In vitro activity of fusidic acid and mupirocin against coagulase-positive staphylococci from pets

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Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) and multiresistant Staphylococcus pseudintermedius (MRSP) have emerged as important pathogens in animal infections. Associated therapeutic problems and the zoonotic potential of staphylococci have renewed interest in topical antibiotics for treatment and carrier decolonization. Fusidic acid and mupirocin are used topically in humans and animals but resistant strains isolated from people are increasing. This study investigates the in vitro activity of fusidic acid and mupirocin against coagulase-positive staphylococci from pets.

Methods: A collection of 287 staphylococci was examined, comprising 102 MRSA, 102 methicillin-susceptible S. aureus, 71 S. pseudintermedius and 12 MRSP from canine and feline infections and carrier sites isolated in the UK and Germany. MICs were determined by the agar dilution method according to CLSI (formerly NCCLS) standards.

Results: The majority (89.7%) of all MICs were ≤0.25 mg/L. High MICs were observed for seven MRSA isolates (five with an MIC of fusidic acid of 512 mg/L, one with an MIC of fusidic acid of 1024 mg/L and one with with an MIC of mupirocin of 16 mg/L). MICs of both antibiotics were ≤2 mg/L for all MRSP. Infection isolates had higher MICs than those isolated from carriage sites for both antibiotics (P < 0.001).

Conclusions: In all but seven MRSA isolates, MICs were below the concentrations achievable experimentally at application sites suggesting therapeutic efficacy of both antibiotics in infections involving multiresistant staphylococci and for decolonization of carriers. However, the seven MRSA with high MICs, all of the dominant UK human hospital lineages, highlight the importance of monitoring treatment success as resistant strains may occur in animals.

Keywords: MRSA, multiresistant, Staphylococcus pseudintermedius, dog, cat

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) and multiresistant Staphylococcus pseudintermedius (MRSP) (previously Staphylococcus intermedius) have emerged as important pathogens in pets.¹² MRSP in particular are often resistant to all antimicrobial products licensed for systemic use in pets posing a significant therapeutic problem, often with topical antimicrobial therapy as the best therapeutic option at least for surface infections.³

Fusidic acid and mupirocin are antibiotics with proven efficacy against coagulase-positive staphylococci and both are available for topical therapy. In humans, fusidic acid (Fucidin®; LEO Pharma, Ballerup, Denmark) and mupirocin (Bactroban®; GSK, Uxbridge, UK) preparations have an indication for the treatment of staphylococcal skin infections such as impetigo, the latter with a recommendation to reserve it for eradication of MRSA from carrier sites (decolonization); fusidic acid is also used systemically. In animals, fusidic acid is authorized for use in dogs and cats in several countries including the UK for the treatment of bacterial infections for skin, ear or eye infections (Fuciderm™ Gel, Canaural™, Fucithalmic™, VetXX, Thame, UK). In the USA, mupirocin is available as ointment for use in dogs (Bactoderm®, GSK, NC, USA). There is increasing concern about reduced efficacy of fusidic acid and mupirocin in humans. A reported increase in fusidic...
acid-resistant European epidemic *S. aureus* clones over the past 10 years, and the emergence of mupirocin-resistant methicillin-susceptible *S. aureus* (MSSA) and MRSA in humans and animals typically relate to MICs above 8 mg/L for low-level resistance and above 256 mg/L for high-level resistance. To date, there are no officially accepted breakpoint standards for these two antibiotics used topically, and evidence for clinically relevant resistance with topical use is lacking or inconsistent. High fusidic acid MICs have been recognized among individual *S. intermedius* from animals in Scandinavia but their clinical significance remains unknown.

This report describes the MICs of fusidic acid and mupirocin for a collection of coagulase-positive staphylococci, including MRSA and MRSP, isolated from pets.

### Materials and methods

#### Bacterial isolates

Two-hundred and eighty-seven coagulase-positive staphylococcal isolates collected from dogs and cats between September 2005 and January 2008 were included (102 MRSA, 102 MSSA, 71 non-multidrug-resistant *S. pseudintermedius* (SP) and 12 MRSP). The 11 canine and 1 feline MRSP had been identified by one dermatology referral clinic in Northern Germany, all other staphylococci originated from veterinary clinics all over Britain (126 canine and 69 feline infections, 80 canine mucosal carrier sites). Isolates were grown on sheep blood agar (Oxoid, Basingstoke, UK) from a collection frozen at −80°C and re-identified based on morphological and biochemical assessments as described previously, including Voges–Proskauer reaction, acid production from trehalose, lactose, β-gentiobiose and D-maltose, fermentation of D-mannitol and API ID32-STAPH (bioMérieux; Marcy l’Étoile, France). Staphylococcal species were confirmed by determining the nature of a species-specific thermonuclease gene (*nuc*). All presumed MRSA and MRSP isolates were verified from subcultures on mannitol salt agar (Oxoid) supplemented with 6 mg/L oxacillin (Sigma-Aldrich Inc., St Louis, MO, USA) by disc diffusion tests on Mueller–Hinton agar and by demonstration of the *mecA* gene. All MRSA isolates had been typed previously as representatives of EMRSA-15 (*n* = 100) or EMRSA-16 (*n* = 2).

#### MICs

MICs were determined by the agar dilution method incorporating serial doubling concentrations (from 0.03125 to 1024 mg/L) of fusidic acid (Sigma-Aldrich Inc.) and mupirocin (Mast Diagnostics, Bootle, UK) in Mueller–Hinton agar (Oxoid) according to CLSI (formerly NCCLS) standards. Reference strains were *S. aureus* ATCC 29213 and NCTC 6571.

#### Statistical analysis

The Mann–Whitney test was used for statistical comparison (*P* < 0.05 considered significant) and the Spearman rank correlation coefficient was used to investigate associations between MICs and lineages.

#### Results

Of the 574 MIC values of either fusidic acid or mupirocin, 515 (89.7%) were ≤/C20 0.25 mg/L. MIC frequencies and the MIC50 and MIC90 of fusidic acid and mupirocin for the four staphylococcal groups are shown in Tables 1 and 2. High MICs (≥16 mg/L) were more frequent for MRSP than for MRSA or MSSA.

### Table 1. MICs of fusidic acid for 287 coagulase-positive staphylococcal isolates from dogs and cats

| Staphylococci | 0.0625 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | MIC50 (mg/L) | MIC90 (mg/L) |
|---------------|--------|-------|------|-----|---|---|---|---|----|----|----|-----|------|-----|-----|-----|----------|----------|
| MRSA (n = 102) | 3 | 66 | 21 | — | 1 | 1 | 2 | 2 | — | — | — | — | 5 | 1 | 0.125 | 2 |
| MSSA (n = 102) | 2 | 61 | 25 | 4 | — | 1 | 3 | — | — | — | — | — | — | — | 0.25 | 0.5 |
| SP (n = 71) | 12 | 50 | — | — | — | 4 | 5 | — | — | — | — | — | — | — | 0.125 | 4 |
| MRSP (n = 12) | 6 | 3 | 1 | — | 1 | 1 | — | — | — | — | — | — | — | 0.0625 | 1 |

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; SP, non-multidrug-resistant *S. pseudintermedius*; MRSP, multiresistant, *mecA*-positive *S. pseudintermedius*.

### Table 2. MICs of mupirocin for 287 coagulase-positive staphylococcal isolates from dogs and cats

<table>
<thead>
<tr>
<th>Staphylococci</th>
<th>0.0625</th>
<th>0.125</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>MIC50 (mg/L)</th>
<th>MIC90 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (n = 102)</td>
<td>—</td>
<td>9</td>
<td>80</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>MSSA (n = 102)</td>
<td>5</td>
<td>17</td>
<td>74</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>SP (n = 71)</td>
<td>57</td>
<td>8</td>
<td>4</td>
<td>—</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>0.0625</td>
<td>0.125</td>
</tr>
<tr>
<td>MRSP (n = 12)</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.0625</td>
<td>0.25</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; SP, non-multidrug-resistant *S. pseudintermedius*; MRSP, multiresistant, *mecA*-positive *S. pseudintermedius*. 

1302
Fusidic acid and mupirocin against staphylococci from pets

were observed for seven MRSA isolates (four canine and three feline infections) and all belonged to the EMRSA-15 lineage.

MICs were significantly higher for S. aureus isolates \((n = 204)\) compared with the S. pseudintermedius isolates \((n = 83)\) \((P < 0.001\) for both antibiotics) but there was no difference between MRSA and MSSA or between MRSP and SP. The MICs were low among the 12 MRSP with 2 mg/L fusidic acid as the highest value. MICs for the two reference MSSA strains were 0.125 mg/L fusidic acid and 0.25 mg/L mupirocin.

Infection isolates showed higher MICs than those isolated from carriage sites for both antibiotics \((P = 0.001\) for fusidic acid; \(P < 0.001\) for mupirocin). Among all staphylococci, there was a trend for feline isolates \((n = 70)\) to have higher MICs than canine isolates \((n = 217)\), but this was statistically significant only for mupirocin \((\text{fusidic acid}, P = 0.072; \text{mupirocin}, P < 0.001)\). There was no correlation between MICs and S. aureus lineage.

Discussion

Few studies report MICs of fusidic acid and mupirocin for S. aureus and S. pseudintermedius from dogs and cats, and most reported ranges were between 0.015 and 0.5 mg/L.\(^7\,11,12\) The results from this study are in line with findings from the literature for the majority of isolates \((89.7\%\) of all MICs \(\leq 0.25\) mg/L) and such values have previously been associated with susceptibility in clinical studies.\(^12\) However, with lineages of MRSA and MRSP, and antimicrobial usage patterns differing between countries, conclusions from the results presented should be related to their origin in Britain and Germany.

For MRSA, six isolates \((5.9\%)\) required fusidic acid concentrations above 256 mg/L. As all six were representatives of the dominant UK human hospital-associated clone, represented by EMRSA-15, original transfer of these organisms from humans to pets is highly likely. However, transfer can occur in both directions between hosts, and animals can act as a reservoir for resistant organisms. Those six MICs being consistent with high-level resistance as defined for systemic use of fusidic acid, such isolates may have implications for human health. With topical application though, these higher MICs may still be exceeded leaving topical therapy as a valuable treatment alternative for superficial infections due to multi resistant staphylococci. However, in the absence of clinical studies supporting this hypothesis, careful monitoring of treatment success is important to avoid unnecessary antibiotic use.

The same applies to topical antimicrobial therapy for decolonization of MRSA or MRSP carrier animals. This may be indicated to prevent both animal re-infection and zoonotic transfer of multi resistant staphylococci to susceptible humans in contact. Systemic therapy alone may not eradicate MRSA from carrier sites as indicated by the persistence of staphylococci on healthy skin and mucosae after successful treatment of superficial pyoderma with cefpodoxime.\(^13\) Fusidic acid has been shown to eliminate S. intermedius carriage from skin and mucosae in dogs after 2 days and is likely to be effective in the decolonization of MRSA and MRSP pet carriers.\(^14\) Topical use of fusidic acid in humans, at least long-term, has been associated with an increase in MICs, which is relevant for humans requiring systemic fusidic acid therapy.\(^15,16\)

Thus, animal decolonization should always be performed in an integrated approach including all MRSA carriers and sources, using products with proven efficacy and including owner education and rigorous follow-up examinations.

The higher MICs of mupirocin for one \((1.0\%)\) MRSA isolate would be consistent with values reported for low-level resistance for which successful elimination from carrier sites is still suggested. Although off-license use cannot be excluded, there is no authorized mupirocin formulation for use in pets in either the UK or Germany and any selection pressure developing from previous drug exposure is therefore more likely to have occurred in a human host.

The MIC\(_{50}\) of 4 mg/L fusidic acid for 71 SP isolates is markedly higher than comparable figures of 0.12 and 1 mg/L reported previously.\(^9,10,15\) MICs of 4 and 8 mg/L fusidic acid as seen for SP \((12.7\%)\) have only been associated with individual isolates before and the genetic basis for resistance in this group seems to differ from the more successful fusidic acid resistance genes in S. aureus. As such concentrations are likely to be exceeded by topical therapy with licensed fusidic acid formulations, they are expected to be of epidemiological relevance only.

Higher MICs of both antibiotics for isolates from infection compared with carriage isolates is likely to reflect selection pressure after exposure to antimicrobials in humans or pets and topical compounds should be included in recommendations on responsible use of antimicrobial drugs.

In summary, the results indicate that fusidic acid and mupirocin can be effective for the treatment of superficial staphylococcal infections and for decolonization in dogs and cats even where multi resistant isolates are involved. With some high-level MICs occurring, though, prudent use is indicated as both compounds may be prescribed in humans and animals against staphylococci that can transfer between hosts. However, clinical studies are warranted to define cut-off values for topical antimicrobials in order to limit their unnecessary use.

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Transparency declarations

None to declare.

References


