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Original Research Article

Comparative Study of Different Microscopic Techniques and Culture Media for Identification and Isolation of Dermatophytes

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Abstract

Introduction: Dermatophytes are the most common fungi causing cutaneous fungal infections. There prevalence is common in tropical areas. Rapid and efficient diagnosis is necessary for early treatment. Antifungal susceptibility testing is also in need as there is increase in azole resistance.

Materials and Methods: Skin, hair and nail samples are collected in aseptic conditions. Microscopy is done using KOH and Calcofluor. Samples are inoculated in Sabouraud's Dextrose agar (SDA), SDA with Chloramphenicol, SDA with Actidione (SDA+A) and Dermatophyte Test Medium (DTM). Later dermatophytes are identified by tease mount, hair perforation test and urease test. Antifungal susceptibility testing is done by disc diffusion for azoles.

Results: Out of 100 samples, 55 were positive by both KOH and culture. 44 were positive by only culture. Out of 44, 32 were dermatophytes and 12 were non dermatophytes. On SDA, 21 dermatophytes and 12 non dermatophytes were grown, whereas on SDA with Actidione only dermatophytes were grown. Trichophyton mentagrophytes (37.5%) was the most common isolate. Clotrimazole is the most susceptible antifungal drug.

Conclusion: Calcofluor White staining is best for identification of fungal elements in direct microscopy than KOH, as it is rapid and causes less eye strain. Sabouraud's Dextrose agar with Actidione is the best medium for isolation of dermatophytes as isolation rate is more and it prevents the growth of non dermatophytic fungi. Clotrimazole was the best drug against dermatophytes.

Keywords: Dermatophyte, Calcofluor White, Sabouraud's Dextrose agar with Actidione, Hair Perforation Test.

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Introduction

The prevalence superficial fungal infections vary in different parts of the world, but in many tropical countries they are the most common cause of skin disease. [1] Dermatophytosis is the most common type of cutaneous fungal infection seen in man and animals. [2] They cause infection of the superficial keratinized horny layer of the skin, the hair and the nails. They do not cause systemic infection. [3] Evolutionary development toward an accommodating host-parasite relationship can be seen among the dermatophytes which is absent among other fungal agents of human disease. [4] The clinical features of dermatophyte infections result from a combination of keratin destruction and an inflammatory host response. [5]

Direct microscopy with KOH is an efficient screening technique. Calcofluor white is used in the paper, textile and related industries as agents to whiten and to prevent "yellowing" of papers and fabrics. [6] This stain can be used in fluorescent microscopy for rapid identification of fungal elements. Sabouraud's dextrose agar is the common medium used for isolation. Addition of Chloramphenicol and Cycloheximide to it, makes it more selective by inhibiting bacteria and non dermatophytic moulds. Dermatophyte test media (DTM) was developed during Vietnam War and it is used routinely as a common media for recovery and identification of dermatophytes from clinical specimens depending on colour change into pink.

In 1980s, discovery of azole had significant impact in the treatment. [2] Tests designed to ascertain the minimal amount of drug needed to inhibit the growth of fungal strains in culture (minimum inhibitory concentration or MIC) are generally used to determine the relative effectiveness of different antifungal agents and to detect the development of drug-resistant organisms. [7] The CLSI

subcommittee on Antifungal Susceptibility Testing has developed reference methods for susceptibility testing of moulds (CLSI M38-A document). [8] Dilution tests are widely used in macro- and micro-assays, but these methods are difficult to be used in most laboratories. The agar-based disk diffusion susceptibility method for dermatophytes is simple, inexpensive, and does not require specialized equipment. The disk diffusion method has a good correlation with the reference dilution assay. [9,10,11,12]

The present study was conducted to evaluate different microscopic techniques for identification and different culture media for isolation of dermatophytes. Antifungal susceptibility was also done by a cost-effective disc diffusion method against the azoles.

Materials and Methods

The present study has been carried out on 100 clinically diagnosed cases of dermatophytosis in all age groups and of both sexes, attending the outpatient department of Dermatology, Venereology and Leprosy at Government General Hospital, Kakinada from July 2024 to June 2025

Inclusion Criteria: All skin, hair and nail samples from clinically suspected cases of dermatophytosis.

Exclusion Criteria: Cases of dermatophytosis with secondary bacterial infection. All relevant details like age, sex, occupation, duration of presenting complaints and site of involvement were taken.

Methods [2,13,14,15,16]

Specimen Collection - From the Skin: The affected area was first thoroughly swabbed with 70% alcohol and was allowed to dry. The scrapings were collected from the border of the lesions with a sterile scalpel blade in a sterile black paper.

From the scalp: Hair from the scalp were epilated with a flame sterilized forceps and the active border area was scraped with a scalpel to collect epidermal scales into a sterile black paper.

From the nail: The affected nail was first cleaned with 70% alcohol. The upper portion of the infected nail was scraped away and debris were collected from beneath the nail on to sterile black paper.

Direct Microscopic Examination: Specimen collected was subjected to potassium-hydroxide (KOH) wet preparation of various concentrations (10% and 40%). The fungal elements appear as highly refractile, hyaline septate branching filaments. [Figure 1]. Specimen was also subjected Calcofluor White stain and was observed under fluorescent microscope. The fungal elements show apple green fluorescence. [Figure 2]

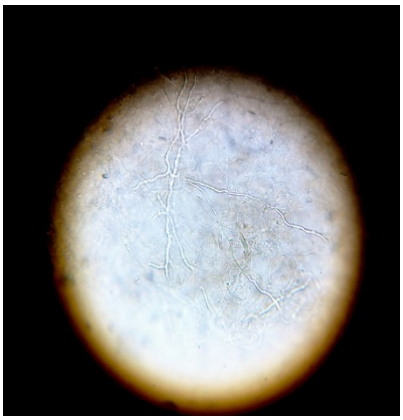

Figure 1: Septate fungal hyphae

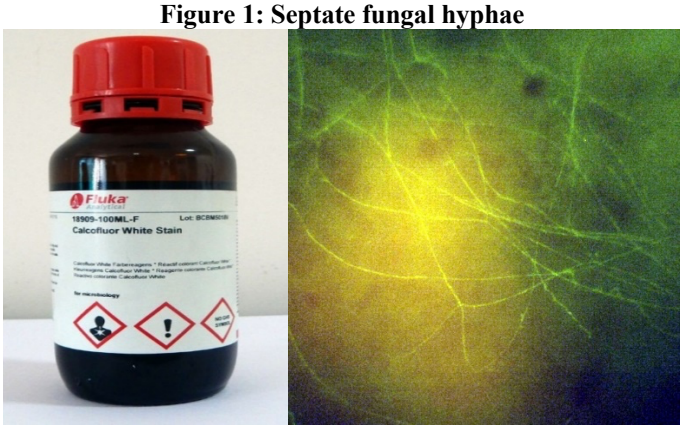

Figure 2: Calcofluor White Stain

Culture: Irrespective of demonstration of fungal elements, the specimen was inoculated onto three sets of test tubes, one containing Sabouraud's dextrose agar with 0.05% Chloramphenicol, Sabouraud's dextrose agar with 0.05% Chloramphenicol and 0.5% Cycloheximide and the other on Dermatophyte test medium.

Tubes were incubated at 28°C for up to four weeks, and was observed periodically for growth. If no growth was found after four weeks, it was taken as negative for the growth of fungi. Dermatophyte test medium was also incubated at 28°C and was observed for colour change. [Figures 3, 4]. If growth was obtained on any test tube, identification was made based on colony morphology, pigmentation, growth rate, microscopy (LPCB) for the presence of hyphae, macroconidia, microconidia and other accessory structures of vegetative hyphae [Figures 5, 6, 7, 8], slide culture [Figure 9], urease test and hair perforation test. The isolates were inoculated on potato dextrose agar for better condition.

Figure 3: Dermatophyte test medium – uninnoculated media and media with growth and colour change.

Figure 4: Growth of Aspergillus Niger on DTM

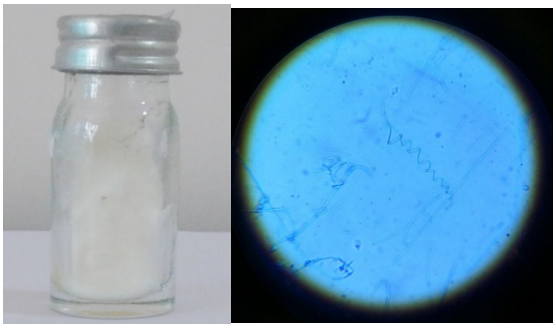

Figure 5: Trichophyton mentagrophytes

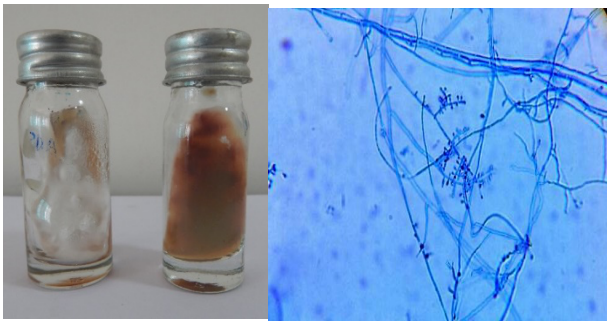

Figure 6: Trichophyton rubrum

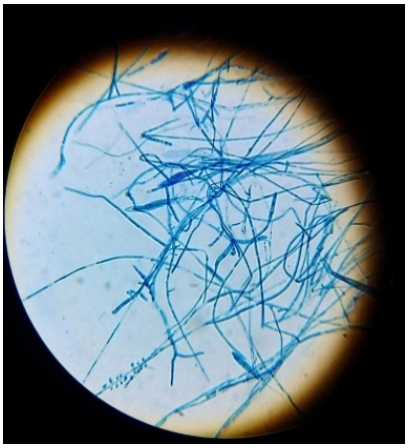

Figure 7: Microsporum gypseum

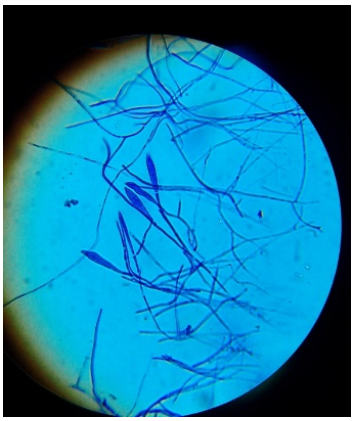

Figure 8: Epidermophyton

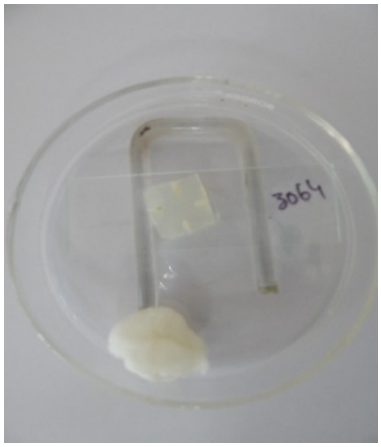

Figure 9: Slide Culture

Antifungal Susceptibility Testing For Dermatophytes

Disc diffusion method: [9,10,11,12,17,18,19,20] Agar based disc diffusion susceptibility method was performed using the antifungal agents such as Fluconazole, Miconazole, Clotrimazole and Ketoconazole. Commercially available discs from HI Media Laboratories, preloaded with Fluconazole (25µ g/disk), Clotrimazole (10µg/disk), Ketoconazole (15µg/disk), and Miconazole (10µg/disk) were used.

Inoculum Preparation: The isolates were sub cultured onto potato dextrose agar to enhance sporulation. Seven day old cultures were covered with 1ml of distilled water and colonies were probed with tip of a sterile Pasteur pipette to obtain a mixture mycelium and conidia.

The suspensions were transferred to sterile tubes and allowed to sediment for 30 minutes and then adjusted with spectrophotometer set at 65% transmittance and 530nm.

Test Procedure: The inoculum was evenly spread on the surface of Petri dishes containing Sabouraud's dextrose agar medium and exposed to air dry. Then, the antifungal disks were applied to the plates, after which the plates were incubated at 25°C for 5-10 days. After the colonies grew, the zones of inhibition around the disks were measured and recorded.[Figure 10]

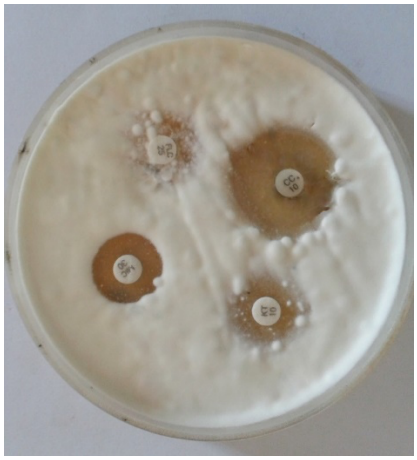

Figure 10: Antifungal Susceptibility Testing – Disc Diffusion

Criteria of susceptibility and resistance of antifungal disks

Antifungal drugs Potency Zone diameter in mm

	S	I	R
Clotrimazole 10 µg	≥20	19-12	≤11
Fluconazole 25 µg	≥22	21-15	≤14
Ketoconazole 15 µg	≥30	29-23	≤22
Miconazole 10 µg	≥20	19-12	≤11

Results

Out of 100 clinical cases of dermatophytosis, skin scrapings were the predominant clinical sample. Males were more commonly effected (62 %) than females (38 %). Highest incidence of cases were between 21 – 30 years age group with 45 %. 55 samples were positive by both KOH and Calcofluor White stain and 44 were positive by culture only.

In culture positive samples, 32 were dermatophytes and 12 were non dermatophytes. Out of 100 samples, 44 were both KOH and culture positive, 45 were both KOH and culture negative.11 were KOH positive and culture negative. [Table 1]

Table 1: Comparison between Direct Microscopy and Culture (N = 100)

KOH	Culture	No. of cases	Percentage
Positive	Positive	44	44%
Negative	Negative	45	45%
Positive	Negative	11	11%

On SDA, 21 dermatophytes and 12 non dermatophytes were isolated. On SDA with Cycloheximide, only dermatophytes were isolated which were 32. On Dermatophyte test medium, 30 dermatophytes and 5 non dermatophytes were isolated. [Table 2]

Table 2: Comparison of Growth on Different Media

	SDA	SDA + A	DTM
Dermatophytes	21	32	30
Non-Dermatophytes	12	0	5
Total	33	32	35

Out of 32 isolated dermatophytes, majority were T. mentagrophytes (37.5 %), followed by T. rubrum (28.1 %), T. verrucosum (9.4 %), M. gypseum (9.4 %), T. tonsurans (6.25 %), E. floccosum (6.25 %) and T. violaceum (3.1 %). [Table 3]. Non dermatophytes isolated were Aspergillus Niger (4), Curvularia (2), Rhizopus (2), and Aspergillus flavus (1), Scopulariopsis (1), Chetomium (1) and Mucor (1). [Table 4]

Table 3: Frequency of Various Species of Dermatophytes [N=32]

Dermatophyte Species	Number	Percentage
T. mentagrophytes	12	37.5 %
T. rubrum	9	28.1 %
T. verrucosum	3	9.4 %
M. gypseum	3	9.4 %
T. tonsurans	2	6.25 %
E. floccosum	2	6.25 %
T. violaceum	1	3.1 %
Total	**32**	**100**

Table 4: Non-Dermatophytes Isolated

Isolate	Number
Aspergillus niger	4
Curvularia	2
Rhizopus	2
Aspergillus flavus	1
Scopulariopsis	1
Chetomium	1
Mucor	1
Total	12

Antifungal susceptibility was done for 31 isolates out of the 32 dermatophytes isolated. [Table V].This could not be done for T. violaceum as it did not grow on subculture. Majority of the isolates were sensitive to Clotrimazole (96.8 %), followed by Ketoconazole (77.4 %) and Miconazole (74.2 %). Most of the samples were resistant to Fluconazole (54.8 %).

Table 5: Anti-Fungal Susceptibility N = 31

Drug	Sensitive	Intermediate	Resistant
Clotrimazole	30 (96.8%)	1 (3.2%)	0
Miconazole	23 (74.2%)	7 (22.6%)	1 (3.2%)
Ketoconazole	24 (77.4%)	6 (19.4%)	1 (3.2%)
Fluconazole	10 (32.2%)	4 (13%)	17 (54.8%)

Discussion

This study was undertaken to compare different microscopic methods like KOH and Calcofluor White staining for identification of dermatophytes and compare different culture media for isolation and identification of dermatophytes. Out of 100 samples 75 were skin scrapings, 20 were nail clippings and 5 were hair samples. Out of these samples, dermatophytes were isolated in 32 cases. Among 32 dermatophytes isolated, T. mentagrophytes was the commonest species 12 (37.5%) followed by T. rubrum 9 (28.1%), M. gypseum 3 (9.4%), T.verrucosum 3 (9.4%), E. floccosum 2 (6.25%), T. tonsurans 2 (6.25%) and T. violaceum 1 (3.1%). The overall isolation rate of dermatophytes was 32 %. The age group commonly affected was 21-30 years, males are affected more than females. In the present study out of 100 samples, 62 % were males and 38 % were females which correlates with Sadiqa et al (2024) and Tasmin et al (2017). Male predominance could be due to increased outdoor physical activities and increased opportunity for exposure to infection than females. Another reason is males may visit the hospital to a greater for dermatological infections.21, 22 In the present study the common age group was 21 – 30 years which correlates with studies done by Tasmin et al (2017), Nikitha et al (2023) and Swetha et al (2020).

The higher occurrence in the age group of 20-40 years could be due to greater physical activity with increased sweating and increased opportunity for exposure. Out of 100 samples, 55 % were positive by direct microscopy using KOH mount which correlates with study done by Nikitha et al (2023). In the present study both KOH and Calcofluor showed 55 % positives. So, according to this study both KOH and Calcofluor are equally useful, but Calcofluor White can be used for rapid diagnosis and less eye strain. But its cost and need for fluorescent microscope are the limitations. In the present study, culture was positive in 44 % and out of these 32 % were dermatophytes and rest were non dermatophytes. This correlates with the study done by Swetha et al (2020). The isolation rate of dermatophytes on SDA with actidione is 100 % and on Dermatophyte test medium is 93.75 % which correlates with studies done by Sadiqa et al (2024). Out of 32 isolates, two isolates did not grow on DTM, but grew on SDA with actidione. One isolate of T. rubrum and one isolate of T. violaceum did not grew on DTM. False positive results were obtained in DTM due to the colour change produced by the growth of non-dermatophytes such as Aspergillus sp., Curvularia sp. which was similar to study done by Salkin et al which showed colour change in several non-dermatophytes. [23] The most common dermatophyte isolated in this study was T. mentagrophytes (37.5 %) which correlates with the study done by Nikitha et al (2023). On SDA, 21 dermatophytes and 12 non dermatophytes were isolated. On SDA with actidione, only dermatophytes were isolated which were 32. On Dermatophyte test medium, 30 dermatophytes and 5 non dermatophytes were isolated. SDA with actidione inhibited the growth of non dermatophytic fungi and the isolation rate was 100%. Therefore SDA with actidione is the best medium for isolation of dermatophytes.

Anti-fungal susceptibility against dermatophytes by disc diffusion: Shalini Gupta et al (2015), showed good correlation between MIC and disc diffusion. It was seen that if MIC was low for a particular isolate a larger zone of inhibition was seen. [12] Nweze et al. (2010), Esteban et al (2005) and Venugopal and Venugopal et al (1995) have also reported good correlation between MIC and inhibitory zone diameter. In the present study, Clotrimazole was 96.8 % sensitive, 3.2 % intermediate and 0 % resistant, Miconazole is 74.2 % sensitive, 22.6 % intermediate and 3.2 % resistant, Ketoconazole sensitivity was 77.4 %, Fluconazole sensitivity was 32.2 % and resistance was 54.8 %. Although there was significant difference in the percentage of Fluconazole resistance when compared to other studies, it was the most common resistant drug in all the studies. This is perhaps because fluconazole is a triazole, and Sabouraud dextrose agar has components that can interfere with the test. [20]

Conclusion

Calcofluor White staining is best for identification of fungal elements in direct microscopy than KOH, as it is rapid and causes less eye strain. Sabouraud's Dextrose agar with Actidione is the best medium for isolation of dermatophytes as isolation rate is more and it prevents the growth of non dermatophytic fungi. Although colour change occurs in Dermatophyte Test medium for easy identification dermatophyte growth, this can also be observed after the growth of some non dermatophytic fungi like Aspergillus species. Antifungal susceptibility was done by disc diffusion method against azoles. As MIC determination by broth dilution method is costly and difficult in many labs, disc diffusion can be used in resource constrained labs. Clotrimazole was the best drug against dermatophytes and Fluconazole was least useful.

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