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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 $\geq$8 μg/ml but $\leq$256 μg/ml (5). Conversely, high-level mupirocin resistance (MIC of $\geq$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. pseudintermedius* at the time of collection based on Gram stain reaction, colony color, and biochemical tests. Tests measured the ability of the isolates to produce hemolysis on Trypticase soy agar supplemented with 5% sheep blood agar (blood agar plates) (BD Diagnostic Systems, Sparks, MD), to produce coagulase, to produce catalase, and to grow on salt-mannitol agar. Isolates were also tested for resistance to polymyxin B, ability to utilize trehalose, and production of urease. At the time of initial collection, isolates were tested for antimicrobial susceptibility using commercially available systems (GPS card, Vitek; bioMérieux, Durham, NC; COMPAN1F and COMPAN2F panels, Trek Sensititre; Trek Diagnostics, Cleveland, OH) and additionally tested for methicillin resistance by oxacillin disk diffusion testing and PCR for the presence of *mecA*. The *mecA* PCR used a previously published protocol with methicillin-resistant *S. aureus* (ATCC 33591) and methicillin-susceptible *S. aureus* (ATCC 29213 or ATCC 25923) as positive and negative controls, respectively (7). Isolates were stored frozen in 10% glycerol at −80°C in 96-well deep-well plates and later inoculated aseptically using a 96-pin replicator onto Mueller-Hinton agar (BD Diagnostic Systems) and onto Mueller-Hinton agar supplemented with 8 μg/ml mupirocin (Sigma-Aldrich, St. Louis, MO) (here referred to as the mupirocin plate) to screen for low-level resistance to mupirocin. *Pseudomonas aeruginosa* (ATCC 27853) was used as a positive control for mupirocin resistance (8). The bacterial concentrations were not standardized prior to screening. Plates were incubated for 24 h at 37°C and then examined.

Colonies were screened by PCR for the presence of the *ileS2* gene using the previously published primers *mupA* and *mupB* to amplify a 458-bp fragment of the *ileS2* gene (9) and primers M1 and M2 to amplify a 237-bp fragment of the gene (Table 1) (10). A total reaction volume of 50 μl was used with the final concentrations of reagents as follows: 2.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (dNTPs), 2.5 pmol of each primer, and 2.5 U *Taq* polymerase per reaction (Lucigen, Middleton, WI). Three to

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TABLE 1 Primers used in this study

Target gene	Primer	Sequence (5′ to 3′)	Reference
ileS2	*mupA*	TATATTATGCGATGGAAGGTTGG	9
	mupB	AATAAAATCAGCTGGAAAGTGTTG	9
	M1	GTTTATCTTCTGATGCTGAG	10
	M2	CCCCAGTTACACCGATATAA	10
IS*257*-*ileS2* junctions	IS257$_{\mathrm{F}}$	GGCATGGCGAAAATCCGTAG	11
	IS257$_{\mathrm{R}}$	TGGCGTATTGATGAGACGTACATC	11
	ileS2-3′	TCGGTGTAACTGGGGAATTA	11
	ileS2-5′	CCATGTCAACCCAGTATCC	11
ileS	*ileS*-F1	CGTGACCGTGGCGAATGGGT	6
	ileS-R1	GTATGCGGAATGATTGGCG	6
mecA	*mecA* F	CTCAGGTACTGCTATCCACC	7
	mecA R	CACTTGGTATATCTTCACC	7

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 30 s, and 72°C for 30 s, followed by 72°C for 7 min; and then a hold at 4°C. Negative controls included water with no-template DNA and DNA from a known mupirocin-sensitive, methicillin-sensitive *S. aureus* strain (ATCC 29213). No positive control was available. The products were then run on a 2% agarose gel for 2 h at 70 V, visualized with GelRed (Phenix Research, Candler, NC), and compared to a 100-bp molecular size marker (Invitrogen, Grand Island, NY).

Plasmid purification was performed using 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 IS*257*-*ileS2* spacer regions using a previously published molecular classification system (11). The primers IS257$_{\mathrm{F}}$, *ileS2*-5′, *ileS2*-3′, and IS257$_{\mathrm{R}}$ (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, 55°C for 30 s, 72°C for 60 s; then 72°C for 7 min; and 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 plasmid vector using the Novagen pT7Blue Perfectly Blunt cloning kit (EMD Chemicals, Inc., Darmstadt, Germany) according to the manufacturer's protocol. Resultant plasmids containing the upstream IS*257*-*ileS2* junction, the downstream *ileS2*-IS*257* 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 pansusceptible 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 for the IS*257*-*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 IS257$_{\mathrm{F}}$ and between 2,000 and 3,054 bp for primers IS257$_{\mathrm{R}}$ 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 MEGA5.1 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

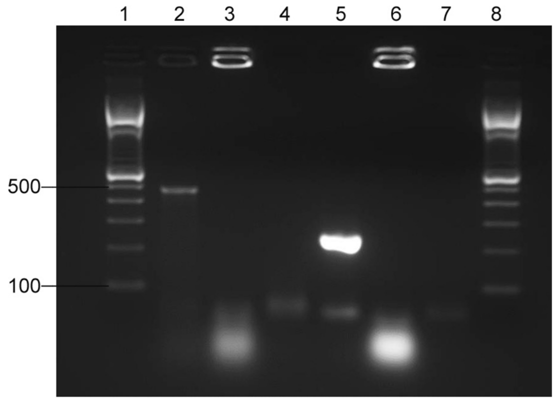

FIG 1 Detection of *ileS2* using PCR. Lanes 1 to 3 include PCR products amplified with *mupA* and *mupB* primers (9). Lanes 4 to 6 include PCR products amplified with M1 and M2 primers (10). The molecular size marker used in lanes 1 and 8 was a 100-bp DNA ladder (Invitrogen, Grand Island, NY). Numbers 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.

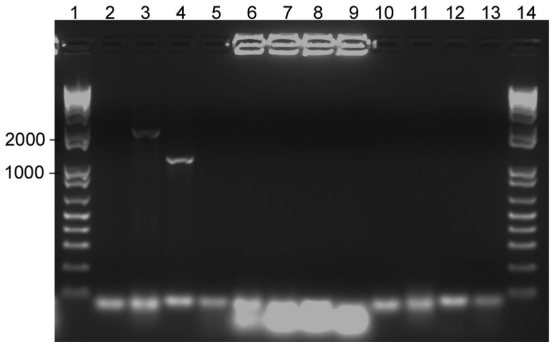

FIG 2 Detection of the *ileS2*-IS*257* junctions in isolate 39-045 using *ileS2*-5′, IS257$_{\mathrm{F}}$, *ileS2*-3′, and IS257$_{\mathrm{R}}$ primers (11). Primer pairs for each reaction were as follows: PCR 1, *ileS2*-5′ and IS257$_{\mathrm{F}}$; PCR 2, *ileS2*-5′ and IS257$_{\mathrm{R}}$; PCR 3, *ileS2*-3′ and IS257$_{\mathrm{F}}$; and PCR 4, *ileS2*-3′ and IS257$_{\mathrm{R}}$. Lanes 2, 6, and 10 are products from PCR 1; lanes 3, 7, and 11 are products from PCR 2; lanes 4, 8, and 12 are products from PCR 3; lanes 5, 9, and 13 are products from PCR 4; and lanes 1 and 14 are a 1-kb DNA ladder (Invitrogen, Grand Island, NY). Template DNA in lanes 2 to 5 is plasmid DNA from isolate 39-045. Template DNA in lanes 6 to 9 is genomic DNA from ATCC 29213 used as a negative control. In lanes 10 to 13, water was substituted for template DNA as a negative control. No positive controls were available. Numbers at left are sizes in bases.

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. Analysis indicated a 99% similarity between isolate 39-045 and the previously published sequences of the *S. pseudintermedius* chromosomal *ileS* gene: JX186511, JX186512, JX186513, and JX186514 (6).

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 of *Staphylococcus* 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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