

Evaluation of Culture Media for the Rapid Isolation of Dermatophytes

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Abstract: Dermatophytes are prevalent causes of cutaneous mycoses and, unlike many other fungal pathogens, are able to cause disease in immunocompetent individuals. They infect keratinized tissue such as skin, hair, and nails, resulting in tinea infections, including ringworm. The dermatophytes include three genera of molds in the class Euscomycetes: Trichophyton, Microsporum, and Epidermophyton. Dermatophytes are grouped according to their habitat as being either anthropophilic (human associated), zoophilic (animal associated), or geophilic (soil dwelling). Anthropophilic species are responsible for the majority of human infections; however, species from all three groups of dermatophytes have been associated with clinical disease. Human infections caused by anthropophiles tend to be chronic, with little inflammation, whereas infections caused by geophiles and zoophiles are often associated with acute inflammation and are self-healing. . The clinical presentation, though very typical of ringworm infection, is very often confused with other skin disorders particularly due to rampant application of broad-spectrum steroid containing skin ointments and creams leading to further misdiagnosis and mismanagement. So there is a need for correct, rapid and efficient laboratory methods for diagnosis of dermatophytosis.

I. Introduction

Superficial fungal infections are among the most common human infections. Although rarely life-threatening, they are associated with considerable inconvenience and discomfort. Many superficial fungal infections are chronic or recurrent, therefore simple incidence figures are not the most useful means of understanding the burden of disease. Dermatophytes are the most important causative agent of superficial mycosis.

The dermatophytes are a group of fungi that invade the superficial layer of the epidermis and degrade the keratinized tissues of skin, hair, and nails.^{1,2} Species belonging to the genera Trichophyton, Microsporum and Epidermophyton are responsible for dermatophytosis.³ Infections caused by these fungi are also known by the names "Tinea" and "Ringworm."⁴ Clinically, ringworm can be classified depending on the site involved. These include Tinea capitis (scalp), Tinea corporis (non-hairy skin of the body), Tinea cruris (groin), Tinea pedis (foot) or athlete's foot and Tinea barbae or barber's itch (bearded areas of the face and neck). Favus is a chronic type of ringworm involving the hair follicles. Depending on their habitat, dermatophytes are described as anthropophilic (human), zoophilic (animal) or geophilic (soil). Anthropophilic dermatophytes are the most common sources of Tinea infections.^{5,6}

Dermatophytosis is common in tropical countries like India and may reach epidemic proportions in areas with high rate of humidity and over population and poor hygienic conditions⁷. The clinical presentation, though very typical of ringworm infection, is very often confused with other skin disorders particularly due to rampant application of broad-spectrum steroid containing skin ointments and creams leading to further misdiagnosis and mismanagement. So there is a need for correct, rapid and efficient laboratory methods for diagnosis of dermatophytosis.

The study was carried out with followed objectives in mind.

1. To isolate and identify dermatophytes
2. To compare KOH and culture positivity
3. To evaluate role of DTM for early isolation of dermatophytes.

II. Materials And Methods

Ninety patients who were clinically diagnosed as cases of dermatophytosis formed the study group. First the details and history of the patients were recorded which included age, sex, duration of the complaints, distribution of the lesions and history of treatment of similar episodes in the past. Samples were collected from affected lesions.

Collection of samples from the skin:

The affected area was swabbed with 70% alcohol and the active edge of lesion scraped with a flame sterilized blunt scalpel. The scrapings were collected from the margins of the lesion without injuring the skin surface .

From the scalp : The same procedure was followed as for scrapings, in addition a few affected hairs were also epilated and collected with a pair of flame sterilized tweezers . Care was taken to collect the basal portion of the hair as the fungus is usually found in this area.

From the nails : The affected nail was swabbed with 70% alcohol after which the nails were scraped deeply enough to obtain recently invaded nail tissue. The samples were collected in paper sachets for transport to the laboratory. The specimens were processed by microscopy and culture.

Microscopic examination was done using 10% KOH solution to see hyphal segments in skin scrapings or either ectothrix or endothrix invasion of infected hairs . The samples were then inoculated on two slopes of Sabouraud’s Dextrose Agar containing chloramphenicol (50mg/liter) one of which also contained cycloheximide (500mg/liter) and a slope of Dermatophyte test medium (DTM). Himedia with supplement . Dermatophyte Test Medium (DTM) is a selective medium recommended for the isolation and cultivation of pathogenic dermatophytic fungi. Nitrogenous and carbonaceous compounds essential for microbial growth are provided by soy peptone. Dextrose serves as the energy source for metabolism. Chloramphenicol acts as a broad spectrum antibiotic which inhibits a wide range of Gram-positive and Gram-negative bacteria. Cycloheximide is added to inhibit saprophytic fungi. Phenol red, the pH indicator, in the medium is affected by the presence of dermatophytes (Epidermophyton, Microsporum, and Trichophyton spp.), which all produce alkaline metabolites. Production of alkali results in the medium changing from yellow orange to red in color. Other organisms that may grow on the medium can be recognized as non- dermatophytes by their color and colony morphology. Contaminating saprophytes can turn Dermatophyte Test Medium from its yellow-orange color to red, but can be ruled out due to the green to black hyphae produced. Dermatophytes typically produce white aerial hyphae.

The slopes were incubated at room temperature and examined at intervals for evidence of fungal growth. Slopes not showing growth for four weeks were discarded. Any visible growth was examined for colony morphology, texture, surface pigmentation and pigmentation on the reverse.

Microscopic examination of colony was done by doing a lactophenol cotton blue mount to examine the hyphal structure, microconidia and macroconidia .Slide culture was also done to study the undisturbed morphology of the fungal structures .Urea hydrolysis was used to further distinguish T. mentagrophytes from T. rubrum.

III. Results

In the present study 90 samples from clinically suspected cases of dermatophytosis were processed. Highest numbers (33.3%) of the patients were in the third decade of life. Males outnumbered females with a ratio of 1.57:1. Out of these 90 cases 66 skin samples, 15 hair samples & 9 nail samples were processed.

A comparison of the direct microscopy and culture results were shown in table 1. It is evident from the table that out of total 90 samples examined, 48 (53.33%) showed the evidence of fungal elements on direct microscopy, and 54 (60%) showed positive culture on DTM and 50 (55.5%) on SDA. 41 on DTM, 39 on SDA were both KOH & CULTURE positive. Out of all sample studied 29 on DTM , 31 on SDA did not show evidence of the fungi either on direct microscopy or culture.

Table 1 : Correlation of results of KOH preparation and culture

	KOH +VE	CULTURE +VE	KOH +VE & CULTURE +VE	KOH +VE & CULTURE -VE	KOH -VE & CULTURE +VE	KOH -VE & CULTURE -VE
DTM	48	54	41	07	13	29
SDA	48	50	39	09	11	31

Most common clinical presentation in the study was T.corporis followed by T.capitis & T.cruis. All isolates obtained were species of Trichophyton namely rubrum (70.37%), mentagrophytes(16.66%) & violaceum (12.96%), . T.rubrum was the predominant isolate accounting for 70.37% of the isolates. T.rubrum was also the most common organism affecting multiple sites. (Table2)

Table 2: Dermatophytes isolated from different clinical types

Clinical type	Total number	T. rubrum	T. mentagrophytes	T. violaceum
T. corporis	40	25	06	01
T. cruris	14	03	02	00
T. pedis	06	01	00	00
T. barbae	02	00	00	00
T. manum	02	00	00	00
T. intertrigo	02	02	00	00
T. capitis	15	03	00	06
T. unguium	09	04	01	00
TOTAL	90	38	09	07

Two media, SDA & DTM was used for the culture of dermatophytes. All the isolates were isolated on DTM while 92.59 % were isolated on SDA . Two T.rubrum & two T.mentagropytes did not grow on SDA. Results are shown in table 3.

Table 3 showing comparison of Dermatophyte growth on SDA-AC & DTM

Type of dermatophyte	Total isolates	Growth on SDA-AC	Growth on DTM
T.rubrum	38	36	38
T.mentagrophyte	09	07	09
T.violaceum	07	07	07
TOTAL	54	50	54

64.81% of dermatophytes were isolated on DTM in the first 5 to 10 days after inoculation while 44% of the dermatophytes were isolated on the SDA after 10 days of inoculation. The minimum incubation period was more than a week for SDA, whereas DTM gave positive results on culture within 5 to 10 days of inoculation. SDA required to be incubated at least for four weeks before being reported as negative. (Table 4)

Table 4 showing comparison of Dermatophyte growth rate on SDA-AC & DTM

Time duration	Growth on DTM	Growth on SDA-AC
5-10 days	35	18
11-15 days	11	22
>15 days	08	16
TOTAL	54	50

IV. Discussion

In the present study out of 90 cases examined highest incidence (31.3%) of the patients were in the third decade of life. Males outnumbered females with a ratio of 1.57:1. These observations are similar to findings of other authors like S Madhavi et al , SS Singh et al and BV Peerapur et al.^{7,8,9} .The higher incidence in young males could be due to greater physical activity and increased sweating .

Most common clinical sample studied in the study was skin(73.33%) followed by hair (16.66%) and nail(10%).Similarly Kumaran et al¹⁰ also studied 64% skin sample, followed by 39% hair sample, & 21% nail samples

Tinea corporis was the commonest lesion(Table 2) accounting for 44.44% cases , followed by Tinea capitis(16.66%) and Tinea cruris (15.55%) . These findings are in agreement with other workers, SS Sen et al, DDBelurkar et al and V Bindu et al who also found Tinea corporis to be the commonest lesion^{11,12,13}

Out of 90 samples screened by direct microscopy 48(53.33%) samples were positive for fungal elements, 54(60%) samples yielded positive culture on DTM or SDA. Overall 41 (45.55%) samples were both KOH & CULTURE positive. 7 (7.77%) samples were positive by KOH negative by culture , probable reasons for culture negativity could be insufficient amount of fungal elements in sample used for culture or patient on antifungal treatment. 32.22% did not show any evidence of fungi either on microscopy or on culture. These results are mostly comparable with the results of Sen SS, Rasul Assam¹¹, which showed- Forty nine (49%) cases were positive for fungal elements by direct microscopic examination. Culture was positive in 51 (51%)

T.rubrum was the predominant isolate accounting for 51.72% of the isolates which is in conformity with other reports^{11,14,15} T.rubrum which also the one mostly affecting multiple sites. George¹⁶ has suggested that both the predominantly chronic nature of the infection and the adaptation of the dermatophyte to the human skin can explain the higher predominance of T. rubrum in India.

All the isolates were isolated on DTM while 92.59 % were isolated on SDA . The comparative evaluation of the isolation of dermatophytes on SDA and DTM has been reported by Singh S, Beena PM et al[8] found SDA to be 96.55% and DTM 98.27% effective in isolation of dermatophytes., Mashkoor Ahmed et al¹⁷ reported SDA 100% and DTM 75% effective in isolation of dermatophytes from onychomycosis cases , while Yavuzdemir¹⁸ found no significant difference in the isolation rates of these media.. The effectiveness of SDA was 93.5% and that of DTM was 95.4% in his study of 225 samples.

86.2% of dermatophytes were isolated on DTM within 5-10 days after inoculation while 47.5% of the dermatophytes were isolated on the SDA within the same period. In a study by Amodkumar yadav et al [4] 30.84% isolates grew faster on DTM when compared to SDA in which only 10.53% grew within the same period

V. Conclusion

Dermatophyte infections are very common in our country where hot and humid climate in association with poor hygienic conditions play an important role in the growth of these fungi. The present study gives an insight about the etiological agents of dermatophytosis in this part of Karnataka. Study signifies the importance of mycological examination in the diagnosis of various dermatophytosis for their effective management. DTM can be used as a rapid screening medium for the isolation and identification of dermatophytes compared to SDA. However it is recommended that biochemical and serological tests be performed on growth from SDA for complete identification.

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