ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 11 | November - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

AN IN VITRO ANTIFUNGAL SUSCEPTIBILITY PATTERNS OF DERMATOPHYTIC FUNGI ISOLATED IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

INTRODUCTION: Fungal infections are of mainly three clinical types namely superficial, subcutaneous and systemic mycoses in which the prevalence of superficial mycotic infections are increasing worldwide which are mainly caused by dermatophytes called dermatomycosis. The antifungal susceptibility differs in various geographical locations and resistance is on rise in clinically diagnosed or suspected cases of dermatomycosis leading to treatment failures. **Objective:** Identification of clinico-mycological pattern of infections caused by dermatophytes and to obtain their in vitro antifungal susceptibility against four antifungals (Terbinafine, Griseofulvin, Itraconazole and Ketoconazole). **Results:** Out of 100 samples collected from clinically diagnosed or suspected cases of dermatomycoses majority of them were from males(73%) followed by females(27%) and among dermatophytes isolated majority were *Trichophyton mentagrophytes*(88.37%) followed by *Tirichophyton rubrum*(6.97%), *Trichophyton tonsurans*(2.33%) and *Microsporum gypseum*(2.33%) in which majority are isolated from cases of *Tinea corporis*(46.52%) followed by *Tinea cruris*(34.88%), *Tinea capitis*(6.97%), *Tinea unguium*(6.97%), *Tinea manuum*(2.33%) and *Tinea faciei*(2.33%). Dermatophytes isolated in our study showed lowest MIC for Itraconazole followed by Ketoconazole, Terbinafine and Griseofulvin respectively. **Conclusion**: The study gives insight into clinico-mycological pattern and the in vitro antifungal susceptibility of dermatophytes among which broth microdilution method is preferred for testing filamentous fungi.

KEYWORDS

Superficial fungal infections, Dermatophytes, Antifungal agents, Antifungal susceptibility testing, Broth microdilution method.

INTRODUCTION

Infectious diseases are one of the leading causes of death worldwide followed by cardiovascular diseases in which fungi are being recognised as causative agents of human diseases as early as in 1839 but are uncommon in healthy individuals when compared to bacterial, viral and parasitic infections.[1,2] As per the literature human infections with true and opportunistic fungi are being increased worldwide over the last decade. Although there are many fungal species which may not cause infections in humans, a small number of fungal species cause human infections, especially in immunocompromised patients and their number is constantly increasing.[3,4] Even though as postulated earlier that the thermal barrier in humans is responsible for the limit of fungal infections, many fungal species have adapted to become infectious to humans. Although described decades earlier, the study of pathogenic fungi did not receive any attention compared to other pathogens which may be due to the benign nature. The majority of the fungi infecting humans are soil saprophytes and mainly cause opportunistic infections and are most prevalent in subtropical and tropical countries like India where the humid and hot climates may aggravate the fungal infections.[1] Invasive and nosocomial fungal infections are also becoming more prevalent due to immunosuppression, cancer, bone marrow or solid organ transplantation, use of broad-spectrum antibiotics[5] etc; The fungal infections are of mainly 3 clinical types i.e., superficial, subcutaneous and systemic mycoses where the prevalence of superficial mycotic infections has been found to be 20-25% worldwide which are caused by hyaline septate molds, confined to the keratin layer of the skin which are called dermatophytes, which infect skin, hair and nails in humans and the condition is called dermatomycosis.[6]

Raymond Sabouraud while studying dermatophytes classified them into four genera - Achorion, Epidermophyton, Microsporum and Trichophyton based on clinical disease, microscopic and cultural characteristics but in 1934 Chester Emmons has modified the taxonomic scheme where the genus Achorion is being eliminated and the rest of the three genera are being confined containing 24 *Trichophyton* species, 16 *Microsporum* species and 2 *Epidermophyton* species.[7,8,9,10]

Based on their ecological characters and habitat dermatophytes are divided into three types namely Anthropophilic which affects humans, Zoophilic habituating animals and birds are Geophilic which habitat the soil.[7]

Definitive diagnosis must be established before starting treatment for dermatomycosis where various topical preparations are available like Whitfield's ointment, Tolnaftate, Griseofulvin and azole derivatives are available. But the emergences of resistance in these groups of drugs have been reported since 2003. These agents should be ideally given on the basis of in vitro antifungal susceptibility testing or MIC(Minimum Inhibitory Concentration) of the fungal isolates to the particular antifungal agent suggested as fresh corps of lesions are reported even in patients who are on antifungal therapy without performing Antifungal susceptibility testing (AFST) and also their antifungal susceptibility may differ in various geographical locations.[1] The in vivo and in vitro resistance discordance can be understood by the "90-60 rule" according to which susceptible strains respond to therapy in 90% of cases and resistant strains respond in 60% of cases.[11,12]

The present study was undertaken at department of microbiology in collaboration with department of Dermatology, Venereology and Leprosy (DVL) at our institution to monitor the pattern of dermatomycosis by isolating and identifying the etiological agents and obtain their antifungal susceptibility pattern in clinically diagnosed or suspected cases attending to department of dermatology against four antifungal agents namely Terbinafine, Griseofulvin, Itraconazole and Ketoconazole.

MATERIALS AND METHODS

The present study is a cross-sectional observational study carried out for seven months where100 clinically diagnosed or suspected cases of dermatophytosis in patients attending to the department of dermatology were included in the study.

The samples from these cases were obtained and processed at the Department of Microbiology, where in case of skin sampling, the skin scrapings were taken with the help of a scalpel blade with the blunt edge and sample was scraped from the edge of the lesion on to sterile black-coloured envelope or filter papers after cleaning the area with 70% alcohol. Hair sampling was done by epilation of hair stubs with the tip of a forceps and the hair routes and crusts are taken and in cases of onychomycosis, nails are cleaned with 70% alcohol and is allowed to dry, and the sample was collected near the nail bed, particularly at the edge of the lesion or scratching the nail at the surface for thin nail fragments or nail clippings are obtained.

These samples were subjected to microscopy by treating them with dissociating agents like 10% and 40% Potassium Hydroxide (KOH) and were observed for presence of highly refractile, large, branched hyphae and also for barrel-shaped arthroconidia.[13,14]

The samples were subjected to culture by using Sabouraud dextrose agar (SDA) and SDA with antibiotics (Fungibiotic) and incubated in a Biological Oxygen Demand(BOD) incubator at 25-30^0 C for 5-7 days

and as long as 4 weeks. The culture positive specimens were identified by macroscopic examination, tease mount preparation and slide culture up to species level.[1,15]

The culture positive specimens were further subjected to Antifungal susceptibility testing by using broth microdilution method.[16]

Stock inoculum preparation:
The culture positive isolates were obtained from SDA agar slants and the colony growth was covered with 5 ml of sterile saline (0.85%) and scraped with a sterile spud and the slants were kept untouched for 10-15 minutes allowing heavy particles to settle down.

The clear suspension was aspirated with a sterile syringe and was taken into a sterile test tube and the turbidity was adjusted to 0.5 Mac Farland standards by spectrophotometry at 530 nm to a final optical density range of 0.09-0.11 solutions were diluted in RPMI-1640 medium (Hi-media Labs, Mumbai, India) in 1:50 dilutions.

Inoculation procedure:
96 well microtiter plates (Hi-media Labs, Mumbai, India) having 12 columns and 8 rows were taken and 100µl stock solution was taken in each well to perform antifungal susceptibility testing.

Antifungal drug stock solution preparation:
Dimethyl sulfoxide (DMSO) was used for diluting the four drugs - Terbinafine, Griseofulvin, Itraconazole and Ketoconazole as they are not water soluble and 2 fold dilutions of drug stock solution were prepared by diluting with RPMI-1640 medium (Hi-media labs, Mumbai, India).

Final concentrations of drugs tested:
Terbinafine: 0.004µg/ml to 1 µg/ml
Griseofulvin: 0.03 µg/ml to 4 µg/ml
Itraconazole: 0.06 µg/ml to 32 µg/ml
Ketoconazole: 0.06 µg/ml to 32 µg/ml

Test procedure:
100 µl of each antifungal drug dilution was added into 100µl of stock inoculum into the microtiter plates into the first 10 columns 11th and 12th columns were kept as positive and negative controls and were incubated at 30^0 C for 96 hours for optimum growth of the dermatophyte in the control well (*Trichophyton mentagrophytes*) for comparison and after the incubation period, the dilution at which the growth is not seen visibly was considered as the minimum inhibitory concentration (MIC) of the drug and the MIC value of each drug for each isolate was recorded.[17,18,19,20]

RESULTS

Clinical samples of skin scrapings nail clippings and hair stubs collected from 100 clinically diagnosed and suspected cases of dermatophytosis from the out-patients attending to OPD of the department of dermatology were processed in the department of microbiology.

Table-1: Showing gender distribution of collected samples

Gender	No. of samples	Percent
Male	73	73%
Female	27	27%
Total samples	100	100%

Out of 100 samples collected, the majority were from males (73%) followed by females (27%).

Table-2: Showing the age distribution of collected samples

Age group	No. of samples	Percent
≤15 years	7	7%
16-30 years	41	41%
31-45 years	38	38%
46-60 years	11	11%
≥61 years	3	3%
Total	100	100%

The majority of the samples collected were from the 16 to 30 years age group (41%) followed by 31 to 45 years (38%), 46 to 60 years (11%), less than 15 years (7%), more than 61 years (3%).

Table-3: Showing different types of collected samples

Sample type	No. of samples	Percent
Skin scrapings	61	61%
Nail clippings	32	32%
Hair	7	7%
Total	100	100%

Among all the samples, 61% samples were skin scrapings, followed by nail clippings 32% and hair plucking 7%.

Table-4: Showing the interpretation by direct microscopy and fungal culture of specimens processed.

Test	Number	Percent
KOH mount positive	39	39%
Fungal culture positive	43	43%
KOH mount negative and fungal culture positive	8	8%
KOH mount positive and fungal culture negative	11	11%
Both KOH mount and fungal culture positive	21	21%
Both KOH mount & fungal culture negative	26	26%
Non-dermatophytic fungi isolated	17	17%

Out of all processed samples 39% are KOH mount positive and 43% are fungal culture positive. Samples which are KOH mount negative and culture positive are 8%, KOH mount positive and culture negative are 11%. Both KOH mount and fungal culture positive are 21%, both KOH mount and fungal culture negative are 26% and 17% of isolates are non-dermatophytic fungi.

Table-5: Showing different dermatophyte isolates from the culture samples.

Fungal Isolates	No. of isolates	Percentage of isolates
Trichophyton mentagrophytes	38	88.37%
Trichophyton rubrum	3	6.97%
Trichophyton tonsurans	1	2.33%
Microsporum gypseum	1	2.33%
Total	43	100%

Among the dermatophytes, isolated majority were *Trichophyton mentagrophytes* (88.37%), *T.rubrum* (6.97%), *T.tonsurans* (2.33%) and *Microsporum gypseum* (2.33%).

Table-6: Showing frequency of presentation of dermatophyte isolates.

Fungal Isolates	*Tinea corporis*	*Tinea cruris*	*Tinea capitis*	*Tinea unguium*	*Tinea manuum*	*Tinea faciei*	*Total*
Trichophyton mentagrophytes	18	12	3	3	1	1	38
Trichophyton rubrum	1	2	-	-	-	-	3
Trichophyton tonsurans	1	-	-	-	-	-	1
Microsporum gypseum	-	1	-	-	-	-	1
Total	20(46.52%)	15(34.88%)	3(6.97%)	3(6.97%)	1 (2.33%)	1(2.33%)	43(100%)

Among the isolates, the majority of them belongs to *Tinea corporis* (46.52%) followed by *Tinea cruris* (34.88%), *Tinea capitis* (6.97%), *Tinea unguium* (6.97%), *Tinea manuum* (2.33%), *Tinea faciei* (2.33%).

All the positive samples were subjected to Antifungal susceptibility testing by micro-broth dilution method to four antifungal agents namely Terbinafine, Griseofulvin, Itraconazole and Ketoconazole.

Table-7: Showing antifungal susceptibility of dermatophytic isolates to Terbinafine

Species	0.004 µg/ml	0.008 µg/ml	0.015µg/ml	0.03 µg/ml	0.06 µg/ml	0.125 µg/ml	0.25 µg/ml	0.5 µg/ml	1 µg/ml	MIC 50	MIC 90

T.mentagrophytes	-	1 (2.63%)	2 (5.62%)	4 (10.52%)	14 (36.84%)	12 (31.57%)	4 (10.5%)	1 (2.63%)	-	0.06	0.25
T.rubrum	2 (66.66%)	-	-	1 (33.33%)	-	-	-	-	-	0.004	0.06
T.tonsurans	-	-	-	-	1 (100%)	-	-	-	-	0.06	0.06
M.gypseum	-	-	-	-	1 (100%)	-	-	-	-	0.06	0.06

Among the isolates tested with Terbinafine, *T.mentagrophytes* showed MIC 50 of 0.06µg/ml & MIC 90 of 0.25 µg/ml, *T.rubrum* showed MIC 50 of 0.004 µg/ml & MIC 90 of 0.06 µg/ml, *T.tonsurans* showed MIC 50 of 0.06 µg/ml & MIC 90 of 0.06µg/ml, and *M.gypseum* showed MIC 50 of 0.06 µg/ml & MIC 90 of 0.06 µg/ml.

Table-8: Showing antifungal susceptibility of dermatophytic isolates to Griseofulvin

Species	0.03 µg/ml	0.06 µg/ml	0.125 µg/ml	0.25 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	MIC 50	MIC 90
T.mentagrophytes	6 (15.78%)	2 (5.26%)	5 (13.15%)	8 (21.05%)	8 (21.05%)	9 (23.68%)	-	-	0.25	1
T.rubrum	-	1(33.33%)	-	1(33.33%)	1(33.33%)	-	-	-	0.25	0.5
T.tonsurans	-	-	-	-	1(100%)	-	-	-	0.5	0.5
M.gypseum	-	-	-	1 (100%)	-	-	-	-	0.25	0.25

Among the isolates tested with Griseofulvin, *T.mentagrophytes* showed MIC 50 of 0.25µg/ml & MIC 90 of 1 µg/ml, *T.rubrum* showed MIC 50 of 0.25 µg/ml & MIC 90 of 0.5 µg/ml, *T.tonsurans* showed MIC 50 of 0.5 µg/ml & MIC 90 of 0.5 µg/ml, and *M.gypseum* showed MIC 50 of 0.25 µg/ml & MIC 90 of 0.25 µg/ml.

Table-9: Showing antifungal susceptibility of dermatophytic isolates to Itraconazole

Species	0.06 µg/ml	0.125 µg/ml	0.25 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	MIC 50	MIC 90
T.mentagrophytes	22 (57.89%)	12 (31.57%)	3(7.89%)	1 (2.63%)	-	-	-	-	-	0.06	0.125
T.rubrum	-	2 (66.66%)	1(33.33%)	-	-	-	-	-	-	0.125	0.25
T.tonsurans	1(100%)	-	-	-	-	-	-	-	-	0.06	0.06
M.gypseum	1 (100%)	-	-	-	-	-	-	-	-	0.06	0.06

Among the isolates tested with Itraconazole, *T.mentagrophytes* showed MIC 50 of 0.06µg/ml & MIC 90 of 0.125 µg/ml, *T.rubrum* showed MIC 50 of 0.125 µg/ml & MIC 90 of 0.25 µg/ml, *T.tonsurans* showed MIC 50 of 0.06 µg/ml & MIC 90 of 0.06 µg/ml, and *M.gypseum* showed MIC 50 of 0.06 µg/ml & MIC 90 of 0.06 µg/ml.

Table-10: Showing antifungal susceptibility of dermatophytic isolates to Ketoconazole

Species	0.06 µg/ml	0.125 µg/ml	0.25 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	MIC 50	MIC 90
T.mentagrophytes	19(50%)	12(31.57%)	2(5.26%)	5(13.15%)	-	-	-	-	-	0.06	0.125
T.rubrum	2 (66.66%)	1(33.33%)	-	-	-	-	-	-	-	0.06	0.125
T.tonsurans	-	-	1(100%)	-	-	-	-	-	-	0.25	0.25
M. gypseum	1(100%)	-	-	-	-	-	-	-	-	0.06	0.06

Among the isolates tested with Ketoconazole, *T.mentagrophytes* showed MIC 50 of 0.06µg/ml & MIC 90 of 0.125 µg/ml, *T.rubrum* showed MIC 50 of 0.06 µg/ml & MIC 90 of 0.125 µg/ml, *T.tonsurans* showed MIC 50 of 0.25 µg/ml & MIC 90 of 0.25 µg/ml, and *M.gypseum* showed MIC 50 of 0.06 µg/ml & MIC 90 of 0.06 µg/ml.

DISCUSSION

Dermatophytosis refers to the disease of skin, hair and nails which is caused by a group of fungi called dermatophytes which are pathogenic fungi with high affinity to keratinised structures causing infections in patients with reduced immunity or suffering from various illnesses like cancer, autoimmune diseases and in patients on post-organ transplant immuno-suppression.

In this study, a total of hundred clinically diagnosed or suspected cases of dermatophytosis attending the department of Dermatology were being collected and processed in the department of Microbiology.

Of these specimens collected 73% were from males and 27% were from females. The majority of the samples which were collected are from 16-30 years age group (41%) followed by 31-45 years (38%), 46-60 years (11%), below 15 years (7%) and above 61 years (3%) in which majority were skin scrapings (61%) followed by Nail clippings (32%) and hair stubs (7%).

Out of all the processed samples, 39% are KOH mount positive, 43% are fungal culture positive, 8% are KOH mount negative & fungal culture positive, 11% are KOH mount positive & fungal culture negative, 21% are both KOH mount and fungal culture positive and 26% are both KOH mount and fungal culture negative.Our findings correlate with the findings of Shambel araya et al.[21] who reported KOH mount positive 41.8%, fungal culture positive 46.5%, KOH mount negative & culture positive 22.3%, KOH mount positive & culture negative 16.9%, both KOH mount and fungal culture positive 23.8% and both KOH mount and fungal culture negative 34.9%.[21]

Out of the total dermatophytic isolates cultured and identified are *T.mentagrophytes* (88.37%) correlating with the findings of Sachin Sharma et al. (92.8%)[22] , Mahajan et al. (75.9%)[19], Bhatia et al.(64.15%)[20], Budhiraja RK et al. (60.98%)[23] followed by *T.rubrum* (6.97%) correlating with the findings of Sachin Sharma et al.(3%)[22], but according to some authors the isolation rates of *T.rubrum* is more as observed by Mahajan et al.(21.9%)[19], Bhatia et al.(33.96%)[20], Budhiraja RK et al. (36.58%)[23], *T.tonsurans* isolation rate is 2.33% which correlates with the findings of Sachin Sharma et al.(1.2%)[22], Mahajan et al.(0.7%)[19], Vanathi Sabtharishi et al. (1.25%)[17], Pathania et al.(3.3%)[24] and *M.gypseum* isolation rate is 2.33% correlating with the findings of Bhatia et al.(1.88%)[20], Budhiraja RK et al.(2.44%)[23], Vanathi Sabtharishi et al.(1.25%)[17].

In our study, most prevalent type of infection out of all the cases was *Tinea corporis* (46.51%) correlating with the findings of Vyoma chudasam et al. (48%)[25] and Uma P et al.(44%)[26] followed by *Tinea cruris* 34.88% correlating with the observations of Poojary et al.(37.29%)[27] followed by *Tinea capitis* 6.97% correlating with the findings of Vyoma chudasam et al.(5%)[25] & Uma P et al.(4%)[26] and *Tinea unguium* 6.97% correlating with the findings of Lakshmanan et al.(6.7%)[27] followed by *Tinea manuum* & *Tinea faciei* 2.33% & 2.33% correlating with the observations of Lakshmanan et al.(2.5% &1.8%)[30] respectively.

In our study, antifungal susceptibility was performed for all the dermatophytic isolates against 4 drugs namely Terbinafine, Griseofulvin, Itraconazole and Ketoconazole by micro broth dilution test according to CLSI guidelines M38-A2.[16]

When tested with Terbinafine, *T.mentagrophytes* showed MIC 50 of 0.06 µg/ml & MIC 90 of 0.25 µg/ml correlating with the findings of Sachin Sharma et al.[22] *T.rubrum* showed MIC 50 of 0.004 µg/ml & MIC 90 of 0.06 µg/ml correlating with the observations of Sachin Sharma et al.,[22] *T.tonsurans* showed both MIC 50 & MIC 90 of 0.06 µg/ml correlating with the findings of Soumya et al.[28] and *M.gypseum* showed MIC 50 & MIC 90 of 0.06µg/ml correlating with the findings of Maurya et al.[29] & Budhiraja RK et al.[23]

When tested with Griseofulvin *T.mentagrophytes* showed MIC 50 of 0.25µg/ml and MIC 90 of 1µg/ml correlating with the findings of Sachin Sharma et al.,[22] and Budhiraja RK et al.[23] *T.rubrum* showed MIC 50 of 0.25µg/ml & MIC 90 of 0.5 µg/ml correlating with the

observations of Vanathi sabtharish et al.[17] *T.tonsurans* showed both MIC 50 of 0.5µg/ml & MIC 90 of 0.5µg/ml correlating with the observations of Sachin Sharma et al.[22] and *M.gypseum* showed both MIC 50 of 0.25µg/ml & MIC 90 of 0.25µg/ml correlating with the observations of Budhiraja RK et al.[23]

When tested with Itraconazole *T.mentagrophytes* showed MIC 50 of 0.06µg/ml and MIC 90 of 0.125µg/ml correlating with the findings of Vanathi Sabtharishi et al.[17] *T.rubrum* showed MIC 50 of 0.125µg/ml & MIC 90 of 0.25µg/ml correlating with the observations of Vanathi Sabtharishi et al.,[17] *T.tonsurans* showed both MIC 50 & MIC 90 of 0.06µg/ml correlating with the observations of Sachin Sharma et al.[22] and *M.gypseum* showed both MIC 50 & MIC 90 of 0.06µg/ml correlating with the findings of Maurya et al.[29] & Budhiraja RK et al.[23]

When tested with Ketoconazole *T.mentagrophytes* showed MIC 50 of 0.06µg/ml and MIC 90 of 0.125µg/ml correlating with the observations of Maurya et al.[29] *T.rubrum* showed MIC 50 of 0.06µg/ml & MIC 90 of 0.125µg/ml correlating with the findings of Maurya et al.[29] *T.tonsurans* showed both MIC 50 & MIC 90 of 0.25µg/ml correlating with the observations of Sowmya et al.[28] and *M.gypseum* showed both MIC 50 & MIC 90 of 0.06µg/ml correlating with the findings of Maurya et al.[29] & Vanathi sabtharishi et al.[17]

CONCLUSION

The present study was carried out at the department of microbiology to know the clinico-mycological pattern of infections caused by dermatophytes and to obtain in vitro antifungal susceptibility pattern of dermatophytic fungi against four antifungal agents (Terbinafine,Griseofulvin, Itraconazole and Ketoconazole).

A total of 100 clinical samples of skin scrapings, nail clippings and hair stubs are processed in which 39% are KOH mount positive and 43% are fungal culture positive and the majority of the dermatophytes isolated are *T.mentagrophytes* 88.37% followed by *T.rubrum* 6.97%, *T.tonsurans* 2.33% and *M.gypseum* 2.33%. The majority of the infections found are *Tinea corporis* 46.5% followed by *Tinea cruris* 34.88%.

Anti-fungal susceptibility testing for all the isolates was done by broth microdilution method as per CLSI guidelines M38-A2 protocol which revealed that most of the dermatophytes isolated in our study showed lower MIC for Itraconazole (MIC 50 - 0.06µg/ml & MIC 90 - 0.125 µg/ml) followed by Ketoconazole (MIC 50 - 0.06µg/ml & MIC 90 - 0.125 µg/ml), Terbinafine (MIC 50 - 0.06µg/ml & MIC 90 - 0.25 µg/ml) and Griseofulvin (MIC 50 - 0.25µg/ml & MIC 90 - 1 µg/ml).

Dermatophytes are the fungi which are considered to be the most prevalent superficial fungal infections worldwide affecting humans and they pose a major problem to microbiologists, researchers and clinicians due to their increasing frequency, changing trends in identification, long duration of treatment and limited number of antifungal drugs and their resistance to antifungals.

So strategical developments of uniform diagnostic methods and implementation of rapid diagnostic methods like molecular detection methods of dermatophytic infections are needed.

Various techniques are used by different laboratories to test for antifungal susceptibility of dermatophytes, in which broth microdilution technique is preferred for filamentous fungi but till now MIC values are not clearly defined. So clinical breakpoint (CBP) value has to be determined by evaluation of epidemiological cut-off value (ECV) to appropriately advise the clinician to treat resistant strains of dermatophytes.

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