Susceptibility in vitro of canine methicillin-resistant and -susceptible staphylococcal isolates to fusidic acid, chlorhexidine and miconazole: opportunities for topical therapy of canine superficial pyoderma

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Objectives: Increasing multidrug resistance amongst canine pathogenic staphylococci has renewed interest in topical antibacterial therapy for skin infections in the context of responsible veterinary prescribing. We therefore determined the activity in vitro of three clinically relevant topical agents and synergism between two of them against Staphylococcus pseudintermedius and Staphylococcus aureus.

Methods: The MICs of fusidic acid (n = 199), chlorhexidine (n = 198), miconazole (n = 198) and a 1:1 combination of miconazole/chlorhexidine (n = 198) were determined for canine isolates [50 MRSA and 49 methicillin-resistant S. pseudintermedius (MRSP), 50 MSSA and 50 methicillin-susceptible S. pseudintermedius (MSSP)] collected from the UK and Germany using an agar dilution method (CLSI VET01-A4). Fractional inhibitory concentration (FIC) indices were calculated to assess the interaction of miconazole with chlorhexidine.

Results: MICs of each drug/combination were significantly (P < 0.0005) higher for S. aureus when compared with S. pseudintermedius. Most strains (n = 172) had an MIC of fusidic acid of ≤0.03 mg/L (MIC > 64 mg/L, n = 5 MRSA). All strains had MICs of chlorhexidine of 0.5–4 mg/L, except for one MRSA (MIC = 8 mg/L). All but four strains had MICs of miconazole of 1–4 mg/L (MIC = 16 mg/L, n = 3; MIC = 256 mg/L, n = 1). Miconazole/chlorhexidine (1:1 ratio) had a synergistic effect against 49/50 MRSA, 31/50 MSSA, 12/49 MRSP and 23/49 MSSP.

Conclusions: Since the majority of these staphylococci, including methicillin-resistant isolates, had MICs that should be readily exceeded by topical skin application of these agents, their therapeutic efficacy for canine superficial pyoderma should be assessed. The synergistic interaction shown in vitro supports further clinical evaluation of miconazole/chlorhexidine combination therapy for staphylococcal infection.

Keywords: MRSA, Staphylococcus pseudintermedius, dogs, synergy

Introduction

The alarming increase in canine skin infections caused by methicillin-resistant Staphylococcus pseudintermedius (MRSP) and MRSA1 paralleled by recognition of zoonotic (and reverse-zoonotic) infections,2 highlights the urgent need to develop strategies to limit further emergence of MDR strains. Topical antibacterial therapy can provide an alternative treatment option for many dogs with bacterial skin infections and thus limit the need for oral antibiotics.3 Licensed drugs include fusidic acid, chlorhexidine and a 1:1 shampoo combination of miconazole and chlorhexidine.

Although there is concern in human medicine over reduced efficacy of agents such as chlorhexidine and fusidic acid,4,5 clinical evidence for treatment failure remains inconsistent. Assessment of resistance is hampered by lack of breakpoint standards for agents used topically. In dog-derived staphylococci, MICs of fusidic acid, chlorhexidine and miconazole have been low,6–8 but geographical differences can be expected.9,10 Since previous MIC studies have evaluated mainly North American staphylococcal isolates,7,8 we determined current susceptibility in vitro of dog-derived European MRSA and MRSP strains and their methicillin-susceptible counterparts to fusidic acid, chlorhexidine and miconazole, and investigated...
Susceptibility in vitro of canine staphylococci to fusidic acid, chlorhexidine and miconazole

the potential for synergistic interaction between miconazole and chlorhexidine.

Materials and methods

Bacterial isolates

Coagulase-positive staphylococci (50 MRSA and 50 MSSA obtained in 2005–07 and 49 MRSP and 50 methicillin-susceptible S. pseudintermedius (MSSP) obtained in 2010–13) isolated from dogs were randomly selected from our collection. Staphylococcus aureus isolates were from canine infections in a UK-wide study. Examples of S. pseudintermedius comprised clinical isolates (Germany, 25 MRSP and 25 MSSP; UK, 24 MRSP) and carriage isolates (UK, 25 MSSP). Species identification and methicillin resistance were confirmed using both phenotypic and genotypic (nuc, mecA) methods.

MIC determination

MICs were determined by agar dilution (CLSI VET01-A4). Prior to MIC determination, strains were sub cultured twice on blood agar base (CM0271, Oxoid) containing 5% sheep blood (TCS Biosciences, Buckingham, UK) at 35°C for 24 h. Stock solutions were prepared at 10× final concentration in distilled water (fusidic acid sodium salt F0881; chlorhexidine digluconate C9394; Sigma-Aldrich Inc.) or 1% DMSO (miconazole nitrate PFR1163; Sigma) adjusted for drug potency. Final concentrations of the active fraction ranged from 0.015 to 2048 mg/L for fusidic acid and from 0.03 to 256 mg/L for chlorhexidine, miconazole and a 1:1 combination of chlorhexidine and miconazole. Discrepancy between duplicate MICs was accepted provided they varied by only one dilution; in such cases, the higher value was identified as the final MIC. S. aureus ATCC 25923, S. aureus ATCC 29663 and S. pseudintermedius LMG 22219 were included for quality control purposes. MSSR strains previously reported with low, medium and high MICs of fusidic acid were also included for comparative purposes.

Fractional inhibitory concentration (FIC) indices

The FIC index was calculated to analyse drug interaction of chlorhexidine and miconazole when used in the 1:1 combination, using the formula FIC = FICchlorhexidine + FICmiconazole = (MICchlorhexidine + MICmiconazole) / (MICchlorhexidine + MICmiconazole), where MICchlorhexidine and MICmiconazole are the MICs of chlorhexidine and miconazole alone, respectively, and MICchlorhexidine + MICmiconazole is the MIC of the two drugs in combination. An FIC index of <0.5 represented synergy, an FIC index of >4 represented antagonism and an FIC index of >0.5–4 represented no interaction.

Statistical analysis

SPSS version 21 (IBM UK Ltd, Portsmouth, UK) was used, with P < 0.05 for significance. Since data were not normally distributed (Shapiro–Wilk test), the MIC values for different bacterial groups were compared using the Kruskal–Wallis test. Post hoc comparisons were performed using Mann–Whitney U-tests with Holm–Bonferroni adjustments.

Results

One MSSP strain failed to grow in studies of MICs of chlorhexidine, miconazole and miconazole plus chlorhexidine. MICs varied by a single dilution between replicates on only five occasions.

Fusidic acid

The MICs of fusidic acid amongst the field collection ranged from ≤0.015 to 384 mg/L (median ≤0.015 mg/L); 172 of 199 isolates had MICs ≤0.03 mg/L and 192 isolates had MICs ≤4 mg/L (Table 1). Seven isolates had higher MICs of 16–384 mg/L, comprising five MRSA (384 mg/L, n = 4; 64 mg/L, n = 1) and single examples of MSSA and MRSP (both 16 mg/L). Overall, MICs were higher (P < 0.0005) for S. aureus isolates (median 0.03 mg/L, n = 100) than S. pseudintermedius isolates (median ≤0.015 mg/L, n = 99), though there was no significant difference in MICs between methicillin-susceptible and resistant isolates within each species. MICs for reference isolates were low (ATCC 25932, 0.03 mg/L; ATCC 29663 and LMG 22219, ≤0.015 mg/L), as expected. The isolates included for internal comparative purposes had MICs comparable to previous determinations (A004, 0.12 (previous) and 0.03 (present) mg/L; A013, 2 and 1 mg/L; and A016, >128 and 384 mg/L).

Chlorhexidine

The MICs of chlorhexidine ranged from 0.5 to 8 mg/L (median 2 mg/L; Table 1). The MICs for MRSA (n = 50) were remarkably uniform, with 49 out of 50 isolates having an MIC of 4 mg/L; these values were higher (P < 0.0005) than those for all other bacterial groups. Overall, the S. aureus isolates (median 4 mg/L, n = 100) had higher MICs than the S. pseudintermedius isolates (median 1 mg/L, n = 98) (P < 0.0005). The MICs did not vary significantly between MRSP (n = 49) and MSSP (n = 49) (P = 0.55).

Miconazole

The MICs of miconazole ranged from 1 to 256 mg/L (median 2 mg/L); 175 of 198 isolates had MICs of 1 or 2 mg/L and 19 had MICs of 4 mg/L (Table 1). Four isolates had higher MICs, comprising single examples of MRSA, MSSA and MRSP with MICs of 16 mg/L and one MSSA with an MIC of 256 mg/L. S. aureus isolates (median 2 mg/L, n = 100) had higher MICs than S. pseudintermedius isolates (median 1 mg/L, n = 98) (P < 0.0005). There was no difference (P = 0.415) in MIC between the MRSA and MSSA groups; however, the MRSP group (median 2 mg/L, n = 49) had a significantly (P < 0.0005) higher MIC than the MSSP group (median 1 mg/L, n = 49).

Miconazole and chlorhexidine in combination

The MICs of the 1:1 combination of miconazole and chlorhexidine ranged from 0.25 to 8 mg/L (median 0.5 mg/L; Table 1); 190 of 198 isolates had an MIC of 0.25 or 0.5 mg/L and all but one MSSA isolate had an MIC of ≤2 mg/L. The MICs for the S. aureus isolates (median 0.5 mg/L) exceeded (P < 0.0005) those for the S. pseudintermedius isolates (median 0.5 mg/L), and the MICs for the MSSP group (median 0.25 mg/L) were lower (P < 0.0005) than for the other three groups. The MSSA isolate whose miconazole MIC was 256 mg/L had an MIC of 8 mg/L for the combination.

A synergistic interaction between miconazole and chlorhexidine (FIC index ≤0.5) was observed for 80 out of 100 S. aureus isolates and 35 out of 98 S. pseudintermedius isolates (Table 2). Antagonistic interactions were not observed; the other strains fell into the ‘no interaction’ group as defined by Odds. EUCAST guidelines consider that FIC indices from 0.5 to 1 indicate additivity; by this definition a further 62 strains demonstrated additive effects (MRSA, n = 1; MSSA, n = 18; MRSP, n = 19; MSSP, n = 24).
Table 1. MICs of fusidic acid for 199 coagulase-positive staphylococcal isolates from dogs and MICs of chlorhexidine, miconazole and a 1:1 combination of miconazole/chlorhexidine for 198 coagulase-positive staphylococcal isolates from dogs

| Drug                        | Bacterial type | MIC (mg/L) | Median (IQR) | MIC<sub>50</sub> | MIC<sub>90</sub> |
|-----------------------------|----------------|------------|--------------|------------------|------------------|
| Fusidic acid                | MRSA           | 34         | 0            | 0                | 0.015 (0.03–0.03)| 0.03             | 0.25             |
|                             | MSSA           | 24         | 0            | 0                | 0.015 (0.015–0.03125)| 0.03             | 0.25             |
|                             | MRSP           | 46         | 1            | 0                | ≤0.015 (≤0.015 to ≤0.015)| ≤0.015           | ≤0.015           |
|                             | MSSP           | 41         | 5            | 0                | ≤0.015 (≤0.015 to ≤0.015)| ≤0.015           | 0.03             |
| Chlorhexidine               | MRSA           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MSSA           | not tested | 0            | 1                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MRSP           | not tested | 0            | 1                | ≤0.015 (≤0.015)  | ≤0.015           | ≤0.015           |
|                             | MSSP           | not tested | 0            | 1                | ≤0.015 (≤0.015)  | ≤0.015           | 0.03             |
| Miconazole                  | MRSA           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MSSA           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MRSP           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MSSP           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
| Miconazole plus chlorhexidine (1:1) | MRSA           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MSSA           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MRSP           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |
|                             | MSSP           | not tested | 0            | 0                | 0.03 (0.03–0.03) | 0.03             | 0.25             |

MRSA, n=50; MSSA, n=50; MRSP, n=49; and MSSP, n=50 for fusidic acid and n=49 for chlorhexidine, miconazole and miconazole plus chlorhexidine.
Susceptibility in vitro of canine staphylococci to fusidic acid, chlorhexidine and miconazole

Table 2. FIC indices for a 1:1 combination of chlorhexidine and miconazole

| Bacterial group | Synergy (≤0.5) | No interaction (>0.5 - 1) | Antagonism (>4) |
|-----------------|---------------|--------------------------|----------------|
| MRSA (n = 50)   | 49            | 1                        | 0              |
| MSSA (n = 50)   | 31            | 18                       | 1              |
| MRSP (n = 49)   | 12            | 19                       | 0              |
| MSSP (n = 49)   | 23            | 24                       | 2              |
| Total           | 115           | 62                       | 20             | 1 | 0

Discussion

The low MICs of fusidic acid, miconazole and chlorhexidine for the great majority of these canine staphylococcal isolates in vitro provide strong support for their use topically in clinical cases of surface and superficial pyoderma, including cases caused by methicillin-resistant bacteria.

The interpretation of MIC values in the context of susceptibility/resistance is hindered by the absence of defined breakpoints; whilst EUCAST guidelines indicate a value of 2 mg/L for fusidic acid when used against S. aureus, this is of questionable value for canine skin infections treated topically rather than systemically.

The prevalence of high fusidic acid MICs for human-derived S. aureus appears related to geographical variation in prescribing practice in human medicine.13 10.7% of human-derived European S. aureus had MICs ≥ 2 mg/L compared with only 0.3% of isolates from North America, where fusidic acid usage is infrequent.10 The comparable frequency of fusidic acid MICs ≥ 2 mg/L amongst our European canine MRSA isolates (10.0%) is perhaps not surprising since these strains likely originate from human sources.6 The strikingly uniform MICs of chlorhexidine, almost exclusively in the narrow range of 0.5–4 mg/L, are remarkably similar to those reported previously for MRSA isolates from human sources worldwide.18 Similarly, the MICs of miconazole in the present study are comparable to those in previous reports indicating good efficacy against staphylococci.6

The frequent synergistic activity of the combination of chlorhexidine and miconazole indicates potential clinical value as a combination treatment in dogs, especially as they lack a critical role in human medicine.

Monitoring for the presence, and potential for transfer, of resistance between populations of MRSA on dogs and humans, and between canine S. pseudintermedius and human S. aureus strains is warranted. Genetic studies are also required to explain the rare higher MICs that were identified in individual isolates. Previous authors have reported maximum values in the order of 16 mg/L miconazole for S. aureus and 8 mg/L fusidic acid for S. pseudintermedius; the value of 16 mg/L fusidic acid seen for a single MRSP strain might correspond to ‘low-level fusidic acid resistance’13 and explanation is needed for the single high MIC of miconazole of 256 mg/L.

This study provides further evidence to support topical therapies as an alternative to systemic antibacterial drugs in superficial pyoderma. Further work is needed to confirm the clinical efficacy of these topical treatments.

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References

1 Loeffler A, Linek M, Moodley A et al. First report of multiresistant, mecA-positive Staphylococcus intermedii in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. Vet Dermatol 2007; 18: 412–21.

2 Guardabassi L, Loeber ME, Jacobson A. Transmission of multiple antimicrobial-resistant Staphylococcus intermedii between dogs affected by deep pyoderma and their owners. Vet Microbiol 2004; 98: 23–7.

3 Loeffler A, Cobb MA, Band R. Comparison of a chlorhexidine and a benzoyl peroxide shampoo as sole treatment in canine superficial pyoderma. Vet Rec 2011; 169: 249.

4 Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? J Antimicrob Chemother 2012; 67: 2547–59.

5 Brown EM, Thomas P. Fusidic acid resistance in Staphylococcus aureus isolates. Lancet 2002; 359: 803.

6 Loeffler A, Baines SJ, Taleman MS et al. In vitro activity of fusidic acid and mupirocin against coagulase-positive staphylococci from pets. J Antimicrob Chemother 2008; 62: 1301–14.

7 Valentine BK, Dew W, Yu A et al. In vitro evaluation of topical biocide and antimicrobial susceptibility of Staphylococcus pseudintermedius from dogs. Vet Dermatol 2012; 23: 493–5.

8 Weese JS, Walker M, Lowe T. In vitro miconazole susceptibility of meticillin-resistant Staphylococcus pseudintermedius and Staphylococcus aureus. Vet Dermatol 2012; 23: 400–e74.

9 Castanheira M, Watters AA, Mendres RE et al. Occurrence and molecular characterization of fusidic acid resistance mechanisms among Staphylococcus spp. from European countries (2008). J Antimicrob Chemother 2010; 65: 1353–8.

10 Castanheira M, Watters AA, Bell JM et al. Fusidic acid resistance rates and prevalence of resistance mechanisms among Staphylococcus spp. isolated in North America and Australia, 2007–2008. Antimicrob Agents Chemother 2010; 54: 3614–7.

11 Soares Magalhães RJ, Loeffler A, Lindsay J et al. Risk factors for methicillin-resistant Staphylococcus aureus (MRSA) infection in dogs and cats: a case–control study. Vet Res 2010; 41: 55.
12 Barrow GI, Feltham RKA. Cowan and Steel’s Manual for the Identification of Medical Bacteria. Cambridge: Cambridge University Press, 2003.

13 Becker K, von Eiff C, Keller B et al. Thermonuclease gene as a target for specific identification of Staphylococcus intermedius isolates: use of a PCR-DNA enzyme immunoassay. Diagn Microbiol Infect Dis 2005; 51: 237–44.

14 Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals—Fourth Edition: Approved Standard VET01-A4. CLSI, Wayne, PA, USA, 2013.

15 Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother 2003; 52: 1.

16 European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. Clin Microbiol Infect 2000; 6: 503–8.

17 Farrell DJ, Castanheira M, Chopra I. Characterization of global patterns and the genetics of fusidic acid resistance. Clin Infect Dis 2011; 52 Suppl 7: S487–92.

18 McDanel JS, Murphy CR, Diekema DJ et al. Chlorhexidine and mupirocin susceptibilities of methicillin-resistant Staphylococcus aureus from colonized nursing home residents. Antimicrob Agents Chemother 2013; 57: 552–8.