Prevalence of Mupirocin Resistance in *Staphylococcus pseudintermedius*

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In the United States, veterinary use of mupirocin is primarily limited to the treatment of canine pyoderma caused by methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). In this study, only 1 of 581 *S. pseudintermedius* isolates tested was resistant to mupirocin and carried the high-level mupirocin resistance gene, *ileS2*, on a plasmid.

*Staphylococcus pseudintermedius* is the primary bacterial pathogen isolated from canine pyoderma and also causes postsurgical infections in dogs (1, 2). Methicillin resistance and multidrug resistance are increasing in *S. pseudintermedius*, thus limiting the options for therapeutic treatment of canine skin infections (2). Mupirocin is a bacteriostatic antibiotic that reversibly binds to isoleucyl-tRNA synthetase to disrupt protein synthesis and is widely used to eliminate nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in human MRSA carriers (3). Mupirocin has been used on only a limited basis in veterinary medicine but is approved in the United States for the treatment of bacterial skin infections and superficial pyoderma in dogs (4).

In *S. aureus*, two levels of mupirocin resistance have been identified. Low-level mupirocin resistance occurs due to a point mutation to the chromosomal *ileS* gene that encodes the native isoleucyl-tRNA synthetase. The MIC for mupirocin for *Staphylococci* carrying the low-level resistance is ≥8 μg/ml but ≤256 μg/ml (5). Conversely, high-level mupirocin resistance (MIC of ≥512 μg/ml) is usually conferred by the plasmid-borne *ileS2*, although a chromosomal location of *ileS2* has been reported (5). Recently, *ileS2* plasmid-mediated mupirocin resistance was found in a mupirocin-resistant, methicillin-susceptible *S. pseudintermedius* strain isolated from a dog in Croatia (6). The goal of the present study was to determine the prevalence of mupirocin resistance in *S. pseudintermedius* isolated from patients presented to a veterinary hospital in Texas.

In this study, 581 isolates of *S. pseudintermedius* were screened for phenotypic low-level mupirocin resistance. Isolates were collected from veterinary patients, predominantly dogs (*n* = 446), but also included isolates from cats (*n* = 9). Some patients were cultured at multiple sites and contributed more than one isolate, and of these, 21 patients contributed more than two isolates. The isolates included a historical collection of 403 isolates from clinical infections and contained both methicillin-resistant *S. pseudintermedius* (MRSP) isolates (*n* = 153) and methicillin-susceptible *S. pseudintermedius* (MSSP) isolates (*n* = 250). The isolates from clinical infections were collected from the following anatomic sites: skin (*n* = 96), external ear canal (*n* = 31), wounds (*n* = 79), postoperative infections (*n* = 33), urine or the urinary tract (*n* = 87), and other sources (*n* = 77). Additional isolates were collected during a study of MRSP prevalence in canine patients without clinical staphylococcal infection that presented for elective orthopedic procedures. The MRSP prevalence study yielded 178 *S. pseudintermedius* isolates (13 MRSP and 165 MSSP isolates) collected from the nares or perineum of 129 dogs.

All isolates were presumptively identified as *S. pseudinterme-

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five colonies isolated from the mupirocin plate were used for colony PCR. Reactions were run in a 2720 thermal cycler (Applied Biosystems, Grand Island, NY) using the following settings: 95°C for 5 min; 35 cycles of 95°C for 30 s, 57°C for 40 s, and 72°C for 60 s; then 72°C for 10 min; and then a hold at 4°C. Negative controls included water with no-template DNA and DNA from a S. aureus strain (ATCC 29213). No positive control was available. The products for concentration and quality using a NanoDrop spectrometer (Thermo Scientific, Waltham, MA) prior to PCR testing. PCR was performed to identify the native ileS gene using the previously published molecular classification system (11). The primers IS257R and IS257F (Table 1) were used in various combinations as previously described under the following conditions: 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 40 s, and 72°C for 60 s; then 72°C for 10 min; and then a hold at 4°C (11). PCR was performed to identify the native ileS gene using the primers ileS-F1 and ileS-R1 (Table 1) (6). Conditions for the thermal cycler were as follows: 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 40 s, 55°C for 30 s, 72°C for 60 s; then 72°C for 7 min; and then a hold at 4°C.

PCR products were purified using either the QIAprep Spin Miniprep plasmid purification kit according to the manufacturer’s instructions (Qiagen, Valencia, CA). Samples were evaluated for concentration and quality using a NanoDrop spectrometer (Thermo Scientific, Waltham, MA) prior to PCR testing. PCR was used to evaluate the IS257-ileS2 spacer regions using a previously published molecular classification system (11). The primers IS257R, ileS2-5’, ileS2-3’, and IS257F (Table 1) were used in various combinations as previously described under the following conditions: 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 40 s, and 72°C for 60 s; then 72°C for 10 min; and then a hold at 4°C (11). PCR was performed to identify the native ileS gene using the primers ileS-F1 and ileS-R1 (Table 1) (6). Conditions for the thermal cycler were as follows: 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 40 s, 55°C for 30 s, 72°C for 60 s; then 72°C for 7 min; and then a hold at 4°C.

PCR products were purified using either the QIAprep gel purification kit (Qiagen, Valencia, CA) or the Zymoclean gel DNA recovery kit (Zymo Research, Irvine, CA) according to the manufacturers’ protocols. Purified PCR products were then cloned into pT7Blue kit (EMD Chemicals, Inc., Darmstadt, Germany) according to the manufacturer’s protocol. Resultant plasmids containing the up- and down-stream IS257-ileS2 junction, the downstream ileS2-IS257 junction, and the 945-bp fragment of the native ileS gene were submitted to the Texas A&M Gene Technologies Laboratory for sequencing. Resultant sequences were compared to sequences in GenBank (JX186508, JX186509, JX186511, JX186512, JX186513, and JX186514) using MEGA5.1 software (6, 12). Of the 581 isolates tested, only one isolate was resistant to mupirocin. The isolate, 39-045, was originally cultured from the nares of a healthy, 1-year-old, castrated, male, Bernese mountain dog presenting for an orthopedic evaluation. This isolate was pan-susceptible to all antimicrobials tested using the COMPAN2F drug panel and negative for the presence of the mecA gene via PCR analysis. The prevalence of mupirocin resistance in dogs without clinical staphylococcal infections that presented for elective orthopedic procedures was 1 in 129, or 0.8%. An additional 194 S. pseudintermedius isolates were collected from 158 dogs with clinical infections during the same period of collection (22 September 2010 to 8 February 2012), resulting in a total of 372 S. pseudintermedius isolates from 287 dogs. The prevalence of mupirocin-resistant S. pseudintermedius in dogs cultured between 22 September 2010 and 8 February 2012 was therefore 1 in 287 dogs, or 0.3%.

The mupirocin-resistant isolate was analyzed for the presence of high-level mupirocin resistance by plasmid DNA isolation followed by PCR amplification of two different regions of the plasmid-borne ileS2 gene. The presence of a 458-bp band with mupA and mupB primers and a 237-bp band with M1 and M2 primers indicates that the isolate contains the ileS2 gene (Fig. 1).

To further determine the structural type of the plasmid, PCR was performed to determine whether isolate 39-045 had both an ileS mutation and the ileS2 plasmid simultaneously. PCR amplification of the chromosomal ileS gene was also performed using previously reported 39-045S and 39-045M primers (9). Lanes 4 to 6 include PCR products amplified with mupA and mupB primers (9). Lanes 4 to 6 include PCR products amplified with M1 and M2 primers (10). The molecular size marker used in lanes 1 and 8 was a 100-bp DNA ladder (Invitrogen, Grand Island, NY). Numbers at left are molecular sizes in bp. Template DNA used for PCR was plasmid DNA from isolate 39-045 (lanes 2 and 3) or genomic DNA from ATCC 29213 (lanes 3 and 6). Water was substituted for DNA in lanes 4 and 7.

### TABLE 1 Primers used in this study

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ileS2</td>
<td>mupA</td>
<td>TATATTATGAGATTGGAGTGG</td>
<td>9</td>
</tr>
<tr>
<td>ileS2</td>
<td>mupB</td>
<td>AATTAAACGCTGGAAAGGTTG</td>
<td>9</td>
</tr>
<tr>
<td>ileS2</td>
<td>M1</td>
<td>GTTTATCTGTGATGTGAGG</td>
<td>10</td>
</tr>
<tr>
<td>ileS2</td>
<td>M2</td>
<td>CCCAGATTAGACCCGATATA</td>
<td>10</td>
</tr>
<tr>
<td>IS257-ileS2 junctions</td>
<td>IS257R</td>
<td>GGGATGCGGAAATAATCGTAG</td>
<td>11</td>
</tr>
<tr>
<td>IS257-ileS2 junctions</td>
<td>IS257F</td>
<td>TGCGTTATGGTAGACGGTACATC</td>
<td>11</td>
</tr>
<tr>
<td>ileS</td>
<td>ileS-F1</td>
<td>CGTGACCGTGGCGAATGGGT</td>
<td>6</td>
</tr>
<tr>
<td>ileS</td>
<td>ileS-R1</td>
<td>GTATGGCGGATGTTGCGGG</td>
<td>6</td>
</tr>
<tr>
<td>mecA</td>
<td>mecA-F</td>
<td>CTACAGTTACTGCTATCCACC</td>
<td>7</td>
</tr>
<tr>
<td>mecA</td>
<td>mecA-R</td>
<td>CACTTGTTATATCATCCACC</td>
<td>7</td>
</tr>
</tbody>
</table>

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**FIG 1 Detection of ileS2 using PCR.** Lanes 1 to 3 include PCR products amplified with mupA and mupB primers (9). Lanes 4 to 6 include PCR products amplified with M1 and M2 primers (10). The molecular size marker used in lanes 1 and 8 was a 100-bp DNA ladder (Invitrogen, Grand Island, NY). Numbers at left are molecular sizes in bp. Template DNA used for PCR was plasmid DNA from isolate 39-045 (lanes 2 and 3) or genomic DNA from ATCC 29213 (lanes 3 and 6). Water was substituted for DNA in lanes 4 and 7.
published primers (6). The resultant 945-bp product was sequenced and analyzed using MEGA5.1 software, and the sequence was deposited in GenBank as KJ000544, KJ000545, KJ000546, and KJ000547.

Nucleotide sequence accession numbers. Sequences from this study were deposited in GenBank as KJ000544, KJ000545, KJ000546, and KJ000547.

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