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.

*S. 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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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>
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<tbody>
<tr>
<td>ileS2</td>
<td>mupA</td>
<td>TATATTATGGGATGGAGGGTGG</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>mupB</td>
<td>AAATATTATGGGATGGAGGGTGG</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>GTTATCTCAGTGATCTGGGAGGC</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>CCCAGGTTACACGGCTATATTA</td>
<td>10</td>
</tr>
<tr>
<td>IS257-ileS2 junctions</td>
<td>IS257R</td>
<td>GCGATGGCGGAAATAGCGTACGGATGAG</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>IS257L</td>
<td>TGGGCGAATCGAGCATCGATTCACTGAA</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>ileS2-3′</td>
<td>TGGGCGAATCGAGCATCGATTCACTGAA</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>ileS2-5′</td>
<td>CGATGCGAATCGAGCATCGATTCACTGAA</td>
<td>11</td>
</tr>
<tr>
<td>ileS</td>
<td>ileS-F1</td>
<td>CGTGAGCGGCTGGCAGAGGTTGGT</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>ileS-R1</td>
<td>GTATGGGAAATATATTCTTCCACC</td>
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</tr>
<tr>
<td>mecA</td>
<td>mecA-F</td>
<td>CTCAAGGTACTGCTATCCACC</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>mecA-R</td>
<td>CACTTGTATATATATCCACC</td>
<td>7</td>
</tr>
</tbody>
</table>

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 for the IS257-ileS2 spacer regions was performed according to a previously published molecular classification system (11). The fragments are similar to the amplification for structural group S2 ileS2 plasmids found in S. aureus pattern II, with bands sized between 1,000 bp and 1,650 bp for primers ileS2-3′ and IS257L, and between 2,000 and 3,054 bp for primers IS257R and ileS2-5′ (Fig. 2). This structural group is similar to the structure previously reported for the plasmid-borne ileS2 gene identified in S. pseudintermedius isolated from a dog with pyoderma in Croatia (6). The resultant PCR products were sequenced and compared to the previously published ileS2 sequences from S. pseudintermedius, JX186508 and JX186509 (6). Sequences from this study were deposited in GenBank as KJ000545, KJ000546, and KJ000547. Comparison of JX186509 with KJ000545 using MEGAS.1 software indicated 99% similarity between the two sequences. Comparison of JX186508 with KJ000546 and KJ000547 indicated 100% and 99% similarity between the sequences, respectively.

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 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%.

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 5) 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. Accession numbers.

In summary, this study found that the prevalence of mupirocin resistance in *S. pseudintermedius* isolated from dogs was 0.3% (1/287) or 0.8% (1/129) in healthy dogs without active, clinical staphylococcal infections. While no mupirocin-resistant isolates were found in our collection of isolates from dogs with clinical disease, the presence of plasmid-mediated mupirocin resistance is of concern as previous work has demonstrated that mupirocin resistance can be transmitted from one species to another in vivo (13). Increased rates of methicillin resistance and multidrug resistance in *S. pseudintermedius* and approval of mupirocin for use in dogs have made mupirocin an attractive alternative for topical use in canine pyoderma (2). This could result in increased mupirocin resistance in *S. pseudintermedius* over time. Although our study found only one mupirocin-resistant *S. pseudintermedius* isolate, 36.5% of U.S. households own a dog (14), and there is the potential for transmission of mupirocin resistance from canine isolates of *S. pseudintermedius* to human isolates of *S. aureus* or vice versa. This could have implications for public health. For these reasons, mupirocin resistance should be monitored and carefully considered before mupirocin is used in canine patients.

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

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