Original Article

Determination of antifungal minimum inhibitory concentration and its clinical correlation among treatment failure cases of dermatophytosis

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Abstract

Introduction: Dermatophytes are most common infectious agents causing superficial mycosis worldwide. A number of topical as well as systemic antifungal drugs are available for treatment of dermatophytosis. Superficial mycosis caused by dermatophytes can be easily treated by topical or oral antifungal drugs, but in the course of time, an increased number of treatment failure cases are appearing. Possible cause for treatment failure could be poor patient compliance, poor drug penetration into affected lesion, and also drug resistance in dermatophytes. The aim of this study is to investigate minimum inhibitory concentration and clinical correlation in treatment failure cases of dermatophytosis. **Methods:** Skin, hair and nail samples were collected from treatment failure cases of dermatophytosis. A total 75 isolates were tested for MIC against four antifungal drugs in the study. Fluconazole, itraconazole, ketoconazole and terbinafine were the antifungal drugs tested using broth microdilution method. MIC_{50} and MIC_{90} values were recorded. **Results:** A total of 75 dermatophytic isolates were tested. Dermatophytic isolates in this study were Trichophyton mentagrophytes (n = 31), T. rubrum (n = 13), T. tonsurans (n = 12), T. verrucosum (n = 9), M. gypseum (n = 5), E. floccosum (n = 4) and T. violaceum (n = 1). MIC_{90} value for fluconazole and terbinafine was significantly higher. **Conclusion:** MIC of 17.33% isolates for fluconazole and 33.33% isolates for terbinafine were lower than cut-off value, which indicates that not all treatment failure cases are due to drug resistance.

Keywords: Antifungals, broth microdilution, dermatophytosis, minimum inhibitory concentration

Introduction

Dermatophytes are a group of fungi that have special ability to obtain nutrient from keratin layer of skin, hair and nail. Although, the infection is not life threatening, it may lead to local allergic reactions like pruritus, erythema, pustular lesions and can also lead to secondary bacterial infections. Dermatophytes are prevalent worldwide but more common in areas with humidity, overpopulation and poor personnel hygiene. Occurrence of dermatophytes is estimated to be 20%.[1,2]

Dermatophytes are classified under three genera namely *Trichophyton, Epidermophyton* and *Microsporum*. Dermatophytosis can be treated with topical application as well as systemic administration of antifungal drugs. Combination therapy with topical along with systemic antifungal drugs are also prescribed. Initially, griseofulvin was used for treatment of dermatophytosis but was soon replaced by the azole group of antifungal agents due to lesser side effects. However,

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at present, topical antifungal agents like clotrimazole, naftifine, ciclopirox olamine, and systemic antifungal agents like itraconazole, fluconazole and terbinafine have been introduced into clinical practice during last 5-10 years for effectively treating dermatophytosis.[3,4] Despite the availability of the wide range of antifungal drugs for dermatophytosis, the treatment failure has been reported worldwide.[4-7] This might be due to non-compliance of patients, patient co-morbidities (immunosuppression, diabetes mellitus), inappropriate drug administration, discontinuation of therapy and infection with non-dermatophyte fungi that are non-responsive to antifungal treatment like *Scopulariopsis, Fusarium* and *Neoscytalidium sp.*[8,9]

Different species of dermatophytes may have different pattern of susceptibility to different antifungal agents.[10-12] In-vitro antifungal susceptibility testing is therefore required and may be helpful in management of dermatophytosis not responding to treatment. Broth dilution, agar dilution and disc diffusion methods have been used for determining antifungal susceptibility pattern.[10,13] Clinical and Laboratory Standards Institute (CLSI) has published approved protocol M38-A2 in year 2008 for filamentous fungi including dermatophytes.[14]

The aim of this study was to isolate and identify etiological agents of dermatophytosis and to investigate minimum inhibitory concentration for fluconazole, itraconazole, ketoconazole, and terbinafine along with its clinical correlation among treatment failure cases of dermatophytosis.

Methods

Test isolates

Skin, hair and nail samples were collected from affected patients attending outdoor department of Skin and VD at our tertiary care hospital. This study was conducted from August 2014 to February 2017. A total of 75 isolates were tested for MIC against 4 antifungal drugs in this study. Skin, hair or nail samples were collected depending upon type of infection. The selection criteria of these patients was whether they were refractory to routinely administered antifungal therapy (i.e. one systemic antifungal and one topical antifungal with different mechanism of action), or had relapse within one month after completion of full course of antifungal treatment. Patients with history of diabetes mellitus, immunosuppression or systemic steroid therapy were excluded. Identification of species was done using conventional methods by correlating colony morphology, lactophenol cotton blue microscopy and certain biochemical tests like urease test and hair perforation test.

Antifungal sensitivity testing

Antifungal susceptibility were performed using broth microdilution method as per Clinical Laboratory Standards Institute (CLSI) approved standard M38-A2 guidelines suggested for molds.[14] Quality control isolates *Aspergillus flavus* ATCC 204304, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included. MIC_{50} and MIC_{90} values for isolates were also recorded.

Antifungal agents

The antifungal agents used in this study were fluconazole, itraconazole, ketoconazole, and terbinafine in powdered form. Stock solutions of itraconazole, ketoconazole and terbinafine were prepared in dimethyl sulfoxide, and fluconazole was dissolved in distilled water. Two-fold dilutions of stock solution were further prepared in RPMI 1640 with L-glutamine without sodium bicarbonate and were buffered at pH of 7.0 ± 0.1 with 0.165M 3-(N-morpholino) propanesulfonic buffer along with 1N NaOH. Concentration used for fluconazole was from 0.125-64 μg/ml, and for other drugs was 0.03-16 μg/ml.

Preparation of inoculum

Cultures of dermatophyte species (7-8 days old) grown on potato dextrose agar slants at 27°C were used to prepare inoculums. The clear suspension of inoculum having conidia was transferred to fresh tube, and its optical density was set equal to 0.5 McFarland standards. The final inoculum was set from 1×10^3 to 3×10^3 colony forming units per ml which was used in the sensitivity testing.

Results

In this study, out of 75 cases, 50 cases presented with tinea corporis, 10 cases with tinea cruris, 2 cases with tinea capitis, and 13 cases presented with complaints of onychomycosis. Patients were in the age group of 5-74 years. Out of the 75 cases, 49 were males and 26 were females. Dermatophytic isolates were *Trichophyton mentagrophytes* (n = 31), *T. rubrum* (n = 13), *T. tonsurans* (n = 12), *T. verrucosum* (n = 9), *M. gypseum* (n = 5), *E. floccosum* (n = 4) and *T. violaceum* (n = 1) were tested against four antifungal drugs fluconazole, itraconazole, ketoconazole, and terbinafine. [Table 1].

Majority of the *T. mentagrophytes* had higher MIC values for fluconazole which ranged from 0.2-64 μg/ml. while Itraconazole was observed to be inhibiting *T. mentagrophytes* effectively even at lower MIC which ranged from 0.03-0.25 μg/ml. Ketoconazole also showed similar MIC range as that of itraconazole. Terbinafine also had higher MIC ranging from 0.03-16 μg/ml.

MIC range of *T. rubrum* for fluconazole was 2-64 μg/ml. Itraconazole showed lower MIC values for *T. rubrum* which was 0.03-0.5 μg/ml. Ketoconazole showed MIC range between 0.03-0.125 μg/ml. MIC range of terbinafine was 0.125-8 μg/ml which was higher than that of itraconazole and ketoconazole.

All the 12 isolates of *T. tonsurans*, showed higher MIC against fluconazole ranging from 1-64 μg/ml. while MIC range for itraconazole and ketoconazole were similar to other isolates. MIC range for itraconazole and ketoconazole were 0.03-0.125 μg/ml and 0.03-0.06 μg/ml, respectively. MIC range

Table 1: Minimum inhibitory concentration of dermatophytes for antifungal drugs

Isolates	Number of isolates	MIC range tested											
Fluconazole	N	0.125	0.25	0.5	1	2	4	8	16	32	64	MIC 50	MIC 90
Trichophyton mentagrophytes	31	0	0	0	0	3	19	5	2	1	1	4	16
Trichophyton rubrum	13	0	0	0	0	1	6	2	2	1	1	4	32
Trichophyton tonsurans	12	0	0	0	2	2	1	1	3	1	2	8	64
Trichophyton verrucosum	9	0	0	0	0	0	2	3	3	0	1	8	16
Microsporum gypseum	5	0	0	1	0	2	1	0	0	1	0	2	32
Epidermophyton floccosum	4	0	0	0	2	0	0	0	1	1	0	1	32
Trichophyton violaceum	1	0	0	0	0	0	0	1	0	0	0	8	8
Itraconazole	N	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	MIC 50	MIC 90
Trichophyton mentagrophytes	31	4	8	15	4	0	0	0	0	0	0	0.125	0.25
Trichophyton rubrum	13	3	3	4	1	2	0	0	0	0	0	0.125	0.25
Trichophyton tonsurans	12	8	3	1	0	0	0	0	0	0	0	0.03	0.06
Trichophyton verrucosum	9	5	3	1	0	0	0	0	0	0	0	0.03	0.06
Microsporum gypseum	5	2	2	1	0	0	0	0	0	0	0	0.06	0.125
Epidermophyton floccosum	4	2	2	0	0	0	0	0	0	0	0	0.03	0.06
Trichophyton violaceum	1	1	0	0	0	0	0	0	0	0	0	0.03	0.03
Ketoconazole	N	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	MIC 50	MIC 90
Trichophyton mentagrophytes	31	7	18	4	2	0	0	0	0	0	0	0.06	0.125
Trichophyton rubrum	13	4	8	1	0	0	0	0	0	0	0	0.06	0.06
Trichophyton tonsurans	12	9	3	0	0	0	0	0	0	0	0	0.03	0.06
Trichophyton verrucosum	9	0	3	3	1	2	0	0	0	0	0	0.125	0.5
Microsporum gypseum	5	1	3	1	0	0	0	0	0	0	0	0.06	0.125
Epidermophyton floccosum	4	1	2	0	1	0	0	0	0	0	0	0.06	0.125
Trichophyton violaceum	1	0	0	0	1	0	0	0	0	0	0	0.25	0.25
Terbinafine	N	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	MIC 50	MIC 90
Trichophyton mentagrophytes	31	1	2	2	0	2	1	8	13	1	1	2	4
Trichophyton rubrum	13	0	0	1	2	2	1	4	2	1	0	2	4
Trichophyton tonsurans	12	0	0	1	0	1	0	2	4	3	1	4	8
Trichophyton verrucosum	9	0	0	0	0	1	0	2	1	3	2	8	16
Microsporum gypseum	5	2	1	1	0	0	1	0	0	0	0	0.06	1
Epidermophyton floccosum	4	0	0	0	0	1	1	0	2	0	0	1	4
Trichophyton violaceum	1	1	0	0	0	0	0	0	0	0	0	0.03	0.03

of terbinafine was again higher for most of the isolates ranging from 0.125-16 μg/ml.

Nine isolates of *T. verrucosum* were also subjected to antifungal MIC testing. Similar to other isolates, fluconazole and terbinafine exhibited higher MIC ranging from 4-64 μg/ml and 0.5-16 μg/ml respectively. Itraconazole and ketoconazole again showed MIC towards lower side. MIC range for itraconazole and ketoconazole were 0.03-0.125 μg/ml and 0.06-0.5 μg/ml, respectively.

Among five isolates of *Microsporum gypseum* MIC ranges for fluconazole was 0.5-32 μg/ml and for itraconazole, ketoconazole and terbinafine 0.03-.0125 μg/ml. In case of *Epidermophyton floccosum*, the MIC range for Fluconazole was 1-32 μg/ml, 0.03-0.06 μg/ml for itraconazole, 0.03-0.25 for ketoconazole and 0.5-4 μg/ml for terbinafine. The only isolate of *Trichophyton violaceum* tested exhibited MIC values for fluconazole, itraconazole, ketoconazole and terbinafine to be 8 μg/ml, 0.03 μg/ml, 0.25 μg/ml and 0.03 μg/ml, respectively.

Isolates with MIC values of >2 μg/ml for fluconazole and >1 μg/ml for itraconazole, ketoconazole and terbinafine, were classified as resistant.[15-17] Isolates resistant to fluconazole and itraconazole were 82.66% and 66.66%, respectively. While isolates which were sensitive to fluconazole and terbinafine were 17.33% and 33.33%, respectively. MIC values for itraconazole and ketoconazole were <1 μg/ml for 100% of isolates [Table 2].

Discussion

In this study, we isolated *T. mentagrophytes, T. rubrum, T. tonsurans, T. verrucosum, M. gypseum* and *T. violaceum* from patients who were refractory to routinely administered antifungal agents. In our institution, one topical antifungal either miconazole or clotrimazole and fluconazole orally are prescribed to patients presenting with dermatophytosis. Patients who do not respond to this treatment are switched over to terbinafine, itraconazole, griseofulvin or ketoconazole.

T. mentagrophytes was the most frequently isolated dermatophyte in our study which is similar to study conducted by other authors.[5,9,16,18] In contrast, *T. rubrum* was the most commonly isolated dermatophyte in several other studies.[19-21]

Table 2: Table showing number of isolates as per cut-off value

Antifungals	No. of isolates below cut-off value	No. of isolates above cut-off value
Fluconazole	13 (17.33%)	62 (82.66%)
Itraconazole	75 (100%)	0 (0%)
Ketoconazole	75 (100%)	0 (0%)
Terbinafine	25 (33.33%)	50 (66.66%)

In this study, all dermatophytic isolates of *T. mentagrophytes, T. rubrum, T. tonsurans, and T. verrucosum* showed higher MIC_{90} values against fluconazole and terbinafine indicating higher chances of treatment failure when treated with these drugs. Higher MIC values for fluconazole have also been reported by some authors previously.[5,16,22,23] Clinical inefficacy with the treatment of terbinafine have been reported by many authors.[7,24,25] Similar to our study, higher MIC against terbinafine has also been reported from India.[5,8] Itraconazole and ketoconazole had lower MIC for all species of dermatophytes, which indicates that these drugs could be the better choice for successful treatment of dermatophytic infections. Many authors from India and abroad have reported similar findings with itraconazole and ketoconazole.[5,11,12,15,16,22,23]

62 isolates (82.66%) showed higher MIC against fluconazole (i.e. cut-off MIC > 2 μg/ml) and 50 isolates (66.66%) against terbinafine (i.e. cut off MIC >1 μg/ml). Patients with these isolates were switched over to itraconazole, as it carried fewer adverse effects compared to others. No patient was switched over to ketoconazole. Patients with isolates having lower MIC values for fluconazole or terbinafine were advised to continue same treatment and were advised to keep personnel hygiene and affected area dry. With implementation of above strategies all treatment failure cases of dermatophytosis were treated successfully.

In our study, we concluded that not all treatment failure cases were associated with antifungal drug resistance in dermatophytes. These treatment failure cases might be managed by properly educating patient about personal hygiene and by keeping the affected area dry. We also concluded that drug resistance against fluconazole and terbinafine are higher in this geographical region. Knowing the resistance pattern of antifungal drugs will guide the family physicians and medical officers working in peripheral regions to decide the appropriate empirical therapy for better patient outcome.

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Conflicts of interest

There are no conflicts of interest.

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