ORIGINAL ARTICLE

Evaluation of Dermatophyte Test Medium and Sabouraud Dextrose Agar for Isolation of Dermatophyte Species

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ABSTRACT

Background and Objective: Dermatophyte infections require laboratory diagnosis before the treatment is started. Although direct microscopy is routinely performed but culture of dermatophytes is the gold standard. However, it takes about 4 weeks for species identification on primary media. Our aim was to compare dermatophyte test medium (DTM) as a screening medium for the isolation of dermatophytes in comparison with sabouraud dextrose agar (SDA).

Methods: It was a comparative study carried out at the Department of Microbiology of Post Graduate Medical Institute, Lahore over a period of nine months. Samples were collected from one hundred patients with clinically suspected dermatophytoses after taking informed written consent. The samples were examined microscopically and then inoculated on two types of culture media, one Sabouraud dextrose agar (SDA) with added chloramphenicol, gentacin and cycloheximide and other dermatophyte test medium (DTM) with added chlortetracycline, gentacin and cyclohexamide.

Results: Fungal growth was observed in fifty-six samples on culture. Out of the fifty-six positive on cultures, nineteen were that of dermatophytes. Out of n = 100 patients, ten were positive on SDA while n = 14 dermatophyte species were able to grow on DTM. A significantly higher positivity ($P \geq 0.05$) for isolating dermatophytes was observed by DTM as compared to SDA. DTM was able to isolate (71%) of the dermatophytes in first 10 days. Isolation rate of dermatophyte species was higher (73.68%) on DTM as compared to SDA which was 52.6%.

Conclusion: Authors recommend the use of dermatophyte test medium for the primary isolation and identification of dermatophyte species to be more effective and time saving.

KEYWORDS: Dermatophytes, Sabouraud's dextrose agar, Dermatophyte test medium.

How to Cite This: *Ahmad MR, Javed I, Mushtaq S, Hafeez R, Cheema KH. Evaluation of dermatophyte test medium and sabouraud dextrose agar for isolation of dermatophyte species. Biomedica. 2020; 36 (4): 362-6. doi: https://doi.org/10.51441/BioMedica/5-80.*

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- Received for publication: 22-10-2020
- Revision received: 02-12-2020
- Accepted for publication: 14-12-2020

INTRODUCTION

Dermatophytes are a group of pathogenic fungi that cause mycotic infections called dermatophytoses. They infect tissues rich in keratin, including skin, hair and nails.[1] Based on different microscopic morphologies and varying modes of sporulation, the dermatophytes are classified into three genera: Microsporum, Trichophyton, and Epidermophyton.[2]

Fungal culture along with direct microscopy is critical for a conclusive diagnosis and proper treatment of causative fungal infection. Culture is considered the gold standard in the laboratory diagnosis of dermatophytoses.[3] Direct microscopy findings indicate a high incidence false negatives, varying from 5% to 15%, due to low visibility and sparsely distributed hyphae on the slide.[4] Sabouraud dextrose agar (SDA) is a commonly used and effective media which supports the growth of pathogenic fungi including dermatophytes and yeasts. However, it is not a differential medium.[5]

Dermatophyte test medium (DTM) culture has chlortetracycline and gentamicin to avoid bacterial contamination, cyclohexamide to inhibit growth of contaminant fungi and phenol red, a pH indicator.[6] Dermatophytes tend to metabolize protein in the culture medium, releasing alkaline metabolites which turn the yellowish DTM medium red at about the same time as the dermatophyte colony appears. Non-dermatophytes can also grow but are recognized due to a lack of color change. Similarly, saprophytic fungi can be ruled out due to green to black hyphae produced by them. Dermatophyte species usually produce white aerial hyphae on DTM.[7] The present study was designed to assess the efficacy of DTM media as a useful screening medium while comparing to SDA for the isolation and identification of dermatophytes.

METHODS

This comparative study was conducted at the Department of Microbiology, Post Graduate Medical Institute, Lahore over a period of nine months from July 2013 to March 2014. The study was approved by the Institutional Ethical Review Committee. Sampling was done by nonprobability convenient technique. Patients diagnosed clinically by dermatologist to have dermatophytoses were included in the study. Patients who used topical antifungal drugs or being treated for dermatophytoses were excluded. Samples were collected from one hundred patients who were suspected to have dermatophytoses. Samples of skin crusts, hair and nail clippings were collected on a clean piece of square black paper 5cm in size which was then neatly folded, labelled and sent to laboratory. The samples were prepared for laboratory examination by reducing them into small pieces with the help of sterile scissors and scalpel blade.

Microscopic examination of fungal hyphae was done by preparing 20% potassium hydroxide wet mount. The specimens were subsequently inoculated on Sabouraud dextrose agar with added chloramphenicol (0.05 g/liter)/ gentamicin (5 ml/liter) /cyclohexamide (0.5 g/liter) and dermatophyte test medium (chlortetracycline/ gentamicin/cyclohexamide added).

Four inoculums of fungal specimen measuring 1mm were then placed on agar of each plate at well-spaced intervals. Some of the fragments were submerged beneath the agar. Loose adhesive tapes were applied to petri dishes. All the culture plates were incubated aerobically at 28-30ºC. The culture plates having SDA were checked twice weekly for a maximum of 30 days while DTM was checked daily for up to 14 days.

Dermatophyte species were identified by observing pigmentation both on surface and on reverse. Their gross colonial morphology and texture were also noted. Dermatophyte colonies on SDA were recognized as light colored, often blue and light shades of white. Colonies which were blue, black or dark green were regarded as non-dermatophytes. On Dermatophyte test medium, the dermatophyte species were identified on the basis of colonial morphology and change incolor of the medium from yellow to pink red.

Microscopic examination of colony was done by preparing a lactophenol cotton blue mount to examine the fungal hyphal structure and spores called microconidia and macroconidia.

STATISTICAL ANALYSIS

Data was collected and entered in Statistical Package for the Social Sciences (SPSS) version 20.0. Student T-test was applied to determine the comparison of positive detected cases with dermatophyte test medium (DTM) and Sabouraud dextrose agar (SDA). The P-value ≤ 0.05 was considered significant.

RESULTS

Out of a total of hundred patients, 52 (52%) were female and 48 (48%) were male. The dermatophytic infections were categorized as tinea

corporis, tinea capitis, tinea pedis, tineaunguium, tinea faciei, tinea cruris and tinea mannum according to the sites of lesions involved. (Table-1).

Table-1: Distribution of patients with dermatophytoses according to their clinical types (n = 100).

Sr. No.	*Clinical Type of Dermatophytes*	*No. of Cases*
1.	Tinea Corporis	31
2.	Tinea Pedis	18
3.	Tinea Unguium	17
4.	Tinea Capitis	13
5.	Tinea Faciei	10
6.	Tinea Cruris	1
7.	Tinea Mannum	5
8.	Mixed Infections	5

A significantly higher positivity ($P < 0.05$) was observed by DTM as compared to SDA (Table-2).

Table-2: Comparison between SDA and DTM.

Culture Technique	*DTM Positive*	*DTM Negative*	*Total*
SDA+C+G+A positive	5	5	10
SDA+C+G+A negative	*8	82	90
Total	13	87	100

*P < 0.05

A comparison of average time period for culture to become positive between SDA and DTM indicates less number of days taken by DTM as compared to SDA (Fig.1).

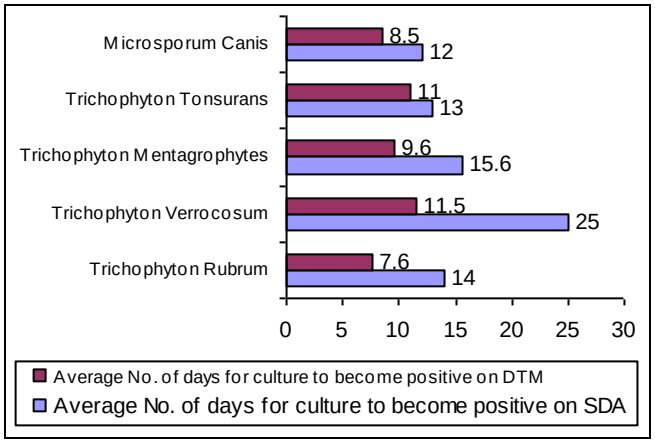

Fig.1: Comparison of average time period for culture to become positive between SDA and DTM.

Out of total n = 19 isolates, 10 (52.6%) grew on SDA while 14 (73.68%) of them were isolated on DTM (Fig.2).

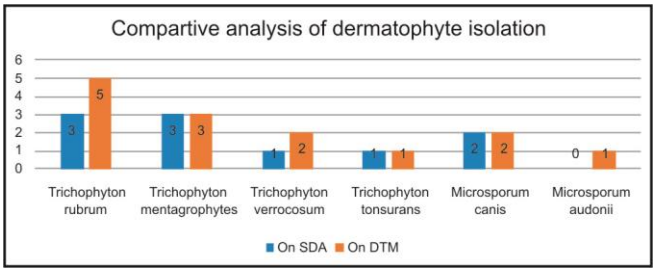

Fig.2: Comparative analysis of dermatophyte isolation on SDA and DTM (n=19).

Fig.3: Surface view of growth of Trichophyton mentagrophytes on dermatophyte test medium.

A) Surface view **B)** Reverse view

Fig.4: Surface view (A) and reverse view (B) of Trichophyton mentagrophytes on Sabouraud dextrose agar with chloramphenicol, gentamicin and cyclohexamide added.

DISCUSSION

Dermatophytes causing chronic infections (dermatophytoses) are generally known as tinea infections. They infect superficial keratinized tissues i.e., skin, hair, and nails. These infections constitute one of the major public health problems in tropical and subtropical areas.[8] The laboratory diagnosis of dermatophytes involves direct microscopy followed by culture of specimen.[9] Despite the important role of microscopy in diagnosis of fungal infections, cultures provide conclusive and accurate identification of the dermatophyte species. Culture of dermatophytes requires longer incubation periods of almost 4 weeks on primary culture medias.[10]

In the present study, out of one hundred samples, fifty-six were positive for fungus on culture while forty-four samples did not grow on culture. Out of fifty-six fungal cultures, nineteen were of dermatophytes. In a study conducted by Deeparsi et al.[6] (11.59%) cases of dermatophytosis were positive on culture. Similarly, other studies from Karachi and Bangladesh reported 26 (20.97%) and 31 (38.75%) cases of dermatophytes positive on culture respectively.[11,12]

According to another study conducted by Majeed et al.[13] a total of one hundred and fifty suspected cases of dermatophytosis were studied, out of which 99 (66%) cases were positive by direct microscopy while only 74 (49.33%) were positive by culture. Pandit and Mehta found 32.1% positive cases on culture out of n = 530 patients with clinically diagnosed dermatophytoses.[14] Mahajan et al.[15] found culture positivity in (52.4%) cases among n = 265.

The explanation of inconsistency in the outcome of microscopic results and culture are the non-viability of dermatophytes in the clinical specimen during inoculation and use of conventional remedies by some of the patients. On the other hand, samples with negative microscopic results but positive cultures can be explained by the fact that the fungus might be in an inactive sporulating phase of sporulation that might have been difficult to detect by direct microscopy but can grow into molds when inoculated on culture media.[12]

The objective of this study was to compare DTM as a screening medium in comparison with SDA to have a better idea of the efficacy of DTM for early identification and isolation of dermatophytes. SDA is the most used culture medium for the primary isolation of dermatophyte species.[16] DTM is an alternative selective medium which tends to change color within 3-7 days on growth of dermatophytes.[6]

In the present study, 71.43% of the dermatophyte species were isolated on DTM in the first 10 days. Average number of days taken by Trichophyton rubrum to grow on DTM was 7.6 days as compared to 14 days taken by SDA (Fig.1). The current study agrees with the findings of Rao et al.[7] in which 4.8% of dermatophytes were isolated on dermatophyte test medium in the first week of incubation.

In present study, the effectiveness of DTM in isolating dermatophyte species was 73.68% as compared to 52.6% for SDA. These results are similar to a study conducted by Majeed et al.[13] in which the effectiveness of SDA was 91.8% as compared to 97.3% for DTM. Rao et al.[7] reported DTM as 100% effective in isolation of dermatophytes. Overall comparison between culture examination of the clinical specimens by SDA and DTM showed a significantly higher positivity ($P < 0.05$) for isolating dermatophytes by DTM as compared to SDA.

The current study found DTM to be more efficient in the primary isolation of dermatophyte species while comparing to SDA and also enabled the laboratories to convey reports earlier to the dermatologist. Nevertheless, the benefit of using SDA as primary culture medium cannot be overlooked as the colonial morphological characteristics i.e., color, texture and topography can only be seen on it. It is therefore proposed that DTM be used together with SDA for the earlier isolation and identification of dermatophytes from fungal cultures.

CONCLUSION

The early laboratory diagnosis of dermatophytoses is desirable so that proper treatment can be started without delay. The use of DTM is more effective and time saving in the isolation of dermatophyte species. DTM can be used and interpreted very easily in simple laboratory setups in Pakistan.

LIMITATIONS OF THE STUDY

This study may be supplemented with larger sample size in order to strengthen the conclusions.

ACKNOWLEDGEMENT

The authors wish to acknowledge the kind guidance provided by Professor Dr. Saeed Anwar, Department of Pathology, CMH Lahore and Professor Dr. Atif Kazmi, Head of Dermatology Department, Mayo Hospital, Lahore.

CONFLICT OF INTEREST

None to declare.

GRANT SUPPORT & FINANCIAL DISCLOSURE

None to disclose.

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Author's Contribution

MRA: Conception of study, acquisition of data, interpretation of data and drafting the article.

IJ: Supervisor, design and concept of study, acquisition, analysis and interpretation of data.

SM: Co-Supervisor, analysis, and interpretation of data for the research work, drafting the paper.

RH: Concept and design of the work.

KHC: Critical revision of article for important intellectual content.

ALL AUTHORS: Approval of the final version of the manuscript to be published.