Mupirocin resistance in methicillin-resistant *Staphylococcus pseudintermedius* from canine and feline clinical samples

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**Introduction**

Mupirocin is one of effective topical antimicrobial agents for treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) decolonization or infection in human medicine (5). The drug arrests bacterial protein synthesis by inhibiting isoleucyl-tRNA synthetase (IleS) enzyme. Staphylococci developed mupirocin resistance (MupR) by mutation of *ileS*, which encodes IleS, presenting low-level MupR (Lo-MupR). Additionally, staphylococci may acquire *ileS2* gene on mobile genetic elements, and exhibit high-level MupR (Hi-MupR) by production of alternative IleS2 (12). The resistance causes failure of treatment and limits a choice of topical antimicrobial selection. (10). In veterinary medicine, mupirocin is recommended for topical treatment of methicillin-resistant *S. pseudintermedius* (MRSP) infections (8). Previous reports showed low prevalences of MupR in *S. pseudintermedius* (2, 7, 11). To reduce the irrational use of a systemic antimicrobial, mupirocin is one of topical drugs that are widely used for localized infections (8). Conversely, the inappropriate use of antimicrobial is a selective pressure of developing and contributing the spread of antimicrobial resistance bacteria. This study aimed to determine the rate of MupR in MRSP isolated from canine and feline clinical samples with naturally-occurring infections and to detect the presence of *ileS2* in MupR MRSP.

**Materials and Methods**

Clinical samples suspected bacterial infection were obtained from veterinary hospitals in Bangkok from April to December 2016 and submitted to Veterinary Diagnostic Laboratories, Chulalongkorn University for culture and antimicrobial susceptibility testing (AST). Bacterial species, and antimicrobial susceptibility profiles were phenotypically identified by VITEK® automated instrument using ID GP and AST-GP76 cards (bioMérieux, France). Isolates were kept in 10% glycerol freezing media at -80°C. MRSP were recovered on tryptic soy agar (Difco, France) with 5% sheep blood at 37°C for 24 h. The cultures were phenotypically confirmed the methicillin resistance by oxacillin disk diffusion test (3). Bacterial DNA was extracted using lysostaphin lysis (6) to further genotypic identification of resistance genes. *nuc* and *mecA* were amplified by PCR for the MRSP confirmation (13, 14). MupR was identified in MRSP by using 5-μg and 200-μg mupirocin disk diffusion assay on Müller-Hinton Agar (Difco, France). Isolates presenting no inhibition zone with 200-μg mupirocin were classified as Hi-MupR (4). *ileS2* was detected by PCR (4).

**Results and Discussion**

A total of 81 MRSP were isolated from dogs (n=78) and cats (n=3). Twenty-two MRSP, representing the rate of Hi-MupR 27.2%, exhibited no inhibition zone (0 mm) with both 5-μg and 200-μg mupirocin disks. Twenty isolates were from dogs with clinical lesions including: open wound (n=10), pyoderma (n=7), abscess (n=1), skin mass (n=1) and urinary bladder mucosa (n=1). The others were two feline isolates from open wound (n=1) and nasal discharge (n=1). All Hi-MupR isolates were positive to *nuc*, *mecA* and *ileS2* amplification and identified to be Hi-MupR MRSP. AST profiles of the Hi-MupR MRSP presented resistance to various antimicrobial classes (Table 1). No isolate was resistant to vancomycin, rifampicin and fusidic acid.

This study demonstrated a high rate of Hi-MupR in MRSP from diseased dogs and cats. In our previous study in 2014, 3.2% (2/64) MRSP isolated from canine carriage sites expressed Hi-MupR (2). Two studies reported MupR *S. pseudintermedius* clinical isolates from USA (1/129) and Croatia (1/106) (7, 11). Only one isolate of Hi-MupR MRSP from urinary tract infection, the history of mupirocin treatment is still questionable. Regarding the emergence of MRSP, mupirocin is more commonly used for treatment of staphylococcal infections in dogs without evidence support of infections or cultures and AST results. The excessive use might contribute the selective pressure for MupR MRSP population. To limit the spread of resistance, mupirocin should be rationally considered based on AST results and restricted only for multidrug-resistant (MDR) MRSP infections. The presence of
ileS2 confirmed the genetic determinant conferring Hi-Mup\textsuperscript{R}. MICs of mupirocin were not available from this routine diagnosis, but all isolates showed resistance by 200-\mu g mupirocin disk diffusion test, identified as Hi-Mup\textsuperscript{R}. The ileS2-carrying staphylococci generally express MIC of mupirocin \( \geq 512 \) \( \mu g/mL \). (12,7,11). This resistance phenotype frequently associated with mupirocin decolonization failure in human MRSA carrier (10). ileS2 gene is commonly located on a pSK41 multi-resistance plasmid in staphylococcal species which facilitate a horizontal spread. The insertion sequence (IS) 257 of the plasmid backbone promotes the resistance gene acquisition and developing of resistance (12). This gene was also detected in Thai canine MRSA and human MRSP isolated from nares (2). The \textit{in vivo} evidence of horizontal transfer of the resistance element from \textit{S. epidermidis} to \textit{S. aureus} was demonstrated (10), but this phenomenon has not been elucidated between \textit{S. aureus} and \textit{S. pseudintermedius}. Genetic analysis by means of plasmid and strain typing is useful for tracking the spreads of the resistance determinants and clones. Our further study will focused on molecular characteristics of the resistance elements and strains.

**Conclusion**

This study presents the increased Mup\textsuperscript{R} rate in MRSP from dogs and cats with the presence of ileS2. To promote appropriate use of mupirocin, prescription should be based on AST results for treatment of MDR MRSP infection. Continued monitoring and molecular typing could reflect the changes of resistance population for control the spread.

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**References**

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**Table 1** Origin, Number of isolates and antimicrobial susceptibility profile of high-level mupirocin-resistant MRSP

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of isolate</th>
<th>Antimicrobial resistance profile\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
<tr>
<td>Cat 1</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
<tr>
<td>Dog 2</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
<tr>
<td>Dog 4</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
<tr>
<td>Dog 6</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
<tr>
<td>Dog 1</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
<tr>
<td>Dog 5</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
<tr>
<td>Total (n=22)</td>
<td>OXA PEN TET ENR MAR ERY GEN CLI CHL SXT NIT VAN RIF FUS</td>
<td>22 22 22 22 22 19 15 15 21 1 0 0 0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}shading, intermediate or resistance; OXA, oxacillin; PEN, penicillin; TET, tetracycline; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; CLI, clindamycin; CHL, chloramphenicol; SXT, sulfamethoxazole/trimethoprim; NIT, nitrofurantoin; VAN, vancomycin; RIF, rifampicin; FUS, fusidic acid