



# In vitro susceptibility of canine *Staphylococcus pseudintermedius* and *S. aureus* to miconazole

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## INTRODUCTION

*Staphylococcus pseudintermedius* and to a lesser degree *S. aureus*, are common causes of skin and ear infections in dogs, as well as wound and surgical site infections.

In recent years, there has been a dramatic increase in methicillin-resistant *S. pseudintermedius* (MRSP) infections in dogs, particularly in skin, ear and surgical site infections.<sup>1,2</sup> While less common, methicillin-resistant *S. aureus* (MRSA) causes a similar range of infections.<sup>3</sup>

The high level of antimicrobial resistance that is often present, particularly with MRSP, has led to a resurgence in interest in topical therapies, which are appealing because they can deliver high concentrations of drug to superficial infection sites with little or no systemic exposure.

Miconazole, an imidazole antifungal, has antibacterial properties against a limited range of bacteria, including *S. aureus*,<sup>4,6</sup> does not have the same importance for human medicine and might be another topical option.

## OBJECTIVES

- 1) To evaluate the minimum inhibitory concentration of miconazole for MRSP, MSSP and MRSA from dogs.
- 2) To compare the MICs between *S. pseudintermedius* and *S. aureus*, and between methicillin-resistant and methicillin-susceptible strains.

## MATERIALS AND METHODS

Two hundred two isolates were tested; 112 MRSP, 53 MRSA and 37 MSSP. Isolates were not epidemiologically related and came from 48 dogs with clinical infection (16 MRSA, 32 MRSP) and 154 dogs with nasal or rectal colonization (37 MRSA, 80 MRSP, 37 MSSP).

Isolates were tested using agar dilution. Miconazole nitrate was dissolved in DMSO (16 mg miconazole in 5 ml DMSO) and added to Mueller-Hinton agar plates to achieve final concentrations ranging from 0.03 to 16 ug/ml. Bacterial isolates were grown on blood agar and 24h pure growth was inoculated into phosphate-buffered saline (pH 7.4) to a concentration approximating a 0.5 McFarland standard. These suspensions were spotted onto each miconazole containing plate and incubated aerobically at 35°C for 18h. *Escherichia coli*, a bacterium that is not inhibited by miconazole, was used as a control.

The minimum inhibitory concentration (MIC) was the concentration that completely inhibited visible growth, disregarding a single colony or thin haze.

To assess repeatability, a convenience sample of 35 isolates was tested in duplicate.

Student's t-test, and one-way analysis of variance (ANOVA) with Tukey's test used for post-hoc pair-wise comparisons were used. A P value of <0.05 was considered significant.

## RESULTS

MIC data are presented in Table 1 and Figure 1.

There was a significant effect of bacterial group on MIC, with MSSP having a significantly lower MIC than both MRSP ( $P=0.006$ ) and MRSA ( $P<0.001$ ), and MRSP having a significantly lower MIC than MRSA ( $P=0.001$ ).

There was no difference in MIC between isolates from infection and colonization with MRSP ( $P=0.57$ ), however MRSA colonization isolates had a significantly higher MIC than isolates from infection ( $P=0.004$ ). All MSSP isolates were from colonization.

Twenty-nine of the 35 (83%) isolates tested in duplicate yielded the same MIC and the remaining six differed only by one dilution.

*Escherichia coli* controls were not inhibited by miconazole.

Group	MIC range (ug/ml)	MIC <sub>50</sub> (ug/ml)	MIC <sub>90</sub> (ug/ml)
MSSP	1-4	2	2
MRSP	1-8	2	4
MRSA	1-8	4	6

Table 1: MIC data for MRSP, MSSP and MRSA.

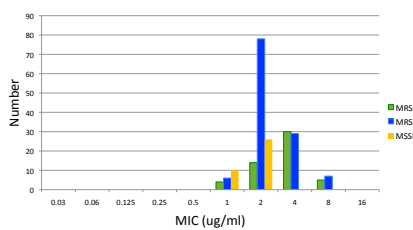


Figure 1: Distribution of miconazole minimum inhibitory concentrations (MICs) for MRSA, MRSP and MSSP

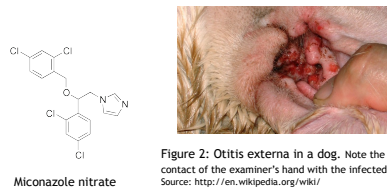


Figure 2: Otitis externa in a dog. Note the close contact of the examiner's hand with the infected site. Source: [http://en.wikipedia.org/wiki/File:Bacterial\\_otitis\\_in\\_dog.jpg](http://en.wikipedia.org/wiki/File:Bacterial_otitis_in_dog.jpg)

Miconazole nitrate

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## CONCLUSIONS

These data are consistent with studies of *S. aureus* that have reported MICs of 1-2 ug/ml,<sup>4,6</sup> and a study of *S. intermedium* (which can be assumed to truly have been *S. pseudintermedius*) that reported an MIC range of 0.37-7.5 ug/ml.<sup>9</sup>

There are no established breakpoints for staphylococci, however the MIC levels reported here are well below concentrations achievable with topical therapy (e.g. 2% commercial products equal 20000 ug/ml).

The statistically significant difference between MSSP, MRSP and MRSA was interesting and a potential mechanism is unclear. Clinically, it is likely of little relevance because of the low magnitude of the difference and the fact that all isolates yielded MICs well below user concentrations.

The mechanism of activity of miconazole against staphylococci is not clearly understood. Its fungicidal action is through inhibition of 14-alpha-sterol demethylase, which is involved in sterol synthesis. This does not directly apply to bacteria, however *Mycobacterium smegmatis*, another bacterium that is susceptible to miconazole, has been shown to contain a cytochrome P450 monooxygenase that is orthologous to 14-alpha-sterol demethylase and strongly binds miconazole.<sup>11</sup> Direct membrane damage has also been suggested as a mechanism of action in staphylococci<sup>6</sup> and it is has recently been reported that miconazole induces intracellular reactive oxygen species in *S. aureus*.<sup>12</sup>

The narrow spectrum of activity, safety, ready availability and low priority in human medicine make miconazole a potentially attractive treatment option for superficial MRSA and MRSP infections. Miconazole use could be accompanied by fewer concerns because of the narrow anti-bacterial spectrum that it possesses and since it is not used in humans for treatment of serious infections.

Clinical study should be undertaken to determine whether miconazole may be an effective treatment of superficial MRSA and MRSP infections.

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