ORIGINAL ARTICLE

Diagnostic Performance of Direct Microscopic Techniques and Culture Media for Fungal Isolates from Hair Specimens

Faiza Zeeshan[1*], Fakhar Uddin[2], Suresh Kumar[1], Shahida Kashif[3], Nazish Jaffar[1]

ABSTRACT

Objective: To compare the diagnostic performance of direct microscopic techniques and culture media for detecting fungal isolates in hair specimens.

Study Design: Cross-sectional study.

Place and Duration of Study: The study was conducted at the Department of Microbiology, Basic Medical Sciences Institute, Karachi, Pakistan, from January 2021 to June 2021.

Materials and Methods: A total of 207 hair specimens were collected from the patients with superficial mycoses of the scalp. The specimens were collected under aseptic techniques using clean glass slides and forceps which were processed for Potassium hydroxide mount and Calcofluor white staining. The mycological cultures were performed on a Dermatophyte test medium and Sabouraud Dextrose agar with and without antibiotics. The data was recorded and analyzed on SPSS version 21. The frequencies, percentages, means, and ratios were calculated using Descriptive statistics. The association between variables were analyzed by the Chi-square test and kappa index.

Results: Potassium hydroxide mount revealed fungal spores and hyphae in 154(74.3%) specimens, while Calcofluor white staining showed fungal elements in 179(86.5%) specimens. Out of 207 specimens, 106(51.2%) were culture positive and 101(48.8%) were declared culture negative. Out of culture-positive specimens, dermatophytes were yielded in 61(57.5%) and non-dermatophytes in 45(42.5%) specimens. Among all culture media, the highest number of dermatophytes was yielded by plain Sabouraud dextrose agar (59; 96.5%), followed by dermatophyte test medium (55; 90%), and Sabouraud dextrose agar with antibiotics (54; 90.1%). On the contrary, the primary isolation of non-dermatophytes on the plain was Sabouraud dextrose agar s 32(71.1%), Sabouraud dextrose agar with antibiotics 31(68.2%), and dermatophyte test medium 13(28.8%).

Conclusion: In our study, we found Calcofluor white staining technique as the most sensitive method to detect fungal elements from the given hair specimens. For mycological culture, all tested culture media were equally effective for the retrieval of dermatophytes. However, for non-dermatophytes, plain SDA and SDA with antibiotics were found to be better media for isolation.

Keywords: *Antimicrobial Agents, Culture Media Dermatomycoses, Fungal Spores, Hyphae.*

How to cite this: *Zeeshan F, Uddin F, Kumar S, Kashif S, Jaffar N. Diagnostic Performance of Direct Microscopic Techniques and Culture Media for Fungal Isolates from Hair Specimens. 2023; 4(3): 236-241. doi: http://doi.org/10.37185/LnS.1.1.316*

[1]*Department of Pathology*
Jinnah Sindh Medical University, Karachi, Pakistan
[2]*Department of Microbiology*
Basic Medical Sciences Institute, Karachi, Pakistan
[3]*Department of Pathology*
Liaquat College of Medicine and Dentistry, Karachi, Pakistan

Correspondence:
Dr. Faiza Zeeshan
Lecturer, Pathology
Jinnah Sindh Medical University, Karachi, Pakistan
E-mail: dr.faizazeeshan@gmail.com

Introduction

Fungal diseases constitute a massive burden among morbidities worldwide. It is estimated that over one billion people suffer from fungal diseases annually.[1] The diagnosis of cutaneous fungal infections can be made clinically; however, due to rising cases of resistant and recalcitrant strains of fungal pathogens, the importance of laboratory diagnosis has escalated exponentially. Direct microscopic techniques have an important diagnostic role as they

Funding Source: NIL; Conflict of Interest: NIL
Received: Nov 24, 2022; Revised: Apr 13, 2023
Accepted: Jun 10, 2023

provide an immediate clue for the presence or absence of fungal components in a given dermatological sample and different sterile fluids. The commonest techniques are 10% potassium hydroxide mount, Gram staining, India ink preparation, Calcofluor white staining, and partial acid-fast staining.[2] These techniques are simple, time-saving, and cost-effective. The potassium hydroxide mount is one of the most useful methods to detect fungal elements. In this technique, a 10% potassium hydroxide solution is used to detect fungal hyphae and spores at high power magnification lens and is ideal for skin, hair, and nail specimens. This method possesses 75% to 85% sensitivity. On the other hand, Calcofluor white staining provides much higher sensitivity.[3] It requires a fluorescent microscope with emission filters of 320-380 nm and excitation filters >480 nm. The stain binds with the chitin of the fungal cell wall and fluoresces. India ink preparations are ideal for the identification of capsulated Cryptococcus species from cerebrospinal fluid, tracheal and bronchial aspirates. This negative staining technique provides sufficient visualization of capsules of Cryptococcus against a dark background. Gram staining and partial acid-fast stains are competent enough to identify branching filaments of Nocardia and Actinomyces.[4]

Various culture media have been used for mycological practices in the laboratory, including Sabouraud dextrose agar (SDA), potato dextrose agar (PDA), Mycosel agar, inhibitory mold agar, dermatophyte test medium (DTM) and chormagar. Incorporating cycloheximide, chloramphenicol, tetracycline, and gentamicin, potentiates pure fungal yielding.[4] Certain culture media are diagnostic for various fungal species; the classic example is Trichophyton agar which provides an unambiguous diagnosis of Trichophyton species. Similarly, on chromogenic BIGGY agar, the pigmented colonies of various candidal species give diagnostic clues usually, a battery of media is inoculated, including an enriched media, a selective media, one differential medium, and one media for sporulation.[5] However, inoculation on several media can be laborious, time-consuming, and uneconomical for routine testing. Therefore, this study is designed to provide ins and outs of the diagnostic performance of different culture media along with direct microscopic techniques, to provide a rapid and authentic diagnosis of fungal species.

Materials and Methods

This cross-sectional study was conducted in the Department of Microbiology, Basic Medical Sciences Institute, Karachi, Pakistan, from January 2021 to June 2021 after getting approval from the Institutional review board (F.2-81/2019-GENL/33047/JPMC). A total of 207 hair samples were collected from the patients with superficial mycoses of the scalp from the dermatology outpatient department. The samples were collected after following an aseptic protocol with the help of sterilized forceps and glass slides. The hair shafts and scales were packaged in filter paper and labeled according to their specified case number. Direct microscopy was performed by 10% potassium hydroxide (KOH) wet mount and Calcofluor white (CFW) staining. To perform the KOH wet mount, about 1ml of KOH was placed on the slide, and hair shafts were added. The slide was coverslipped and allowed to stand for 3-5 minutes. The specimens were observed under low and high magnification power to visualize fungal hyphae and spores. The endothrix, exothrix, favus, and mixed pattern of hair invasion were observed. For Calcofluor white staining, one drop of CFW stain along with one drop of 10% KOH was placed on a glass slide. The specimens were observed after 5 minutes under the fluorescence microscope (Nikon, Japan). The fungal elements emitted apple green fluorescence at the emission filter 340nm and excitation filter 420nm. Different patterns of hair invasion were observed.

The specimens were cultured on dermatophyte test medium (DTM, Thermo Fisher, USA), and Sabouraud dextrose agar (SDA, Thermo Fisher, USA) with and without antibiotics. In the dermatophyte test medium, cycloheximide 500mg, chlortetracycline 100mg, and gentamicin 100mg were incorporated. In Sabouraud dextrose agar, cycloheximide 500mg and chloramphenicol 50mg were added to inhibit the growth of saprobes and bacteria, respectively. Two vials of each medium were incubated at 25° C and

35 C for four weeks. The vials were checked daily for the first week, and then periodic checking was made for the next three weeks to observe colonial morphology, pigmentation, and pigmentation on the reverse.[6] The arrangement of hyphae and spores was observed using a Lactophenol cotton blue (LPCB) mount. The species were identified by LPCB staining and biochemical testing (urease test).[7]

Results

Potassium hydroxide mount revealed fungal spores and hyphae in 154(74.3%) specimens, out of them, 98(63.6%) showed endothrix pattern followed by ectothrix in 30(19.4%), mixed in 18(11.8%) and favus in 8(5.2%) hair samples (figure 1). The Calcofluor white staining (CFW) technique was found to be more sensitive in detecting fungal elements in given samples, provided that 179(86.5%) samples yielded fungal spores and hyphae (*p-value=0.000*). The most common patterns of hair invasion on CFW staining were endothrix (98; 54.7%), ectothrix (55; 30.7%), and mixed (26; 14.5%). Out of 207 specimens, 106(51.3%) were culture positive while 101(48.8%) were declared culture negative. Table 1 shows the fungal culture and direct microscopic results, sensitivities, and specificities of the staining techniques relative to the culture results. In the current study, the mycological culture was considered the gold standard; on account of this, the CFW staining technique (97.2%) was found to be more sensitive than the KOH mount (84.9%). The kappa index statistics (0.415) showed a moderate degree of agreement between the findings of both direct microscopic techniques.

Table 1: Comparison of Direct mi croscopic techniques relative to culture

	Potassium Hydroxide mount (KOH)		Calcofluor white staining (CFW)	
Fungal culture	**Positive (n)**	**Negative (n)**	**Positive (n)**	**Negative (n)**
Positive	90	16	103	03
Negative	64	37	76	25
Sensitivity (%)	84.9		97.2	
Specificity (%)	36.6		24.8	
Kappa index	0.415*			

*indicates moderate agreement between the results of KOH mount and CFW staining

The culture media were also tested for viable growth of different fungal species. Out of culture-positive specimens, dermatophytes were yielded in 61(57.5%) and non-dermatophytes in 45(42.5%) specimens (Table 2). Among all culture media, the highest number of dermatophytes was yielded by plain SDA (59; 96.5%), followed by DTM (55; 90%), and SDA with antibiotics (54; 90.1%). On the contrary, the primary isolation of non-dermatophytes on plain SDA was 32; 71.1% SDA with antibiotics 31; 68.2% and DTM 13; 28.8%. *T. violaceum* was the most common isolated dermatophyte in our study (21;10.1%). Plain SDA was positive for 20 isolates of *T. violaceum*, SDA with antibiotics showed the growth of 19 isolates, and on DTM, 17 isolates of *T. violaceum* grew well (Table 3). Among non-dermatophytes, the most common isolated fungus was *Aspergillus spp* (27;13%). SDA with antibiotics was able to retrieve 21 *Aspergillus spp*, followed by plain SDA (19), and on DTM, 9 species of *Aspergillus* were isolated. The rest of the details are shown in Table 2.

Discussion

Dermatophytes are the keratinolytic fungi which are the etiologic agent of tinea. They can be detected in clinical specimens by direct microscopic techniques. The diagnostic performance of direct microscopic techniques was compared in the current study. According to our findings, Calcofluor white staining was found to be the most sensitive diagnostic method for the detection of fungal elements from specimens, in comparison with the potassium hydroxide mount method. Mourad et al. and and Dass et al. demonstrated similar findings in their study.[8,9] The dermatophytes tend to invade hair follicles by releasing several proteolytic enzymes. These enzymes degrade the keratin and give the pathogen access to the hair shaft. The current study highlighted the different patterns of hair invasion by dermatophytes and non-dermatophytes. The most

Table 2: Frequency of fungal isolates retrieved from the culture -positive specimens (n =106)

Isolated species	Frequency (n)	Percentage (%)
Alternaria	04	3.8
Aspergillus	27	25.7
Candida	02	1.9
Curvularia	05	4.8
M.canis	03	2.9
M.gypseum	02	1.9
Mucor	01	1.0
Penicillum	04	3.8
Scopularis	01	1.0
T.mentagrophytes	21	19.0
T.rubrum	04	3.8
T.soudanense	03	2.9
T.tonsurans	08	7.6
T.violaecum	21	20.0

Table 3: Culture results on Dermatophyte Test Medium (DTM), Sabouraud's Dextrose Agar (SDA) with and without antibiotics

Fungal isolates	Sabouraud Dextrose Agar (n)	Sabouraud Dextrose Agar with antibiotics (n)	Dermatophyte Test Medium (n)	*p-value*
T. violaceum	20	19	17	
T. mentagrophytes	20	18	18	
T. tonsurans	08	08	08	
T. soudanense	03	02	03	*>0.05*
T. rubrum	04	03	04	
T. gypseum	01	01	02	
T. canis	01	03	03	
Aspergillus spp	19	21	09	*<0.05*
Alternaria	04	03	01	
Curvularia	03	04	01	
Penicillium	03	00	01	*>0.05*
Mucor	01	01	01	
Candida	01	01	00	
Scopularis	01	01	00	
Total	92	85	68	*>0.05*

common pattern was endothrix which was observed in 131, 63.6% of cases of KOH mount and in 113; 54.7% of cases of CFW staining (figure 1).

The endothrix mode of hair invasion is related to anthropophilic species of genera *Trichophyton.*[10] The findings reported by Attal et al. are in line with our results.[11] As in our study, the primary reported dermatophytes are from genera *Trichophyton*; therefore, the predominance of endothrix pattern was not surprising. The ectothrix pattern of hair invasion is mostly related to *Microsporum species* and *T. verrucosum.*[12]

Various culture media are used for mycological culture. In our study, plain SDA, SDA with antibiotics, and DTM were used. According to our study, out of 61 isolates of dermatophytes, plain SDA retrieved 59;96.7% strains, followed by SDA with antibiotics (54;88.5%) and DTM (55;90.1%), however, there is

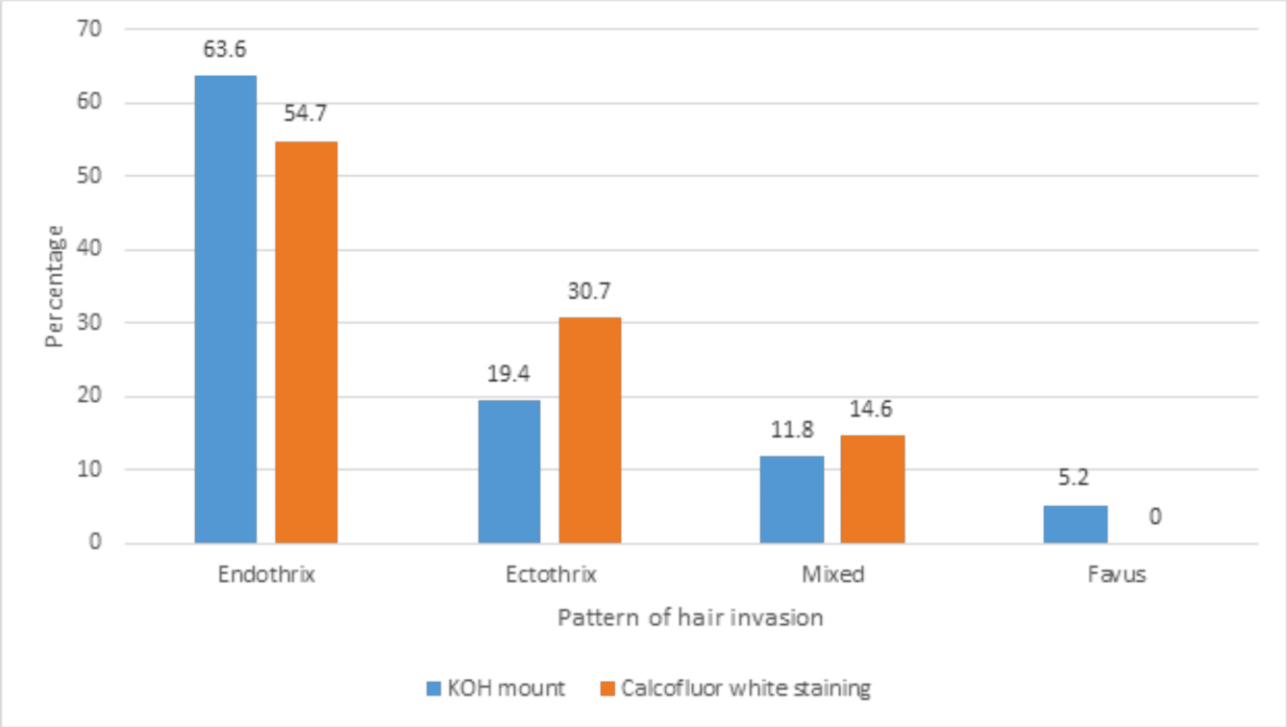

Fig 1: Pattern of hair invasion on direct microscopic techniques

no statistical difference between the performance of three media for the yielding of dermatophytes(*p*-value>0.05). These results are in accordance with Naseemudin et al[13] who also reported no statistical difference between plain SDA and SDA with antibiotics and DTM. However, Alzubaidy et al. and Geetalakshmi et al. reported contradictory results, according to them, dermatophytes did not grow on SDA (*p*-value<0.05), and their growth was significant on DTM.[14-15] Azubaidy et al. reported that 52.2% of dermatophytes had no growth on SDA. Similarly, Geetalakshmi et al. stipulated only 31% positive growth cases on SDA. The reported reasons for the difference in growth rate on SDA and DTM are due to the growth of saprophytic fungi which inhibited the growth of true pathogen.[14-15] This could be associated with inappropriate inoculation of the specimen and a contaminated working environment. Among non-dermatophytes, plain SDA yielded 32; 71.1% isolates, and on SDA with antibiotics, 31; 68.2% specimens were isolated. Similar results were reported by Ali et al.[16] According to them, SDA without antibiotics was the better medium to isolate non-dermatophytes. DTM is an enriched differential media for dermatophytes but in our study 13; 28.8% pure isolates were recovered from DTM. However, there was no statistically significant difference between the growth on plain
SDA and SDA with antibiotics (*p-value>0.05).*

Conclusion

Direct microscopic techniques for fungal isolation carries pivotal importance. The study found that the Calcofluor white staining technique is the most sensitive method to detect fungal elements from the given hair specimens. For mycological culture, all tested culture media were equally effective for the retrieval of dermatophytes. *T. violaceum* was the most commonly isolated species among dermatophytes constituting 20% of all isolated species, while among non-dermatophytes, *Aspergillus* was retrieved from 25.7% of all culture-positive specimens. However, for non-dermatophytes, plain SDA and SDA with antibiotics were found to be better media for isolation.

Limitations

The study was self-funded by the author. Therefore, molecular diagnostics techniques could not be performed.

Acknowledgement

We would like to acknowledge Dr. Bahram Khan from

the Department of Dermatology, Jinnah Postgraduate Medical Centre, Karachi, Pakistan to facilitate sample collection.

REFERENCES

1. Almeida F, Rodrigues ML, Coelho C. The still underestimated problem of fungal diseases worldwide. Frontiers in microbiology. 2019; 10: 214. doi: 10.3389/fmicb.2019.00214
2. Prakash R, Prashanth HV, Ragunatha S, Kapoor M, Anitha TK, Krishnamurthy V. Comparative study of efficacy, rapidity of detection, and cost-effectiveness of potassium hydroxide, calcofluor white, and Chicago sky blue stains in the diagnosis of dermatophytoses. International journal of dermatology. 2016; 55: e172-5. doi: 10.1111/ijd.13037
3. Baddireddy K, Poojary S. A novel contrast stain for the rapid diagnosis of dermatophytoses: A cross-sectional comparative study of Chicago sky blue 6b stain, potassium hydroxide mount, and culture. Indian Journal of Dermatology. 2019; 64: 311. doi: 10.4103/ijd.IJD_401_18
4. Acharya T, Hare J. Sabouraud agar and other fungal growth media. InLaboratory Protocols in Fungal Biology: Current Methods in Fungal Biology. Cham: Springer International Publishing. 2022: 69-86. doi: 10.1007/978-3-030-83749-5
5. Postigo V, Sánchez A, Cabellos JM, Arroyo T. New Approaches for the Fermentation of Beer: Non-Saccharomyces Yeasts from Wine. Fermentation. 2022; 8: 280. doi: 10.3390/fermentation8060280
6. Zafar A, Jabeen K, Farooqi J. (Eds.). (2017). A practical guide and atlas for the diagnosis of fungal infections. Available at: https://ecommons.aku.edu/books/62
7. Larone DH, Walsh TJ, Hayden RT. Larone's Medically Important Fungi: A Guide to Identification. ASM Press; 2018.
8. Mourad B, Ismail M, Hawwam S, Msseha M, Hassan R. Evaluation of the efficacy of fluorescent staining and Chicago sky blue staining as methods for diagnosis of dermatophytosis in hair and nails. Clinical, Cosmetic, and Investigational Dermatology. 2019: 751-8. doi: 10.2147/CCID.S215661
9. Dass SM, Vinayaraj E, Pavavni K, Pallam A, Rao MS. Comparison of KOH, Calcofluor white and fungal culture for diagnosing fungal onychomycosis in an urban teaching hospital, Hyderabad. Indian Journal of Microbiology Research. 2015; 2: 148-53. doi: 10.5958/2394-5478.2015.00004.7
10. Brito-Santos F, Figueiredo-Carvalho MH, Coelho RA, Sales A, Almeida-Paes R. Tinea capitis by Microsporum audouinii: case reports and review of published global literature 2000–2016. Mycopathologia. 2017; 182: 1053-60. doi: 10.1007/s11046-017-0181-1
11. Attal RO, Deotale V, Yadav A. Tinea capitis among primary school children: A clinicomycological study in a rural hospital in central India. International Journal of Current Research and Review. 2017; 9: 25-31. doi: 10.7324/IJCRR.2017.9236
12. Dhurat R, Shukla D, Agrawal S, Chitalia J, Ghate S, Jage M. Tinea capitis presenting as diffuse hair loss and significance of trichoscopy: four case reports. Skin appendage disorders. 2021; 7: 286-91. doi: 10.1159/000513315
13. Nasimuddin S, 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 Journal of Dental and Medical Sciences (IOSR-JDMS). 2014; 13: 68-73.
14. Alzubaidy TS, Mohammed AJ, Al-Gburi AA. Comparison of two conventional methods for identification of dermatophyte fungi. Ibn AL-Haitham Journal for Pure and Applied Sciences. 2018; 31: 21-30. doi: 10.30526/31.2.1958
15. Geethalakshmi V, Jasmine K, John AP, Prathap P. Effectiveness of Sabouraud's Dextrose Agar and Dermatophyte Test Medium in Detection of Candidiasis and Dermatophytosis in Superficial Skin Lesion. Journal of Clinical & Diagnostic Research. 2021; 15: 11-5. doi: 10.7860/JCDR/2021/49630.15252
16. Ali SR, Fradi AJ, Al-Aaraji AM. Comparison between different cultural mediums on the growth of five Aspergillus species. World Journal of Pharmaceutical Research. 2016; 5:9-16. doi: 10.20959/wjpr20168-6623