicillin resistance and multi-drug resistance in *Staphylococcus pseudintermedius* has resulted in greater use of clindamycin to treat canine pyoderma because of its perceived clinical efficacy and good distribution to the skin (1, 2). Clindamycin is a
lincomamide 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 macrolides, lincosamides, and streptogramin B antibiotics (MLS\textsubscript{B} phenotype) can occur through acquisition of a methylase enzyme that removes a methyl group from an adenine residue in the 23S ribosomal RNA 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 \textit{msrA} gene also confers resistance to macrolides and streptogramin antibiotics but not lincosamides such as clindamycin (MS phenotype) (6).

Approximately forty \textit{erm} genes that encode methylases have been reported in different bacterial genera, with \textit{ermA}, \textit{ermB}, and \textit{ermC} the most commonly found among staphylococci (7). In \textit{S. aureus}, \textit{ermA} and \textit{ermC} confer erythromycin resistance in 94-98\% of isolates (8). In \textit{S. pseudintermedius}, \textit{ermB} is primarily responsible for MLS\textsubscript{B} resistance, but its expression can be constitutive or inducible (9). Detailed descriptions of the mechanisms of \textit{erm} gene expression and mutations leading to constitutive MLS\textsubscript{B} resistance have been previously published (10, 11). Mutation in the macrolide-inducible DNA sequence preceding \textit{ermB} genes can alter resistance from inducible to constitutive (10). These mutations occur at a rate of about one in every $2 \times 10^6$ 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\textsubscript{B} resistance and strains carrying the mutation will dominate strain within the
bacterial population at the site of infection, particularly in the presence of antimicrobial selection pressure (10). Therefore, if bacteria carrying inducible MLS\textsubscript{B} resistance are present in an infection, mutations may result in constitutive MLS\textsubscript{B} resistance leading to treatment failure. Routine antimicrobial susceptibility testing can detect constitutive MLS\textsubscript{B} resistance but fails to detect inducible resistance (13). Inducible clindamycin resistance should be suspected in isolates that are erythromycin-resistant but clindamycin-susceptible on \textit{in vitro} antimicrobial susceptibility testing. In this study we evaluated the frequency of inducible clindamycin resistance in \textit{S. pseudintermedius} from patients presented to the Texas A&M Veterinary Medical Teaching Hospital (VMTH) by double disk diffusion testing (“D-test”) for inducible clindamycin resistance and the presence of \textit{ermB} by polymerase chain reaction (PCR).

A total of 608 canine \textit{Staphylococcus pseudintermedius} isolates collected from the VMTH between 2007-2012 were screened for inducible clindamycin resistance. At the time of initial collection, all isolates were presumptively identified as \textit{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 \textit{S. aureus} ATCC 43300, ATCC 25923, and ATCC 29213. Quality control strains for the D-test included \textit{S. aureus} BAA-977 and BAA-976. All quality control strains were obtained from the
American Type Culture Collection (ATCC) (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 \textit{ermB} PCR using a DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD) according to the manufacturer’s instructions for gram-positive bacteria. PCR amplification of a 639 base pair product specific for \textit{ermB} was performed using primers (5) and methods (17) previously described with an alteration in the annealing temperature to $46^\circ$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 \textit{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 pre-scrubbed surgical site for a torn cranial cruciate ligament repair. The remaining two isolates came from a blood culture and post-surgical 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 six 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 \textit{in vitro} tested positive for inducible clindamycin resistance by D-test and the presence of \textit{ermB} associated with MLS\textsubscript{B} resistance (Table 1, Fig. 1). Two of the isolates were methicillin-susceptible (25%) while the remaining six were methicillin-resistant (75%).

\textit{S. pseudintermedius} is the most common bacterial agent isolated from canine pyoderma and surgical and non-surgical 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 \textbeta-lactam antibiotics such as penicillins and cephalosporins. With increased prevalence of methicillin resistance, alternatives to \textbeta-lactam antibiotics have been sought (1, 20). In addition to being resistant to \textbeta-lactam antibiotics, methicillin-resistant \textit{S. pseudintermedius} (MRSP) strains are increasingly resistant to other antibiotics. A recent multi-center 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 \textit{Staphylococcus} from North America collected from 2006-2008, 17.7% (11/62) of \textit{S. aureus} isolates carried inducible clindamycin resistance vs. 0% (0/46) of \textit{S. pseudintermedius} isolates (22). In MRSP isolates from Europe and North America collected from 2004-2009 1.9% (2/103) of isolates were positive for \textit{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 canine 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 identical *S. pseudintermedius* strains 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 empiric, 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 empiric 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 a S.
pseudintermedius isolate is reported as susceptible (or intermediate) to clindamycin while
resistant (or intermediate) to erythromycin on in vitro antimicrobial susceptibility tests (27).

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FIGURES

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) ATCC 25923 *Staphylococcus aureus*, erythromycin- and clindamycin-susceptible, negative D-test. (B) *S. pseudintermedius* clinical isolate 11-012, erythromycin- and clindamycin-resistant, negative D-test; (C) BAA-976 *S. aureus*, erythromycin-resistant, clindamycin-susceptible, negative D-test; (D) BAA-977 *S. aureus*, erythromycin-resistant, inducible clindamycin-resistant, positive D-test.

TABLES

**Table 1:** Inducible clindamycin resistance, erythromycin resistance, and oxacillin resistance in *Staphylococcus pseudintermedius*.

<table>
<thead>
<tr>
<th>Isolate Number</th>
<th>MIC (µg/mL) with Interpretation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>D-test Result</th>
<th>PCR test for <em>ermB</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clindamycin</td>
<td>Erythromycin</td>
<td>Oxacillin</td>
</tr>
<tr>
<td>11-001</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>≥8 R</td>
</tr>
<tr>
<td>11-025</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>≥8 R</td>
</tr>
<tr>
<td>11-033</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>≥8 R</td>
</tr>
<tr>
<td>11-064</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>≥8 R</td>
</tr>
<tr>
<td>12-012</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>≤0.25 S</td>
</tr>
<tr>
<td>17-016</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>2 R</td>
</tr>
<tr>
<td>18-007</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>≥8 R</td>
</tr>
<tr>
<td>24-014</td>
<td>≤0.5 S</td>
<td>≥8 R</td>
<td>≤0.25 S</td>
</tr>
</tbody>
</table>

<sup>a</sup>Minimum Inhibitory Concentration (MIC) for clindamycin, erythromycin, and oxacillin.

Breakpoints for antimicrobials were from CLSI **VET01-A4** (15). Abbreviations for interpretations were 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).