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New insights on the antibacterial efficacy of miconazole in vitro

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Summary

Miconazole is a broad-spectrum antifungal used in topical preparations. In the present investigation the minimal inhibitory concentration (MIC) of miconazole for eighty wild type strains of gram-positive and gram-negative bacteria isolated from infected skin lesions was assessed using a modified agar dilution test (adapted to CLSI, Clinical Laboratory Standards Institute). 14 ATCC reference strains served as controls. Miconazole was found efficacious against gram-positive aerobic bacteria (n=62 species), the MICs against *Staphylococcus (S.) aureus*, *S.* spp., *Streptococcus* spp. und *Enterococcus* spp. ranged between 0.78 and 6.25 μg/mL. Interestingly, there were no differences in susceptibility between methicillin-susceptible (MSSA, 3) methicillin-resistant (MRSA, 6) and fusidic acid-resistant *(FRSA*, 2) *S. aureus* isolates. Strains of *Streptococcus pyogenes* (A-streptococci) (8) were found to be slightly more sensitive (0.78-1.563 μg/mL), while for gram-negative bacteria, no efficacy was found within the concentrations tested (MIC >200 μg/mL). In conclusion, for the gram-positive aerobic bacteria the MICs of miconazole were found within a range which is much lower than the concentration of miconazole used in topical preparations (2%). Thus topically applied miconazole might be a therapeutic option in skin infections especially caused by gram-positive bacteria even by those strains which are resistant to antibiotics.

KEYWORDS

FRSA, gram-positive bacteria, miconazole, MRSA, topical antibacterial therapy

1 | INTRODUCTION

Topical antimicrobial therapy is an important alternative to systemic antibacterial therapy in particular in the management of superficial bacterial skin infections and superinfected or impetiginised eczema, which are mainly caused by gram-positive bacteria.

In contrast to systemic therapy, advantages of topically applied antibiotics are the immediate onset of action, generation of higher concentrations at the site of infection and the reduction or lack of systemic side effects.[1,2] On the other hand topical therapy with antibiotics is seen very critical because of the risk of contact sensitisation, delayed wound repair, resorptive toxicity and promotion of bacterial resistance.[1,4] Another option for topical antimicrobial therapy could be the use of antiseptics. The effect of antiseptics is based on a physico-chemical destruction of cell walls or denaturisation of proteins. They have a broader spectrum and a faster onset of action than topically applied antibiotics. Development of resistance is seen very rarely.[1] On the contrary they are only effective within a narrow therapeutic range: lower concentrations lack efficacy and higher concentrations may cause toxic effects and delayed wound healing.[1]

Interestingly azole antimycotics have been shown to also exert antibacterial effects [5] and new formulations for skin diseases have been suggested.[6] Especially the broad-spectrum imidazole antifungal miconazole was found to be effective against skin infections caused by gram-positive bacteria both in vitro and in vivo.[7–17] The present investigation aimed to determine the efficacy of miconazole against bacteria currently isolated from superficial skin lesions and infections using a modified standardised agar dilution test in order to show whether miconazole could be a therapeutic alternative even in the management of difficult to treat bacteria, esp. methicillin- and fusidic acid-resistant *Staphylococcus aureus* isolates.

2 | MATERIALS AND METHODS

Miconazole pure substance was provided by Almirall Hermal GmbH, Reinbek, Germany. To prepare a stock solution of 4 mg/mL for further testing 20 mg of miconazole were dissolved in a mixture of 2 mL dimethylsulfoxide (DMSO, Hollborn, Leipzig, Germany) and 3 mL sterile aqua destillata.

2.1 | Strains and media

In order to determine the antibacterial activity of miconazole eighty wild type strains as well as fourteen reference strains as controls were included (Tables 1 and 2). The wild type strains have been isolated from materials sent for routine diagnostics to the Laboratory for medical Microbiology, Mölbis, Germany, in spring and summer 2016. The isolates originated from superficial skin infections (i.e. impetigo, folliculitis, panaritium, pyoderma, superinfected eczema, intertrigo) as well as from chronic wounds, in particular ulcera crura. The reference strains were purchased from ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110 USA).

The following strains were investigated as detailed in Table 1.

TABLE 1 Wild type strains (number of strains in brackets)

Gram-positive bacteria (n=62)	
Staphylococcus aureus (12)	Coagulase-negative Staphylococci (2)
MRSA (Methicillin-resistant *Staphylococcus aureus*) (6)	*Streptococcus pyogenes* (A-Streptokokken) (8)
MSSA (Methicillin-sensitive *Staphylococcus aureus*) (3)	B-Streptococci (*Streptococcus agalactiae*) (7)
FRSA (fusidic acid-resistant *Staphylococcus aureus*) (2)	*Enterococcus faecalis* (5)
Staphylococcus epidermidis (4)	*Micrococcus luteus* (1)
Staphylococcus haemolyticus (1)	*Corynebacterium* spp. (11)
Gram-negative bacteria (n=18)	
Escherichia coli (3)	*Enterobacter cloacae* (3)
ESBL (Extended spectrum beta lactamase forming*) Escherichia coli* (3)	*Pseudomonas aeruginosa* (3)
Klebsiella oxytoca (4)	*Acinetobacter baumannii* (2)

The 14 ATCC reference strains used as controls are summarised in Table 2.

For antimicrobial susceptibility testing Mueller-HintonAgar (Becton-Dickinson, Heidelberg, Germany) without antibiotics and for the anaerobes Gifu Anaerobic Medium Agar (G.A.M. Agar Nissui, HyServe, Uffing, FRG) were used.

2.2 | Agar dilution test

Agar dilution test was performed as described by Nenoff[18] and Arendrup.[19,20] The latter describes a standardised method for sensitivity testing of fungi to antimycotics according to EUCAST but the media applied for this test did not allow the growth of bacteria. No standardised method was available for testing the antimicrobial activity of an antifungal agent against bacteria. Therefore testing was performed according to the Clinical and Laboratory Standards Institute (CLSI)[21–24] with the restriction that this method was standardised for sensitivity testing of bacteria against antibiotics. In more detail the agar dilution test was based on the method recently described by Clark et al. [16].

From the stock solution (4 mg/mL) 14 concentrations in a range from 0.488 to 4 mg/mL were prepared by serial dilution. A quantity of 1 mL of each concentration was mixed with 19 mL freshly prepared agar (Mueller-Hinton Agar or G.A.M. Agar Nissui, cooled down to about 60°C) resulting in a miconazole concentration of 0.0244 to 200 μg/mL in the final agar based media.

2.3 | Inocula

Bacteria were suspended in sterile saline to get a density of 10^7 colony forming units (CFU) per mL by comparing with the McFarland Standard 0.5 (bioMérieux SA, Marcy l'Etoile, France). Accordingly, approximately 10^4 CFU (1 μL) were applied per inoculation point.

Incubation was performed at a temperature of 37°C. Results were obtained by visual assessment after 24 and 48 h. The minimal inhibitory concentration (MIC) was defined as the lowest concentration where no growth was observed. With each attempt growth controls were carried out by inoculating the bacteria on a medium without the active substance miconazole. Furthermore the colony count was controlled by smears on Columbia blood agar (Becton-Dickinson, Heidelberg, Germany). All tests were performed in duplicate.

3 | RESULTS

Table 3 summarises the MICs of miconazole in μg/mL for the different bacteria obtained after 24 and 48 h of incubation (in brackets number of test strains). Miconazole was found efficacious against gram-positive aerobic bacteria (n=62 species), the MICs against *Staphylococcus (S.) aureus*, *S.* spp., *Streptococcus* spp. und *Enterococcus* spp. ranged between 0.78 and 6.25 μg/mL. Interestingly, there were no differences in susceptibility between methicillin-susceptible (MSSA, 3) methicillin-resistant (MRSA, 6) and fusidic acid-resistant *(FRSA, 2) S. aureus* isolates. Strains of *Streptococcus pyogenes*

TABLE 2 ATCC reference strains tested

Enterococcus faecalis	*Escherichia coli*	*Stenotrophomonas maltophilia*
ATCC 29212TM	ATCC 35218TM	ATCC 17666TM
Staphylococcus aureus	*Escherichia coli*	*Enterococcus casseliflavus*
ATCC BAA977TM	ATCC 25922TM	ATCC700327TM
Staphylococcus aureus	*Pseudomonas aeruginosa*	*Staphylococcus saprophyticus*
ATCC BAA976TM	ATCC27853TM	ATCC BAA750TM
Staphylococcus aureus	*Klebsiella pneumoniae*	*Enterococcus faecalis*
ATCC 29213TM	ATCC700603TM	ATCC 51299TM
Staphylococcus aureus	*Enterobacter hormaechei*	
ATCC BAA1026TM	ATCC 700323TM	

TABLE 3 MICs (median) of miconazole against gram-positive bacteria (μg/mL)

	24 h of incubation			48 h of incubation		
	n	Median	Range	n	Median	Range
Staphylococcus aureus						
S. aureus	12	3.125	1.563-3.125	12	3.125	1.563-3.125
ATCC-reference strains	4	2.3[a]	1.563-3.125	4	2.3[a]	1.563-3.125
MRSA	6	3.125	1.563-3.125	6	3.125	1.563-3.125
MSSA	3	3.125	3.125	3	3.125	3.125
FRSA	2	3.125	3.125	2	3.125	3.125
Staphylococcus div.						
S. epidermidis	4	1.563	0.78-1.563	4	1.563	0.78-1.563
S. haemolyticus	1	-	3.125	1	-	6.25
S. coagulase neg.	2	1.563	1.563	2	1.563	1.563
S. saprophyticus ATCC	1	-	3.125	1	-	3.125
Streptococcus						
St. pyogenes	8	0.78	0.78	8	1.563	0.78-1.563
St. agalactiae	7	3.125	1.563-3.125	7	3.125	1.563-6.25
Enterococcus						
Enterococcus faecalis	5	6.25	3.125-6.25	5	6.25	3.125-6.25
Enterococcus faecalis ATCC reference	2	3.125	3.125	2	6.25	3.125-6.25
Enterococcus casseliflavus ATCC	1	-	3.125	1	-	3.125
Diverse						
Micrococcus luteus	1	-	0.78	1	-	0.78
Corynebacteria spp.	7[b]	0.39	<0.024-0.78	11	0.78	0.095-3.125

n, number of strains; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; FRSA, fusidic acid-resistant *S. aureus*.
[a]mean value. [b]four strains without growth at 24 h.

(A-streptococci) (8) were found to be slightly more sensitive (0.78-1.563 μg/mL). Among the species tested *Corynebacteria* spp. were found to be most sensitive.

Figure 1 comprises the medians of MICs (median; μg/mL) of miconazole against all gram-positive species (two and more strains tested) after 24 and 48 h of incubation. With the exception of the strains of *St. pyogenes* and *Corynebacteria* spp. the results were identical.

All gram-negative bacteria tested showed growth at the maximum inhibitory concentration tested (200 μg/mL) after 24 h as well as after 48 h. The results were confirmed with the gram-negative ATCC reference strains (n=6).

4 | DISCUSSION

The imidazole antifungal miconazole is a broad-spectrum antifungal agent widely used in the topical therapy of superficial mycotic infections.[7–10] In the present in vitro study this azole was found to

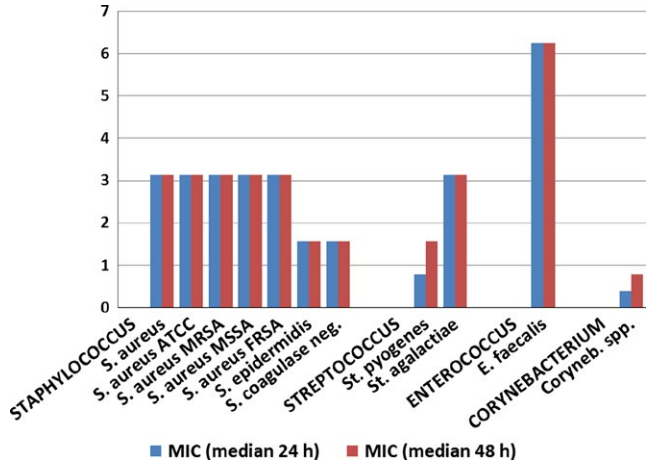

FIGURE 1 Medians of MICs (median; μg/mL) of miconazole against all gram-positive species (two and more strains tested) after 24 and 48 h of incubation

be efficacious against different species of gram-positive bacteria (*Staphylococcus aureus*, *S.* spp., *Streptococcus* und *Enterococcus* spp.) with MICs ranging from 0.78 to 6.25 μg/mL. The results for the resistant *S. aureus* strains are most interesting. The MICs obtained for MRSA (Methicillin-resistant *S. aureus*), MSSA (Methicillin-sensitive *S. aureus*) and FRSA (Fusidic acid-resistant *S. aureus*) were identical to those without the problems of resistance (Table 3, Figure 1). With a MIC median of 6.25 μg/mL the five strains of *Enterococcus faecalis* tested were also within this range, while strains of *Streptococcus pyogenes* (A-Streptococci) were found to be slightly more susceptible (0.78-1.563 μg/mL).

The results are well in line with previous results.[11–17] As in veterinary medicine, emergence and dissemination of methicillin-resistant staphylococci are of rising importance. Boyen et al. determined the in vitro antimicrobial activity of miconazole, polymyxin B and a combination of both against 24 canine MRSA and 50 canine methicillin resistant *S. pseudintermedius* (MRSP) isolates using a broth microdilution assay.[13] The MIC values for miconazole and polymyxin B against MRSA were in the range of 4-8 and 8-64 μg/mL, respectively, while those against MRSP were in the range of 1-2 and 0.25-4 μg/mL. Weese et al. assessed the in-vitro susceptibility of 112 methicillin-resistant MRSP, 53 methicillin-resistant MRSA and 37 methicillin-susceptible *S. pseudintermedius* (MSSP) to miconazole using agar dilution.[12] The minimal inhibitory concentration (MIC) ranges, MIC(50) and MIC(90) for MRSP were 1-8, 2 and 4 μg/mL, respectively. Corresponding results for MRSA were 1-8, 2 and 6 μg/mL, and for MSSP 1-4, 2 and 2 μg/mL. Clark et al. determined the MICs of fusidic acid (n=99), chlorhexidine (n=98), miconazole (n=198) and a 1:1 combination of miconazole/chlorhexidine (n=98) for canine isolates (50 MRSA, 50 MSSA and 49 MRSP and 50 MSSP) collected from the UK and Germany using an agar dilution method.[16] All but four strains had MICs of miconazole of 1-4 mg/L (MIC=6 mg/L, n=3; MIC=256 mg/L, n=1). For fusidic acid most strains (n=172) had an MIC ≤0.03 mg/L (MIC ≥64 mg/L, n=5 MRSA). Miconazole/chlorhexidine (1:1 ratio) had a synergistic effect against 49/50 MRSA, 31/50 MSSA, 12/49 MRSP and 23/49 MSSP.

The MIC values for fusidic acid as one of the gold standards in topical antibacterial therapy and miconazole against staphylococci (*S. aureus* and *Staphylococcus epidermidis*) were also directly compared in the study by Alsterholm et al. [5]. For fusidic acid the MIC values against staphyococci were 0.13 μg/mL in comparison to 1.563 μg/mL obtained with miconazole. For miconazole this matches with our results. However, in this in vitro study fusidic acid was less effective than in the study by Clark et al. [16] and only in a power of ten more effective than miconazole. In a further study Clark et al. assessed the MICs of combinations of chlorhexidine/miconazole and chlorhexidine/trisEDTA in vitro in 196 canine *Staphylococcus pseudintermedius* isolates.[17] TrisEDTA alone did not inhibit growth. The Chlorhexidine/miconazole MIC_{90} (0.5 mg/L) was lower than those of either drug alone (chlorhexidine MIC_{90} 2 mg/L; miconazole MIC_{90} 1 mg/L; $P<.05$) and lower than chlorhexidine/trisEDTA MICs (median=1 mg/L; $P<.0005$).

In this study some of the *Corynebacteria* spp. showed excellent sensitivity against miconazole with MICs ranged from (<0.024) 0.049 to 0.78 μg/mL after 24 h of incubation and 0.097 to 3.125 μg/mL after 48 h (Table 3, Figure 1A,B). These results are in accordance with the data published by Nenoff et al., [18] who found *Corynebacteria* to be susceptible to bifonazole with MICs of 0.05 to 1.56 μg/mL.

In our study no inhibitory effect of miconazole was found against the gram-negative bacteria within the concentrations and incubation periods tested (MIC >200 μg/mL). The results were confirmed by the MICs determined with the ATCC reference strains and are well in line with the data published by Pietschmann et al. [14,15].

Azoles like the imidazole miconazole exert their antifungal effects by inhibition of the cytochrome p450 dependent enzyme lanosterol-14-demethylase which is essential for the ergosterol synthesis in fungi.[7–9,25] The antibacterial effect might be based on the presence of 14α-sterol-demethylase-homologues in staphylococci.[5,25] More recent data correlated the antibacterial activity with the inhibition of bacterial flavohaemoglobins.[26,27] They play a key role in bacterial resistance to nitrosative stress and NO signalling modulation. Remarkably, not all azole derivatives exhibit similar effects. Thus in a study by Sugita et al. on the in vitro activities of azole antifungal agents against *propionibacterium acnes*, fluconazole and voriconazole showed no anti-*P. acnes* activity with the concentrations tested.[11]

In this study a broad spectrum of bacterial pathogens important for skin diseases was tested. Miconazole was found to have antibacterial properties that include antistaphylococcal activity. Most interestingly, there were no differences in susceptibility between *Staphylococcus (S.) aureus*, methicillin-susceptible (MSSA) methicillin-resistant (MRSA) and fusidic acid-resistant (FRSA) *S. aureus* isolates. In dermatology, the resistance pattern of *S. aureus*, one of the most important skin pathogens, is of special interest. Methicillin-resistant *S. aureus* (MRSA) strains have spread successfully from hospitals into the community. Furthermore a fusidic acid-resistant clone of *S. aureus* (FRSA) has been responsible for outbreaks of bullous impetigo among children in Sweden and Norway and is now reported frequently in patients with atopic dermatitis.[3,28–31] To the best of our best knowledge

our report is the first to show that miconazole is also effective against FRSA.

Especially in inflammatory skin disease, such as atopic dermatitis, seborrhoeic dermatitis and psoriasis, microbes are believed to trigger, exacerbate or sustain the pathological processes.[3,4,6] Azoles with their combined antifungal and antibacterial effects against staphylococci, streptococci, dermatophytes and yeasts can be of great use in dermatology where infections are often mixed.[6] As topical skin medications with a broad, non-resistance-promoting activity, they have a very low potency of causing contact allergy in contrast to antibiotics.[32,33] Furthermore, in combination with topical corticosteroids miconazole can be a valuable option in the treatment of limited superficial infections such as *S. aureus*-mediated flare-ups of atopic dermatitis or other kind of superinfected eczema by restoring the defective skin barrier more rapidly.[2]

One problem with the present and other MIC studies is the difficulty of interpreting and applying in vitro data to the in vivo situation. Specifically, it is hard to transform a MIC value in µg/mL obtained with the agar dilution assay into dosing suggestions for the amount of cream to be applied in order to reach a similar concentration on the skin.[5]

The in vitro data suggest that miconazole could be a useful therapeutic option for superficial infections also caused by resistant staphylococci. However, in vivo environments can differ greatly influenced by several factors such as pH, salt concentrations and temperature. Therefore a proper clinical investigation is required. However, the low MICs against staphylococci and corynebacteria are likely to be exceeded by topical therapy. This may also apply to anaerobes esp. *propionibacterium acnes*, although the MIC found is somewhat higher. Miconazole in a 2% cream formulation generates relatively high local concentrations of the active substance.

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