Received: 27 February 2022 | Revised: 3 May 2022 | Accepted: 7 May 2022

DOI: 10.1111/myc.13468

ORIGINAL ARTICLE

mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases WILEY

Disinfection trials with terbinafine-susceptible and terbinafine-resistant dermatophytes

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Funding information
Electrolux; GRUNDIG NORDIC

Abstract

Background: Treatment of tinea pedis and onychomycosis is complicated by high rates of reinfection and the emergence of terbinafine-resistant strains of *Trichophyton* spp. Effective disinfection of contaminated socks is an important measure. Appropriate washing reduces the risk of reinfection and is paramount in treating tinea pedis and onychomycosis.

Objectives: The aim of this study was to describe the effect of commonplace disinfection methods using socks pieces inoculated with terbinafine-resistant or terbinafine-susceptible isolates of *Trichophyton* spp.

Methods: Sock pieces were inoculated with seven terbinafine-resistant isolates of *Trichophyton* spp. with known mutations in the SQLE-gene (*T. rubrum* ($n = 3$), *T. interdigitale* ($n = 1$) and *T. indotineae* ($n = 3$)) and six terbinafine-susceptible isolates of *Trichophyton* spp. (*T. rubrum* ($n = 3$) and *T. interdigitale* ($n = 3$)). Methods of disinfection included soaking in a quaternary ammonium (QAC) detergent (0.5, 2 and 24 h), freezing at −20°C (0.5, 12 and 24 h), domestic and steam washing (both at 40°C with detergent). Sock pieces were cultured for 4 weeks following disinfection. The primary end point was no growth at the end of week 4.

Results: Soaking in a QAC-detergent for 24 h procured at disinfectant rate of 100% (13/13), whilst soaking in 0.5 and 2 h had a disinfectant rate of 46.2% (6/13) and 84.6% (11/13), respectively. Domestic washing (40°C with detergent) produced a disinfectant rate of 7.7% (1/13). Freezing at −20°C (0.5, 12 and 24 h) and steam washing (40°C with detergent) had no disinfectant properties.

Conclusions: Soaking in a QAC-detergent for 24 h effectively disinfected sock pieces contaminated with dermatophytes.

KEYWORDS
terbinafine resistance, tinea, dermatophytosis, onychomycosis, *Trichophyton*

1 | INTRODUCTION

The anthropophilic dermatophytes *Trichophyton (T.) rubrum* and *T. interdigitale* are the main pathogens causing tinea pedis and onychomycosis (tinea unguium).[1] These highly prevalent mycoses constitute common complaints in general and dermatological practice.[2,3] The high prevalence of tinea pedis is attributable to multiple factors: A: The anatomy of the nail that lacks the adaptive immune response, which facilitates reinfection of the skin from a dermatophyte reservoir.[4,5] B: Co-morbidities (e.g. diabetes).[6] C: Environmental factors (e.g. humidity from occlusive footwear and the use of public bathing facilities).[6–8] The first-line and generally effective agent for *Trichophyton* infections is terbinafine.[9,10] Terbinafine inhibits the squalene epoxidase (SQLE), an important enzyme in the biosynthesis of ergosterol, leading to a toxic intracellular accumulation of squalene.[11,12] Point mutations in the SQLE-gene have been found in terbinafine-resistant strains of *T. interdigitale* and *T. rubrum*.[1,13,14] Moreover, extensive use of topical formulations containing terbinafine in Asia has been associated with the emergence and spread of terbinafine-resistant *T. indotineae*.[15,16]

Hence, tinea pedis and onychomycosis represent a significant health problem due to their highly infectious nature and rising rate of terbinafine resistance particularly not only in India but also elsewhere.[17–20] A multifaceted approach is needed to not only focus on the medical treatment of the infection but also at eliminating fungal reservoirs such as those in contaminated textiles. Domestic laundering at 60°C of textiles contaminated with *T. rubrum* has been documented to be effective, but is not always feasible, whereas studies on washing at lower temperatures show conflicting results.[21,22]

This implies that there is a continued need for effective and practical disinfection of fomites involved in the transmission of fungal infections. Other sanitation methods published for *Scabies*, *Toxoplasma gondii*, yeast and *E. coli* include cryogenic disinfection at −20°C[23–25] and quaternary ammonium compounds (QACs)[26] but have to our knowledge not been systematically tested for *Trichophyton* spp. Furthermore, not all textiles tolerate laundering above 60°C warranting research into alternative methods of disinfection.

The aim of this study was to study additional disinfection methods for both terbinafine-resistant and terbinafine-susceptible strains of *Trichophyton* spp. providing data for rational handling of relevant fomites, *in casu* socks.

2 | MATERIALS AND METHODS

2.1 | Ethics

Full ethics approval was waived by the local committee of research ethics (EMN-2021-03052).

2.2 | Dermatophyte isolates

Thirteen dermatophyte isolates were retrieved from the Statens Serum Institute (SSI) strain collection and sequenced for known mutations in the SQLE-gene (six *T. rubrum* (three terbinafine-susceptible [SQLE: WT] and three terbinafine-resistant [SQLE: L393S, L393F and F397L]), four *T. interdigitale* (three terbinafine-susceptible [SQLE: WT] and one terbinafine-resistant [SQLE: L393F]), and three *T. indotineae* (terbinafine-resistant [SQLE: two F397L and one L393S]). Isolates of *T. interdigitale* and *T. indotineae* were ITS sequenced to ensure correct species identification.[16]

2.3 | Inoculum preparation

Dermatophyte isolates were incubated at 25°C for at 2–4 weeks on Sabouraud agar supplemented with cycloheximide and chloramphenicol (SSI Diagnostica A/S). The inoculum was prepared by covering the isolates with 8 ml of sterile water supplemented with 0.1% tween 20 (SSI Diagnostica). The mycelium was rubbed with a sterile cotton swab (Cotton Tipped Applicator, OneMed Group Oy) to release conidia. A sterile syringe (Syringe, BD Emerald™) was utilised to retransfer the suspension to the test tube through a 11 µm sterile filter (Millipore Nylon Net Filter 11 µm NY11, Merck Millipore Ltd) ensuring that the suspension was solely composed of conidia. The suspension was vortexed for 15 s with a gyratory vortex mixer at 2000 revolutions per minute. A spectrophotometer (Densimat, bioMérieux, Marcy-l'Étoile, France) was used to adjust the suspension into a final working inoculum of 1.0 OD. A positive control was produced to ensure that the final working inoculum was viable.

2.4 | Inoculation of sock pieces

Cotton sock pieces measuring 4 cm^2 were autoclaved prior to inoculation with 400 µl (OD 1.0) of fungal suspension as this was the minimal volume needed to soak the entire sock piece. The sock was then incubated for 2 weeks at 25°C on Sabouraud agar supplemented with chloramphenicol and cycloheximide (SSI Diagnostica A/S). Mycelium growth covering the sock piece was visually noted. A positive control was included to assess the viability of the inoculum for each isolate and two negative controls (one autoclaved and one not subjected to any disinfection) to rule out cross-contamination.

2.5 | Disinfection methods

2.5.1 | Quaternary ammonium compound

Dermatophyte inoculated sock pieces were soaked according to the manufacturer's instructions in a 1:10 solution of QAC-detergent

TABLE 1 Disinfection effect of quaternary ammonium detergent exposure (0.5, 2 and 24 h) expressed as number of negative cultures/total number of tested isolates

Disinfection method	0.5 h % (*n*/total)	2 h % (*n*/total)	24 h % (*n*/total)
Species			
T. rubrum (TBR)	66.7% (2/3)	100.0% (3/3)	100.0% (3/3)
T. rubrum (WT)	66.7% (2/3)	100.0% (3/3)	100.0% (3/3)
T. interdigitale (TBR)	0% (0/1)	0% (0/1)	100.0% (1/1)
T. interdigitale (WT)	66.7% (2/3)	66.7% (2/3)	100.0% (3/3)
T. indotineae (TBR)	0% (0/3)	100.0% (3/3)	100.0%, (3/3)
Overall efficacy of disinfection rate (% (*n*/total))	46.2% (6/13)	84,6% (11/13)	100.0% (13/13)

Abbreviations: h, hours; *n*, number; TBR, terbinafine-resistant isolate; WT, wild-type-isolate.

(Rodalon INDENDØRS ®, Brenntag Nordic A/S) in sterile water for 0.5, 2 and 24 h before incubation. The QAC-detergent contained three chemically active QAC-compounds (Alkyl (C12-16) dimethylbenzylammonium chloride(≤1%), didecyldimethylammonium chloride (≤1%), C12-C14-Alkyl(ethylbenzyl)dimethylammonium chloride (≤1%)).[27] The final concentration of QAC was 0.3% in the soaking solution. Rodalon INDENDØRS® is commercially available, but the company producing it (Brenntag Nordic A/S) was not informed about the current study.

2.5.2 | Freezing

Inoculated sock pieces were placed in freezer bags and frozen at −20°C for 30 min, 12 h and 24 h, respectively.

2.5.3 | Domestic and steam washing

Inoculated sock pieces were placed in tube gauze to avoid being flushed out during the washing process and washed at 40°C for approximately 1 h and 45 min according to a cotton washing programme using a domestic detergent. Additionally, the effect of adding a steam wash cycle for approx. 50 min (according to the washing programme) was examined. The drum of the washing machine was cleaned with ethanol (70%) (Ethanol Disinfection Disposable wipes with ethanol, WET WIPE A/S) and chlorine-based (1000 ppm) (WipeClean Chlorine Disinfection, Plum A/S) wipes to prevent potential contamination from the previous disinfection test.

2.6 | Efficacy evaluation of disinfection

The treated sock pieces were incubated on Sabouraud agar supplemented with chloramphenicol and cycloheximide (SSI Diagnostica A/S) at 25°C for 4 weeks after disinfection to evaluate the disinfection efficacy. A fungal imprint was made by placing the side of the sock piece containing most mycelium face-down for 10 min on the agar prior to re-location of the sock pieces to an adjacent site face-up for final evaluation of growth. The fungal imprint was marked with an 'X' to enable assessment of growth. Photo documentation was performed weekly for 4 weeks for subsequent efficacy evaluation and comparison. The primary end point was no growth at both the imprint site and on the sock piece at week 4. All *Trichophyton* isolates were individually tested with each of the eight disinfection methods.

3 | RESULTS

3.1 | Viability of the inoculum and negative controls

All positive controls displayed growth after 4 weeks confirming the viability of inocula for all experiments. None of the negative controls showed growth after 4 weeks of incubation.

3.2 | Efficacy of disinfection methods

3.2.1 | Quaternary ammonium detergent (Rodalon INDENDØRS ®)

No growth was seen following soaking of inoculated sock pieces in the QAC-detergent (Rodalon INDENDØRS ®, Brenntag Nordic A/S) for 24 h (Table 1). The disinfection rate was thus 100.0% (13/13). Soaking for 2 h leads to growth one (1/3) terbinafine-resistant and 1/3 terbinafine-susceptible *T. interdigitale*. Thus, the overall disinfection rate was 84.6% (11/13) (Table 1). Soaking for 0.5 h was notably less efficacious (overall 46.2%) (6/13) (Table 1).

3.2.2 | Freezing

Growth was observed at both the imprint site and on the sock piece for all isolates of *Trichophyton* spp. after 4 weeks of incubation resulting in a disinfection rate of 0% (0/13).

3.2.3 | Domestic washing (40°C with detergent)

One (1/3) isolate of terbinafine-susceptible *T. rubrum* was disinfected by domestic washing at 40°C with detergent. Additionally, no growth was observed at the imprint site of one terbinafine-resistant *T. rubrum* following disinfection. Thus, the overall disinfection rate was 7.7% (1/13).

3.2.4 | Steam washing (40°C with detergent)

Growth was detected for all tested terbinafine-susceptible and terbinafine-resistant *Trichophyton* spp. Consequently, the overall disinfection rate was 0% (0/13).

4 | DISCUSSION

Efficient control of fomites for the prevention of reinfection is of great importance not only for patients suffering from tinea pedis and onychomycosis, but also to halt the emerging spread of terbinafine-resistant infections. Socks are one important fomite in this context. Patient advice on efficient sanitisation of socks must be based on systematic evidence-based research. We evaluated and compared different approaches for this purpose: soaking in a QAC-detergent, freezing, steam washing and domestic washing with the aim to find an easily implementable and effective method of disinfection of socks inoculated with dermatophytes.

We demonstrated that soaking in a QAC-based detergent for 24 h was 100% effective in eradicating all viable isolates of *Trichophyton* spp. from the inoculated sock pieces, irrespective of resistance pattern. QACs exercise their antifungal effect on yeast by disorganising the cell membrane inducing a biocidal cascade culminating in lysis of the fungal cell wall.[26] Yeasts and dermatophytes share some similarities in cell wall morphology, and the mechanism of action is thus expected to be the same for dermatophytes. The difference in efficacy exhibited by soaking for 0.5, 2 and 24 h is possibly due to insufficient penetration of the detergent into the large quantities of mycelium when soaking for shorter time periods. Soaking for 24 h in a QAC-based detergent seems preferable, but further studies are needed to assess the minimum time needed for disinfection. The highly concentrated inoculum adopted in this study is expected to greatly exceed the quantity of fungal material in socks of tinea pedis patients, and further studies are needed to explore whether a shorter soaking time may be sufficient when disinfecting socks from patients with a lower fungal load even though the content of arthrocondia, which is supposed to be more resistant, are unknown. Moreover, conidia or hyphae contained within skin scales or nail pieces may prove more difficult to disinfectant using QAC-detergents than fungal material from inoculated sock pieces. Also, this study did not explore whether washing of the sock pieces after soaking in the QAC-detergent would affect the demonstrated disinfectant properties. Studies on the QAC-concentrations needed to elicit a fungicidal effect on dermatophytes show inconsistent results.[28,29] Previously, it has been reported that QAC-concentrations of less than 0.5% only elicited a fungistatic effect on terbinafine-sensitive strains of *T. mentagrophytes*, *T. raubitschekii* and *T. tonsurans*.[29] However, we found that QAC-concentrations of 0.3% are sufficient to disinfect dermatophytes thus aligning with what has been reported for *T. mentagrophytes* elsewhere.[28] QAC-based sanitation of textiles has many advantages, including low toxicity, low risk of resistance, no discolouring of fabrics, and easy implementation by patients.[26,30]

Rapid freezing is necessary to facilitate cytotoxic formation of intracellular ice crystals.[31] Cryogenic disinfection is achieved by a freezing rate above of 20°C per min in yeast,[31] but quantification of the cooling rate falls outside the scope of this paper. However, our results indicate that the freezing rate produced by domestic freezing at −20°C is inefficient in forming intracellular ice crystals and cell death in dermatophytes rendering cryogenic disinfection inadvisable. Freezing below −20°C was not investigated given that domestic freezers generally do not freeze below this temperature.

The effect of laundering at temperatures below 60°C has previously been investigated with conflicting results. This may be due to the lack of methodological standardisation.[21,22] Our findings suggest that laundering textiles contaminated with *Trichophyton* spp. at 40°C using domestic detergent is an ineffective disinfection method. These results align with previous findings for terbinafine-susceptible strains of *T. rubrum*[22] and is here documented to be true also for terbinafine-susceptible strains of *T. interdigitale* and terbinafine-resistant strains of *T. rubrum*, *T. interdigitale*, and *T. indotineae*. Given that previous studies have suggested that high humidity and temperatures of 31.8°C ± 2.7°C at the inner side of footwear contribute to the risk of tinea pedis,[6] it is perhaps not surprising that laundering at 40°C is ineffective as a disinfectant method since these conditions are somewhat mimicked by this method.

In conclusion, we propose that patients should be advised to either launder their socks at a minimum of 60°C[21,22] or soak their socks in a QAC-based detergent for at least 24 h prior to laundering to effectively reduce the risk of reinfection. Our findings suggest that laundering at 40°C with domestic detergent, steam washing at 40°C with domestic detergent and freezing at −20°C are all ineffective methods for disinfection of contaminated fabrics. The high rate of reinfection from tinea pedis and onychomycosis underscores the need for evidence-based recommendations. In light of the current epidemic of terbinafine-resistant infections, this has become even more important as effective disinfection of textiles may contribute to a lower transmission and reinfection rate.

ACKNOWLEDGEMENTS

The authors would like to thank Birgit Brandt and Karin Jørgensen for their help pertaining to all aspects of the laboratory work and Rasmus Krøger Hare for ITS sequencing. The authors would

also like to express their appreciation to GRUNDIG NORDIC and Electrolux for donation of washing machines. Statens Serum Institut funded laboratory material. All other expenses were covered by the Dept. of Dermatology, Zealand University Hospital, Roskilde, Research Fund.

CONFLICT OF INTEREST

Kristoffer Nagy Skaastrup has no conflict of interests. Karen Marie Thyssen Astvad has within the past 5 years received travel grants from Pfizer and Gilead and a speaker honoraria (personal fees) from Pfizer and Gilead. Maiken Cavling Arendrup has, over the past 5 years, received research grants/contract work (paid to the SSI) from Amplyx, Basilea, Cidara, F2G, Gilead, Novabiotics and Scynexis, and speaker honoraria (personal fee) from Astellas, Chiesi, Gilead, MSD and SEGES. She is the current chairman of the EUCAST-AFST. Gregor Borut Jemec reports grants from Leo research foundation, during the conduct of the study; personal fees from Abbvie, Chemocentryx, Kymera, Leo Pharma, Coloplast, grants; and personal fees from Novartis, UCB, Inflarx, grants from Janssen-Cilag, Serono and Regeneron, outside the submitted work. Ditte Marie Lindhardt Saunte was paid as a consultant for advisory board meeting by AbbVie, Janssen, Sanofi, Leo Pharma, Norvatis and received speaker's honoraria and/or received grants from the following companies: Abbvie, Pfizer, Galderma, Novartis and Leo Pharma during the last 5 years.

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How to cite this article: Skaastrup KN, Astvad KMT, Arendrup MC, Jemec GBE, Saunte DML. Disinfection trials with terbinafine-susceptible and terbinafine-resistant dermatophytes. *Mycoses*. 2022;65:741-746. doi: 10.1111/myc.13468