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Original Research Article

Clinicomycological study on identification and antifungal susceptibility testing of dermatophytes at a tertiary care hospital in Tamil Nadu

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Abstract

Background and Aim: The most prevalent cutaneous fungal infection is dermatophytosis, which is caused by a class of keratinophilic filamentous fungus known as dermatophytes. There is dramatic rise in recalcitrant and extensive dermatophytosis associated with antifungal resistance. So early and accurate diagnosis is essential for timely administration of anti-fungal agents in dermatophytosis. The present study aimed to isolate and identify dermatophytes and to study the antifungal susceptibility pattern.

Materials and Methods: Prospective Cross sectional study conducted for 18 months (Nov 2022 - April 2024). 250 clinical isolates from patients with clinical diagnosis of Superficial Mycoses attending OPD Dermatology were collected, initial KOH mount was done. The identification was carried out using both traditional methods and Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF MS). The isolates were tested for their antifungal susceptibility using the micro broth dilution technique according to the Clinical and Laboratory Standards Institute (CLSI) M38 - A2(2008).

Results: Among 250 clinical specimens, all 103 dermatophyte isolated in conventional culture methods, Genus was *Trichophyton*, of which *Trichophyton mentagrophytes* was 88.3% as the predominant isolate, followed by *Trichophyton rubrum* with 11.7% isolates. In MALDITOF-MS identification, out of 50 isolates, 66% correlated at Genus level and 62% correlated at species level. Out of 45 *Trichophyton mentagrophyte* isolates tested for Antifungal susceptibility testing (AFST), 97.80% showed resistance to Fluconazole and 31.10% to Itraconazole. 5 isolates of *Trichophyton rubrum* showed resistance to 80% of Fluconazole and 40% of Itraconazole.

Conclusion: To improve patient outcomes in the future, it is important to diagnose dermatophytosis early and accurately, utilize newer antifungal medicines with caution, and practice antifungal stewardship.

Keywords: Tinea, Cutaneous mycoses, Dermatophytes, *Trichophyton*, Antifungal susceptibility, Dermatophytosis

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1. Introduction

With an infection prevalence of up to 20%- 25% globally.[1] Dermatophytes are by far the most prevalent filamentous fungus that cause disease. The incidence of dermatophytosis might reach 40 -60% in areas where there is higher prevalence. (1) Superficial dermatophytosis, more often known as tinea, was once thought to be a mild skin illness. There is a shift seen recently in the clinical presentation of extensive and recalcitrant dermatophytosis.[2] Dermatophytosis has a recent incidence in India ranging from 36.6 -78.4%, with *Trichophyton mentagrophytes* and *Trichophyton rubrum* being the most common isolates. As contrast to previous times, the contemporary dermatophytosis situation is marked by several unusual symptoms, including epidemiological, clinical, and mycological presentations.[3] Although the disease can be diagnosed based on the typical clinical presentation, laboratory confirmation may be necessary in patients with rare symptoms. The significance of laboratory diagnostics for isolation, identification, and in vitro susceptibility testing is further enhanced by the relapse or recurrence issue in India.[4] Traditional approaches need a great deal of knowledge, skill, and time. In order to quickly, easily, and reliably identify dermatophytes, MALDITOF MS has recently seen extensive application.[5] Antifungal resistance has been on the rise due to the growing usage of antifungal medications in therapy, in

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recent years. Antifungal susceptibility testing has recently received a lot of interest and several studies have attempted to quantify the actual load of resistance.[6] The current investigation is carried out with the purpose of isolating and species-identifying dermatophytes in order to shed light on the changing trend in prevalence, confirm their sensitivity to antifungals, and emphasize their resistance pattern.

2. Materials and Methods

2.1. Study design

Cross sectional Descriptive study.

2.2. Study period

18 months [Nov 2022 - April 2024]

2.3. Place of study

Department of Microbiology, A.C.S Medical College & Hospital, Chennai. MALDI-TOF MS was performed in Dr. Rela Institute & Medical Centre, Chromepet.

2.4. Study population

Patients with clinical diagnosis of Superficial Mycoses attending Out Patient Department of Dermatology.

2.5. Sample size

250 clinical samples

2.6. Ethics approval

Obtained from ACS Medical College and Hospital Ethics committee. Project number (No.582/2022/IEC/ACSMCH).

2.7. Informed consent

Obtained from all patients

2.8. Microbiological analysis

1. Sample collection: Prior to sample collection, the lesion and its surrounding area were wiped with 70% ethyl alcohol and left to dry for few minutes. Samples collected were skin scrapings, nail bits and infected hairs and transferred in sterile black cardboard paper.
2. Direct microscopy with 10% potassium hydroxide (KOH) mount for skin scrapings and 40% KOH mount for nail and hair specimens was used for the primary dermatophyte identification. The wet mount was directly examined under a microscope at 10x and 40x magnification to look for yeast cells, spores, or fungal hyphae.
3. Culturing: Separate sets of samples were inoculated onto Sabouraud's Dextrose Agar with chloramphenicol and cycloheximide gradients. They were both placed in an incubator set at 25˚C and 37˚C, which measure biological oxygen demand (BOD). Colony morphology was evaluated in relation to the growth on both slopes. After the first week of incubation, the cultures were checked daily; after that, they were checked twice weekly for another four to six weeks.
4. Colony features, growth rate, and pigment production were used for macroscopical identification when growth was detected on Sabouraud Dextrose agar. Microscopical analysis was performed using Lactophenol cotton blue mount. Species identification was conducted based on the characteristics of hyphal structure, as well as the number and arrangement of macro and micro conidia.
5. Vitek –MS – BioMérieux MALDI-TOF MS identification was done for 50 dermatophyte isolates by Protein extraction method using ethanol, formic acid and acetonitrile.
6. Antifungal susceptibility testing for Dermatophytes was done for 50 dermatophyte isolates using Microbroth dilution method and Minimum Inhibitory Concentration (MIC) was reported. The procedure followed was according to the Clinical and Laboratory Standards Institute (CLSI) M38 - A2(2008).[7]
7. Drug dilutions: A solution of dimethyl sulfoxide was prepared with each of the medications (Griseofulvin, Fluconazole, Itraconazole, and Terbinafine). The drug stock dilutions used in the microdilution test were 100 times stronger than the final drug concentration. The stock medication solutions were kept at a temperature of -80 ˚C. They were first diluted in RPMI 1640 at a ratio of 1:50 before usage.
8. Preparation of inoculum: The dermatophyte colonies on Potato Dextrose agar were supplemented with 1 ml of sterile 0.85% normal saline after they had been developing for 7 days. A Pasteur pipette was used to delicately scrape the colonies with a loop before transferring them to a sterile tube. After 3–5 minutes of undisturbed time, the tube was allowed to settle for the heavier particles. After transferring the leftover solution to a fresh sterile tube, it was vortexed for 15 seconds to further modify the density of the conidial suspension to 0.5 McFarland Turbidity standard.
9. Microbroth dilution test: Microplates with a sterile bottom and 96 wells were used. Each well contained 100 µl of inoculum and 100 µl of drug dilution, and inoculum and serial dilution of the produced medicines were introduced in a sequential manner in wells 1 through 10. As a drug control, the eleventh well included 100 µl of RPMI-1640 and 100 µl of antifungal agent. The growth control well, which was the 12th and final well, had 100 µl of inoculum and 100 µl of RPMI 1640. After that, the microplates were kept at 25 ˚C for a duration of 7 days.
10. Tests results were recorded by comparing the growth in the well with growth control well. For each isolate, minimal inhibitory concentration (MIC) value was recorded for all antifungals. Additionally, MIC 50 was calculated for each antifungal against all dermatophyte isolates that exhibited growth.

3. Results

Dermatophytosis was most common in patients aged 19–30 (29.6% of the total) and 31–40 (22.8%), while the age range of the patients included in the study was 8–88 years. With 22 participants, the pediatric category made up 8.8% of the total. As shown in **Table 1**, there were 114 men and 136 females out of 250 patients, for a male to female ratio of 1:1.2. Out of 250 samples, a total of 89 (35.6%) samples were positive in both direct KOH microscopic examination and culture and 83 (33. 2%) samples were negative by both the techniques (**Table 2**). All samples were cultured regardless of whether they tested positive for KOH. Of the clinical specimens, 103(41.2%) dermatophytes were isolated .147(58.8%) had negative Dermatophyte cultures.

Every culture isolation was a member of the *Trichophyton* genus. 91 (88.3%) isolates of *Trichophyton mentagrophytes* were the most common, followed by 12 (11.7%) isolates of *Trichophyton rubrum*. *Trichophyton mentagrophytes* was isolated in 33 patients, *Trichophyton rubrum* in 8 patients out of the 41 clinical cases of Tinea Corporis, respectively. Two patients with Tinea Cruris were involved in this investigation, and *Trichophyton mentagrophytes* was isolated from both of them. Out of 54 patients with Mixed dermatophytosis, *Trichophyton mentagrophytes* was isolated in 50 patients, *Trichophyton rubrum* in 4 patients (**Table 3**). 50 isolates, with 45 *Trichophyton mentagrophytes* and 5 isolates of *Trichophyton rubrum* identified by conventional methods were subjected to MALDI-TOF MS identification. 31 out of 50(62%) isolates done by MALDI - TOF MS correlated with conventional fungal culture methods.16% of isolates were reported as no ID in MALDI-TOF MS (**Table 4**).

Table 1: Age and sex distribution of study population (n = 250)

Age group	Male	Female	Total (%)
<=30	49	47	96(38.4)
31-40	22	35	57(22.8)
41-50	18	31	49(19.6)
51-60	15	12	27(10.8)
>60	10	11	21(8.4)
Total	114	136	Total 250(100)

Table 2: Correlation between KOH mount and culture (n = 250)

	n	%
KOH (+ve), Culture (+ve)	89	35.6
KOH (+ve), Culture (- ve)	64	25.6
KOH (- ve), Culture (+ve)	14	5.6
KOH (- ve), Culture (-ve)	83	33.2
Total	250	100

Table 3: Distribution of dermatophytes among the various isolated dermatophytosis by Conventional culture methods (n = 103)

	Trichophyton mentagrophytes	*Trichophyton rubrum*	Total	%
Tinea Corporis	33	8	41	40
Tinea Cruris	2	-	2	2
Tinea Glutealis	6	-	6	6
Mixed Dermatophytosis	50	4	54	52
Total	91	12	103	100

Table 4: Identification of dermatophytes by MALDI -TOF MS (n = 50)

	n	%
Trichophyton mentagrophytes/ interdigitale	28	56.0
Trichophyton rubrum	3	6.0
Rasamsonia argillacea complex	1	2.0
Trichophyton benhamiae	1	2.0
Trichophyton erinacei	1	2.0
No identification	16	32.0
Total	50	100.0

Table 5: Determination of MIC values (MIC 50) of antifungal agents against *Trichophyton mentagrophytes* and *Trichophyton rubrum*

S.No.	Isolates	Values	Fluconazole	I traconazole	Griseofulvin	Terbinafine
1	*Trichophyton mentagrophytes* (n = 45)	MIC range	1 - 64	0.06 - 8	0.03 - 16	0.03 - 16
		MIC 50	16	0.5	0.125	0.06
2	*Trichophyton rubrum* (n = 5)	MIC range	1 - 32	0.125 - 8	0.03 – 0.06	0.03 – 0.125
		MIC 50	4	1	0.06	0.06

Table 6: Comparative study MIC 50 of *Trichophyton mentagrophytes*

	Fluconazole	Itraconazole	Griseofulvin	Terbinafine
Indira G *et al*[23]	1. 28 mcg/ ml	0. 24 mcg/ ml	1. 28 mcg/ ml	0. 06 mcg/ ml
Vanathi Sabtharishi *et al*[6]	2 mcg/ ml	0. 03 mcg/ ml	0. 12 mcg/ ml	
Pathania *et al*[24]	4 mcg/ ml	0. 30 mcg/ ml	16 mcg/ ml	0. 125mcg/ ml
Gnanasuriyan *et al*[25]	2 mcg/ ml	0. 25 mcg/ ml		1 mcg/ ml

Table 7: Comparative study MIC 50 of *Trichophyton rubrum*

	Fluconazole	Itraconazole	Griseofulvin	Terbinafine
Indira G *et al*[23]	1.28 mcg/ml	0.24 mcg/ml	1.28 mcg/ml	0.05 mcg/ml
Vanathi Sabtharishi *et al*[6]	1 mcg/ml	0.12 mcg/ml	0.25 mcg/ml	
Pathania *et al*[24]	4 mcg/ml	0.03 mcg/ml	32 mcg/ml	0.06mcg/ml
Gnanasuriyan *et al*[25]	2 mcg/ml	0.25 mcg/ml		0.5 mcg/ml

Using CLSI recommendations, antifungal susceptibility testing was conducted on fifty isolates. MIC 50 of *Trichophyton mentagrophytes* was calculated, Terbinafine had a concentration of 0.06 mcg/ml, Griseofulvin of 0.125 mcg/ml, Fluconazole of 0.5 mcg/ml, and Itraconazole of 16 mcg/ml. A MIC 50 of 4 mcg/ml for Fluconazole, 1 mcg/ml for Itraconazole, 0.06 mcg/ml for Griseofulvin, and 0.06 mcg/ml for Terbinafine were determined for *Trichophyton rubrum*. Among the *Trichophyton* tested, 97.8% of the mentagrophytes and 80% of the rubrum were resistant to Fluconazole. A significant percentage of *Trichophyton mentagrophytes* (31.1%) and *Trichophyton rubrum* (40.0%) were shown to be resistant to Itraconazole.

4. Discussion

India, like many other tropical and subtropical nations, has the serious public health issue of dermatophytosis. The need for accurate and quick fungal pathogen identification as well as antifungal susceptibility testing is paramount in light of the rising tide of dermatophytes that are resistant to standard antifungal treatments.

Ages of study participants varied widely, from eight years old to eighty-eight years old. People in the 19–30 age group had 29.6% of all cases of Dermatophytosis in this study, followed by those in the 31–40 age group with 22.8% and seen in similar studies.[8,9] It is possible that the higher rates of physical activity and perspiration in this age group, together with the tropical climate, contribute to the higher occurrence of Dermatophytosis. Consistent with the findings of Gupta *et al*.[9] which also found 8.1% of participants to be children, 8.8% of the current study's participants are children.

Of the 250 patients diagnosed with dermatophytosis, 54.4% were females and 45. 6% were males. This distribution corresponds to a male to female ratio of 1:1. 2. This ratio aligns with findings from studies, where dermatophytic infections were more common in females with a male to female ratio of 0.58:1[10] and 1: 1.1.[11] In contrast, certain studies they observed a male predominance with male to female ratios of 1.3:1[12] and 1.2:1.[13]

Out of 250 samples, 89 (or 35.6% of the total) tested positive by culture and direct KOH microscopic analysis, whereas 83 (or 33.2% of the total) tested negative by both analyses. For a total of 64 samples, or 25.6%, the results were negative when cultured but positive when examined directly. This could be due to inappropriate use of antifungal or mixed treatment before sampling. The significant fluctuation in the positive rate could result from variations in sample methods and selection criteria. This highlights how crucial it is to perform culture even on KOH-negative samples.

In conventional culture identification, genus *Trichophyton* was represented by all 103 dermatophyte isolates. The most common isolate, accounting for 91 (88.3%) was *Trichophyton mentagrophytes*, whereas *Trichophyton rubrum* accounted for 12 (11.7 %). This is well corroborated with studies[13,14] and contrast to the studies[15,16] *Trichophyton rubrum* was the commonest fungus isolated. With an increasing percentage of *Trichophyton mentagrophytes* complex, which seems to be the foundation for the recent exponential spread of superficial dermatophytosis in India. There was a time when *Trichophyton rubrum* was considered as the prototypical Dermatophyte.

It takes many weeks for discriminative traits to show for conventional dermatophyte identification. Thanks to MALDITOF MS's widespread availability in diagnostic settings, patients may be quickly diagnosed.[17] Improving laboratory diagnostic capabilities, such as updating and expanding MALDI-TOF MS databases, would enhance the speed and accuracy of identifying dermatophytes at the species level.[18] In this study, 31 out of 50 isolates (62%) identified by MALDI -TOF done by Vitek MS correlated with conventional fungal culture methods.16% of isolates were reported as no ID in MALDI - TOF MS. Rare zoophilic species like *Trichophyton benhamiae* and *Trichophyton erinacei* were reported with confidence interval of 49%.In the study conducted by Hedayati MT *et al*[19] 47% isolate identification was done at species level and 13% had no Identification. Similarly, De Respinis *et al*[20] reported 59.6% species identification and reported identification of rare species of. *Trichophyton benhamiae* and *Trichophyton erinacei.* In contrast, Jin Shao *et al*[21] reported 90. 1% identification at species level.

The inoculum size, incubation temperature and duration, endpoint determination, medium for conidiation, and medium for inoculation are some of the factors that must be addressed while establishing a standard procedure for antifungal susceptibility testing of dermatophytes.[7] The rising resistance profile for Fluconazole is a result of the drug's universal use, which is a result of its cheap cost, dose, and extensive availability at all levels of health care facilities. Low MIC value of *Trichophyton mentagrophytes* for Fluconazole, Itraconazole, Griseofulvin and Terbinafine was reported in study conducted by Singh et al.[22]

The MIC 50 was calculated by finding the medication concentration that inhibits 50% of the isolates. The recurrence in dermatophyte infections could not be explained by a high MIC of antifungal medications alone. As the minimal inhibitory concentration of commonly used antifungal drugs is going up, dermatophyte organisms are likely becoming more harmful. This poses a significant rising health risk to the population due to aspects including hosts, environments, and treatments.

Given the growing resistance to commonly used antifungals like fluconazole and itraconazole, routine antifungal susceptibility testing ought to be included into clinical practice to direct targeted therapy. To prevent the indiscriminate use of antifungal agents, which leads to the development of resistance, antifungal stewardship programs must be put in place. Furthermore, the burden of extensive and recurrent dermatophytosis may be lessened by public health education that emphasizes early detection and treatment compliance. Lastly, in order to overcome resistance and enhance patient outcomes in the management of dermatophytosis, it may be necessary to investigate newer antifungal agents and combination therapies.

5. Conclusion

All of the dermatophyte isolates in this investigation were members of the genus *Trichophyton*, with *Trichophyton mentagrophytes* and *Trichophyton rubrum* being the most common isolates.Fluconazole was the most resistant medication, followed by Itraconazole.

The main causes of the rising incidence of dermatophytosis and treatment failure are the emergence of antifungal resistance and patient non-compliance. A better cure for the patient is promised by the prudent use of more recent antifungal medications, antifungal stewardship and a focus on patient compliance.

6. Limitations

MALDITOF MS detection of dermatophytes was done only for 50 isolates. Detection of MIC was done for 50 isolates for commonly used four drugs - Fluconazole, Itraconazole, Griseofulvin and Terbinafine due to limited resources.

7. Ethics Approval

Ethics approval obtained from ACS Medical College and Hospital Ethics committee. Project number (No.582/2022/IEC/ACSMCH).

8. Informed Consent

Informed consent was obtained from all patients.

9. Source of Funding

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10. Conflict of Interest

The authors declare no conflict of interest.

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