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Evaluation of Culture Methods for Identification of Dermatophytes

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ABSTRACT

Dermatophytosisare of worldwide distribution. Epidemiological studies concerning dermatophytes infection have been performed and the differences in the etiological agents have been noted. The present study was undertaken to isolate & detect the etiological agents of dermatophytosis up to species level by microscopy & culture, & to evaluate the culture methods. A total of 72 clinically diagnosed untreated cases of dermatophytosis attending the Dermatology outpatient of a tertiary care hospital, Mysore, constituted the material for the study. Specimens such as hair, nail and skin scrapings were collected from suspected cases. Direct microscopy was done using potassium hydroxide preparation and culture was done on Saboraud's dextrose agar and Dermatophyte test medium was done. Isolates were identified up to species level following conventional methods.72 samples from suspected cases of dermatophytosis were processed. Dermatophytes were isolated from 38 (52.77%) samples. Trichophyton rubrum was the commonest isolate 26 (36.11%). Male to female ratio was 2.3:1.21-40 years was the most common age group involved. Tinea corporis (22.22%) was the commonest clinical type of dermatophytosis.

Conclusion: The present study provided data regarding the etiological agents of dermatophytosis in this part of Karnataka. DTM is very useful as a screening medium and the isolation of dermatophytes is rapid when compared to SDA. Early diagnosis is essential to prevent transmission of infection.

Key words: *Dermatophytes, Trichophyton, Tinea corporis, Dermatophyte test medium.*

INTRODUCTION

Dermatophytes are common cutaneous fungal skin infections seen in man which mainly invade the dead keratin (which mainly include the skin, hair &nails). They do not invade the living tissues[1].Hot & humid climate in tropical & subtropical countries like India makes dermatophytosis or ring worm a very common superficial fungal skin infection. Dermatophytosis remains a significant health problem affecting children, adolescents & adults & is also of cosmetic importance[2].

Prevalence of infection with different species of dermatophytes varies with geographical locations & conditions[3]. The most common organism seen is *T.rubrum, T.mentagrophytes*, and *T.tonsurans*. Most of the patients acquiring dermatophytic infection are diagnosed & treated by clinicians without any laboratory confirmation[3].

Dermatophytes require long incubation period of growth[2]. Hence usage of a medium which produces rapid growth is essential. A study of dermatophytosis in a population is important as it may reflect the climatic condition, customs, hygienic & socio-economic status of the people[4].

Hence the present study was taken up to study the incidence of dermatophytes & evaluate the microscopic & culture methods for the isolation & identification of Dermatophytes.

REVIEW OF LITERATURE

Mycology the study of fungi came in to existence before bacteriology. The history of human Medical Mycology started with discovery and incrimination of etiologic agents of dermatophytosis. Growth of dermatophytes is more or less equal in all directions & lesions produced tend to creep in a circular or ring form. For this reason, the Greeks named the disease Herpes. The Romans associated the lesions with insects & named the disease 'tinea' meaning any small insect larva[5].

A study by Hanumanthappa et al on Clinicomycological aspect of dermatophytosis in a tertiary care centre in South India studied 150 clinically diagnosed randomly selected cases. Tinea corporis was the most common clinical type followed by tineacapitis. The most common affected age group was 21-30 years (24%) with a male to female ratio of 1.94:1. *T.rubrum* was the most frequently isolated dermatophytes followed by T.mentagrophytes[6].

A study on the profile of dermatophytes infections in Baroda by Suman Singh studied 260 clinically suspected cases of dermatophytosis of which 157 (60.38%) showed fungal elements in microscopy while 116 (44.62%) were culture positive. Tines corporis was the most common clinical presentation followed by tineacruris. Young adults in the age group of 16-30 years were the most affected. The male to female ratio was 1.57:1. *T.rubrum* was the most common isolate followed by distant second by *T.mentagrophytes* (17.24%), *E.flocossum* (7.75%) &*T.violaceum* (1.72%)[7].

A comparative study of different microscopic techniques & culture methods for the isolation of dermatophytes detected fungal elements in 157 samples out of 260 clinically suspected cases of dermatophytosis. They noted that better visualization was noted with 40% DMSO added to 10% KOH. In this study fungi were recovered

from SDA, DTM, and EDM in 96.5%, 98.3% & 85.3% of the cases respectively[8].

A study conducted in Calicut showed that out of 150 cases, the maximum number of patients was seen in the age group of 11-20 years (23.3%). Male to female ratio was 2.06:1. Tinea corporis was the commonest clinical type followed by Tinea cruris. Tines capitis was the predominant dermatophytosis in children. Among the associated diseases, Diabetes mellitus was seen in 10.6%, atopic diathesis in 10% & HIV infection in 2%. Recurrence of disease was noted in 32% of cases[9].

A study in the rural population of Hyderabad by Madhavi et al on Mycological study of dermatophytosis in a rural population studied 100 clinically suspected cases of dermatophytosis of which 43% cases were positive on microscopy & 58% in culture. 21-30 years was the most common age group affected and males outnumbered females 1.3:1.Tinea corporis was the commonest clinical lesion followed by Tine pedis&T.unguium. *T.rubrum* was the commonest dermatophytes isolated followed by *T.mentagrophytes, T.violaceum&T.verrucosum.* They were of the opinion that DTM is a better screening media as opposed to identification medium of dermatophytes[10].

AIMS & OBJECTIVES

1] Isolation of dermatophytes.

2] Evaluation by culture and microscopic methods.

3] Evaluation of role of DTM for early identification of dermatophytes

MATERIAL & METHODS

The present study was carried in the department of Microbiology over a period of two months- August 2014 & September 2014.

A total of 72 clinically diagnosed untreated cases of dermatophytosis attending the Dermatology outpatient of JSS Hospital, Mysore, constituted the material for the study.

COLLECTION OF MATERIAL

Dermatophytes can invade skin, scalp and nails. Collection was done accordingly-

FROM THE SKIN

The affected area was thoroughly cleaned with 70% alcohol to remove surface contaminants. After it has dried the active edge of the lesion was scraped with a flame sterilized blunt scalpel. The scrapings were collected from the margins of the lesion without causing injury to the skin surface. The scrapings thus received were directly placed on the slide or sterile paper packet.

FROM THE SCALP

Same procedure as above was followed but here few affected hairs will be epilated (removal of hair by its root) with a sterile epilation forceps. Care was taken to collect the basal portion of the hair as the fungus is usually found in this area.

FROM THE NAIL

The affected nail was cleaned with 70% alcohol and allowed to dry. Scrapings were obtained by lifting the nail. Scrapings were taken with the help of scalpel or scissors.

COLLECTION OF SAMPLE IN PAPER SACHET

The samples or scrapings were collected in paper sachets both for microscopy and culture. These were packed into square packets and were clipped together.

These packets were preferred to bottles as they reduce humidity and multiplication of bacteria.

MICROSCOPY

These sterilized specimens was transferred aseptically from the paper sachets or directly collected onto a sterile microscopic slide. To this a drop of 10% KOH was added if it is specimen of the skin, 20% for hair and 40% KOH was added if it is specimen of nails.

The preparation was examined under low power and high power objectives and looked for fungal elements.

CULTURE

Culture media SDA (Sabouraud's Dextrose Agar) with cycloheximide and DTM (Dermatophyte Test Medium) were used for identification of dermatophytes. The samples or the specimen was transferred to SDA-AC and DTM media.

Tubes & plates were incubated at 37ºC and room temperature. The sterile tubes & plates were discarded after four weeks.

Once growth has occurred the fungi was identified by topography, rate of growth, surface texture, surface pigmentation, pigmentation on reverse on SDA, on DTM growth was identified by colour change and microscopically by Lacto-phenol cotton blue preparation of the growth.

Fungal hyphae, size, shape and arrangement of micro & macro conidia and presence of other special structures like racquet hyphae, spiral hyphae etc. were observed. Slide culture was also done to appreciate better morphology. *T.rubrum* was differentiated from *T.mentagrophytes* by urease test, hair perforation test and pigment production.

RESULTS

Table 1: Age Wise Distribution

Age group	Total no of patients in the age group
0-20	15 (20.83%)
21-40	34 (47.22%)
>41	23 (31.94%)

\In the present study maximum cases of dermatophytosis was seen in the age group of 21-40 years (47.22%).

Table 2: Sex Wise Distribution Of Cases

No of cases	Males	Females
72	49 (68.05%)	23 (31.94%)

Males were affected more with a male to female ratio of 2.13:1

Table 3: Clinical Sample Distribution

Clinical sample	No of pt's	Male	Female
Skin	56 (77.77%)	41 (56.94%)	16 (22.22%)
Hair	6 (8.33%)	4(5.55%)	2(2.77%)
Nail	10 (12.5%)	4 (5.55%)	5 (6.94%)
Total	72	49 (68.05%)	23 (31.94%)

In the present study skin scrapings were the predominant clinical sample obtained followed by nail clippings & Hair.

Table 4: Table Showing KOH Positivity

No of cases	+ ve by KOH	+ve by culture
72	32 (44.44%)	38 (52.77%)

Fungal elements by microscopy were positive in 32 (44.44%) of the samples and Dermatophytes were isolated from 38 (52.77%) samples out of the 72 samples received.

Table 5: Table showing KOH & Conventional SDA-AC culture

KOH pos, Culture pos	KOH pos, Culture neg	KOH neg, culture pos	KOH neg, Culture neg
22 (30.55%)	12 (16.66%)	7 (9.72%)	3 1(43.05%)

It was observed that 22 (30.55%) samples showed fungal elements in KOH & grew dermatophytes in SDA – AC medium.

12 samples were KOH positive but failed to produce growth on SDA-AC.

7 samples which were KOH negative were picked up by culture on SDA-AC.

31 samples were neither positive on microscopy or on culture.

Table 6: showing KOH & DTM culture findings

KOH pos, Culture pos	KOH pos, Culture neg	KOH neg, culture pos	KOH neg, Culture neg
27 (37.5%)	7 (9.72%)	11 (15.27%)	27 (37.5%)

27 samples in the present study were positive both by microscopy & culture.

Fungal elements were detected by microscopy in 7 samples but failed to grow on DTM.

11 samples which did not show fungal elements on microscopy were positive by culture.

Negativity in both microscopy & culture was observed in 27 samples.

Table 7: Distribution of different clinical type of Dermatophytic infections.

Clinical type	No of cases	KOH positive	Dermatophyte Growth	Non Dermatophyte
T.capitis	6	4	4	-
T.corporis	23	18	21	-
T.cruris	3	1	-	-
T.mannum	2	1	1	-
T.pedis	4	-	1	-
T.unguium	10	4	3	4
T.barbae	1	-	-	-
T.cruris	7	4	4	-

,T.corporis				
T.intertrigo	1	-	-	-
No diag	15	-	-	-
TOTAL	72	32	34	4

T.corporis (31.94%) was the most common dermatophytosis observed in the present study, followed by T.unguium (13.88%) and T.corporis with T.cruris (9.72%).

Table 8: Dermatophytes isolated from different clinical types

Clin type/Species	*T.rubrum*	*T.mentagrophytes*	*T.violaceum*	TOTAL
T.capitis	3	-	1	4
T.corporis	16	4	1	21
T.mannum	1	-	-	1
T.pedis	-	1	-	1
T.unguium	3	-	-	3
T.cruris with corporis	3	1	-	4
TOTAL	26	6	2	34

T.rubrum (36.11%) was the most common dermatophytes isolated in the present study followed by *T.mentagrophytes* (8.33%) followed by *T.violaceum.*

Table 9: Comparison of rate of growth on SDA-AC & DTM

Time duration	No of isolates on DTM	No of isolates on SDA-AC
5-10 days	16	6
11-15 days	11	11
>16 days	7	8
Total	34	25

16 isolates produced growth on DTM within 10 days of inoculation on DTM, whereas only 6 days produced growth in this period on SDA-AC. 11 isolates grew between 11-15 days on both SDA-AC & DTM.
Dermatophytes were isolated from 34 (47.22%) clinical samples inoculated on DTM, whereas only 25 (34.72%) samples yielded growth on SDA-AC.

DISCUSSION

Dermatophytosis is the commonest group of superficial fungal infection seen in the tropics. All races are usually are usually affected and the clinical varieties and prevalence appear to depend mainly on environmental factors[3].

Dermatophytes are widely prevalent in our part of the world. In our study dermatophytosis was found to be commonest in the age group of 21-40 years in accordance with other studies[9,11,12].

The higher incidence may be attributable to higher freedom of movement, carelessness and perhaps lack of guidance regarding personal hygiene could be the factors responsible for higher incidence of tinea infection[13].

Male to female ratio in the present study was 2.13:1. The reason behind high incidence in male may be due to maceration effect of hyperhidrosis in comparison to females. The males are usually

more exposed to infection during their outdoor occupation & females, especially of rural background rarely seek medical advice for a fungal infection. In case of T.pedis, higher incidence in male is seen probably due to their heavy, closed footwear while females mostly use open ventilated footwear[14,15,16].

The most prevalent clinical type was Tinea corporis (31.94%) followed by Tinea unguium (13.88%). The highest incidence of Tinea corporis among cases of dermatophytosis was also noted by several other researchers[16, 17].

The main reason behind high prevalence of T.corporis &T.cruris is the sever itching which induces the patient to seek medical advice[3].

Direct microscopy revealed fungal elements in 44.44% of cases similar to study done by Madhavi et al. of these 38 (52.77%) were culture positive. Out of the 38 culture positive, 11(15.27%) were negative by microscopy. Out of the 72 clinical samples studied 27(37.5%) did not show evidence of fungi either on direct microscopy or culture. The results are comparable with results of Madhavi eta al which showed 43% cases were positive for fungal elements by direct microscopic examination. Culture positivity in their study was 58% and of these 11 had no evidence of fungus by direct microscopy while 7 out of 27 culture negative cases were positive by direct microscopy[10].

Most of the isolates obtained were species of *Trichophyton* namely *rubrum* (36.11%) *mentagrophytes*(8.33%)*violaceum* (2.77%). *T.rubrum* was the predominant isolate accounting for 36.11% of the isolates which is in conformity with other reports. George has suggested that both the predominantly chronic nature of the infection and the adaptation of the dermatophytes to the human skin can explain the higher predominance of *T.rubrum* in India[18].

SDA-AC and DTM were used for the culture of the samples. DTM was found better medium in primary isolation of dermatophytes. All the isolates were isolated on DTM while 73.5% were isolated on SDA-AC. Similar findings was observed by Singh S &Beena P M who found SDA to be 96.55% & DTM 98.27% effective in isolation of dermatophytes[8]. Mashkoor Ahmed et al reported SDA 100% & DTM 75% effective in isolation of dermatophytes[19]. While Yavuzdemir in their study reported no significant difference in the isolation rates of dermatophytes from both the media where growth on SDA was 93.5% & DTM was 95.4% out of 225 samples studied[20].

47% of dermatophytes were isolated o DTM within 10 days after inoculation while on SDA-AC 17.6% of the dermatophytes were isolated in the first 10days. After 10 days, no significant difference was observed between both the media. DTM gave positive results on culture within 15 days of inoculation, whereas SDA was incubated for at least three weeks before reporting culture as negative.

CONCLUSION

The present study provided data regarding the etiological agents of dermatophytosis in this part of Karnataka. Commonest lesion, species isolated & other variables is in concordance to studies done in other parts of India.

DTM is very useful as a screening medium and the isolation of dermatophytes is rapid when compared to SDA. However, it is recommended that biochemical and/or serological tests be performed on growth for complete identification.

SUMMARY

- Dermatophytosis is prevalent in our settings also.
- The most common age affected was 21-40 years of age.
- The male to female ratio was 2.13:1.
- T.corporis was the commonest clinical type of dermatophytosis in the present study.
- 44.44% of samples were positive by microscopy, 52.77% were positive by culture.
- *T.rubrum* (36.11%) was the predominant fungus isolated, followed by *T.mentagrophytes* (8.33%).
- Isolation rate on SDA was 34.72% whereas on DTM was 47.22%.
- DTM was found to be better medium for screening and rapid isolation of dermatophytes in our study.
- In our study, the combined sensitivity of direct microscopy & culture was greater than those of direct microscopy & culture alone, emphasising the need for both tests.
- Early accurate mycological diagnosis up to species level is an important tool to control and reduce the incidence of dermatophytosis and some species show slower response to azole derivatives.
- Periodic epidemiological analysis of dermatophytosis is essential to ensure efficacious control.

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