

# Comparing the Effectiveness of Sabouraud Dextrose Agar and Dermatophytes Test Medium for Isolation of Dermatophytes.

Parameswari Katay<sup>1</sup>, Sravya Ravi<sup>2</sup>, Geethanjali Anke<sup>3</sup>

<sup>1</sup>Associate Professor, Department of Microbiology, Siddhartha Medical College, Vijayawada.

<sup>2</sup>House Surgeon, Siddhartha Medical College, Vijayawada.

<sup>3</sup>Senior Resident, Department of Microbiology, Government Medical College, Ananthapuramu.

Received: April 2016

Accepted: May 2016

**Copyright:** © the author(s), publisher. It is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

**Background:** Dermatophytes affects more than 30% of the population, usually as superficial mycosis but also present as deeper subcutaneous tissue infection in rare occasions. Because of ambiguous clinical presentations of dermatophytosis need to diagnose accurately to avoid mismanagement. The present study was selected to know the significance of KOH (Potassium Hydroxide) in diagnosis of dermatophytosis and to compare Sabouraud Dextrose Agar (SDA) with Dermatophytes Test Medium (DTM) in isolation of dermatophytes. **Methods:** A total of 124 patients were included in this study who was diagnosed as clinically suspected dermatophytosis at Department of DVL. Samples were collected and inoculated in to Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide, Dermatophytes Test Medium and also KOH mount was done. **Results:** Out of 124 clinically suspected dermatophytosis studied population, 78 (62.9%) were culture positive. Tinea corporis (29.4%) was affected predominantly followed by Tinea cruris (21.7%) and Tinea capitis (17.9%). On correlation of culture positivity with KOH microscopy, 15 patients (12.09%) were culture positive and KOH negative. 12 (9.6%) patients out of 124 were KOH positive and culture negative. The isolation rate in this study from SDA was 93.5% and that of DTM was 100%. On comparing of dermatophytes isolation from clinical samples among DTM and SDA, shown statistically insignificant ( $P>0.05$ ). **Conclusion:** KOH gives rapid probable diagnosis to start empirical therapy, lesser sensitive than culture media. Both SDA and DTM gives good isolation results, where as DTM is superior than SDA in isolation and aid in easy interpretation.

**Keywords:** Dermatophytes, Potassium Hydroxide, Sabouraud Dextrose Agar, Dermatophytes Test Medium.

## INTRODUCTION

Dermatophytes are hyaline narrow septate fungi with branching and conidia, which cause infection called "Dermatophytosis". Dermatophytes are well known for its keratophilic nature, generally limited to non living keratinized layers of skin, hairs and nails.

### Name & Address of Corresponding Author

Dr. K. Parameswari,  
Associate Professor, Dept of Microbiology,  
Siddhartha Medical College, Vijayawada, India  
E-mail: drkparameswari3@gmail.com

Dermatophytes cause superficial mycosis affecting skin, hair, nails and its appendages. It has a major impact on public health worldwide affecting millions of people and is also of cosmetic importance.<sup>[1]</sup> Dermatophytes include Trichophyton, Microsporum and Epidermophyton. Trichophyton species infect skin, hair & nails, Microsporum species infect skin & hair, Epidermophyton species infect skin & nails.<sup>[2]</sup>

Dermatophytes affected more than 30% of the population<sup>[3,4]</sup>, usually as superficial mycosis but also presents as deeper subcutaneous tissue infection in rare occasions.<sup>[5]</sup>

Diagnosis of a typical dermatophytic lesions can be easily done by many clinicians but few lesions are confusing, need of diagnostic methods for accurate diagnosis. Misdiagnosis of dermatophytic lesions leading to altered treatment such as steroid application, which cause varied clinical presentation. There is a need of rapid accurate diagnostic methods to avoid misdiagnosed cases and mismanagement.

For rapid diagnosis KOH will help in screening of dermatophytes<sup>[2]</sup> and Culture media such as Dermatophyte test medium (DTM), Sabouraud dextrose agar (SDA) with chloramphenicol and cycloheximide helps us in accurate diagnosis.<sup>[6]</sup> The present study was selected to know the significance of KOH in diagnosis of dermatophytosis and to compare SDA with DTM in isolation of dermatophytes.

## MATERIALS AND METHODS

This prospective study has done in the Department of Microbiology for one year at Siddhartha Medical College, Vijayawada. A total of 124 patients were included in the study who was diagnosed as clinically suspected dermatophytosis at Department of DVL. Informed consent form was taken from patients.

**Sample Collection:** Lesions were cleaned with spirit (70% alcohol) to avoid contamination of bacteria. After few seconds samples were collected from lesions of skin, hair and nails in to a clean black paper, folded and sent to the laboratory for processing.<sup>[1]</sup> All the samples were tested for KOH (Potassium Hydroxide) mount and inoculated into media within 24 hours from the time of collection.

**KOH Mount:** Samples (skin, hair, nail) which have scraped or cut from patient was kept on a clean grease free slide, a drop of 10% KOH was added and covered with cover slip. This slide was passed under the flame slightly to hasten the disintegration of tissue. Slide was kept for observation for 30 minutes, in between every 5 minutes slide was examined for narrow, septate branching hyphae with or without arthrospores.

**Media Inoculation:** All samples were inoculated in to Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide, Dermatophyte Test Medium. Inoculated media was kept at 22°C in BOD (Biological Oxygen Demand) and observed daily for any growth, if there was no growth even after 6 weeks then it was finally reported as negative. At 3 weeks preliminary report was given.

**Slide culture:** All the samples which were grown in various media were processed for slide culture to know the exact morphology of fungus. Growth from slide culture was taken and mounted with LPCB (Lacto Phenol Cotton Blue) stain to observe the hyphal structures, conidia and their arrangement.

Clinical types of Dermatophytes, growth on various media and KOH examination were analyzed and tabulated.

## RESULTS

Out of 124 clinically suspected dermatophytosis studied population, 78 (62.9%) were culture positive. Among 78 culture positive patients, 57 (73.1%) were males and 21 (26.9%) were females. Skin lesions were observed most commonly followed by hair and nails. Tinea corporis (29.4%) was affected predominantly followed by Tinea

cruris (21.7%) and Tinea capitis (17.9%). Among studied population varied clinical presentations were observed [Table 1].

**Table 1:** Various Clinical types of Dermatophytes among studied population

Dermatophyte infection	Number of cases affected	Percentage
Tinea corporis	23	29.4%
Tinea cruris	17	21.7%
Tinea capitis	14	17.9%
Tinea unguim	6	7.6%
Tinea pedis	6	7.6%
T.corporis & T.cruis	8	10.2%
Tinea capitis & Tinea unguim	4	5.1%
Total	78	100%

All the samples were observed for narrow, septate hyphae with branching under microscopy using 10% KOH. On correlation of culture positivity with KOH microscopy, 15 patients (12.09%) were culture positive and KOH negative. 12 (9.6%) patients out of 124 were KOH positive and culture negative [Table 2].

**Table 2:** Correlation of Culture positivity with KOH Microscopy

	Culture Positive	Culture Negative	Total
<b>KOH Positive</b>	63	12	75 (60.4%)
<b>KOH Negative</b>	15	34	49 (39.5%)
<b>Total</b>	78 (62.9%)	46 (37%)	124

Isolation of dermatophytes was observed in 73 (97.3%) patients in both Sabouraud Dextrose Agar and Dermatophyte test medium. 5 (6.4%) dermatophyte isolates were observed only in Dermatophyte test medium [Table 3]. On comparing the isolation of dermatophytes on SDA and DTM, was statistically insignificant (P>0.05).

**Table 3:** Number of Dermatophyte isolates on SDA and DTM

SDA	DTM	Total	78 dermatophyte isolates
+	+	73	
-	+	5	
+	-	0	

Various dermatophytes isolated on sabouraud dextrose agar and dermatophyte test medium and were identified using slide culture. [Figure 1]. Species identification was done by observing pigmentation in SDA, sporulation in slide culture using LPCB (Lacto Phenol Cotton Blue) and urease test [Figure 2-5]. Species of various dermatophytes were represented in Pie diagram [Figure 1].

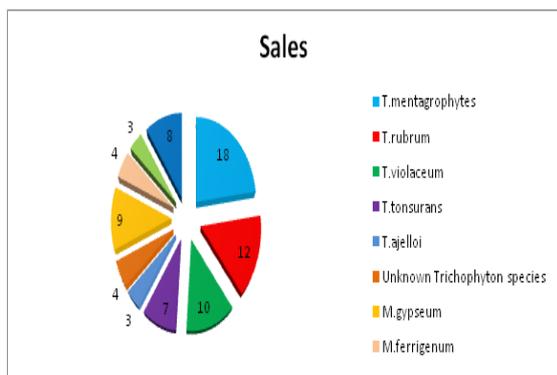


Figure 1: Percentage of Various Dermatophytes.



Figure 5: Showing sporulation of Trichophyton mentagrophytes using LPCB stain.



Figure 2: Showing the growth of Microsporum gypseum in Sabouraud dextrose agar.

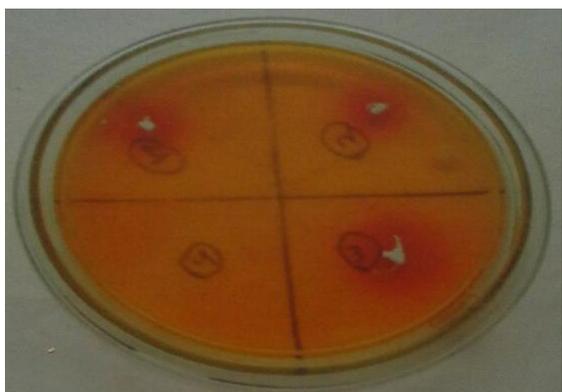


Figure 3: Showing Dermatophytes growth on Dermatophyte test medium.



Figure 4: Showing pigmentation of Trichophyton violaceum on reverse side of Sabouraud dextrose agar.

## DISCUSSION

The incidence of Dermatophytes is increasing, also becoming resistance to many anti-fungal and has invasive nature (deep infections), became more problematic in immunocompromised people. This is also causing much cosmetic problem. Ambiguous clinical presentations of dermatophytes are causing much problem in diagnosis and treatment; there is a need of efficient, rapid, cost effective diagnostic methods.

The prevalence of dermatophytes isolation among clinically suspected dermatophytosis cases was 62.9%, male predominance was observed. Male and female ratio was about 3:1, whereas Male-Female ratio of 2:1 observed in Amin AG et al<sup>[7]</sup>, Rao et al.<sup>[8]</sup>

Sowmya N<sup>[9]</sup>, Kannan et al<sup>[10]</sup> and Singh et al<sup>[11]</sup> reported the prevalence of dermatophytes as 43%, 48.5% and 44.61% respectively. Our study shown higher prevalence which may be due to varied geographical area with its climatic conditions, also depends on tight clothing and sweating among people and various other factors like urbanization, unhygienic practices, occupational exposure.

In this study Tinea corporis (29.4%) was affected predominantly followed by Tinea cruris (21.7%) and Tinea capitis (17.9%). T. corporis was found to be predominant clinical presentation by Sumana et al<sup>[12]</sup>, BK Gupta et al<sup>[13]</sup>, Verenkar et al<sup>[14]</sup>, Banerjee et al<sup>[15]</sup>. In contrast to this Kumar et al<sup>[16]</sup>, Nagarkatti et al<sup>[17]</sup> had reported T. cruris as commonest clinical presentation followed by T. corporis.

As per this study on correlation of culture positivity with KOH microscopy, 15 patients (12.09%) were culture positive and KOH negative. 12 (9.6%) patients out of 124 were KOH positive and culture negative. 60.4% were positive by KOH and 62.9% were culture positive in this study. Singh et al<sup>[11]</sup> also documented that microscopy (40.76%) was less sensitive than culture (44.61%).

In this study Isolation of dermatophytes was observed in 73 (58.8%) out of 124 patients in both Sabouraud Dextrose Agar and Dermatophyte test

medium. 5 (4%) dermatophyte isolates were observed only in Dermatophyte test medium. The isolation rate in this study from SDA was 93.5% and that of DTM was 100%. On comparing of dermatophyte isolation from clinical samples among DTM and SDA, shown no significance statistically.

Similar to this study Singh et al<sup>[11]</sup> observed dermatophytes isolation from DTM and SDA was good and not statistically significant. Yavuzdemir et al<sup>[18]</sup> was also documented that isolation rate of SDA was 93.5% and DTM was 95.4%, shown no statistical significant difference in isolation rates of these media. Dermatophyte test medium shown good isolation rate than Sabouraud dextrose agar, change in color to red indicates growth of dermatophytes, so DTM interpretation is easy. Main disadvantage of DTM is even though it is a selective medium for dermatophytes, non dermatophyte fungi also grows occasionally. Growth on DTM has to confirm as dermatophytes by slide culture, in which we can observe exact morphology of fungus.

In the present study among various dermatophytes, Trichophyton species was 69.2% and found to be a predominant pathogen followed by 20.5% Microsporum species and 10.2% Epidermophyton species. Species identification was done by slide culture and noting down the pigmentation in SDA. Pigmentation of Dermatophytes can easily observed in Sabouraud dextrose agar. Most common isolate was *T. mentagrophytes* (23%) followed by *T. rubrum* (15.3%). In line with this study Sowmya N et al<sup>[9]</sup> observed *T. mentagrophytes* followed by *T. rubrum* and few other studies reported that most common isolate was *T. rubrum* followed by *T. mentagrophytes* [10,11,19,20].

## CONCLUSION

Ambiguous dermatophytic lesions need to diagnose in a accurate and rapid way using various diagnostic methods to avoid mismanagement. New addition for diagnosis are MALDI TOF, PCR which gives effective and fast result but those are expensive and also not available for routine purpose in most of the medical institutes and laboratories. So there is a need to diagnose dermatophytes at all health care facilities by using simple KOH mount and various culture media. KOH gives rapid probable diagnosis to start empirical therapy, lesser sensitive than culture media. Both SDA and DTM gives good isolation results, where as DTM is superior than SDA in isolation and aid in easy interpretation.

## REFERENCES

1. Jagdish Chander. Textbook of Mycology. 2009, 3rd Ed. Mehta publishers; pp. 122-142; 266 283: 508- 516.
2. Rippon JW. 3rd ed. Philadelphia: Saunders. Medical Mycology: The pathogenic Fungi and the Pathogenic Actinomycetes ; 1988. pp. 140-275.
3. Rippon JW. The changing epidemiology and emerging patterns of dermatophyte species. In: McGinnis MR, ed. Current Topics in Medical Mycology, Vol 1. New York; Springer Verlag: 1985. 208-234.
4. Noguchi H, Hiruma M, Kawada A, Ishibashi A, Kono S. Tinea pedis in members of the Japanese self-defense forces: relationships of its prevalence and its severity with length of military service and width of inter digital spaces. Mycoses. 1995; 38: 494-499.
5. Gong JQ, Liu XQ, Xu HB, Zeng XS, Chen W, Li X F. Deep dermatophytosis caused by *Trichophyton rubrum*: report of two cases. Mycoses. 2007; 50: 102-108.
6. Robert R, Pihet M. Conventional Methods for the Diagnosis of Dermatophytosis. Mycopathologia. 2008; 166:295-306.
7. Amin AG, Shah CF, Shjan HS. Analysis of 141 cases of dermatophytosis. Indian J Dermatol Venereol. 1971; 31(4): 123-128.
8. Rao BR, Annapurna E. Dermatophytosis in vishakapatnam. Indian J Dermatology Venereol. 1973; 39(5): 209-212.
9. Sowmya N, Appalaraju B, Surendran P, Srinivas CR. Isolation, Identification and comparative analysis of SDA and DTM for dermatophytes from clinical samples in a tertiary care hospital, IOSR-JDMS. 2014;13(11):68-73.
10. Kannan P, Janaki C, Selvi GS. Prevalence of dermatophytes and other fungal agents isolated from clinical samples. Ind J Med Microbiol. 2006;24(3) :212-215.
11. Singh S, Beena PM. Comparative study of different microscopic techniques and culture media for the isolation of dermatophytes. Ind J Med Microbiol. 2003; 21(1):21-24.
12. Sumana V, Singaracharya MA. Dermatophytes in Khammam. Indian J Pathol Microbiol. 2004; 47:287-289.
13. Gupta BK, Kumar S, Khurana S. Mycological aspects of Dermatophytosis in Zudhiana. Indian J Pathol Microbiol. 1993; 36(3):233-237.
14. Verenkar MP, Pinto MJ, Rodrigues S, Roque WP, Singh I. Clinicomicrobiological study of dermatophytoses. Indian J Pathol Microbiol. 1991;34:186-192.
15. Banerjee U, Pasricha JS. Observation of Tinea corporis in Delhi. Indian J Pathol Microbiol. 1987; 207-212.
16. Kumar AG, Lakshmi N. Tinea capitis in Tirupathi. Indian J Pathol microbial. 1990;33(4):360-363.
17. Nagarkatti PG, Souzan D, Ramachandraiah V. Dermatophytosis in North Karnataka. Indian J Pathol Bacteriol. 1975;18:26-31.
18. Yavuzdemir S. Comparative evaluation of the isolation of the dermatophytes by direct laboratory evidence and MSDA with MDTM culture media. Microbiol Bulletin. 1992; 26(4):367-72.
19. Rajesh R, Subramainam K, Padmavathy BK, Vasanthi S. Prevalence and species profile of Dermatophytosis among HIV positive patients in ruran referral centre. Indian J Transm disease. 2006; 27(2)70 - 74.
20. Bindu V, Pavithran K. Clinico - mycological study of dermatophytosis in Calicut. Indian J Dermatol Venereol Leprol. 2002; 68:259-61.

**How to cite this article:** Katay P, Ravi S, Anke G. Comparing the Effectiveness of Sabouraud Dextrose Agar and Dermatophytes Test Medium for Isolation of Dermatophytes. Ann. Int. Med. Den. Res. 2016;2(4):174-77.

**Source of Support:** Nil, **Conflict of Interest:** None declared