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Original Research Article

Dermatophytosis Susceptibility to Antifungal Drugs

A Prospective Study On Microbiological Profile of Dermatophytosis and Its Susceptibility to Common Antifungal Drugs

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Abstract: Diagnosing the superficial fungal infection is important for initiating appropriate treatment and epidemiological purposes. Dermatophytes cause the majority of these infections. The rising prevalence of these infections worldwide, increased and uncontrolled use of antifungals and corticosteroids, rising burden of the immune-compromised population, and antifungal drug resistance pose therapeutic challenges to practitioners. The study's main objective was to isolate, identify and determine the antifungal susceptibility of the dermatophytes causing superficial mycosis. This was a cross-sectional study from a tertiary care centre in South India. Samples were collected from 130 patients clinically diagnosed to have dermatophytosis. All the samples were examined by microscopy using a KOH mount and culture. Species identification was done by slide culture and Lactophenol Cotton Blue mount, and performed antifungal susceptibility testing against common antifungals by broth microdilution method. Descriptive statistics expressed the nominal variables' number of cases and percentage (%). On analysis, the males to females' ratio was 1.7:1. Adults were affected more than children. Tineacorporis was the commonest clinical type (65.3%), followed by tineacruris (26.2%). Among the clinically diagnosed dermatophytosis, KOH mount had a higher positivity rate over yield in culture (69.2% versus 48.4%). *Trichophyton mentagrophytes* were the commonest isolate, 38 (60.3%), followed by *Trichophyton rubrum* 23 (36.5%). Voriconazole was found to have low MIC values for most of the isolates tested compared to the standard strain. The percentage of isolates having higher MIC than the standard for Itraconazole, amphotericin B, terbinafine, and fluconazole were 11.6%, 20.9%, 32.5%, and 41.8%, respectively. Most isolates had higher MIC for griseofulvin, suggesting resistance in South India. The findings conclude that the direct microscopic examination by KOH mount was more sensitive than culture, and the most common fungal pathogen isolated was *Trichophyton mentagrophytes.* Griseofulvin showed the least activity among the six antifungals tested, and voriconazole performed best with the least number of isolates with high MIC.

Keywords: Superficial mycosis, dermatophytes, KOH mount, antifungal susceptibility testing.

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1. INTRODUCTION

Favourable climatic conditions like temperature, humidity, and varied topography in a country like India highly favor the acquisition of fungal infections of the skin and its appendages. The cause of dermatophytosis is also adversely influenced by economic factors like poverty, poor hygiene, and social conditions like overcrowding. But with time, lifestyle changes in populations, the evolution of preventive measures, and hygienic practices in societies, the nature of these fungal infections also tend to change.[1,2]. Frequent and unchecked usage of antibiotics, immunosuppressive drugs, and various conditions like organ transplantations, lymphomas, leukemia, and human immunodeficiency virus (HIV) infections may all have contributed to the recent increase in these infections. [3]A definite challenge to humanity in the years to come would be the management of dermatophytosis because of the degree of immunosuppression and the number of immunosuppressed patients, which are increasing at an alarming rate.[4]Irrational use of antifungal agents in superficial cutaneous mycoses has resulted in the emergence of antifungal resistance. According to various studies worldwide, common antifungal drugs used to treat dermatophytosis are increasingly becoming less effective. [5-7] A correct diagnosis for the fungal infection is important to initiate appropriate treatment as well as important for epidemiological purposes. Detection of these agents becomes mandatory for effective management and to prevent further invasions and spread, especially in the case of immunosuppressed. Even though there is a rising trend of relapses, early cessation of antifungal therapy did not contribute to developing resistance. Other factors like host immune response and impaired barrier function of the epidermis may play a role in the relapse of the infection. Another possible cause for the relapse might be the availability of fixed-dose combinations with antifungals and their overuse. [8]The present study was taken to identify the microbiological profile of dermatophytes causing superficial fungal infection in the patients attending the dermatology outpatient department. Given the rising antifungal resistance, the study aimed to determine the prevailing resistance status among dermatophytes in South India. The present study's findings may serve as usable data for clinicians to formulate empirical therapy for these infections. It may also help anticipate our region's most likely etiological agents causing these infections.

2. MATERIALS AND METHODS

The study was conducted in the Department of Microbiology in a tertiary care medical college in South India between 2017 to 2018. The institutional ethics committee approval was obtained before the start of the study. A total of 130 clinically diagnosed cases of dermatophytosis who attended the dermatology outpatient department were included in this cross-sectional study after obtaining consent. Patient details such as age, sex, site, and clinical examination of cutaneous lesions were recorded. Patients of all age groups who were clinically diagnosed with dermatophytosis were included in this study. Patients with preexisting skin morbidities like psoriasis, scabies, burns, leprosy, and secondary infections were excluded from the study.The samples were collected with adequate sterile precautions from the active and advancing edge of the lesion in a clean black paper, folded to form a packet, and transported to the laboratory. Nail clippings were also collected after adequate sterilization.All the samples were divided into two parts for direct microscopy by KOH mount and culture. Potassium hydroxide (KOH) mounting was performed by direct microscopic visualization of fungal elements present in the specimen. For this, 10% and 20% KOH was used for skin and nail specimens, respectively.All samples were cultured on Sabouraud's Dextrose Agar(SDA). The slopes were incubated at a temperature of 30^0C for 1-3 weeks and examined at regular intervals for evidence of fungal growth (dermatophytes). Slopes not showing growth after 4 weeks were considered negative. The results obtained from microscopy and cultures were analyzed.Their morphological appearance and pigmentation made species identification of the dermatophytes. Slide cultures and lactophenol cotton blue mount(LPCB) were prepared to observe the microscopic morphology of the fungal growth. Presumptive pathogen identification was made based on the macroscopic observation of fungal growth as per standard norms. Urease test and in-vitro hair perforation tests were done to distinguish between *Trichophytonmentagrophytes* and *Trichophytonrubrum.* [9]

2.1 Antifungal Susceptibility Testing

Dermatophytes were grown on oatmeal agar slants and were incubated at 30^0C until sufficient conidia were formed (7-10 days). Conidia were taken at 0.5 X 10^4- 4.0 X 10^4 CFU/ml concentration in the Rosewell Park Memorial Institute (RPMI) media to achieve double-concentrated inoculum suspensions. The antifungal drugs were tested over the following range of concentrations: Amphotericin B (64 - 0.25 µg/ml); Fluconazole (64 - 0.25 µg/ml); Itraconazole (16 – 0.06 µg/ml); Voriconazole (32 – 0.125 µg/ml); Griseofulvin (16 – 0.06 µg/ml); Terbinafine (16 – 0.06 µg/ml). Antifungal stock solutions were prepared at a concentration 100 times more than the highest concentration tested. Aliquots of 100 µl conidial suspension are inoculated with 100 µl drug with increasing dilutions added correspondingly in microtitre wells. Growth control was 100 µl of inoculum and 100 µl of drug-free RPMI 1640 medium. The microtitre plates were incubated at 30^0C and read after a minimum of 4 days of incubation (5 days for *Trichophyton nrubrum, Trichophyton mentagrophytes,* and *Trichophyton tonsurans* and 7 days for *Epidermophyton flocossum*). The MIC was defined (except for amphotericin) as the point at which there was 80% inhibition of growth compared to growth control when read visually by two observers. Amphotericin B's MIC was measured at 100% growth inhibition compared to growth control. MIC results were recorded in µg/ml. [10] MIC ranges of antifungals against all the tested species were noted. *Trichophyton mentagrophytes* ATCC 4439 was used as control strain.

2.2 Ethical Considerations

The Institutional Ethics Committee (IEC) approved the present study through Ref. No: KMC/PO/Ethics Cert/Crs 17-03-03 before the start of the study.

3. STATISTICS

Data analysis was performed with the SPSS for Windows version 20. Descriptive statistics expressed the nominal variables' number of cases and percentage (%).

4. RESULTS

Among the clinically diagnosed cases of dermatophytosis, the highest incidence (38%) was found in the age group between

41- 60 years with male preponderance (1.7:1). Most common clinical types were *Tinea corporis* 85(65.3%), followed by *Tinea cruris* 34 (26.2%). Direct microscopic examination by KOH mount was positive in 90 (69.2%) cases (Table 1 and Fig 1). Dermatophytes grew in culture in 63(48.4%) isolates only. Among the 130 specimens, 59 (45.4%) were positive in direct microscopy and culture. In 31 (23.8%) cases, they were positive in the direct microscopic examination but did not yield any growth in culture.

Table 1: Correlation between clinical diagnosis, KOH mount, and culture in dermatophytosis

KOH	Culture		Total
	Positive	Negative	
Positive	59 (45.4%)	31(23.8%)	90(69.2%)
Negative	4 (3%)	36 (27.8%)	40 (30.8%)
Total	63 (48.4%)	67 (51.6%)	130 (100%)

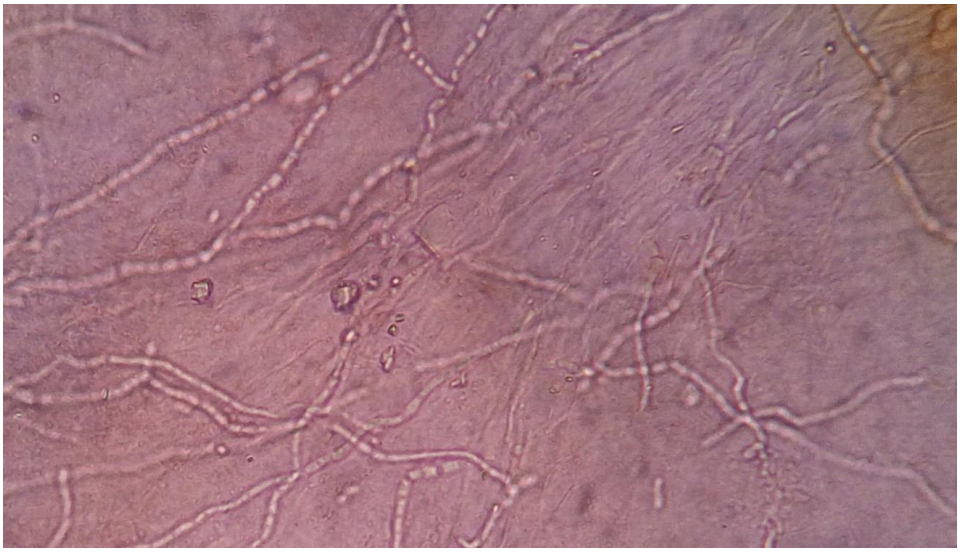

Fig 1: KOH mount showing fungal hyphae in dermatophytes (40X)

Trichophyton mentagrophytes (Fig 2a,2b)were the most common isolate 38(60.3%). T The second common isolate was*Trichophyton rubrum* 23(36.5%) (Fig 3a, 3b). There was a single (1.6%) isolate of an *Epidermophyton floccosum* and *Microsporum gypseum*each.

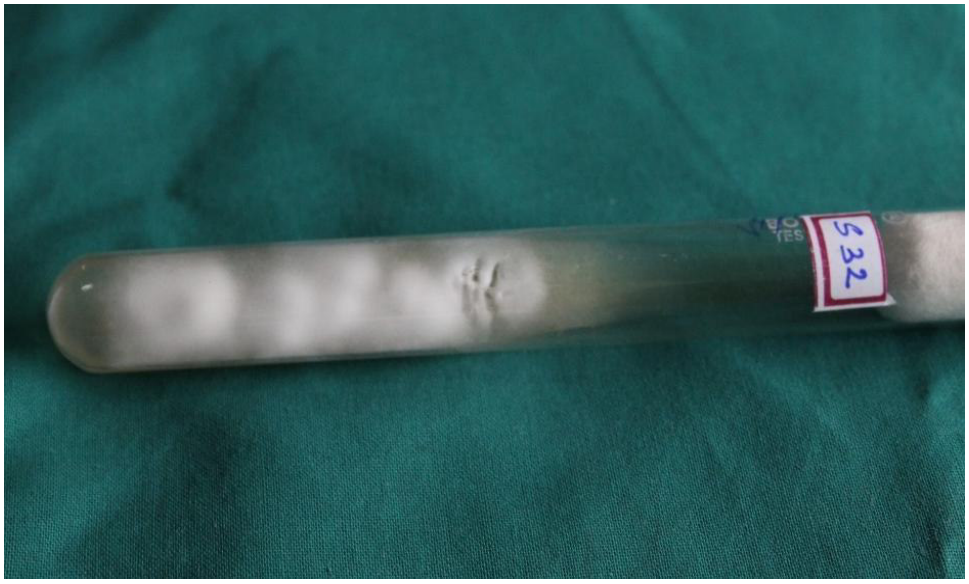

Fig 2a: *Trichophyton mentagrophytes* grown in SDA tube

Fig 2b: LPCB mount of *Trichophyton mentagrophytes*(40X)

Fig 3a: Trichophyton rubrum grown in SDA tube

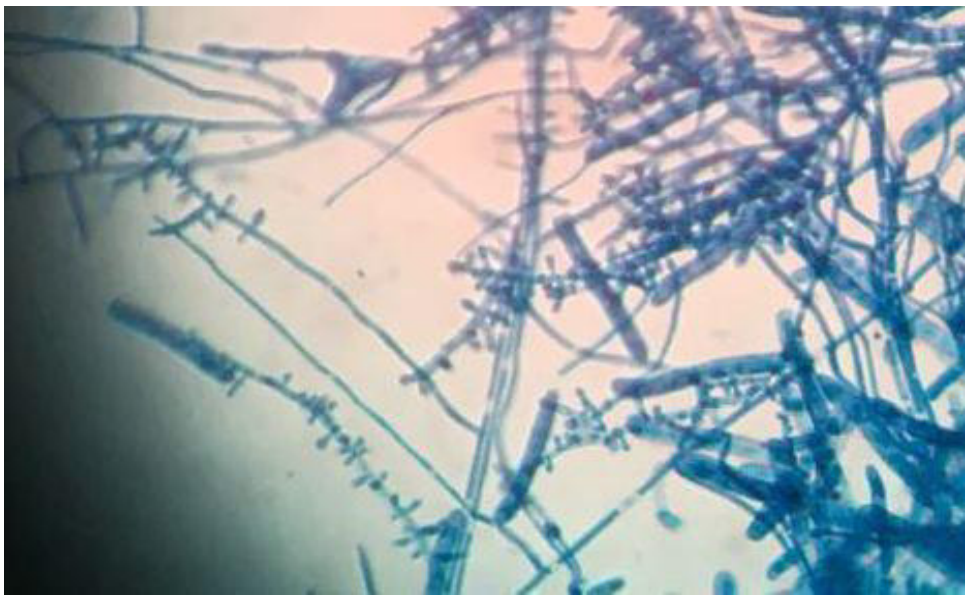

Fig 3b: LPCB mount of *Trichophyton rubrum* (40X)

4.1 Antifungal Susceptibility Testing

Among the 63 isolates of dermatophytes grown in culture, only 43 were subjected to anti-fungal susceptibility testing. The rest 20 isolates were not tested because of contamination. All the isolates showed detectable growth after 4-5 days of incubation. Table 2 displays the MIC range of six antifungal drugs against all isolates.

Table 2: MIC range obtained for various isolates against six antifungal drugs.

Amphotericin B (ATCC:4 µg/ml)	Conc	2µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	64 µg/ml	>64 µg/ml
	n(%)	3 (7)	31 (72)	8(18)			1 (2)
Fluconazole (ATCC:4 µg/ml)	Conc	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	64 µg/ml	>64 µg/ml
	n(%)	11 (26)	14 (33)	12 (28)	2 (5)	1 (2)	3 (7)
Itraconazole (ATCC:<0.06µg/ml)	Conc	<0.06 µg/ml	0.06 µg/ml	0.5 µg/ml	8 µg/ml	16 µg/ml	
	n(%)	32(74)	6 (14)	1(2)	3 (7)	1 (2)	
Voriconazole (ATCC:<0.125 µg/ml)	Conc	<0.125 µg/ml	0.125 µg/ml	0.5 µg/ml	4 µg/ml		
	n(%)	29 (67)	10 (23)	3 (7)	1 (2)		
Terbinafine (ATCC:<0.06 µg/ml)	Conc	<0.06 µg/ml	0.06 µg/ml	0.25 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml
	n(%)	15 (35)	14 (33)	1 (2)	1 (2)	10 (23)	2 (5)
Griseofulvin (ATCC: 0.125 µg/ml)	Conc	0.125 µg/ml	0.25 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	16 µg/ml
	n(%)	1 (2)	9 (21)	23 (53)	8 (19)	1 (2)	1 (2)

Conc: Concentration

The drug concentrations tested for MIC are shown in Table 3a concerning ATCC. Table 3b describes the MIC-90 range of isolated dermatophytes for the drugs tested.

Table 3a: MIC range of isolated dermatophytes for the drugs tested.

	Amphotericin B	Fluconazole	Itraconazaole	Terbinafine	Griesofulvin	Voriconazole
T mentagrophytes	2 - >64	2 - >64	<0.06 – 16	<0.06 – 1	0.125-16	<0.125 - 4
T. rubrum	2 - 8	2 - >64	<0.06 - 8	<0.06 – 2	0.25-1	<0.125- 0.5
E. flocossum	4	2	<0.06	<0.06	0.25	<0.125
M. gypseum	4	>64	8	0.06	0.5	0.5

Trichophyton mentagrophytes; Trichophyton rubrum; Epidermophyton floccosum; Microsporum gypseum

Table 3b: MIC -90 range of isolated dermatophytes for the drugs tested.

	Amphotericin B	Fluconazole	Itraconazole	Terbinafine	Griesofulvin	Voriconazole
All isolates	4-8 µg /ml	16 µg /ml	0.5 µg /ml	0.5-1µg /ml	0.5-1µg /ml	0.125µg /ml

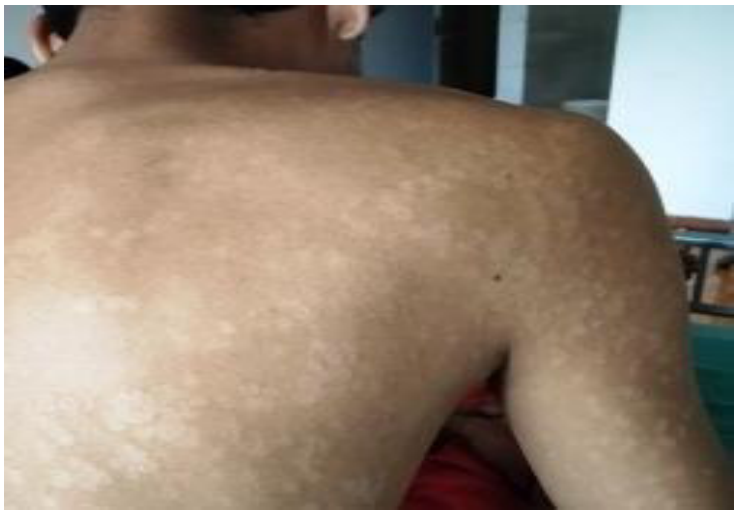

Fig 4: Extensive distribution of tinea versicolor

5. DISCUSSION

The present study highlights that *Trichophyton mentagrophytes* had a higher prevalence among the dermatophytes implicated in superficial skin infections. Dermatophytes, non-dermatophytic molds, and commensal yeasts are well-known causes of superficial fungal infections, of which dermatophytes are the most prevalent and therefore assume

high significance, particularly in developing countries like India. [11] South India, especially Kerala, have a hot and humid environment favourable for the development of these infections. Fig 4 displays the extensive lesions of *Tinea versicolor* in a patient. Even though most superficial infections are easily diagnosed and readily amenable to treatment, frequent relapses, as well as increasing resistance to antifungal drugs, have become a major concern of dermatologists, microbiologists, and patients. [12] Males were found to be more affected (1.7:1), which was consistent with other Indian studies (Male to Female 1.57 to 2.14:1). [13-16] The higher incidence in males may be explained by their increased exposure to environmental conditions conducive to the spread of these organisms and increased sweating due to physical activity. Studies have shown males have five times more chance of having a fungal infection than females.[15,17] The lower incidence in females may be due to non-reporting to the hospital, probably due to social stigma, especially in rural areas [4]Superficial mycosis affects all age groups. Many studies reported a higher incidence of superficial mycoses in young adults. The high incidence of dermatophytosis in young patients may be due to greater incidence of trauma, physical activity, and increased sweating and retention of moisture in these groups in addition to the tropical climatic conditions.[18,19] However, in the present study, the highest incidence was found in the age group 41- 60 years. Occlusive or synthetic fabric dressing and contact with soil regularly because of their occupation and frequent human-to-human interaction also increase the frequency of dermatophytic infections in adults. [15]Tinea corporis was the most common clinical type (65.3%), followed by Tinea cruris (26.2%). These findings are consistent with many studies from India, with Tinea corporis consistently being the most prevalent type of superficial fungal infection, probably due to their symptomatic nature (pruritus), which leads the patient to seek medical advice.[13,14,20,21]The KOH mount and culture results in 130 patients with clinical dermatophytosis were compared. Dermatophytes grew in 63 (48%) cultures. This starkly contrasted with the KOH mount, which had 90 (69.3%) samples. The low sensitivity of culture *vis-à-vis* KOH mount is well documented. Based on these figures, we could estimate the positive predictive value of KOH mount to be 61% and the negative predictive value of 90%. Experts are against the routine use of culture in diagnosing dermatophytosis, even though it is the gold standard. Positive KOH mount clinches the diagnosis of superficial mycosis. [22]Culture should be done only in recalcitrant and multiple site infections. These findings more or less echo those of many other studies. Patel P et al., in their study, also reported that the detection rate was more by direct microscopy using KOH preparation (62.12%) than culture (29.29%).[11]In making an etiological diagnosis, culture might contribute to the clinical epidemiology of dermatophytosis, distinguishing between clinical conditions that mimic dermatophytosis and atypical presentations. Non-availability of antifungal susceptibility testing in practically all clinical labs finally seals the feasibility of ordering for culture as a routine diagnostic method. Four of the culture-positive samples in our study showed no fungal elements on direct microscopy. This could be because the fungus could have been in an inactive sporulating phase difficult to be seen by microscopy but able to grow in appropriate media. [23]The distribution of dermatophytosis and its etiological agents varies with geographical location, the lifestyle of people living in communities, and socioeconomic conditions. In our study, *Trichophytonmentagrophytes* were the most commonest (60.3%), followed by *Trichophyton rubrum* (36.5%). In contrast, most studies conducted in India and elsewhere have shown *Trichophyton rubrum* as the predominant pathogen, followed by *Trichophyton mentagrophytes.* [14,24]However, more recently, *Trichophyton mentagrophytes* appears to be the major pathogen from South India. [8,25,26]The incidence of Trichophyton *rubrum* as a dermatophytic pathogen is perhaps understated. *Trichophyton rubrum* shows a predilection towards chronic forms of the disease. [4] Criteria for case selections and sampling employed in most studies automatically exclude these cases. This may impact the results of the studies in terms of the relative incidence of causative agents. Secondly, chronicity of infections also reduces the possibility of culture in these cases owing to fewer organisms in the specimens. Thirdly, *Trichophyton rubrum* grows slowly in culture. The growth might thus be missed or masked by the growth of other co-dermatophyte spp. The frequency and appropriateness of clinical usage informed us in selecting the tested antifungal agents. MICs for *Trichophyton mentagrophytes* ATCC 4439 were within the established range. As shown in the table/figure3, approximately 20% and 11.6% of the dermatophytes tested showed a high MIC against the reference strain for *Amphotericin*-B and itraconazole, respectively. Close to one-third of isolates exhibited higher MIC for terbinafine (32.5%) and fluconazole (42%). In our study, almost all (97.7%) isolates showed a high MIC for griseofulvin. Among the six antifungal drugs tested for sensitivity, voriconazole showed the least number of strains (9.3%) having a high MIC value. MIC breakpoints to categorize an isolate as sensitive, intermediate, or resistant to a particular antifungal drug are unavailable. The guidelines by "The Clinical and Laboratory Standards Institute" point towards a lack of consistent correlation between in vitro antifungal sensitivity data and clinical outcomes. [27] Progress towards clarity in interpreting susceptibility data, formulating treatment, and predicting clinical outcomes is awaited. Owing to these limitations, routine antifungal testing of dermatophytes is presently not recommended. Limitations of the study – As the number of isolates tested for sensitivity was not very large, the appropriateness of applying these data to a larger patient population may be low from an epidemiological standpoint. More extended studies with more isolates are needed to clarify the prevailing conditions and trends, ultimately enhancing their clinical and epidemiological relevance.

6. CONCLUSION

The study concluded that diagnosis of dermatophytosis was better detected using KOH mount than culture (69% vs 48%). Griseofulvin showed the least activity among the six antifungals tested, and voriconazole performed best with the least number of isolates with high MIC.

7. AUTHORS CONTRIBUTION STATEMENT

Dr. Sarath KE and Dr. Gufran Ahmed Bijapur conceived and designed the study, data collection, data analysis, and paper writing.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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