Susceptibility of dermatophytic fungi to commonly used disinfectants*

Susceptibilidade de fungos dermatófitos a desinfetantes comumente utilizados

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Abstract

This study aimed to evaluate the antidermatophytic activity of three commercial disinfectants commonly used for environmental control of microorganisms in veterinary medicine. Sodium hypochlorite at 40 μL/mL, chloro-phenol derived at 30 μL/mL and chlorhexidine digluconate at 66.7 μL/mL were tested against 14 strains of dermatophytes, identified as Microsporum canis (n: 3) and Microsporum gypseum (n: 11). The tests were performed in accordance with guidelines of the Clinical and Laboratory Standards Institute (CLSI), documents M38-A2 and M51-A, adapted to disinfectants. In the microdilution broth test, chlorhexidine digluconate had MIC values (Minimum Inhibitory Concentration) of 4.16 μL/mL and MCF (Minimum Fungicidal Concentration) from 4.16 to 8.33 μL/mL, while chloro-phenol derived obtained MIC and MCF of 1.87 μL/mL, and both disinfectants had fungicidal activity at concentrations below the recommended. Sodium hypochlorite obtained MIC from 10 to 80 μL/mL and MFC of 40 to 80 μL/mL, requiring at most isolates twice the recommended concentration to achieve same activity. In the disc diffusion test, the mean inhibition zones for chlorhexidine digluconate was 10.53 mm, for chloro-phenol of 9.9 mm and for sodium hypochlorite was 6.2 mm. Chlorhexidine digluconate and chloro-phenol presented a significant reduction in the growth of dermatophytes, while sodium hypochlorite in concentration recommended showed a low antifungal activity against tested isolates.

Keywords: Antidermatophytic activity, Disinfection, Microdilution broth technique, Disc diffusion.

Introduction

Annually millions of superficial mycoses are diagnosed in humans and animals, most caused by Trichophyton sp., Epidermophyton sp. and Microsporum sp. dermatophytes, a group of filamentous fungi that infect keratinized tissue (Grumbt et al., 2011). Dermatophytosis is a contagious and zoonotic dermatomycosis which leads to costly treatment, requiring control measures and prevention (Cafarchia et al., 2006; Chermette et al., 2008). The control of this mycosis is hampered by the presence of arthroconidia in the environment for a long period of time (Rycroft and McLary, 1991; Chermette et al., 2008). Additionally to treatment with antifungal medication, one of the most important
measures in controlling dermatophytosis in veterinary cases is
the environmental disinfection, failures in disinfection can result
in chronic disease, infection and reinfection (Chernette et al.,
2008).

However, there are few informations regarding the use of
disinfectants in controlling dermatophytosis, and the dilution of
1:10 household bleach on nonporous surfaces is recommended
(Moriello and Newbury, 2006). Chlorine at 1% had a high level
disinfectant bringing about a rapid inactivation of *Trichophyton*
species, followed by 5% phenol and 0.5% quartenary ammonium
compounds (Gupta et al., 2001).

In Brazil, the analysis of the antifungal activity of chemicals
disinfectants is not standardized, using different methods
(Estrela et al., 2003; Menezes et al., 2008; Madrid et al., 2012)
in accordance with the standards of Association of Official
Analytical Chemists (AOAC) (Horwitz and Latmer Jr., 2010) and
the National Health Surveillance Agency (ANVISA).

The objective of this study was evaluate the antidermatophytic
susceptibility to three commercial disinfectants – sodium
hypochlorite, chlorhexidine gluconate and chlorophenol
derivatized – commonly used for environmental control of
dermatophytes in veterinary medicine.

**Materials and methods**

Fourteen dermatophytes isolates of *Microsporum canis* (*n*: 3) and
*Microsporum gypseum* (*n*: 11) previously isolated from clinical
cases of feline and canine dermatophytosis were subcultured on
Potato Dextrose Agar (PDA; Difco Laboratories, Detroit, MI, USA)
and incubated at 25°C for seven days. Inoculum suspensions
were obtained by scraping the agar surface with 1% Tween 20
in saline solution. The inoculum consisting of conidia and fungal
hyphae was standardized at McFarland scale 1, at a final cell
density of approximately 1 to 5 x 10³ CFU ml⁻¹.

For disinfectant tests, sodium hypochlorite solution at 4% (40
μL/mL) (QBoa® Indústria Anhembi S/A, São Paulo, Brazil),
chlorhexidine digluconate at 6.6% (66.7 μL/mL) (Cloroxidina-
Cetrimida Chemitec®, Chemitec Agro-veterinária, São Paulo,
Brazil) and chloro-phenol derivatize at 3% (30 μL/mL) (Pinho Sol®,
orto-benzil p-clorofenol 0.25%, Colgate-Palmolive Indústria e
Comércio Ltd, São Paulo, Brazil) according to the manufacturer’s
recommendations were evaluated using the disc diffusion method. Both
microdilution method was also utilized for test six dilutions in log₂,
varying from 2 to 0.062 times the concentrations recommended by the
manufacturers.

The broth microdilution method was performed in accordance with Clinical
and Laboratory Standard Institute (CLSI) guidelines, document M38-A2
(CLSI, 2008), with modifications adapted to disinfectants as follows. A
fungical inoculum aliquot diluted in RPMI-1640 (Roswell Park Memorial Institute - Sigma Chemical Co.,
Steinheim, Germany) (1:50) buffered to a pH 7.0 with MOPS
(3-morpholin-4-yl-propane-1-sulfonic acid) was added to each
microdilution well containing the disinfectant previously diluted in
RPMI-1640. The microplates were incubated at 25°C for seven
days. The Minimal Inhibitory Concentration (MIC) was defined
as the lowest disinfectant concentration at which no growth
could be seen. To determine Minimal Fungicidal Concentration
(MFC), 10 μl aliquots from each well were spread on SDA (SDA,
Neogen Acumedia®, Michigan, USA) Petri dishes and incubated
at 25°C until thirty days.

Disc diffusion assays were performed according to the guidelines
provided by the CLSI document M51-A (CLSI, 2010), adapted
to disinfectants as follows. Briefly, sterile filter papers discs
(Whatman® Whatman International Ltd., number 1 with 5
mm diameter) were impregnated with the disinfectant solution
and sterile distilled water (control). The discs were placed in
duplicates on SDA added with chloramphenicol (Neogen
Acumedia®, Michigan, USA) previously inoculated with 0.1
mL of each fungal isolate. The plate was incubated at 25°C
for seven days. The antifungal activity of each disinfectant was
assessed by measuring the inhibition growth around the discs
and compared to the control.

For MIC the average of duplicates from the reciprocal of the
lowest dilution where there was inhibition of microbial growth
was performed. The diffusion test result was obtained from the
average of duplicates of each microorganism. The comparative
analyses of the data were performed by the Student’s t-test.

**Results and Discussion**

The culture microdilution of the disinfectants chlorhexidine
digluconate and chloro-phenol derivated showed MIC and
MFC values below the concentration recommended by the
manufacturer. Chlorhexidine digluconate reached 0.41% (4.16
μL/mL) of MIC and MFC varied from 0.41% to 3.3% (4.16-8.33
μL/mL) for all isolates, presenting fungicidal action at half
the recommended concentration. Chloro-phenol derivated
presented MIC value 0.18% (1.87 μL/mL) for all isolates and
MFC 0.18% (1.87 μL/mL) having fungicidal activity with 1/8 of
the recommended concentration.

Sodium hypochlorite had the worst performance, requiring twice
the recommended concentration to have antifungal activity. It
presented MIC ranging from 1 to 8% (10- 80 μL/mL) remaining
above the recommended concentration in 40% (*n*: 6) of the
isolates tested, the MFC ranged from 4 to 8% (40-80 μL/mL) in
66% (*n*: 10) of the isolates (Table 1).

**Table 1: Minimal inhibitory concentration (MIC) and minimal fungicidal concentration
(MFC) in μL/mL of chlorhexidine digluconate, sodium hypochlorite and chloro-
phenol derivatized in dermatophytes isolates**

<table>
<thead>
<tr>
<th>Species</th>
<th>Chlorhexidine Digluconate at 66.7 μL/mL*</th>
<th>Sodium Hypochlorite at 40 μL/mL*</th>
<th>Chloro-phenol Derived at 30 μL/mL*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. canis</em></td>
<td>≤4.16</td>
<td>4.16-8.33</td>
<td>10-40</td>
</tr>
<tr>
<td><em>M. gypseum</em></td>
<td>≤4.16</td>
<td>4.16-16.67</td>
<td>10 ≥80</td>
</tr>
</tbody>
</table>

*Concentration recommended by the manufacturer.

In disc diffusion significant difference was observed (p<0.05)
in fungicidal activity between the disinfectants. The highest
inhibition zones means were chlorhexidine digluconate (10.53
mm) and chloro-phenol derivatized (9.9 mm), while sodium
hypochlorite had the lowest (2.06 mm). No zones of inhibition
were observed on the control discs (Table 2).
Chlorhexidine digluconate was effective against dermatophytes (Xavier et al., 2007; Menezes et al., 2008). It is more resistant to disinfectants than yeasts and considerably lower values of MIC and MFC (4.16 μL/mL). Similar research on the effect of disinfectants on fungal organisms is limited and most often involve yeast (McDonnell and Russel, 1999; Bambace et al., 2003) with limited information on filamentous fungi (Xavier et al., 2007; Menezes et al., 2008). It may still be considered the occurrence of response variations of micro-organisms to disinfectants and that filamentous fungi are more resistant to disinfectants than yeasts and considerably more resistant than non sporulated bacteria (Russel, 2003).

Chlorhexidine digluconate was effective against dermatophytes in lower values of MIC and MFC (4.16 μL/mL). Similar research using this disinfectant at 66.7 μL/mL against clinical and environmental isolates of Sporothrix schenckii, dimorphic fungus, obtained inhibitory effect at concentrations below 0.8% (Madrid et al., 2012). The efficacy of chlorhexidine at 4.16 μL/mL was previously demonstrated in filamentous fungi of the genera Aspergillus (Xavier et al., 2007; Xavier et al., 2008) and using a 1% aqueous solution of chlorhexidine against yeasts for the disinfection of surfaces using the technique spray wipe spray (Bambace et al., 2003).

The best performance of the chlorhexidine digluconate in relation to sodium hypochlorite in the broth microdilution is in accordance with the study (Menezes et al., 2008) that used sodium hypochlorite at 5.25% e chlorhexidine digluconate at 2% against Candida albicans. However, there is a report (Estrela et al., 2003) that both disinfectants at 2% were effective against C. albicans, with better performance in the sodium hypochlorite direct exposure test and of the chlorhexidine digluconate in the disc diffusion test.

The poor performance of the sodium hypochlorite in broth microdilution and disk diffusion tests was similar to that found in a study that evaluated the performance of the chlorhexidine digluconate and sodium hypochlorite in isolates of S. schenckii (Madrid et al., 2012). In a trial evaluating the efficacy of disinfectants in bacteria using the disk diffusion technique, the 0.5% chlorhexidine was effective, while sodium hypochlorite only had bactericidal activity at concentrations of 2% or more (Pedrini and Margatho, 2003).

However, it is considered that the growth inhibition zone on the disk diffusion test depends on the solubility and diffusivity of the tested substances, which may not express its full fungicidal potential (Estrela et al., 2003). Thus, underperforming of the sodium hypochlorite can be partly explained by the acidity of the medium and instability of chlorine. However we take into account the ability of dermatophytes to raise the pH of the culture medium (Weitzman and Summerbel, 1995). Thus, sodium hypochlorite used in environment should have greater concentrations than that used for in vitro tests, due to the influences of external factors such as temperature, pH and the presence of organic matter (McDonnell and Russel, 1999).

Contrary to the findings, the solution of sodium hypochlorite at 1% associated with solution spray of enilconazole at 0.6% applied daily for 10 minutes was effective in controlling dermatophytosis in animal shelters (Carlotti et al., 2010). However, enilconazole is not available in Brazil. The same way, the 5% sodium hypochlorite solution was 100% fungicidal at a final dilution of 1:80, while chlorhexidine-diacetate at 2% was ineffective even when used at four times the manufacturer’s recommended dilution (1:100) (Moriello et al., 2004). However, these results were obtained with techniques different from those used in this present study.

Despite the diversity of techniques and results on the fungicidal activity of chloro-phenol derivate, in our study, this disinfectant achieved satisfactory results, in accordance with previous published data which demonstrated that phenolic solution at 2% had fungicidal activity after 15 minutes interaction with the dermatophytes (Terleckyj and Axler, 1993). However, in an experiment using feline hair naturally infected by M. canis, chloro-phenol derivate had a low performance, whereas hypochlorite sodiumachieved better results (Rycroft and McLay, 1991). Similarly, 1% chlorine had a better performance against T. mentagrophytes than 5% phenol (Gupta et al., 2001).

### Table 2: Average diameter (mm) of the inhibition zone of fungal growth in the disc diffusion test

<table>
<thead>
<tr>
<th>Species</th>
<th>Chlorhexidine Digluconate mean±SD</th>
<th>Sodium Hypochlorite mean±SD</th>
<th>Chloro-phenol Derivated mean±SD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsporum canis</td>
<td>10 ± 0.6</td>
<td>7 ± 4.0</td>
<td>12 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>Microsporum gypseum</td>
<td>14.3 ± 5.5</td>
<td>8 ± 2.0</td>
<td>11.4 ± 1</td>
<td>0</td>
</tr>
</tbody>
</table>

SD: standard deviation.

In isolates of M. gypseum, highly significant differences were found in antifungal activity of chlorhexidine digluconate and chloro-phenol derivated in relation to sodium hypochlorite. In isolates of M. canis, only chloro-phenol derivated and sodium hypochlorite differed significantly. However, chlorhexidine digluconate and chloro-phenol derivated had equal antifungal activity in all fungal species evaluated (Table 3).

### Table 3: Comparative analysis of the disinfectants in the disc diffusion among the species Microsporum canis and Microsporum gypseum, by Student’s t test

<table>
<thead>
<tr>
<th>Disinfectants</th>
<th>Microsporum canis</th>
<th>Microsporum gypseum</th>
</tr>
</thead>
<tbody>
<tr>
<td>/CD-SH/</td>
<td>5.67 (p=0.0740)²</td>
<td>12.81 (p=0.0001)¹</td>
</tr>
<tr>
<td>/CD-CPD/</td>
<td>1.17 (p=0.0572)²</td>
<td>3.12 (p=0.1526)³</td>
</tr>
<tr>
<td>/SH-CPD/</td>
<td>6.83 (p=0.0037)¹</td>
<td>9.68 (p=0.0000)²</td>
</tr>
</tbody>
</table>

CD: chlorhexidine digluconate, SH: sodium hypochlorite, CPD: chloro-phenol derivated, /: Value of the difference between disinfectants expressed in module. ¹: Homogeneous variance by F test at 5% probability, ²: Homogeneous variance by F test at 5% probability.

The microdilution culture and disc diffusion techniques showed similar results regarding the fungicidal activity of the three disinfectants tested. In both used techniques the sodium hypochlorite demonstrated the worst performance as a fungicidal agent.

Studies on the effect of disinfectants on fungal organisms are limited and most often involve yeast (McDonnell and Russel, 1999; Bambace et al., 2003) with limited information on filamentous fungi (Xavier et al., 2007; Menezes et al., 2008). It may still be considered the occurrence of response variations of micro-organisms to disinfectants and that filamentous fungi are more resistant to disinfectants than yeasts and considerably more resistant than non sporulated bacteria (Russel, 2003).

## References

- Madrid et al., 2012
- Estrela et al., 2003
- McDonnell and Russel, 1999
- Bambace et al., 2003
- Xavier et al., 2007
- Xavier et al., 2008
- Carlotti et al., 2010
- Rycroft and McLay, 1991
- Gupta et al., 2001
- Madrid et al., 2012
- Estrela et al., 2003
- Pedrini and Margatho, 2003
- Weitzman and Summerbel, 1995
- Carloti et al., 2010
- Terleckyj and Axler, 1993
- Rycroft and McLay, 1991
- Gupta et al., 2001

Conclusion
Chlorhexidine digluconate and chloro-phenol derivate had fungicidal activity against dermatophytes at concentrations below those recommended by the manufacturer. However, the most used and prescribed disinfectant in Brazil is hypochlorite sodium, that was ineffective against most dermatophytes tested, requiring concentrations two times above the recommended to achieve fungicidal activity. Techniques of disc diffusion and broth microdilution presented similar results. Therefore, it demonstrates the need for reassessment of the recommended concentrations as fungicide of the main disinfectants used in Brazil.

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References