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ORIGINAL RESEARCH

Comparative analysis of Sabouraud's Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM) for the early diagnosis of dermatophytoses in a tertiary care center, Shivamogga

S. Sadiqa Begum[1], Rashmi G K[2], Jyothi.R H[3]

[1,2]Assistant Professor, Department of Microbiology, Subbaiah Institute of Medical Science, Shivamogga, Karnataka, India
[3]Assistant Professor, Department of Microbiology, KLE JGMM Medical College, Hubballi, India

Corresponding author
S. Sadiqa Begum
Assistant Professor, Department of Microbiology, Subbaiah Institute of Medical Science, Shivamogga, Karnataka, India
Email: sadiqabegum81@gmail.com

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ABSTRACT

Introduction: Dermatophyte fungi are a common cause of skin, nail and hair infections globally, ranging from mild to debilitating in nature. These are closely related keratinophilic fungi, capable to invade keratinized tissues of skin and appendages causing dermatophytoses, frequently named as tinea or ringworm infections. Overall prevalence of dermatophytoses is around 14%, ranging from mild to debilitating in nature. **Aim:** 1. Isolation and identification of dermatophytes from a clinically suspected cases of dermatophytoses by using SDA and DTM media.2. Comparison of two media for effective isolation and identification of dermatophytes. **Materials & Methods:** A 1 year cross-sectional study conducted at Subbaiah Institute of Medical Sciences from June 2022 to June 2023. All new cases of suspected dermatophytic infections with scaly annular lesions affecting skin, nail and hair were included in this study attending OPD at SUIMS hospital. All old cases with antifungal treatment and Patients having concomitant other skin infections were excluded from this study. Samples were collected after cleaning the affected surface with 70% alcohol. All samples were subjected to microscopic examination by using 10 -20% KOH for the presence of fungal elements. Further inoculated on Sabouraud's Dextrose Agar (SDA) containing chloramphenicol (0.04 g/l) and cycloheximide (0.5 g/l), and on Dermatophyte Test Medium (DTM) with supplement to observe for the growth. Microscopy by LPCB tease mount and urease test performed for species identification. **Results:** Out of 163 clinically suspecteddermatophytoses,145 (88.9%) were culture positive. Among 145 culture positive patients, 99 (68.2%) were males and 46(31.7%) were females.Tineacorporis (58%) infection was found predominantly followed by Tineacruris (12%), Extensive dermatophytosis (6%).On comparing culture positivity and KOH microscopy,37 (22.6%) were culture positive and KOH negative and 4 (2.4%) samples out of 163 were KOH positive and culture negative. On comparing, the isolation of dermatophytes on SDA (88.9%) and DTM(95.8%), was statistically insignificant (P>0.05). **Concluson:** The positive identification of dermatophyte species allows a definitive diagnosis, correct treatment and prevention of possible source of infection. This study shows isolation of dermatophytes on both SDA and DTM were good but DTM showed early growth and easy interpretation was needed. Speciation was better with SDA compared to DTM.

INTRODUCTION

Dermatophyte fungi are a common cause of skin, nail and hair infections globally, ranging from mild to debilitating in nature. These are closely related keratinophilic fungi, capable to invade keratinized tissues of skin and appendages causing dermatophytoses, frequently named as tinea and ringworm infections[(1)].The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*. In addition, dermatophytes can also be divided into

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anthropophilic, zoophilic, and geophilic species on the basis of their primary habitat associations[2].

The clinical importance of identifying species of dermatophyte is to find out the probable source of infection. Also, there are some prognostic considerations as well. The anthrapophilic group causes chronic infection which may be difficult to cure. The zoophilic and geophilic dermatophytes cause inflammatory lesions which easily respond to therapy and occasionally spontaneously heal[3] .

Diagnosis of dermatophytic infection is mostly done clinically, but often confused with other skin infections due to topical application of steroid ointments and creams, leading to further misdiagnosis and mismanagement. Light microscopic mycological examination using KOH mount and fungal culture using SDA and DTM media are required for identification of dermatophyte infections phenotypically[4]. The positive identification of dermatophyte species allows a definitive diagnosis, correct treatment and prevention of possible source of infection[5].

The Purpose of the study was to isolate and identify different dermatophyte species from the clinical isolates obtained from Subbaiah institute of Medical sciences and to compare the effectiveness of two culture media SDA and DTM used for isolation.

MATERIALS & METHODS

A 1 year cross-sectional study conducted at Subbaiah Institute of Medical Sciences from June 2022 to June 2023.

Inclusion criteria: All new cases of suspected dematophytic infections with scaly annular lesions affecting skin,nail and hair were included in this study attending OPD at SUIMS hospital.

Exclusion criteria: All old cases with antifungal treatment and Patients having concomitant other skin infections.

Sample size calculation: Sample size was calculated on the formula Zα2p (100-p)/d2 {d=10-20% of p}, and it was obtained as 163.

A detailed history was taken from all 163 patients using a proforma which included age, sex, occupation, duration of illness, history of any treatment taken and any associated disease.

Samples were collected after cleaning the affected surface with 70% alcohol. The skin scrapings were obtained from the active edge of the lesion, nail clippings and hair pluckings were collected. All samples were subjected to microscopic examination by using 10 -20% KOH for the presence of fungal elements. Remaining specimens were inoculated on Sabouraud's Dextrose Agar (SDA) containing chloramphenicol (0.04 g/l) and cycloheximide (0.5 g/l), and on Dermatophyte Test Medium (DTM) with supplement.

SDA culture tubes were incubated at room temperature. Daily observation of all the inoculated culture tubes were made and tubes were considered negative if there was no fungal growthafter four weeks of incubation. Morphology of the colony, rate of growth and pigment production were recorded. Microscopic characteristics were studied by examining Lactophenol Cotton Blue (LPCB) preparation after teasing a tiny bit of the growth with teasing needle and putting a coverslip.

DTM media was used for presumptive identification of dermatophytes from fungal or bacterial contaminants. Growth of a dermatophyte in DTM was indicated by a change in colour, yellow to red[1].

The three genera of the dermatophytes namely, *Trichophyton, Epidermophyton and Microsporum* were speciated based on the morphology of macroconidia and microconidia. Urease test was done to differentiate between *T. mentagrophytes* and *T.rubrum*. For detailed morphological identification of all isolates, slide culture technique was performed.

RESULTS

Out of 163 clinically suspected dermatophytoses, 145 (88.9%) were culture positive. Among 145 culture positive patients, 99 (68.2%) were males and 46(31.7%) were females. Skin lesions were observed most commonly followed by hair and nails. Tineacorporis (58%) infection was found predominantly followed by Tineacruris (12%), Extensive dermatophytosis (6%) and few others(Graph 1).

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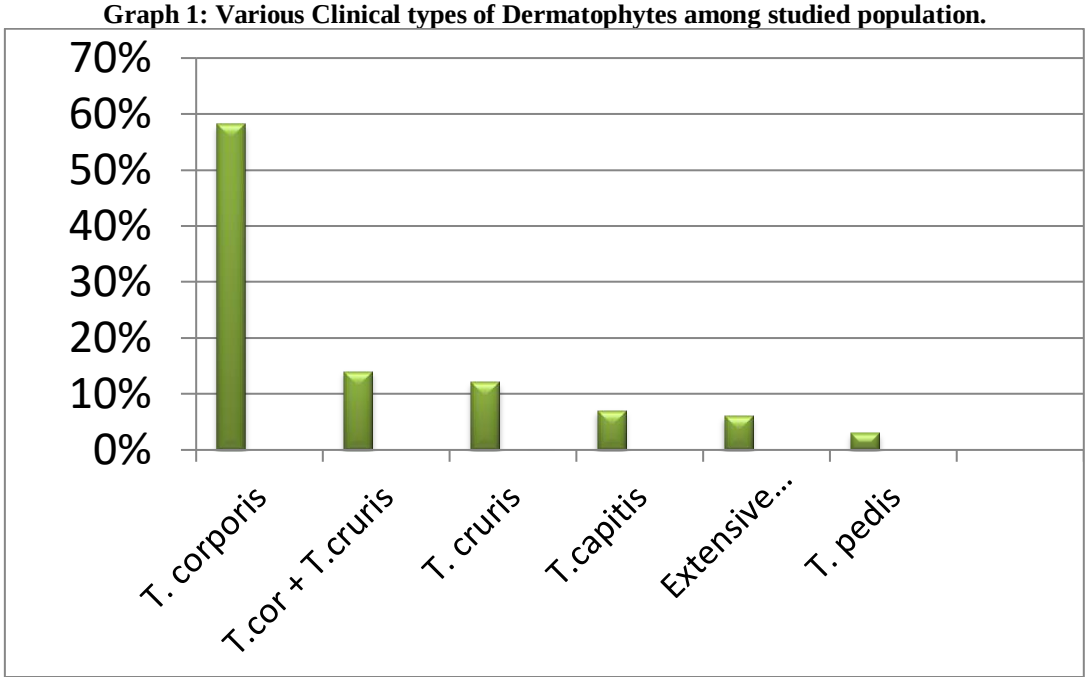
Graph 1: Various Clinical types of Dermatophytes among studied population.



All the samples were observed for narrow, hyaline septate hyphae with branching under microscopy using 10% KOH(Fig 1). On correlation of culture positivity with KOH microscopy, 37 (22.6%) were culture positive and KOH negative. 4 (2.4%) patients out of 163 were KOH positive and culture negative [Table 1].

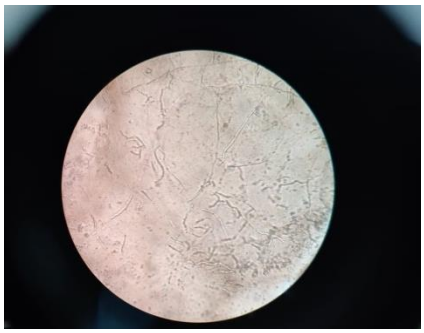


Fig-1 KOH mount showing thin hyaline septate hyphae

Table 1: Correlation of KOH microscopy with culture

	Culture positive	Culture negative	Total
KOH positive	108	04	112
KOH negative	37	14	51
Total	145(88.9%)	18(11.04%)	163

Isolation of dermatophytes was observed in 123 (84.8%) patients in both Sabouraud Dextrose Agar and Dermatophyte test medium. 16 (11.03%) dermatophyte isolates were observed only in Dermatophyte test medium. 6(4%) isolates were observed only in SDA[Table 2]. On comparing, the isolation rate of dermatophytes on SDA and DTM, was statistically insignificant (P>0.05).

Table 2: Number of dermatophyte isolates from SDA and DTM

SDA	DTM	Total	
Growth +	Growth +	123	145 dermatophyte isolates
No growth	Growth +	16	
Growth +	No growth	6	

A comparison of average time period for culture to become positive between SDA and DTMindicates less number of days taken by DTM as compared to SDA (Graph 2).

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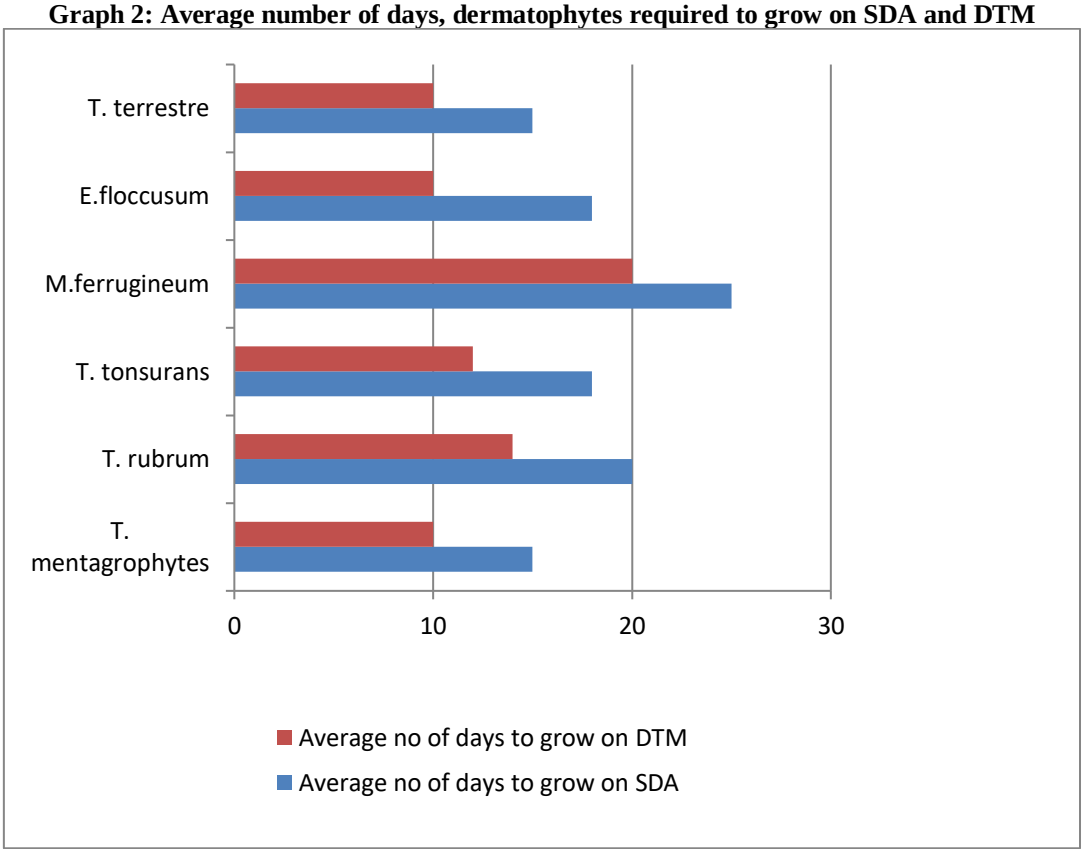
Graph 2: Average number of days, dermatophytes required to grow on SDA and DTM



Overall, *T.mentagrophytes*(Fig-2) was the most common isolate obtained about 81(55%), followed by *T. rubrum*(Fig 4) 52(35.8%), *T.tonsurans*(Fig 4) 4(27%), *E. floccosum* 3(2%). Less commonly isolated species are *T. terrestre, T. megninii, M.ferrugineum, T.verrucosum about 3.4%.*

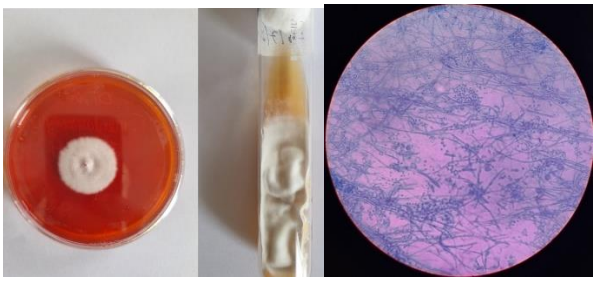


Fig-2 T. mentagrophytes - DTM and SDA.Microscopy(LPCB)

Fig-3 T. rubrum - DTM and SDA.Microscopy (LPCB)

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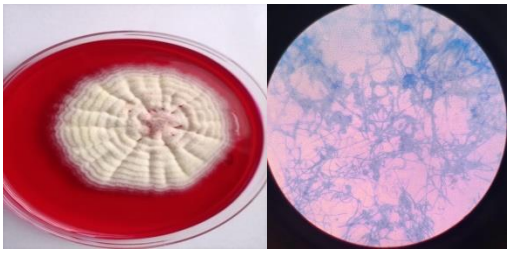
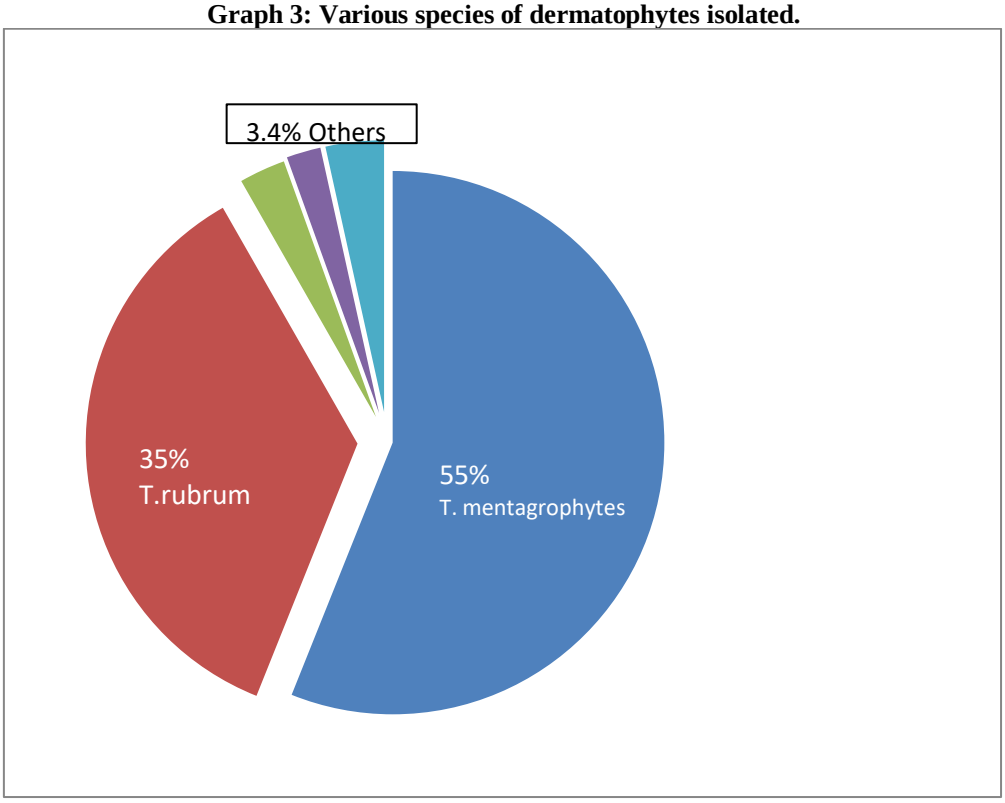


Fig 4 T. tonsurans- DTM Microscopy(LPCB)

Graph 3: Various species of dermatophytes isolated.



DISCUSSION

Dermatophytes are the only fungi that have evolved a dependency on human or animal infections for the survival of the species. It is therefore not surprising that these fungi are not among the most common infectious agents of humans. Cutaneous infections have increased in the last decade and 20–25% of the fungal infections worldwide are caused by dermatophytes[7].

The prevalence of dermatophytes isolation among clinically suspected dermatophytoses cases was 55%, male predominance was observed. Male-Female ratio of 2:1 was observed in Lakshmi et al[8].

Among total of 163 cases, 95(58.2%) were tineacorporis, 20(12.2%) were tineacruris, 23(14.1%) were both tineacorporis&tineacruris. 5(3%) tineapedis and 12(7.3%) tineacapitis. Similar to the study conducted bySamiaAfreen Khan et al[4], Majid Rauf Ahmad et al[9].

Direct microscopy using 10% KOH revealed fungal elements in 112(68.7%) out of 163 suspected samples . Of these 108(96.4%) were culture positive . As per this study, on correlation of culture positivity with KOH microscopy, 37 patients (22.6%) were culture positive and KOH negative. 4(2.4%) patients out of 163 samples were KOH positive and culture negative. In a study conducted by ParameswariKatay et al, 60.4% were positive by KOH and 62.9% were culture positive and 15 patients (12.09%) were culture positive and KOH negative. 12 (9.6%) patients out of 124 were KOH positive and culture negative[10].

Isolation of dermatophytes was observed in 123 (84.8%) samples in both SDA and DTM. 16 (11.03%) dermatophyte isolates were observed only in DTM. 6(4%) isolates were observed only in SDA. On comparing, the isolation of dermatophytes on SDA and DTM was statistically insignificant (P>0.05). This correlates with the previous studies conducted by Lakshmi et al[8], Singh and Beena et al[11]. DTM showed good isolation rate than Sabouraud dextrose agar. DTM interpretation is easyas change in colour to red indicates growth of dermatophytes. Main disadvantage of DTM is even though it is a selective medium for dermatophytes, non dermatophyte fungi also grows occasionally.

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With respect to the time period required for isolation, samples yielded early growth on DTM (10-12 days) than SDA(18-25 days). This correlates with other studies conducted by Lakshmi[8], Madhvi et al[12].

Pigment produced by Dermatophytes was observed on Sabouraud dextrose agar.Species identification was done by slide culture, LPCB mount and Urease test. Among the three species of dermatophytes, *Trichophyton*spp was commonly isolated followed by *Epidermophyton*and *Microsporum* spp. Most common isolate was *T. mentagrophytes* (55%) followed by *T. rubrum* (35.8%). In line with this study Sowmya N et al[13] observed *T. mentagrophytes*isolates followed by T.rubrum and few other studies reported that most common isolate was *T. rubrum* followed by *T.mentagrophytes.*

CONCLUSION

Dermatophytosis presenting in an ambiguous nature among various group of patients with or without debilitating disease need an early and definitive diagnosis. Microscopy and selecting the definitive media will benefit in early diagnosis and correct treatment. Further speciation indeed requiredto know the possible source of infection and prevention of further spread.

This study shows isolation of dermatophytes on both SDA and DTM were good but DTM showed early growth and easy interpretation was needed. Speciation was better with SDA compared to DTM.

This study concludes that dermatophyte prevalence varies and is not constant. KOH microscopy and selection of different culture media will give early and definitive diagnosis.

CONFLICT OF INTEREST: No conflict of interest to declare

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