Original Article

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Microbiology Section

Antifungal Susceptibility Testing of Five Antifungal Agents against Clinically Isolated Dermatophytes Species from a Tertiary Care Hospital in Northern India

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ABSTRACT

Introduction: Dermatophytosis form approximately 15-75% of all the mycological infections. Dermatophytes are closely related keratinolytic fungi with ability to degrade keratin and invade the skin, hair and nails causing dermatophytosis.

Aim: To evaluate the Minimum Inhibitory Concentration (MIC) of antifungal drug against the isolated dermatophytes by broth microdilution method.

Materials and Methods: The present cross-sectional study was conducted in the Department of Microbiology, Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, India. The duration of the study was August 2018-August 2020 and included 245 patients of which 165 samples were culture positive. A 10-20% Potassium Hydroxide (KOH) mount was prepared from the skin scrapings, nail clippings, and hair bits to look for fungal elements. The specimens were also inoculated on Mycosel media and Sabouraud Dextrose Agar (SDA) with chloramphenicol. The dermatophytes were identified on the basis of colony characteristics, Lacto Phenol Cotton Blue (LPCB) mount, nutritional requirement, temperature tolerance, urease production, and in-vitro hair perforation test. Antifungal susceptibility testing was done for all fungal isolates and performed by broth microdilution method. The descriptive statistics were reported as means with their Standard Deviation (SD). Data were statistically evaluated with International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics for Mac, Version 25.0., IBM Corp., Chicago IL.

Results: In this study, the MIC range for all the 165 isolates for dermatophytes tested for antifungal susceptibility showed that itraconazole, terbinafine and voriconazole showed the lowest MIC range of 0.0019-0.5 µg/mL followed by griseofulvin and fluconazole at MIC range of 0.125-32 µg/mL. The MIC50 of itraconazole and terbinafine was seen lowest at 0.0313 µg/mL followed by voriconazole at 0.0625 µg/mL, griseofulvin at 0.25 µg/mL for all isolated dermatophytes. Highest MIC50 with 4 µg/mL was found for fluconazole against *T. mentagrophytes* and *T. violaceum*. MIC90 of terbinafine, itraconazole and voriconazole was seen lowest at 0.25 µg/mL followed by griseofulvin at 1 µg/mL for all isolated dermatophytes. Highest MIC90 of fluconazole was recorded at 16 µg/mL for all isolated dermatophytes.

Conclusion: Highest MIC50 with 8 µg/mL was found for fluconazole against all isolated dermatophytes. MIC90 of terbinafine, itraconazole and voriconazole was seen lowest at 0.25 µg/mL followed by griseofulvin at 1 µg/mL for all isolated dermatophytes. Highest MIC90 of fluconazole was recorded at 16 µg/mL for all isolated dermatophytes.

Keywords: Itraconazole, Minimum inhibitory concentration, *Trichophyton mentagrophytes*, *Trichophyton violaceum*, Voriconazole

INTRODUCTION

Dermatophytosis is a group of keratinophilic fungi that can infect skin, hair, and nails. Dermatophytosis is also called tinea, and the name is according to the site of infection as tinea corporis, involving the arms, trunk and legs; tinea capitis, involving the scalp; tinea pedis involving the foot. Dermatophytes are divided into three closely related genera: *Epidermophyton, Trichophyton*, and *Microsporum*. They are classified based on their habitat; geophilic dermatophytes are naturally present in the soil, zoophilic in animals, and anthropophilic in humans [1]. These infections are usually not life-threatening but occur even in immunocompetent hosts, and in many cases, are long lasting, recurrent, and complicated to cure [2]. Superficial mycoses are among the commonest disease of human, likely to affect more than 20-25% of the world's population, and their incidence is constantly increasing. The prevalence of dermatophytes infection and their causative agents varies with geographical region. It is affected by a variety of factors, such as type of population, lifestyle, migration of people, cultural practices and socio-economic conditions, the incidence of peculiar co-morbidities, and drug therapy [3-5].

Antifungal agents are used to cure fungal infections. Based on the structure and mechanisms of action, antifungal agents are grouped into two large pharmacological groups. These are the azoles and the allylamines. Members of the azole class include clotrimazole, econazole, ketoconazole, fluconazole, itraconazole, oxiconazole, voriconazole, miconazole and sulconazole. These agents have broad spectrum activity. Naftifine and terbinafine are important allylamine compounds. Both groups of antifungal agents have fungicidal activity. Comparatively, terbinafine was found to be more effective than azoles [3].

The skin infections caused by dermatophytes are based on the use of topical and systemic antifungal agents. For localised lesions that are not extensive, topical therapies are usually used. For skin lesions with tinea unguium, tinea capitis, extensive lesions or foliculitis, systemic antimycotic drugs are required. Itraconazole and terbinafine are the most commonly used oral drugs to day to treat severe conditions [4,5].

These fungi are among the most common skin infections in the world, and the current rise in the number of patients with immunocompromised disease, such as acquired immune deficiency syndrome, diabetes mellitus, cancer, and organ transplant patients [6]. Dermatophyte infections can be disruptive, persistent and chronic (especially nail infections) and usually require enduring treatment with antifungal agents. As a result of patient poor drug compliance, infection with a new strain or the development of resistance to antifungals used in treatment can cause inflammation of recurrent dermatitis [7].

Antifungal susceptibility testing is done on the fungi that cause the disease, especially if the infection is severe, refractory to treatment, which occurs in the patient exposed to antifungal agents. Antifungal susceptibility testing is also essential for resistance surveillance, epidemiological studies, and provides information to enable the clinician to select appropriate antifungal agents useful for treating a particular fungal agent.

The present study was designed to evaluate the MIC of antifungal drugs against isolated dermatophytes.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology, Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, India, from August 2018-August 2020. The study protocol was reviewed and approved by the Ethics Committee of Muzaffarnagar Medical College, Muzaffarnagar (MMC/IEC/2016/156, Dated 17.10.2016). Verbal and written consent was taken from all the participants.

Inclusion criteria: All clinically suspected cases of dermatophytosis were included in the present study.

Exclusion criteria: Cases of dermatophytosis with secondary microbial infection, patients already on antifungal drugs were excluded from the present study.

Sample size calculation: The prevalence rate of dermatophytosis in world is around 20% accordingly [4,5], the minimum sample size has been calculated using appropriate sample size formula:

$n=Z^2 \times p(1-p)/d^2$

n=required sample size

Z=confidence level at 95% (t=1.96)

p=Prevalence

d=margin of error at 5% (m=0.05)

$n=1.96^2 \times 0.2(1-0.2)/0.05^2$

=245

Specimen Collection

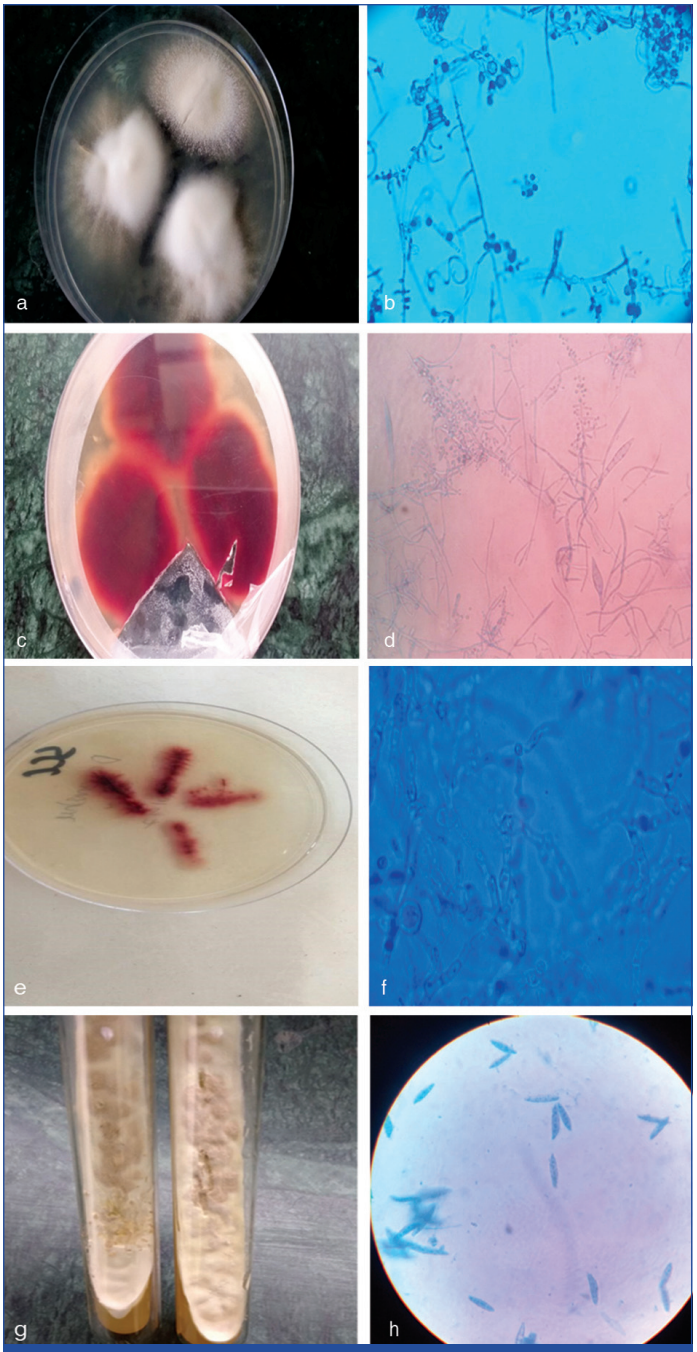
A total of 245 samples were collected in mycology laboratory from patients referred from the dermatology Outpatient Department (OPD) of Muzaffarnagar Medical College and Hospital, Muzaffarnagar, Uttar Pradesh, India. The patients who presented with vesicles, scales on skin and nails and breakdown of hairs, were examined by dermatologist's in the OPD of the hospital. The patients were then sent to the mycology laboratory for collection of samples. Depending upon the site of infection, samples collected were:

- Skin scrapings
- Nail scrapings and clipping
- Hair plucking and scales scrapings

Wet mount of different samples e.g., skin scrapings, hair and nail clipping were prepared using 10% KOH with 40% Dimethyl Sulfoxide (DMSO). This preparation was examined under the microscope to look for the presence of dermatophytic fungi which appear as thin branching fungal hyphae. The presence of fungal hyphae in clinical material was not enough to identify the organisms with certainty. Samples were plated on SDA with chloramphenicol and Mycosel agar [Table/Fig-1a-h]. Plates were incubated at 25°C for upto four weeks. The characteristic features of the colony that were considered were rate of growth, texture, topography and colour of the colony, and the production of pigment on the reverse of the colony. Microscopic morphology was examined by LPCB mount of the colonies and slide culture.

Further identification of dermatophytes included [3,8]:

- Nutritional requirement (such as vitamin and amino acid utilisation) on *Trichophyton* agar;
- Temperature tolerance;



[Table/Fig-1]: a) Colonies of *Trichophyton mentagrophytes* appearing powdery, white and downy on Potato Dextrose Agar (PDA) agar plate; b) LPCB mount showing septate hyphae, coiled spiral hyphae and cluster of conidiophores of *Trichophyton mentagrophytes* (Magnification 40X); c) Colonies of *Trichophyton rubrum* appear fluffy and reverse is deep red color on PDA plates; d) LPCB mount of *Trichophyton rubrum* showing tear drop microconidia and pencil like macroconidia (Magnification 40X); e) Colonies of *Trichophyton violaceum* appearing heaped and deep purplish red on Mycosal agar plate; f) LPCB mount of *Trichophyton violaceum* showing tangled and irregular hyphae, with intercalary chlamydoconidia (Magnification 40X); g) Growth of *Microsporum canis* appearing whitish, coarsely fluffy with yellow pigment on Mycosel agar tubes; h) LPCB mount of *Microsporum canis* showed spindle shaped, rough, and thick walled macroconidia with taper to knob-like ends (Magnification 40X).

- Urease production;
- In-vitro hair perforation test.

Antifungal Susceptibility Testing

Antifungal susceptibility testing was done for all fungal isolates and performed by Broth microdilution method [9].

Antifungal agents: Antifungal drugs such as griseofulvin, terbinafine, itraconazole, fluconazole and voriconazole in powdered form were used. The stock solution of antifungal drugs was prepared according to standard protocol [10]. All drugs were dissolved in DMSO to prepare as stock solutions of 1000 mg/mL. Serial two-fold dilutions were prepared at 100-fold strength of final concentration after further

dilution (1:50) in Roswell Park Memorial Institute (RPMI) 1640 to double the final strength required for the test. A 100 mL aliquots of the two fold drug dilutions were inoculated into the wells with a multichannel pipette. The microtiter plates were stored at-70°C until use. Concentrations ranged from 4-64 µg/mL for fluconazole, from 0.125-4 µg/mL, for griseofulvin, from 0.062 to 2 µg/mL for itraconazole and from 0.007-0.25 µg/mL for terbinafine.

Medium: Broth microdilution tests were carried out in RPMI 1640 medium supplemented with L-glutamine but not sodium bicarbonate and buffered with 0.165 M Morpholinepropanesulfonic (MOPS) acid at pH 7.0.

Inoculation preparation: Isolated fungus was subcultured on Potato Dextrose Agar (PDA) except Trichophyton rubrum which was inoculated on Oatmeal agar plate. The plate was incubated at 30°C for 4-5 days inducing conidia formation. When there was sufficient growth, the plates were covered with 5 mL of sterile 0.85% normal saline and a suspension was prepared by gently probing the colonies with the tip of a transfer pipette. The suspension was left for 10 minutes, and conidia were counted in a haemocytometer. The concentration of the suspension was adjusted according to the spore count.

Inoculum quantitation of dermatophytes: This was performed by plating 0.01 mL dilution of the adjusted inoculum on SDA to confirm the viable number of Colony Forming Unit (CFU) per mL. Plates were incubated at 30°C and observed daily for the presence of fungal colonies [10].

Procedure: Two-fold dilutions of antifungal drugs were prepared in 96 flat bottom microtiter plates and 100 µL of inoculum (2×10^3 to 6×10^3 CFU/mL) was added to each well. Microtiter plate was incubated at 28°C for five days. MIC is the lowest concentration of antifungal agent that substantially inhibits the growth of the organism, as detected visually when testing most antifungal agents. The growth in each MIC for the traditional microdilution process was compared with growth control with the aid of a reading mirror. MIC50 was calculated by taking the drug concentration, where 50% of isolates are inhibited. Similarly, MIC90 was noted with drug concentration where 90% of the isolates were inhibited [9].

STATISTICAL ANALYSIS

The descriptive statistics were reported as means with their SD. The p-value was calculated using one-way Analysis of Variance (ANOVA) test. A p-value <0.05 was considered to be significant. Data were statistically evaluated with IBM SPSS Statistics for Mac, Version 25.0., IBM Corp., Chicago, IL.

RESULTS

A total of 245 patients were enrolled in the study, of which 189 were male patients (77.1%), and 56 were female patients (22.9%). The male to female ratio was 3.4:1. The most common age group was ≤15 years 19 (7.8%), 16-30 years 145 (59.2%) followed by 31-45 years 48 (19.6%), 46-60 years 21 (8.5%), and 61-75 years 12 (4.9%) years. Out of 245 samples, 162 (66.1%) were KOH positive samples, in which 151 were culture positive (93.2%) and 11 samples were culture negative (6.8%). In 83 KOH negative samples (33.9%), 14 were culture positive (16.9%) and rest 69 (83.1%) were negative by culture. A total of 165 samples were culture positive, of which *Trichophyton mentagrophytes (T. mentagrophytes)* was isolated from 153 (92.8%) followed by *Trichophyton rubrum (T. rubrum)* from 5 (3.0%), *Trichophyton violaceum (T. violaceum)* from 3 (1.8%), *Trichophyton tonsurans (T. tonsurans)* and *Microsporum canis (M. canis)* from 2 (1.2%) samples each.

[Table/Fig-2] demonstrates MIC of antifungal drugs for the various dermatophytes. MIC of griseofulvin, fluconazole, itraconazole, terbinafine and voriconazole were determined for 153 (92.8%) *T. mentgrophytes* isolates. The majority of isolates of {53(34.6%)} *T. mentagraphytes* were sensitive to griseofulvin at a concentration of 0.125 µg/mL, and 39 (25.5%) were sensitive to griseofulvin at the concentration of 0.5 µg/mL.

[Table/Fig-3,4] demonstrates MIC (MIC50, MIC 90, MIC range, and geometric mean) and mean MIC of antifungal drugs against dermatophytes species. The MIC range for 165 dermatophyte isolates tested for antifungal susceptibility. Highest MIC50 with 4 µg/mL was found for fluconazole against *T. mentagrophytes* and *T. violaceum*. MIC90 of terbinafine, itraconazole and voriconazole was seen lowest at 0.25 µg/mL followed by griseofulvin at 1 µg/mL for all isolated dermatophytes.

Sr. No.	Species	Antifungal drugs (µg/mL)	0.0019	0.0039	0.0078	0.0156	0.0313	0.0625	0.125	0.25	0.5	1	2	4	8	16	32
1	***T. mentagrophytes* (153)**	Griseofulvin							53 (34.6)	33 (21.6)	39 (25.5)	16 (10.4)	4 (2.6)	5 (3.3)	2 (1.3)	-	1 (0.7)
		Fluconazole							21 (13.7)	4 (2.6)	2 (1.3)	8 (5.2)	13 (8.5)	30 (19.6)	27 (17.7)	41 (26.8)	7 (4.6)
		Terbinafine	34 (22.2)	9 (5.9)	14 (9.1)	14 (9.1)	13 (8.5)	22 (14.4)	24 (15.7)	18 (11.8)	5 (3.3)						
		Itraconazole	33 (21.6)	5 (3.3)	14 (9.2)	16 (10.4)	11 (7.2)	22 (14.4)	21 (13.7)	23 (15.0)	8 (5.2)						
		Voriconazole	27 (17.7)	6 (3.9)	16 (10.5)	12 (7.8)	11 (7.2)	21 (13.7)	26 (17.0)	22 (14.4)	12 (7.8)						
2	***T. rubrum* (5)**	Griseofulvin							1 (20)	4 (80)	0	0	0	0	0	0	0
		Fluconazole							1 (20)	0	0	0	0	0	1 (20)	3 (60)	0
		Terbinafine	1 (20)	2 (40)	0	0	0	2 (40)	0	0	0						
		Itraconazole	1 (20)	0	0	1 (20)	0	1 (20)	0	2 (40)	0						
		Voriconazole	0	0	0	0	0	0	3 (60)	0	2 (40)						
3	***T. violaceum* (3)**	Griseofulvin							0	1 (33.3)	2 (66.7)	0	0	0	0	0	0
		Fluconazole							0	0	0	0	1 (33.3)	1 (33.3)	1 (33.4)	0	0
		Terbinafine	1 (33.3)	0	2 (66.7)	0	0	0	0	0	0						
		Itraconazole	2 (66.7)	0	0	1 (33.3)	0	0	0	0	0						
		Voriconazole	0	1 (33.3)	1 (33.3)	0	0	0	1 (33.4)	0	0						

4	*T. tonsurans* (2)	Griseofulvin							0	1 (50)	1 (50)	0	0	0	0	0	0
		Fluconazole							0	0	0	0	0	0	1 (50)	1 (50)	0
		Terbinafine	0	0	0	0	0	1 (50)	1 (50)	0	0						
		Itraconazole	0	0	0	1 (50)	1 (50)	0	0	0	0						
		Voriconazole	1 (50)	1 (50)	0	0	0	0	0	0	0						
5	*M. canis* (2)	Griseofulvin							0	2 (100)	0	0	0	0	0	0	0
		Fluconazole							0	1 (50)	0	0	0	0	0	1 (50)	0
		Terbinafine	0	0	0	1 (50)	1 (50)	0	0	0	0						
		Itraconazole	0	1 (50)	1 (50)	0	0	0	0	0	0						
		Voriconazole	0	0	0	0	0	0	1 (50)	1 (50)	0						

[Table/Fig-2]: Minimum Inhibitory Concentration (MIC) values of antifungal drugs for the various dermatophytes values presented as n (%).

Dermatophytes	MIC (Mean±SD)				
	Griseofulvin	Fluconazole	Itraconazole	Terbinafine	Voriconazole
T. mentagrophytes	0.83±2.78	8.14±7.96	0.09±0.12	0.07±0.1	0.11±0.14
T. rubrum	0.22±0.05	11.22± 7.11	0.11±0.12	0.02±0.03	0.27±0.21
T. violaceum	0.41±0.14	4.67±3.05	0.01±0.01	0.01±0.01	0.04±0.06
T. tonsurans	0.37±0.17	12 ± 5.65	0.02±0.01	0.09±0.04	0.01±0.01
M. canis	0.25±0.00	8.12±11.13	0.01±0.01	0.02±0.01	0.18±0.08
p-value	0.979	0.773	0.543	0.514	0.067

[Table/Fig-3]: Mean Minimum Inhibitory Concentration (MIC) (µg/mL) of antifungal drugs against dermatophytes species in Mean±SD.
Test applied: One-way ANOVA

Dermatophytes	Griseofulvin				Fluconazole				Itraconazole				Terbinafine				Voriconazole			
	Range (µg/mL)	GM	MIC 50	MIC 90	Range (µg/mL)	GM	MIC 50	MIC 90	Range (µg/mL)	GM	MIC 50	MIC 90	Range (µg/mL)	GM	MIC 50	MIC 90	Range (µg/mL)	GM	MIC 50	MIC 90
T. mentagrophytes	0.125-32	0.3	0.25	1	0.125-32	3.6	4	16	0.0019-0.5	0.03	0.0313	0.25	0.0019-0.5	0.02	0.0313	0.25	0.0019-0.5	0.03	0.0625	0.25
T. rubrum	0.125-0.25	0.2	0.25	0.25	0.125-16	5.3	8	16	0.0019-0.0625	0.1	0.0625	0.25	0.0019-0.0625	0.01	0.0039	0.0625	0.125-0.5	0.2	0.125	0.5
T. violaceum	0.25-0.5	0.4	0.5	0.5	4-8	4	4	8	0.0019-0.0156	0.004	0.0019	0.0156	0.0019-0.0078	0.005	0.0078	0.0078	0.0038-0.125	0.02	0.0078	0.125
T. tonsurans	0.25-0.5	0.4	0.25	0.5	8-16	11.3	8	16	0.0156-0.0313	0.02	0.0156	0.0313	0.0625-0.125	0.09	0.125	0.0625	0.0019-0.0038	0.003	0.0019	0.0039
M. canis	0.125-0.25	0.25	0.25	0.25	0.125-0.5	2	0.25	16	0.0038-0.0078	0.01	0.0039	0.0078	0.0156-0.0313	0.02	0.0156	0.0313	0.125-0.5	0.2	0.125	0.25

[Table/Fig-4]: Minimum Inhibitory Concentration (MIC) (MIC50, MIC90) of antifungal drugs against dermatophytes.
GM: Geometric mean; MIC: Minimum inhibitory concentration

DISCUSSION

In the current study, 165 strains belonging to five dermatophytes species were tested for their susceptibility to five different antifungals. The results of different studies of the in-vitro susceptibility of dermatophytes to antifungals have been variable due to differences in the methodologies used.

The current study revealed that griseofulvin had the lowest MIC (0.125 µg/mL in 32.7%), followed by terbinafine (0.0019 µg/mL in 21.8%), Itraconazole (0.0019 µg/mL in 21.8%), for all isolated dermatophytes. Sharma R et al., showed that griseofulvin had the lowest MIC followed by itraconazole and terbinafine. The MIC of all the isolates against fluconazole was high [11]. Itraconazole and terbinafine had low MICs, but fluconazole had a high MIC, according to a study conducted by Araujo CR et al., [12]. Another study by Bueno JG et al., showed the lowest MICs were obtained with terbinafine, followed by voriconazole against dermatophytes [13]. The present study showed that the highest MIC for itraconazole was 0.5 µg/mL (4.8%), although in other studies it was found to be 8 µg/mL [11], and 1 g/mL [14]. The present study revealed that the isolates showed high MIC for terbinafine was 0.5 µg/mL (3%), griseofulvin was 8 µg/mL (1.2%) and 32 µg/mL (0.6%) and voriconazole was 0.5 µg/mL (8.5%). Other studies reported highest MIC for fluconazole to be 32 µg/mL [12] and 64 µg/mL [15], terbinafine to be 1 µg/mL [12] and 4 µg/mL [14]; griseofulvin to be 8 µg/mL [12] and 16 µg/mL [15]. In a study by Maurya VK et al., majority of the fungal isolates (38.6%) showed MIC of fluconazole at 4 µg/mL, where the maximum MIC of 64 µg/mL was shown by 6.6% isolates, 90.6% isolates showed itraconazole MIC of ≤0.125 µg/mL which is quite low. Regarding ketoconazole all (100%) isolates had MIC ≤ 0.5 µg/mL, whereas for terbinafine the highest MIC seen was 16 µg/mL, while 21.33% and 29.33% isolates had MIC values 2 µg/mL and 4 µg/mL, respectively [16].

In the current study, the mean MIC value of griseofulvin against T. *mentagrophytes* was 0.83±2.78 µg/mL. The mean MIC of fluconazole was 8.14±7.96 µg/mL, of itraconazole was 0.09±0.12 µg/mL, of terbinafine was 0.07±0.1 µg/mL, and mean MIC of voriconazole was 0.11±0.14 µg/mL, which was higher than the study of Sarifakiouglu E et al., done in Turkey, where mean MIC value of fluconazole against T. *mentagrophytes* was 1.27±0.9, the mean MIC value of itraconazole was 0.028±0.02 µg/mL; of terbinafine was 0.0078±0.005 [17].

In the current study, the mean MIC value of griseofulvin against T. *rubrum* was 0.22±0.05 µg/mL. The mean MIC of fluconazole was 11.22±7.11 µg/mL, of itraconazole was 0.11±0.12 µg/mL, and of terbinafine was 0.02±0.03 µg/mL and mean MIC of voriconazole was 0.27±0.21 µg/mL. Sarifakiouglu E et al., showed the mean MIC value of fluconazole against T. *rubrum* was 0.51±0.5.The mean value of itraconazole was 0.022±0.02; of terbinafine was 0.0057±0.003 [17].

In the current study, the mean MIC value of griseofulvin against T. *violaceum* was 0.41±0.14 µg/mL. The mean MIC of fluconazole was 4.67±3.05 µg/mL, of itraconazole and terbinafine were 0.01±0.01 µg/mL, of voriconazole was 0.04±0.06 µg/mL. The mean MIC value of griseofulvin against T. *tonsurans* was 0.37±0.17 µg/mL. The mean MIC value of fluconazole was 12±5.65 µg/mL, of itraconazole was 0.02±0.01 µg/mL, of terbinafine was 0.09±0.04 µg/mL and mean MIC of voriconazole was 0.01±0.1 µg/mL. The mean MIC value of griseofulvin against *M. canis* was 0.25±0.00 µg/mL. The mean MIC of fluconazole was 8.12±11.13 µg/mL, of itraconazole was 0.01±0.01 µg/mL, of terbinafine was 0.02±0.01 µg/mL, and of voriconazole was 0.18±0.08 µg/mL. In a study by Ael AAA and Taha MM done in Egypt Institute in Zagazig University in year 2007, the authors isolated dermatophytes and did Antifungal susceptibility testing. However, the individual dermatophytes antifungal susceptibility data was not done. In their study the mean MIC value of amphotericin B was 10.6±4.6, of fluconazole was 9.8±6.3, of ketoconazole was 14.4±3.5, and of itraconazole was 9.8±5.3 [18].

Different studies have shown the MIC of griseofulvin, fluconazole, itraconazole, terbinafine and voriconazole in the form of MIC values, mean MIC, MIC50, MIC90 etc. In the current study, the MIC ranges for all the 165 isolates for dermatophytes tested for antifungal susceptibility showed that itraconazole, terbinafine and voriconazole showed the lowest MIC range of 0.0019-0.5 µg/mL followed by griseofulvin and fluconazole at MIC range of 0.125-32 µg/mL. The MIC50 of itraconazole and terbinafine was seen lowest at 0.0313 µg/mL followed by voriconazole at 0.0625µg/mL, griseofulvin at 0.25 µg/mL for all isolated dermatophytes. Highest MIC50 with 4 µg/mL was found for fluconazole against *T. mentagrophytes* and *T. violaceum*. In a study by Kurup AS et al., terbinafine exhibited MIC50 at 0.25 µg/mL for *T. interdigitale*, 0.125 µg/mL for *T. rubrum* and 0.0312 µg/mL for *T. mentagrophytes* [19].

The MIC90 of terbinafine, itraconazole and voriconazole was seen lowest at 0.25 µg/mL followed by griseofulvin at 1 µg/mL for all isolated dermatophytes. Highest MIC90 of fluconazole was recorded at 16 µg/ml for *T. mentagrophytes*, *T. rubrum, T. tonsurans. M. canis* and 8 µg/ml for *T. violaceum*. Previous studies could be correlated with present study in which the MIC of terbinafine, itraconazole and voriconazole was significantly higher than fluconazole [Table/Fig-5a,b] [20-23]. The result of current study clearly shows that there is a lot of variation in the dermatophytes profile and antifungal susceptibility of different species of dermatophytes to different antifungal agents. This could reflect the variation in the geographical area selection of patients, susceptibility of patients to different infections empirical use or misuse of antifungal agents.

Breakpoints have not been described for mold testing [9]. However, a comprehensive data on the most common dermatophytes agents

Dermatophytes	Antifungal drug	Present study, Northern India (2022)				Perea S et al., 2001 San Antonio, Texas [20]				Fernandez Torres B et al., 2002 Spain [21]			
		Range (µg/mL)	GM	MIC50	MIC90	Range (µg/mL)	GM	MIC50	MIC90	Range (µg/mL)	GM	MIC50	MIC90
T. mentagrophytes	GRI	0.125-32	0.3	0.25	1	0.125-8	1.16	1	4	-	-	-	-
	FLU	0.125-32	3.6	4	16	1->16	19.39	16	64	0.06-64	15.08	16	>64
	ITR	0.0019-0.5	0.03	0.0313	0.25	<0.0156-2	0.04	0.03	0.125	0.01-2	0.17	0.25	1
	TER	0.0019-0.5	0.02	0.0313	0.25	0.004-0.125	0.04	0.03	0.125	0.007-0.5	0.04	0.06	0.06
	VRI	0.0019-0.5	0.03	0.0625	0.25	<0.125-1	0.46	0.5	1	0.01-1	0.09	0.25	1
T. rubrum	GRI	0.125-0.25	0.2	0.25	0.25	0.5-8	1.95	2	4	-	-	-	-
	FLU	0.125-16	5.3	8	16	02-08	3.31	4	8	0.06->64	2.8	4	16
	ITR	0.0019-0.0625	0.1	0.0625	0.25	0.03-1	0.08	0.06	0.25	0.01-8	0.09	0.125	0.5
	TER	0.0019-0.0625	0.01	0.0039	0.0625	<0.04-0.05	0.01	0.007	0.01	0.007-0.5	0.04	0.06	0.06
	VRI	0.125-0.5	0.2	0.125	0.5	<0.125-1	0.38	0.5	0.5	0.01-1	0.09	0.25	1
T. violaceum	GRI	0.25-0.5	0.4	0.5	0.5	-	-	-	-	-	-	-	-
	FLU	0.125-8	4	4	8	-	-	-	-	0.0625-16	1.91	4	8
	ITR	0.0019-0.0156	0.004	0.0019	0.0156	-	-	-	-	0.0625-0.25	0.01	0.01	0.03
	TER	0.0019-0.0078	0.005	0.0078	0.0078	-	-	-	-	0.007-0.03	0.09	0.007	0.001
	VRI	0.0038-0.125	0.02	0.0078	0.125	-	-	-	-	0.01-0.25	0.04	0.04	0.06
T. tonsurans	GRI	0.25-0.5	0.4	0.25	0.5	01-08	2.44	2	4	-	-	-	-
	FLU	08-16	11.3	8	16	64->64	64	64	64	0.06-16	1.91	4	8
	ITR	0.0156-0.0313	0.02	0.0156	0.0313	0.03-1	0.15	0.06	0.5	0.06-0.25	0.01	0.01	0.03
	TER	0.0625-0.125	0.09	0.125	0.0625	0.007-0.25	0.04	0.01	0.125	0.0078-0.0313	0.09	0.007	0.01
	VRI	0.0019-0.0038	0.003	0.0019	0.0039	0.25-1	0.67	0.5	1	0.0156-0.25	0.04	0.04	0.06
M. canis	GRI	0.125-0.25	0.25	0.25	0.25	0.25-1	0.156	0.5	1	-	-	-	-
	FLU	0.125-0.5	2	0.25	16	02-64	10.07	8	16	0.06->64	5.39	8	16
	ITR	0.0038-0.0078	0.01	0.0039	0.0078	0.0313-2	0.1	0.06	0.125	0.01-4	0.08	0.125	0.5
	TER	0.0156-0.0313	0.02	0.0156	0.0313	0.0313-2	0.06	0.01	0.06	0.007->16	0.04	0.06	0.06
	VRI	0.125-0.5	0.2	0.125	0.25	0.25-0.5	0.4	0.5	0.5	0.01-0.5	0.04	0.06	0.125

[Table/Fig-5a]: Comparison of range, geometric mean, MIC50 and MIC90 with other study [20,21].
GRI: Griseofulvin; FLU: Fluconazole; ITR: Itraconazole; TER: Terbinafine; VRI: Voriconazole

Dermatophytes	Antifungal drug	Present study				Indira G 2014 Telangana, India [22]				Adimi P et al., 2013 Tehran, Iran [23]			
		Range (µg/mL)	GM	MIC50	MIC90	Range (µg/mL)	GM	MIC50	MIC90	Range (µg/mL)	GM	MIC50	MIC90
T. mentagrophytes	GRI	0.125-32	0.3	0.25	1	0.32-5.12	-	1.28	2.56	0.0312-256	2.66	2	256
	FLU	0.125-32	3.6	4	16	0.08-20.48	-	1.28	10.24	0.0625-256	18.8	64	256
	ITR	0.0019-0.5	0.03	0.0313	0.25	0.03-1.92	-	0.24	0.96	0.0009-4	0.045	0.0625	0.5
	TER	0.0019-0.5	0.02	0.0313	0.25	0.002-0.16	-	0.06	0.08	0.0156-8	0.28	0.5	4
	VRI	0.0019-0.5	0.03	0.0625	0.25	-	-	-	-	0.0156-8	0.28	0.5	4

T. rubrum	**GRI**	0.125-0.25	0.2	0.25	0.25	0.16-5.12	-	1.28	2.56	0.0312-256	1.61	2	256
	FLU	0.125-16	5.3	8	16	0.16-20.48	-	1.28	2.56	0.0625-256	11.05	32	256
	ITR	0.0019-0.0625	0.1	0.0625	0.25	0.03-3.84	-	0.24	1.92	0.0009-4	0.06	0.0625	2
	TER	0.0019-0.0625	0.01	0.0039	0.0625	0.001-0.08	-	0.005	0.04	0.0156-16	0.172	0.0312	16
	VRI	0.125-0.5	0.2	0.125	0.5	-	-	-	-	0.0078-8	0.19	0.125	4
T. violaceum	**GRI**	0.25-0.5	0.4	0.5	0.5	0.32-5.12	-	1.28	2.56	16	16	-	-
	FLU	0.125-8	4	4	8	0.03-1.92	-	0.48	0.96	1-128	11.31	-	-
	ITR	0.0019-0.0156	0.004	0.0019	0.0156	0.01-0.96	-	0.12	0.48	0.25	0.25	-	-
	TER	0.0019-0.0078	0.005	0.0078	0.0078	0.001-0.08	-	0.01	0.04	0.0156- 0.125	0.044	-	-
	VRI	0.0038-0.125	0.02	0.0078	0.125	-	-	-	-	0.0312	0.031	-	-
T. tonsurans	**GRI**	0.25-0.5	0.4	0.25	0.5	0.64-5.12	-	1.28	2.56	0.03-256	24.24	128	256
	FLU	08-16	11.3	8	16	0.16-20.48	-	2.56	5.12	0.0625-256	13.92	32	256
	ITR	0.0156-0.0313	0.02	0.0156	0.0313	0.48-7.68	-	1.92	3.84	0.0076-2	0.147	0.375	2
	TER	0.0625-0.125	0.09	0.125	0.0625	0.005-0.04	-	0.01	0.02	0.0156-8	0.088	0.0078	8
	VRI	0.0019-0.0038	0.003	0.0019	0.0039	-	-	-	-	0.0625-4	0.32	0.312	4
M. canis	**GRI**	0.125-0.25	0.25	0.25	0.25	0.64-5.12	-	1.28	5.12	0.02-128	0.16	0.0312	96
	FLU	0.125-0.5	2	0.25	16	0.64-20.48	-	5.12	10.24	0.0625-256	11.98	32	256
	ITR	0.0038-0.0078	0.01	0.0039	0.0078	0.24-3.84	-	0.96	1.92	0.0009-0.5	0.08	0.0312	0.5
	TER	0.0156-0.0313	0.02	0.0156	0.0313	0.02-0.01	-	0.005	0.01	0.0312-8	0.044	0.0312	4
	VRI	0.125-0.5	0.2	0.125	0.25	-	-	-	-	0.0156-8	0.164	0.125	7.5

[Table/Fig-5b]: Comparison of range, geometric mean, MIC50 and MIC90 with other study [22,23].
GRI: Griseofulvin; FLU: Fluconazole; ITR: Itraconazole; TER: Terbinafine; VRI: Voriconazole

is prevalent in a particular area, the risk factors associated with it, and the availability antifungal susceptibility profile of common dermatophytes to the commonly used drugs will go along way in providing holistic therapy to the patients and preventing antifungal resistance. However, more studies are needed to correlate the antifungal MIC with clinical response to the antifungals so that susceptibility breakpoints may be arrived at.

Limitation(s)

In the current study, the patient group was very small. Authors suggest further prospective population-based research on a large population for finding the antifungal susceptibility testing of antifungal agents against clinically isolated dermatophytes in Northern India. Also, a follow-up of patients to correlate clinical response with the MIC values was not done.

CONCLUSION(S)

This study highlights the prevalent dermatophytes in Northern India and their antifungal susceptibility. Dermatophytes take long time to grow, they may not be isolated in all the cases of dermatophytosis and putting up antifungal susceptibility testing routinely may not be feasible. Studies should be taken up to correlate the clinical condition with their most common pathogen and the best antifungal to treat these infections. Since most of the clinicians do not send a sample, rather treat these infections empirically, these studies will go a long way to help the clinicians in choosing the most appropriate therapy. However, more studies will go a long way to help the clinicians in choosing the most appropriate therapy.

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