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Identification and *in vitro* antifungal susceptibility of dermatophyte species isolated from lesions of cutaneous dermatophytosis: A cross-sectional study

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ABSTRACT

Objectives: The objectives of the study were to determine the *in vitro* susceptibility of dermatophyte species, isolated from the clinically diagnosed lesions of cutaneous dermatophytosis to fluconazole, and terbinafine.

Materials and Methods: The skin scrapings from clinically diagnosed lesions of cutaneous dermatophytoses were cultured in Sabouraud dextrose agar to identify the causative dermatophyte. Antifungal susceptibility testing was performed using microbroth dilution assay.

Results: During the study period, 94 specimens from clinically diagnosed lesions of cutaneous dermatophytoses were received for fungal culture. Dermatophytes were identified as the causative agent in 44 specimens (*Trichophyton rubrum* was identified in 18/44 (40.9%), *Trichophyton mentagrophytes* in 17/44 (38.6%), *Trichophyton interdigitale* in 5/44 (11.4%), and *Nannizia gypsea* in 4/44 (9.1%) isolates). Minimum inhibitory concentration (MIC) of fluconazole was ≥64 μg/ml in 22.7% (10/44) and MIC of terbinafine was ≥0.5 μg/ml in 36.4% (16/44) of specimens. When compared to fluconazole, terbinafine showed a lower MIC 50 of 0.0019 μg/ml for *Nannizia gypsea*.

Limitations: Small sample size and lack of clinical correlation were the major limitations of the study. Antifungal susceptibility testing limited to fluconazole and terbinafine was another limitation of the study.

Conclusion: *Trichophyton rubrum* was the most common isolate identified in culture of scrapings from clinically diagnosed lesions of cutaneous dermatophytosis. A rising trend in MIC values of isolates to terbinafine and fluconazole was observed.

Keywords: Cutaneous, Dermatophytosis, Antifungal susceptibility, Terbinafine, Fluconazole

INTRODUCTION

Recent years have witnessed an alarming increase in chronic, recurrent, and recalcitrant dermatophytosis in India.[1] Despite the availability of a wide range of antifungals, treatment failure is often observed. This is often attributed to the probable emergence of drug-resistant strains. We did a cross-sectional study to determine the *in vitro* susceptibility of dermatophyte species isolated from patients with cutaneous dermatophytosis to two commonly used antifungals (terbinafine and fluconazole).

MATERIALS AND METHODS

We included the samples received in the microbiology department of our tertiary care center during a period of 18 months from January 2017 to June 2018 for fungal culture from clinically diagnosed lesions of cutaneous dermatophytosis. The Institutional Ethics Committee approved the study. Individual study participant gave written informed consent.

For fungal culture, the specimens were transported to the department of microbiology in two tubes of Sabouraud dextrose agar (SDA) – one without antibiotics and the other containing cycloheximide and chloramphenicol. The SDA tubes were incubated at 30°C. The cultures were examined daily for 4 weeks. If growth was obtained on SDA, identification was made based on colony morphology, microscopic appearance, and genotyping.

The microbroth dilution assay for antifungal susceptibility testing of dermatophytes was performed according to the Clinical and Laboratory Standards Institute guidelines – document M38-A2 of filamentous fungi.[2] Since terbinafine and fluconazole are water insoluble, dimethyl sulfoxide is used as a solvent. Antifungal drugs were prepared as stock solution and serial 2-fold dilutions were obtained to provide final concentrations that ranged from 0.125 to 64 µg/ml for fluconazole and 0.001 to 0.5 µg/ml for terbinafine which was filtered through a membrane filter.

All organisms were subcultured onto potato dextrose agar and incubated at 30°C for 4–5 days until a good conidial growth was obtained. Conidial suspensions were then prepared so that the concentration of final test inocula was 2 times the density needed for testing (1×10^3–3×10^3 colony-forming units/ml). This was done by adjusting optical densities between 0.102 and 0.192 using a spectrophotometer at 530 nm wavelength.

Aliquots of 100 µl of suspensions were inoculated in wells of microtiter plate containing 100 µl of tested antifungal of specific concentration. Growth and sterility controls were included for each isolate tested. All microdilution trays were incubated at 35°C without agitation and evaluated after 4 days.

For fluconazole and terbinafine, minimum inhibitory concentration (MIC) was defined as the lowest concentration that produced prominent inhibition of growth (approximately 80% or more reduction in growth compared to growth in growth control wells). MIC of fluconazole ranged from 0.125 to 64 microgram/ml. MIC of terbinafine ranged between 0.0009 and 0.5 µg/ml. MIC of >64 µg/ml and ≥0.5 µg/ml was considered resistant to fluconazole and terbinafine, respectively.[2]

MIC 50 was calculated by taking the drug concentration which inhibited 50% of isolates. MIC 90 was calculated by taking the drug concentration that inhibited 90% of the isolates.

All data were entered into Microsoft Excel sheets and analyzed with Inc. IBM company version 18 Chicago, SPSS Inc. (United States of America).

RESULTS

During the study period, 94 samples were received for fungal culture from lesions of clinically diagnosed cutaneous dermatophytoses. Potassium hydroxide (KOH) examination was positive for fungal hyphae in 74.5% (70/94) of skin specimens.

Of the 94 specimens, 46.8% (44/94) and 6.4% (6/94) yielded dermatophytes and other fungi respectively. *Trichophyton rubrum (T.rubrum)* was identified in 40.9% (18/44), *Trichophyton mentagrophytes (T. mentagrophytes)* in 38.6% (17/44), *Trichophyton interdigitale (T. interdigitale)* in 11.4% (5/44), and Nannizia gypsea (*N. gypsea*, formerly *Microsporum gypseum*) in 9.1% (4/44) of specimens from the skin lesions [Figures 1 and 2].

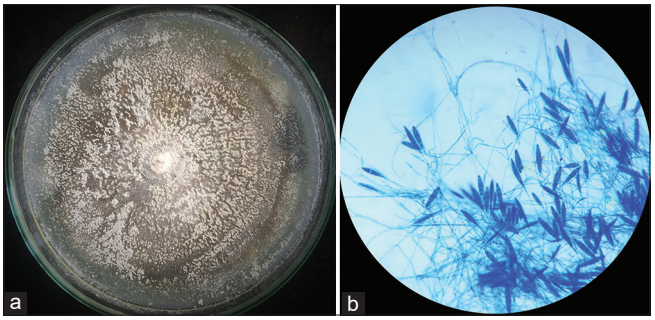

Figure 1: (a) Powdery growth of *Nannizia gypsea* on Sabouraud dextrose agar, obverse (b) *Nannizia gypsea* showing numerous, thin-walled, spindle-shaped macroconidia (lactophenol cotton blue, 400x).

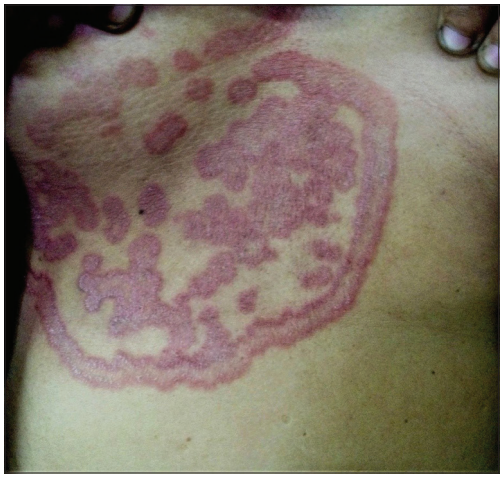

Figure 2: Well-defined, double-margined, annular, and circinate, erythematous plaque of dermatophytosis. *Nannizia gypsea* was isolated from the lesion.

In the present study, 10 isolates (10/44, 22.7%) were resistant to fluconazole and 16 isolates (16/44, 36.4%) were resistant to terbinafine. No significant difference was noted between the two antifungals on antifungal susceptibility testing. Six isolates (6/44, 13.6%) were resistant to fluconazole alone, 12 were (12/44, 27.3%) resistant to terbinafine, and 4 (4/44, 9.1%) were resistant to both fluconazole and terbinafine.

Among the 18 isolates of *T. rubrum* tested, 2 (2/18, 11.1%) were resistant to fluconazole, 6 (6/18, 33.3%) to terbinafine, and 1 (1/18, 5.6%) resistant to both fluconazole and terbinafine.

Among the 17 isolates of *T. mentagrophytes* tested, 2 (11.8%) were resistant to fluconazole, 5 (29.4%) to terbinafine, and 2 (11.8%) resistant to both fluconazole and terbinafine.

One (1/5, 20%) each out of the five isolates of *T. interdigitale* showed resistance to fluconazole and terbinafine, respectively, while 1 other (1/5, 20%) was resistant to both fluconazole and terbinafine.

All four isolates of *N. gypsea* were sensitive to terbinafine (0.001–0.5 μg/ml) where as 1 isolate (1/4, 25%) was resistant to fluconazole.

Fluconazole showed MIC 50 at 16 μg/ml for *T. rubrum*, *T. mentagrophytes,* and *N. gypsea* and at 32 μg/ml for *T. interdigitale*. MIC 90 was >64 μg/ml for all isolates except *T. mentagrophytes* [Table 1].

Terbinafine exhibited MIC 50 at 0.25 μg/ml for *T. interdigitale*, 0.125 μg/ml for *T. rubrum*, 0.0312 μg/ml for *T. mentagrophytes,* and 0.0019 μg/ml for *N. gypsea*. MIC 90 was >0.5 μg/ml for all isolates except *N. gypsea* which was 0.0312 μg/ml.

DISCUSSION

Out of the 94 skin scrapings cultured, 44 were positive for dermatophytes (46.8%), which was consistent with the findings of a previous study conducted in the same institution by Bindu *et al*. where they found a positivity of 45.3%.[3] The culture positivity for dermatophytosis was slightly lower than the 52.4% noted in another, recent Indian study.[4]

Table 1: MIC 50 and MIC 90 of fluconazole and terbinafine against dermatophyte species isolated from the lesions of cutaneous dermatophytosis.

Isolate	MIC	MIC of fluconazole in microgram/ml	MIC of terbinafine in microgram/ml
Trichophyton rubrum	MIC 50	16	0.125
	MIC 90	>64	>0.5
Trichophyton mentagrophytes	MIC 50	16	0.0312
	MIC 90	64	>0.5
Trichophyton interdigitale	MIC 50	32	0.25
	MIC 90	>64	>0.5
Nannizzia gypsea	MIC 50	16	0.0019
	MIC 90	>64	0.0312

MIC: Minimum inhibitory concentration (MIC 50 was calculated by taking the drug concentration which inhibited 50% of isolates. MIC 90 was calculated by taking the drug concentration that inhibited 90% of the isolates).

Out of the 44 isolates of dermatophytes, 40 (90.9%) were *Trichophyton* species. Among them, *T. rubrum* was the most common (18/40, 45%) followed by *T. mentagrophytes* (17/40, 42.5%) which was similar to the observation of earlier studies from South India.[3,5]

Recent mycological studies undertaken across the country have demonstrated *T. mentagrophytes* to be the predominant causative organism.[6,7] The disparity noted in the current study could be due to variations in geographic and climatic conditions.[8]

N. gypsea, a geophilic dermatophyte which rarely causes human infections, was identified in four patients. *N. gypsea* are inhabitants of soil and also dwell in the fur of apparently healthy animals. Clinical manifestations reported include circinate lesions [Figure 2], annular scaly erythematous lesions with pustules, granulomatous and vesicular lesions, seborrheic dermatitis-like lesions, psoriasis-like lesions, and dystrophic onychomycosis.[9]

Antifungal susceptibility testing for fluconazole and terbinafine was done for all isolates. Sensitive strains to fluconazole had MIC that ranged from 0.25 to 64 μg/ml, which was consistent with literature.[4,10,11] In this study, 10 isolates (10/44, 22.7%) showed MIC ≥64 μg/ml to fluconazole. They included 16.7% of T. rubrum [3/18], 23.5% of T. mentagrophytes [4/17], 40% of T. interdigitale [2/5], and 25% of N. gypsea [1/4]. They were considered resistant to fluconazole. Resistance to fluconazole is well documented in many previous studies.[12-15]

Sensitive strains to terbinafine had MIC in the range of 0.001–0.5 μg/ml. About 36.4% isolates (16/44) had MIC ≥0.5 μg/ml in contrast to a previous Indian study where resistance to terbinafine was 18%.[3] Considering individual species, 38.9% of *T. rubrum* (7/18), 41.2% of *T. mentagrophytes* (7/17), and 40% of *T. interdigitale* (2/5) were resistant to terbinafine. Singh *et al*. reported terbinafine resistance in 65.9% of isolates of *T. mentagrophytes* and 100% of *T. rubrum*.[13] Recent Indian studies have reported high terbinafine resistance due to F397L mutation in squalene epoxidase gene.[16,17]

A higher proportion of dermatophyte isolates was above the sensitivity range for terbinafine (16/44, 36.4%) in comparison to fluconazole (10/44, 22.7%) in the current study. Four (4/44, 9.1%) of them were resistant to both fluconazole and terbinafine.

Azoles act in a synergic manner when combined with terbinafine providing good therapeutic results.[18] However, the presence of fungal strains exhibiting resistance to both fluconazole and terbinafine (9% in this study) raises questions about the efficacy of a combination therapy.

MIC 50 and MIC 90 values depict the epidemiological pattern of the susceptibility of any given species and help to select the most effective drug for management. Terbinafine exhibited lowest MIC 50 of 0.0019 μg/ml for *N. gypsea* followed by 0.0312 μg/ml for *T. mentagrophytes*, 0.125 μg/ml for *T. rubrum,* and 0.25 μg/ml for *T. interdigitale*. MIC 90 was >0.5 μg/ml for all except *N. gypsea* which was 0.0312 μg/ml.

Fluconazole showed MIC 50 at 16 μg/ml for *T. rubrum*, *T. Mentagrophytes,* and *N. gypsea* and at 32 μg/ml for *T. interdigitale*. MIC 90 of fluconazole was above 64 μg/ml for all isolates except *T. mentagrophytes*.

Maurya *et al*. in their study on dermatophytoses of skin, hair and nail that showed treatment failure, found that 17.3% of isolates were sensitive to fluconazole and 33.3% to terbinafine. They concluded that not all treatment failures are due to drug resistance.[19]

Limitations

Small sample size and lack of clinical correlation were the major limitations of the study. Antifungal susceptibility testing limited to fluconazole and terbinafine was another limitation of the study. Dermatophytosis often presents with typical clinical features and the diagnosis is made clinically and a fungal culture is usually carried out in recurrent/resistant/atypical disease. Since the study was carried out on specimens received in the microbiology department, it is likely to include more specimens from atypical or recurrent or resistant lesions.

CONCLUSION

T. rubrum was the most common etiological agent isolated from clinically diagnosed lesions of cutaneous dermatophytosis. We observed a rising trend in MIC values to terbinafine and fluconazole. Resistance to both fluconazole and terbinafine was found in 9% of isolates. However, a clear statement about resistance cannot be made due to lack of guidelines defining the drug breakpoints. The higher MIC values noted for the antifungal agents warrant either a higher dosage of drugs or a longer duration of treatment to get the desired clinical response. Although not significant, a higher percentage of isolates was above the sensitive range of MIC for terbinafine than for fluconazole. Whether the low MIC (0.0019 μg/ml) exhibited by *N. gypsea* to terbinafine is suggestive of better efficacy of the latter in treating infections due to *N. gypsea* needs analysis in future studies.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

Dr. Anza Khader is on the editorial board of the Journal.

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