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"A CROSS-SECTIONAL STUDY OF CLINICO-MYCOLOGICAL PROFILE OF DERMATOPHYTES AND ANTIFUNGAL SUSCEPTIBILITY AMONG PATIENTS ATTENDING DERMATOLOGY OPD AT A TERTIARY CARE HOSPITAL IN TELANGANA"

Medical Microbiology

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ABSTRACT

Background: Dermatophytosis is the most common superficial fungal infection worldwide, caused by dermatophytes that cause infections of the skin, hair, and nails due to their ability to invade keratin. Belong to three closely related genera: Trichophyton, Microsporum, and Epidermophyton. It is common in the tropics & subtropical regions and in areas of high humidity. Recent years have seen an alarming increase in chronic, recurrent, and recalcitrant dermatophytosis in India. 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. **Objectives:** To identify the dermatophyte species causing Dermatophytosis and to determine antifungal susceptibility patterns to Fluconazole and Griseofulvin by Broth microdilution. **Method:** A cross-sectional study was conducted over a period of 18 months duration i.e., from June 2021 to November 2022. Skin scrapings, hair plucking, and nail clippings were collected from 200 clinically diagnosed cases of dermatophytosis who attended the outpatient department of Dermatology (DVL) and samples were processed at the upgraded Department of Microbiology at Osmania General Hospital, Afzalgunj, Hyderabad. **Results:** A total of 200 clinically diagnosed dermatophytosis patients were included in our study following inclusion and exclusion criteria. Their demographic details and clinical examination of cutaneous lesions were recorded. In our study, most patients affected were males 57% and the most common age group was 21-30 years seen in 25.5% of patients. Tinea corporis (40%) was the most common dermatophyte lesion followed by Tinea cruris (19.5%) seen in our study. KOH positivity was seen in 42% and culture was positive in 69.5% of cases. Dermatophytes isolated are 42% and non-dermatophytes isolated are 27.5% of cases in our study. The most common dermatophyte isolated in culture was Trichophyton rubrum (45%), followed by Trichophyton mentagrophytes 28 (33.3%). Among isolates of dermatophytes, Fluconazole is sensitive to 33.3% of isolates, whereas Griseofulvin is sensitive to 52.3% of isolates. **Conclusion:** Therapeutic failure is alarmingly common in the current scenario of dermatophytosis in India. Failure is probably seen with all common isolates, younger patients, high contagious nature. This study will help to standardize care, provide guidance on management, and assist in clinical decision-making for healthcare professionals.

KEYWORDS

Clinical features, Dermatophytosis, Antifungal agents, Antifungal susceptibility testing

INTRODUCTION

Dermatophytosis is the most common superficial fungal infection [1] in the dermatology outpatient department worldwide affecting the skin, hair, and nails of human beings. The World Health Organization estimates that dermatophytes affect about 20% to 25% of the world population[2]. It is more prevalent in tropical and subtropical countries like India where heat and moisture play a vital role[3]. The introduction of modern treatment modalities and aggressive use of more chemotherapeutic agents results in promptly expanding chemically induced immunosuppression. These patients are more prone to severe fungal infections. The inadequate and inappropriate usage of antifungal agents and over-the-counter topical corticosteroids and host factors such as non-compliance and immunosuppression result in the emergence of resistance. In recent years there is an alarming increase in chronic, recurrent, and recalcitrant dermatophytosis in India.

Hence, the present study is conducted with an aim to isolate and speciate dermatophytes to reveal the changing trend in the prevalence and to identify their antifungal susceptibility.

AIMS AND OBJECTIVES

AIM:

To study the clinical-mycological profile of Dermatophytes and Antifungal susceptibility pattern among isolates of Dermatophytes.

OBJECTIVES:

1. To identify the Dermatophyte species causing Dermatophytosis
2. To correlate clinical features with the dermatophyte isolated
3. To determine antifungal susceptibility pattern by micro broth dilution method as per CLSI M38-A2 guidelines from isolated dermatophyte species to two antifungal agents: Fluconazole and Griseofulvin.

MATERIALS AND METHOD

It is a cross-sectional study conducted over a period of 18 months duration i.e., from June 2021 to November 2022 in a tertiary health care centre after institutional ethics committee approval. Skin scrapings, hair plucking, and nail clippings were collected from 200 clinically diagnosed cases of dermatophytosis which was calculated using the formula $4pq/l^2$ following inclusion and exclusion criteria who attended the outpatient department of Dermatology (DVL) and samples were processed at the upgraded Department of Microbiology at Osmania General Hospital, Afzalgunj, Hyderabad.

METHODOLOGY

Skin scrapings, Hair plucking, and Nail clippings were collected in autoclaved black craft paper for both microscopy and culture with a sterilized blunt scalpel from the margins of the lesion without injuring the skin surface.

A small portion of the specimen was transferred aseptically from the black craft paper or directly collected onto a sterile microscopic slide to which a drop of 10% KOH (Potassium Hydroxide) was added and covered with a cover slip. In the case of epidermal scales and hairs after applying the cover slip the preparation was gently warmed whereas, in the case of nail clippings, the sample was transferred to a penicillin bottle containing a few drops of 40% KOH and kept for 24 hours before examination for the presence of fungal elements.

All KOH positive and negative samples were inoculated on Sabouraud's dextrose agar containing 0.05% chloramphenicol and 0.5% cycloheximide, incubated in a fungal incubator at 30ºC and then growth is checked daily for three weeks. If growth is observed on SDA, identification is done based on colony morphology of the slant & lactophenol cotton blue mount for speciation.

Antifungal Susceptibility Testing

In-vitro antifungal susceptibility test was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines, document M38-A2 for filamentous fungi. Dermatophytes were subcultured from the primary Sabouraud's dextrose agar to oatmeal agar medium to induce conidiation and were incubated at 35ºC for 7 days or longer until colonies developed abundant spores. The conidial spores were then carefully collected by gently flushing 5ml phosphate buffer saline on top of the colonies and aspirating the suspension into a sterile collection tube.

The fungal spores were enumerated using a hemacytometer and cell numbers were adjusted to 1×10^4cells/ml. The susceptibility of isolates to two antifungals fluconazole and griseofulvin were tested in our study. The antifungal drug powders were purchased from Sigma-Aldrich. The stock solution of griseofulvin was prepared in dimethyl

sulfoxide (DMSO), as it is a water-insoluble drug, at a concentration of 2 mg/ml, except fluconazole which is dissolved in water.

Next, the drug stock solutions were diluted in Roswell Park Memorial Institute (RPMI) 1640 liquid medium buffered with MOPS (3-N-Morpholinopropane sulfonic acid), in twice the final concentration followed by the addition of an equal volume of the preadjusted inoculum of dermatophyte isolates, in 96-well microtiter plates.

The following final concentration ranges were tested: fluconazole (0.125-64µg/ml), griseofulvin (0.03-4µg/ml). All plates were incubated at 35º C for 4 days or longer until sufficient growth (i.e.; confluent hyphal growth covering the bottom of the well) was observed in control wells containing media without antifungal drugs.

Control strains: T. mentagrophytes ATCC MYA-4439 and T. rubrum ATCC MYA-4438 were used as quality control strain which was obtained from the National Culture Collection of Pathogenic Fungi, PGIMER, Chandigarh.

Interpretation: Minimum Inhibitory Concentration (MIC)-90 (i.e.; the concentration of the drug that inhibits 90% of the growth of the fungus), of the drug against the fungus in each well was detected visually by using a magnifying reading glass. The reading was taken at 4 days or until the day of sufficient growth of the fungus in the control well for comparison.

Clinical significance: Since there are no breakpoints established for antifungal agents against dermatophytes, the interpretive criteria were assessed according to breakpoints established in CLSI M38-A2 reference documents for Trichophyton mentagrophytes MRL 1957 ATCC MYA-4439, Trichophyton rubrum MRL 666 ATCC MYA-4438.

For Fluconazole, isolates with MIC 64 ≤µg/ml are categorized as susceptible and ≥ 64 µg/ml as resistant. For Griseofulvin, isolates with MIC ≤1µg/ml are considered susceptible, and ≥1µg/ml is resistant.

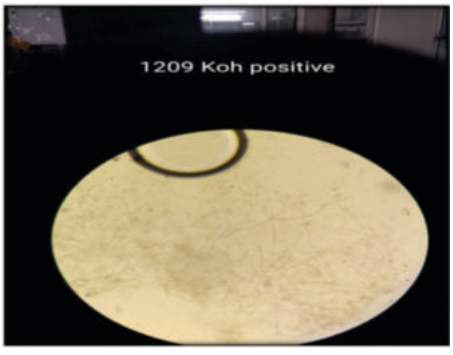

Figure:1 showing KOH Mount positive for fungal elements

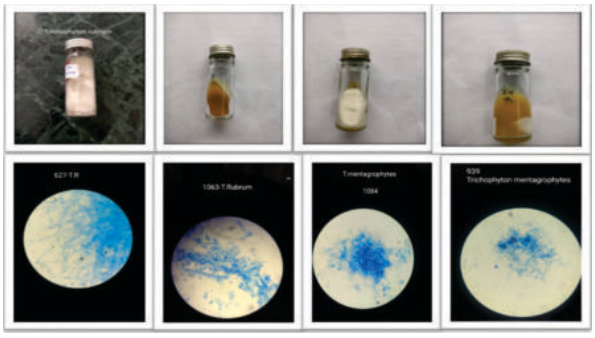

Figure 2 Obverse and reverse, LPCB Images of T.rubrum and T.mentagrophytes

RESULTS

During the study period, 200 samples were collected from skin scraping, hair plucking, and nail clipping from clinically diagnosed dermatophytosis patients were processed. In our study majority of patients affected were males 57% and females 43%. Among different age groups, the most frequently affected age group was 21-30 years (25.5%) followed by the 31-40 years age group with 24% of patients. Diabetes mellitus is 21% the most common risk factor followed by Anaemia 14% seen in our study. The most common clinical type was Tinea corporis (40%) followed by Tinea cruris (19.5%) in our study. KOH positivity was seen in 42% and culture was positive in 69.5% of cases.

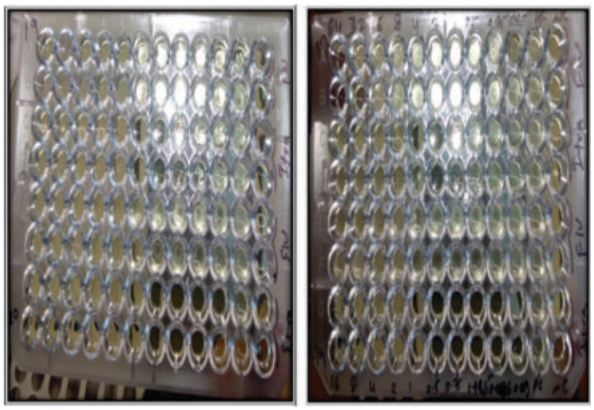

Figure 3: Image showing Antifungal susceptibility of Fluconazole and Griseofulvin

Among 200 cases studied, 37.5% were positive by both KOH and culture, 4.5% were positive by KOH and negative on culture, 32% were negative by KOH but positive on culture whereas 26% cases were negative by both KOH and culture.

Among the 200 samples collected, the total number of dermatophytes isolated was 42%, non-dermatophytes isolated were 27.5% and the samples that did not show any growth on culture was 30.5%.

The most common dermatophyte isolated in culture was Trichophyton rubrum (45%), followed by Trichophyton mentagrophytes 33.3%. Among isolates of dermatophytes, Fluconazole is sensitive to 33.3% of isolates, whereas Griseofulvin is sensitive to 52.3% of isolates.

Table 1: Demographic and clinical data of confirmed cases

Variable	n (%)
Gender	
Male	114 (57%)
Female	86 (43%)
Common Age group	
21-30 years	51 (25.5%)
31-40 years	48 (24%)
Risk factors	
Diabetes mellitus	42 (21%)
Anaemia	28 (14%)
Clinical type	
Tinea corporis	80 (40%)
Tinea cruris	39 (19.5%)
KOH positive	84 (42%)
Culture positive	139 (69.5%)
KOH positive Culture negative	9 (4.5%)
KOH negative Culture Positive	64 (32%)

Table 2: Overview of isolated fungal pathogens

Total number of Dermatophytes	84 (42%)
Total number of non-Dermatophytes	55 (27.5%)
No Growth	61 (30.5%)
Trichophyton rubrum	32 (38%)
Trichophyton mentagrophytes	28 (33.3%)

Table 3: Antifungal susceptibility pattern of Isolated Dermatophytes

Fungal Isolates	Fluconazole sensitive MIC ≤64	Resistant MIC ≥64	Griseofulvin Sensitive MIC ≤1	Resistance MIC≥1
T. rubrum	10 (35.7%)	22 (52.3%)	18 (40.9%)	14 (53.8%)
T. mentagrophytes	12 (42.8%)	16 (38%)	20 (45.4%)	08 (30.7%)
Total	28 (33.3%)	42 (50%)	44 (52.3%)	26 (30.9%)

DISCUSSION

Dermatophytosis is a significant public health problem in tropical and

subtropical countries like India still remains unresolved. Because of the increasing trend of antifungal-resistant dermatophytes, the requirement of rapid and precise identification of causative fungi and antifungal susceptibility testing become vital.

In our study, most patients affected were males 114 (57%) may be due to occupational hazards related to their nature of work, greater physical activity, increased sweating because of environmental conditions such as hot and humid weather, the frequent interaction with different people of the society, poor personal hygiene, and illiteracy. The lower incidence in females 86 (43%) may be due to the non-reporting of female patients to hospitals because of prevailing social stigma in rural populations4. Srinivasan Balakumar et al, ignorance to seek medical advice and less exposure to the environment that helps in the spread of fungi.

In our study, the age groups of the patients were ranging from 5 to 60 years with the highest incidence of dermatophytosis seen in 21 to 30 years in 51 (25.5%) patients followed by 31 to 40 years age group in the 48 (24%) patients.

The increased incidence of dermatophytosis in this age group may be because this age group people only take part in maximum outdoor activities like agriculture and manual labour which were predisposing them to acquire infection from an environment in the body that favours the growth of dermatophytes. This study correlates with the studies of Kucheria et al [5], and Praveen S et al [8] in which the most common age groups affected are 21-30 years, which is like our study.

Comparison of our findings with various studies

Author (year)	Area	Sample size	Male: Female	Common age group
Kucheria et al (2015) 5	North India	100	1.3:1	21-30 years
Naglot et al (2015) 6	North-east India	632	4.4:1	21-40 years
Ramraj et al (2016) 7	South India	210	4:3	21-40years
Praveen S et al (2021) 8	Andhra Pradesh	96	1.08:1	21-30years
Present study	Hyderabad Telangana	200	1.3:1	21-30years

Coexisting diseases play an important role as they are aggravating factors in tinea infections. In our study, Diabetes mellitus was the most common association seen in 42 (21%) patients followed by anaemia which is seen in 28 (14%) of patients. In a study done by Praveen S et al [8] Diabetes was seen in 15.6% of patients, in the study done by Lakshmi Vasantha Poluri et al [9] in which Diabetes accounts for 8.06%, anaemia 6.45% & B.Janardhan et al [10] Diabetes accounts for 22% which is similar to our study.

In our study, among the skin lesions, tinea corporis was the predominant clinical presentation which occurred in 80 (40%) patients followed by tinea cruris in 39 (19.5%) patients, tinea pedis in 10 (5%) patients, tinea manuum in 06 (3%), tinea faciei in 06 (3%) patients.

The higher incidence of tinea corporis followed by tinea cruris was probably due to its symptomatic (pruritic) nature which led the patient to seek medical advice. It was observed that most of the patients with tinea infection were involved in exhausting physical work and prolonged exposure to the sun that result in profuse sweating. The tight fittings and synthetic clothing may also provide damp, sweaty and warm skin conditions that favour dermatophyte infection. The most common site of Tinea corporis involved in females was the waist area due to the patterns of clothing i.e., sarees and salwar which were worn by the women act as predisposing factors because of friction, excessive sweating, collection of dust and fungal spores at the belt line.

In a study done by Praveen S et al [8] Tinea corporis was seen in 28 (29.2%) patients, tinea cruris in 26 (27.1%), tinea faciei in 5 (5.2%), tinea pedis in 7 (7.3%), tinea Mannum in 4 (4.1%).

In this study, the majority 141 (70.5%) of samples were skin scrapings, 34 (17%) were nail Clippings and 25 (12.5%) were epilated hair specimens. These findings are in accordance with the studies done by Dr Raghavendra Rao M et al [11] in which Skin 73.3%, Hair samples 16.66% & Nail clippings 10% and Kumaran et al [12] in which Skin scraping 54%, Hair samples 39% & Nail clippings 7% reported.

In the present study, among 200 samples direct microscopic examination (KOH) was positive in 84 (42%) samples and negative in 116 (58%) samples. In a study done by Praveen S et al [8] KOH was positive in 67.7% of patients, whereas other studies reported KOH positivity from 32.4% to 94.1%[13,14]. These differences could be because of differences in sample collection, processing, and the skill to identify the fungal elements.

In the present study, among 200 samples, 139 (69.5%) samples showed growth on culture i.e., culture positive and 61 (30.5%) samples showed no growth on culture i.e., culture negative. In a study done by Pathania et al [15] culture was positive in 60% and in a study done by Praveen S et al [8] culture was positive in 58.3% of their cases which was like our study.

Comparison of Direct microscopy (KOH) and Culture among various studies.

Author (Year)	KOH+ Culture+	KOH – Culture+	KOH+ Culture-	KOH- Culture-
Nisha Majeed et al (2016) 16 in kerala	36%	13.33%	30%	20.6%
Priyam Basak et al (2019) 17 in Odisha	57.9%	1.9%	13.2%	27%
Dr.Raghavendra Rao M et al (2015) 11 in Mysore	45.55%	14.46%	7.77%	32.22%
Praveen S et al (2021) 8 in Andhra Pradesh	46.8%	11.5%	20%	21.7%
In our study (2022) Hyderabad, Telangana	37.5%	32%	4.5%	30.5%

Among 200 samples, 75 (37.5%) samples were both KOH & Culture positive, 09 (4.5%) samples were positive by KOH & negative by culture, 64 (32%) samples were positive by culture and negative by KOH and 52 (26%) samples did not show any evidence of positivity of fungi either by direct (KOH) microscopy or culture. Sensitivity of KOH =75/139=54%. Specificity of KOH 52/61=85%.

The probable causes for this variation could be due to the non-viability of fungal elements which may be due to the insufficient number of fungal elements in clinical specimens collected from very small lesions used for culture or non-reporting of partial treatment with anti-fungal agents prior to specimen collection. Thus, all KOH-negative samples should be subjected to culture. Non-visualization of hyphae on direct microscopy could possibly be due to a severe inflammatory reaction which obscures them and the inactive sporulating phase of fungi which was very difficult to observe under the microscope.

In the present study, 84 specimens show KOH positivity alone and 139 specimens show culture positivity alone which elucidates the importance of both direct microscopy and culture to establish a definitive diagnosis. These findings of our study correlate with the studies of Nisha Majeed et al [16], Priyam Basak et al [17] and Dr Raghavendra Rao M et al [11].

Comparison of sensitivity and specificity of KOH among various studies.

Author (Year)	Sensitivity of KOH	Specificity of KOH
Nisha Majeed et al (2016) 16 in kerala	27%	40%
Priyam Basak et al (2019) 17 in Odisha	3.1%	67%
Dr.Raghavendra Rao M et al (2015)11 in Mysore	24%	80.5%
Praveen S et al (2021)8 in Andhra Pradesh	19%	52%
In our study (2022) Hyderabad, Telangana	54%	85%

Among fungal isolates 84 (42%) were dermatophytes, 55 (27.5%) were non-dermatophytes and 61 (30.5%) showed no growth on culture.

Out of 84 isolates of Dermatophytes, most of the isolates belonged to the Genus Trichophyton of which Trichophyton rubrum was the predominant isolate 32 (38%) followed by Trichophyton mentagrophytes 28 (33.3%), Trichophyton tonsurans 16 (19%),

Trichophyton verrucosum 5 (5.9%) and the least 1 (1.1%) isolate each of Tichophyton violaceum, Trichophyton schoenleini and Microsporum gypseum.

The high preponderance of Trichophyton rubrum was explained by the persistent characteristic pattern of infection and the variation of dermatophyte to the skin of human beings.

Comparison of our study according to various studies

Author (year)	Area	Sample size	Clinical subtype	Predominant Dermatophyte
Kucheria et al 5 (2015)	North India	100	Tinea corporis (31%)	T. rubrum (46.4%) T. mentagrophytes (30.35%)
Naglot et al 6 (2015)	North-east India	632	Tinea corporis (34.82%)	T. rubrum (50.15%) T. mentagrophytes (30.35%)
Ramaraj et al 7 (2016)	South India	210	Tinea corporis (63.27%)	T. rubrum (48.95%) T. mentagrophytes (44.75%)
Present study (2022)	South India	200	Tinea corporis (40%)	T. rubrum (38%) T.mentagrophytes (33.3%)

Antifungal susceptibility Testing

In the present study, the Minimum Inhibitory Concentration (MIC)-90 and antifungal susceptibility pattern for Fluconazole and Griseofulvin were determined. Out of 84 isolates of dermatophytes, Fluconazole is sensitive to 28 (33.3%) isolates and Resistant to 42 (50%) isolates, whereas Griseofulvin is sensitive to 44 (52.3%) and resistant to 26 (30.9%) of isolates.

R.K. Agarwal et al [18] and other studies indicate that fluconazole had less activity against dermatophytes, which is like our study. Griseofulvin was sensitive to most of the isolates and showed good antifungal activity. Fernandes-Torres et al [19] reported low MIC and higher sensitivity of Griseofulvin showing good antifungal activity which is like our study.

Comparison of Antifungal susceptibility with other studies.

Study	Fluconazole	Griseofulvin
R.K.Agarwal et al 18	Less sensitive	-
Fernandes –Torres et al 19	-	More sensitive (good antifungal)
Present study (2022)	28 (33.3%)	44 (52.3%)

Even though Norries et al and C.J. Jessup[20] et al studies established the inoculum size, optimum condition, optimum medium for conidial formation, incubation time, duration and end point determination, a standard reference method for antifungal susceptibility testing of dermatophyte infection is lacking. In our study, most of the isolates were sensitive to Griseofulvin.

Colin. S. osborne et al [21] have found that some strains of Trichophyton rubrum isolated showed intrinsic resistance to Terbinafine which on prolonged exposure to the drug can raise the MIC Values. Mukharjee et al [22] first confirmed the Terbinafine resistance in superficial fungal infections.

Triazole antifungal resistance is like studies done by Dominique and Pramod [23] Cervelatti EP et al (2006) and Fachin AL et al (2006) [24] has detailed the involvement of ATP Binding Cassette transporter gene in the development of resistance to azoles in Trichophyton rubrum. These findings are substantiated by the studies of Prabhat Kiran Khatri et al [25] (2017) in which the Antifungal Resistance pattern of T.rubrum is 38.46% of Fluconazole, 30.77% Terbinafine.

The repeated exposure to the azoles group of antifungals may be responsible for the development of resistance in dermatophytes. T. rubrum can develop resistance to azoles and Terbinafine after prolonged exposure to a sub-inhibitory concentration of these drugs leading to treatment failures and persistence and chronicity of infections.

The suboptimal quality of many antifungal brands in India may further worsen the situation. In India, inadequate treatment regimen or discontinuation of medication due to cost of treatment, difficulties in eliminating predisposing factors and sources of re-infection may play a vital role in antifungal resistance.

CONCLUSION

In our study, the commonest presentation is Tinea Corporis, the commonest fungal isolate Trichophyton rubrum and most of the isolates are sensitive to Griseofulvin. The use of less efficacious molecules results in clinical failure and drug resistance. Hence, the quality controls of these drugs are a real concern.

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