Dermatophytic Infections and Their Antifungal Susceptibility Pattern in Rajshahi

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Abstract

Background: Dermatophytosis is a common skin disease affecting millions of people worldwide. These infection occur in both healthy and immunocompromised patients. Conventional methods for detecting fungi in clinical specimens are either microscopy or culture. Now a days, resistant to antifungal drugs by the dermatophytes can result in treatment failure. Antifungal susceptibility testing of dermatophytes may help in the management of patients, cases presenting with therapy failure and may help to choose more efficacious antifungal agents. **Objectives:** To detect different dermatophytes and their antifungal susceptibility pattern, and antifungal diagnostic accuracy of microscopy test in comparison of culture. **Methods:** This cross sectional type of descriptive study was conducted among the attending patients at the Department of Microbiology, Rajshahi Medical College and outpatient department of Dermatology and Venereology, Rajshahi Medical College Hospital (RMCH) Different clinical samples (e.g., skin scrapings, nail clipping and hair plucking) were collected under aseptic precautions. A total 171 specimens were collected in this study. The isolation and identification of dermatophytes was performed through microscopic examination using 10% KOH mount, mycological culture and species identification by lactophenol cotton blue mount from positive culture. All dermatophytes isolates were subjected to antifungal susceptibility testing using the agar based disk diffusion method in Mueller Hinton agar media. The sensitivity, specificity and accuracy of microscopy test were calculated in comparison of fungal culture. **Results:** Out of 171 samples, 92 (53.8%%) were positive by direct microscopy with KOH mount microscopy, 106(62%) were positive by culture. Trichophyton rubrum was the predominant dermatophyte species with 76(71.7%) followed by T.mentagrophyte were 15(14.2%). Voriconazole,Clotrimazole and Itraconazole were more effective drugs. Griseofulvin and Fluconazole were the least effective drug. The sensitivity, specificity and accuracy of the KOH mount microscopy were 74.53%, 80.00% and 76.61% respectively. **Conclusion:** Trichophyton rubrum was the commonest dermatophyte species in this study population. Voriconazole, Clotrimazole and Itraconazole were more effective drugs. Antifungal diagnostic accuracy of microscopy test was optimal level specially positive predictive value and specificity. KOH mount microscopy test may be used for the screening and diagnostic purpose because it's

a easy, fast and inexpensive method. When the microscopy test result will not correlate with the clinical findings then physician should be advised for culture.

Key words: Dermatophytes, Antifungal agents, Antifungal susceptibility test.

Introduction

Earth has been documented as a natural territory for fungi which cover individual kingdom with evolution. Among all fungus,- dermatophyte is one of the cutaneous fungus. Dermatophyte, a keratinophilic fungus which can invade kertinized tissue cause dermatophytosis. It is one of the major superficial fungal infection. According to World Health Organization, 20-25 % world population is affected by dermatophytes.[1]

Dermatophytes are taxonomically classified into three genera: Epidermophyton, Microsporum, and Trichophyton. These species are also classified as anthropophilic, geophilic, zoophilic according to their habitat.[2] Prevalance of dermatophyte infection in Bangladesh 37.4%, in India 69.8%, in North America, East Asia and Europe prevalence rate ranging from 14-26.8%.[3,4]

Dermatophytosis can not be easily diagnosed on the basis of clinical manifestation as a number of other conditions mimic the clinical presentation. The differential diagnosis of dermatophytosis includes seborrheic dermatitis,atopic dermatitis, contact dermatitis, psoriasis, eczema etc. Further it is more difficult to diagnose dermatophytosis in immunocompromised patients as clinical presentation is often atypical.[5]

The diagnosis of a dermatophytic infection is mostly done clinically, but often confused with other skin infections due to topical application of steroid ointments and creams, leading to further misdiagnosis and mismanagement. Hence there arises the need for the correct, efficient, and rapid laboratory diagnosis of dermatophytes. The laboratory identification of dermatophytes is typically based on the macroscopic observation of colony morphology (pigmentation, growth rate, texture,etc) grown on selective media, followed by a microscopic examination of conidia.[6] Fungal culture and light microscopic mycological examination are required for identification of dermatophyte infections phenotypically. Microscopic examination is easy, rapid, and inexpensive diagnostic test but it may show high false negative. Culture is a gold standard to identify fungi. While culture of species takes approximately 4 weeks for the growth of the fungus.[1] Sabouraud Chloramphenicol Agar is used for selective cultivation of Yeasts and Moulds. This medium was described originally by Sabouraud for the cultivation of fungi, particularly useful for the fungi associated with skin infections. The medium is often used with antibiotics such as chloramphenicol for the isolation of pathogenic fungi from materials containing large numbers of fungi or bacteria. Incubation times will vary, from approximately 2 days for the growth of yeast colonies such as Malasezzia, 2 to 4 weeks for growth of dermatophytes.[7]

In Bangladesh, dermatophytes cases 51 (46.5%) were positive and common isolates were Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans and Microsporum canis distributed as 86.5%, 6.5%, 4.5% and 2.5% respectively.[8]

There are many antifungal drugs that are used to treat dermatophytosis. However ,not all species of dermatophytes have the same susceptibility pattern and relative or absolute

resistance may occur. The reason of treatment failure and development of resistance is attributed to decreased drug uptake, phenotypic or genotypic alterations or increased in drug efflux.[9]

So, the aims of this study were to isolate and identify the different species of dermatophytes by mycological culture and susceptibility of antifungal drugs by agar based disc diffusion method and to evaluate the diagnostic accuracy of the KOH mount microscopy test to detect dermatophytes considering the mycological culture is the gold standard for diagnosis of fungal infection.

Methods

A cross sectional type of descriptive study was conducted among the attending patients at the Department of Microbiology, Rajshahi Medical College (RMC) and outpatient department of Dermatology and Venereology, Rajshahi Medical College Hospital (RMCH) from January to December 2019. A total of 171 samples were collected from the attending patients in aseptic precautions from infected areas such as skin, nail and hair. Among 171 specimens, 112 were skin, 39 were nail and 20 were hair and scalp. Specimens were processed at department of Microbiology for direct microscopic examination and fungal culture as per standard protocol. Culturing of organisms from skin, nail and hair was done on selective medium as Sabouraud's chloramphenicol agar with supplements for identification of dermatophytes species. The identified fungi were subcultured on Potato dextrose agar media to enhance sporulation and processed for drug susceptibility test. Isolation and identification of dermatophytes was done based on macroscopic observation of fungal colonies as well as lactophenol cotton blue mount microscopic examination. Antifungal susceptibility testing was performed after identifying dermatophytesusing Mueller Hinton agar media. Antifungal disks of Clotrimazole, Fluconazole, Miconazole, Itraconazole, Ketoconazole, Voriconazole, Terbinafine and Griseofulvin were used for susceptibility testing.[10,11,12,13] The accuracy of the KOH mount microscopy test to detect dermatophytes was calculated in comparison with the fungal culture (Gold standard). Data were statistically analyzed using SPSS software version 23.

Results

Table I: Detection of dermatophytes by culture from different site of lesion (n=171).

Site of lesion	Culture positive N (%)	Culture negative N (%)	Total N(%)
Skin	73(65.2)	39(34.8)	112 (65.5)
Nail	23(58.9)	16(41.1)	39 (22.8)
Hair	10(50.0)	10(50.0)	20 (11.7)
Total N (%)	106(62.0	65(38.0)	171(100.0)

A total of 171 specimens, 106 (62.0%) were culture positive and the rest 65 (38.0%) were culture negative. Out of 122 skin specimens, 73 (65.2%) were culture positive. In case of nail, culture positive cases were 23(58.9%). Fifty percent of the hair samples were culture positive (Table I).

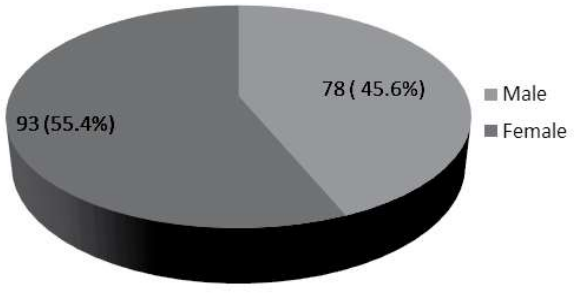

Figure 1: Distribution of study specimens according to gender (N=171)

In the current study, among 171 study specimens, 93(54.4%) were from females and 78(45.6%) were from males, male and female ratio was 1:1.2 (Figure I).

Table II: Correlation between microscopy and culture results in dermatophytosis.

Test Results of KOH mount microscopy	Results of Culture		Total
	Positive N (%)	Negative N (%)	N (%)
Positive	79 (85.9)	13 (14.1)	92 (53.8)
Negative	27 (34.2)	52 (65.8)	79 (46.2)
Total	106 (62.0)	65 (38.0)	171 (100.0)

Out of 171 cases, 106 (62.0%) were diseased persons, i.e., having dermatophytosis and 65 (38.0%) cases were healthy (non diseased), i.e., not having dermatophytosis. Seventy nine (85.9%) cases were positive in both KOH mount microscopy and culture (True positive). Twenty seven (15.8%) cases were positive by culture but negative by KOH mount microscopy (False Negative). Thirteen (14.1%) cases were negative by culture but positive by KOH mount microscopy (False Positive). Fifty two (65.8%) cases were negative in both microscopy and culture (True Negative) (Table II).

Table III: Diagnostic accuracy of KOH mount microscopy test in dermatophytosis.

Statistic	value	95% Confidence Interval
Sensitivity	74.53%	65.14% - 82.49%
Specificity	80.00%	68.23% - 88.90%
Positive Predictive Value (PPV)	85.87%	78.68% - 90.91%
Negative Predictive Value (NPV)	65.82%	57.64 – 73.16%

The diagnostic accuracy indices, i.e., sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the KOH mount microscopy to identify dermatophytes were 74.53%, 80.00%, 85.87% and 65.82% respectively (Table III).

Table IV: Detection of dermatophyte species by lactophenol cotton blue mount microscopy from culture.

Identified species	Skin	Nail	Hair	Total
T. rubrum	52(71.2)	16(69.6)	08(80)	76(71.7)
T.mentagrophyte	11(15.1)	03(13)	01(10)	15(14.2)
E. floccosum	08(11)	04(17.4)	00	12(11.3)
M. canis	02(2.7)	00	01(10)	03(2.8)
Total	73(68.7)	23(21.7)	10(9.4)	106(100)

Table IV: showed isolated dermatophyte species from positive culture by lactophenol cotton blue mount microscopy of different types of clinical lesions. Trichophyton rubrum was predominant dermatophyte 76(71.7%) followed by T.mentagrophyte 15(14.2%), E. floccosum 12(11.3%) and M. canis 03(2.8%).

Table V: Antifungal susceptibility pattern of the isolated dermatophytes species.

Dermatophytes	FLU		ITC		KCA		MCL		VOR		AGF		TRB		CIO	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
T. rubrum n=76	04	72	64	12	55	21	59	17	62	14	03	73	46	30	63	13
T.mentagrophyte n=15	03	12	13	02	11	04	09	06	12	03	01	14	08	07	12	03
E. floccosum n=12	02	10	10	02	09	03	07	05	11	01	01	11	07	05	10	02
M. canis n=3	00	03	03	00	02	01	03	00	03	00	00	03	02	01	03	00

S= Sensitive, R= Resistant

FLU=Fluconazole, ITC=Itraconazole, KCA=Ketoconazole, MCL=Miconazole, VOR=Voriconazole, AGF=Griseofulvin, TRB=Terbinafine, CLO=Clotrimazole

Table V shows susceptibility pattern of antifungal drugs against different species of dermatophytes. Highest sensitivity was shown to Itraconazole (84%), Clotrimazole (83%),Voriconazole (83%) followed by Miconazole (73.6%) and Ketoconazole (72.6%). Highest resistance was shown against Griseofulvin(95.3%) followed by Fluconazole (91.5%).

Discussion

In the present study, 106(62.1%) cases were positive by culture is nearly similar with the studies done by Rao *et al.(2015)*[14] in India and Ahmad *et al.(2010)*[15] in Bangladesh. But dissimilar with the study of Islam *et al.(2018)*[5] and Rahim *et al. (2012)*[16] in Bangladesh. This variation may be due to non-viability of fungal elements in some cases and/or other reasons may be co-existing microbes which may inhibit the growth of pathogenic fungi .

Among 171 study specimens, 78(45.6%) were from male and 93(54.4%) were from female. This study nearly similar with the study of Ghosh *et al.(2009)*[17] and Arammehr *et al.(2020)*[2]. The highest incidence in female may be due to prolonged exposure to water during household works such as exposure to detergents while working as cooking and cleaning. It also may be the body of the females remains covered by wet clothes, which may due to keep the body moist and provides favourable environment for the growth of fungus. But the study of Islam *et al.(2018)*[5] in Bangladesh and Dabas *et al.(2017)*[18] in India showed that prevalence of dermatophytosis was high among males. The higher prevalence amongst males may be due to increased outdoor physical activity, increase sweating and increased opportunity for exposure.

In the present study, out of 171 clinically suspected patients of dermatophytosis, 92(53.8%) cases were positive by direct microscopy with KOH and 106(62.1%) cases were positive by culture. Direct microscopic finding is similar with the other studies done by Niranjan *et al.(2015)*[19] and Dass *et al.(2015)*[20]. But dissimilar with the study of Afshar *et al.(2018)*[21] and Rahman *et al.(2018)*[22]. The negative results of direct microscopic examination may be associated with an inadequate amount and preparation of specimens, skills of observer, a non-suitable temperature of the specimens.

Among the 106 culture positive dermatophytes, Trichophyton rubrum was the commonest (71.7%) isolates followed by Trichophyton mentagrophyte (14.2%). Trichophyton rubrum was found to be the main etiological dermatophyte species responsible for dermatophytosis in the present study which is comparable with the study done by Siddique *et al.(2009)*[23] and Rahim *et al.(2012)*[16] in Bangladesh. But dissimilar with the study of Sharma *et al.(2019)*[24] and Arammehr *et al. (2020)*[2]. This variation may vary depending on the geographical area, social, cultural, environmental and occupational factors.

In this study, eight antifungal drugs named Clotrimazole, Fluconazole, Itraconazole, Miconazole, Ketoconazole, Griseofulvin, Terbinafine and Voriconazole were tested by disc diffusion method against 106 isolates of dermatophytes. Antifungal test results revealed that Clotrimazole, Itraconazole and Voriconazole were the most effective antifungal drugs.

Griseofulvin and Fluconazole the least effective antifungal drugs. This result was comparable to the study done by Agarwal *et al.(2015)*[12], Khatri *et al.(2017)*[25] and Budhiraja *et al.(2018)*[26] in India. According to the study of Alim *et al.(2017)*[27], Rahim *et al.(2011)*[28] and Sabtharishi *et al.(2017)*[29] Griseofulvin and Fluconazole were shown the highest resistance antifungal drugs. Griseofulvin and Fluconazole showed highest resistance because universal usage due to its low cost and dosage and its widespread availability in all level of healthcare centres, which in turn has turned up to increased resistance profile for that drugs.[27]

Culture can be used as a definitive procedure for diagnosis of dermatophytic infection. It is essential that good laboratory methods should be available for rapid and precise identification of the dermatophytes, not only for accurate diagnosis but also for post-therapeutic strategies.[5] There is a need for accurate, reproducible and predictive susceptibility testing of fungal isolates in order to help physicians for choosing of antifungal drugs appropriately. The standard disc diffusion assay can be adapted for assessment of dermatophyte resistance against antifungal drugs.[30]

Findings of diagnostic accuracy analysis in this study suggests, detection of fungus by microscopic examination is less sensitive compared to culture method. But Physicians can be used the KOH microscopy test result for the screening and as well as diagnostic purpose in their clinical practice due to its rapid and cost effective procedure. Because probability that the dermatophytic infections is present more than 85.0% cases when the KOH microscopy test is positive and as well as it ables to identify the healthy individual as healthy in case of 80.0%. When the microscopy test result will not correlate with the clinical findings then physician should be advised for culture.

Ethical approval: Clearance from the Ethical Review Committee of Rajshahi Medical College was taken to carry out this study.

References

1. Khan SA, Shamsuzzaman, SM, Rahman AKMS, Ashekin NAK, Mahmud R, Sharmin R, Siddique MRU, Khan MA, Sultana S, Haque F. Isolation and identification of Dermatophyte causing Dermatophytosisat a Tertiary care Hospital in Bangladesh. Archives of Clinical and Biomedical Research 2021; 5:437-51.
2. Arammehr A, Dehghan P, Chadeganipour M, Katoueezadeh M, Shadzi S. Detection of Dermatophytes from Dermatophytosis-Suspected Cases in Iran, Evaluation of Polymerase Chain Reaction-Sequencing Method. Adv Biomed Res. 2020; 9: 56-62.
3. Khan AH, Khan SMAH, Sarker MR, Hussain RF, Ahmed MU, Joarder Y, Hasan MB. Studies on microscopic technique and culture on Sabouraud's dextrose agar medium for diagnosis of dermatophytes infection. KYAMC Journal 2012; 3: 1-5.
4. Naglot A, Shrimali DD, Nath BK, GogoiHK,Veer V,Chander J, Tewari R. Recent Trends of Dermatophytosis in Northeast India (Assam) And Interpretation With Published Studies. International Journal of Current Microbiology and Applied Sciences 2015; 4 (11):111-20.

5. Islam BAT, Majid F, Ahmed M, Afrin S, Jhumky T, Ferdouse F. Prevalence of Dermatophytic Infection and Detection of Dermatophytes by Microscopic and Culture Methods. J Enam Med Col. 2018; 8(1): 11-5.

6. Shalaby MFM, El-din AN, El-Hamd MAS. Isolation, identification, and in vitro antifungal susceptibility testing of dermatophytes from clinical samples at Sohag University Hospital in Egypt. Electronic physician. 2016; 8(6): 2557-67.

7. Janelle Hare. Sabouraud Agar for Fungal Growth Protocols. American Society for Microbiology. 2016;1-5.

8. Razia S, Anwar S, Miah MRA, Nahar N, Barua R. Evaluation of semisolid agar method for antifungal susceptibility test of T. rubrum. Bangabandhu Sheikh Mujib Medical University Journal 2014;7(1):11-4.

9. Sowmya N, Appalaraju B, Srinivas CR, Surendran P. Antifungal susceptibility testing for dermatophytes isolated from clinical samples by broth dilution method in a tertiary care hospital. The Journal of Medical Research 2015; 1(2): 64-7.

10. Pakshir K, Bahaedinie L, Rezaei Z, Sodaifi M, Zomorodian K. In vitro activity of six antifungal drugs against clinically important dermatophytes. Jundishapur Journal of Microbiology 2009; 2(4): 158-63.

11. Esteban A, Abarca ML,Cabanes FJ. Comparison of disc diffusion method and broth microdilution method for antifungal susceptibility testing of dermatophytes. Med mycol. 2005; 43:61-6.

12. Agarwal RK, Gupta S, Khan F, Roy S, Agarwal A. Antifungal susceptibility testing of dermatophytes by agar based disc diffusion method. Int J currMicrobiol App Sci. 2015; 4(3): 430-6.

13. Nweze EI, Mukherjee PK, Ghannoum MA. Agar-based disc diffusion assay for susceptibility testing of dermatophytes. Journal of clinical microbiology 2010; 48(10): 3750-2.

14. Rao PN, Shah A, Hawelia D, Shah GK, Girdhar M. Etiological prevalence and antifungal Susceptibility pattern of Dermatophytosis in India- A multicentric study. Indian Journal of Dermatology, Venereology and Leprosy 2015; 1(7): 6-10.

15. 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.

16. Rahim MR, Saleh AA, Miah RA, Anwar S, Rahman MM. Pattern of dermatophyte in Bangabandhu Sheikh Mujib Medical University. Bangladesh J Med Microbiol. 2012; 06(02): 11-4

17. Ghosh S. A study on dermatomycoses: TAJ,2009; 22:75.

18. Dabas Y, Xess I, Singh G, Pandey M, Meena S. Molecular Identification and Antifungal Susceptibility Patterns of Clinical Dermatophytes Following CLSI and EUCAST Guidelines. J. Fungi. 2017; 3(2) :17.

19. Niranjan HP. Isolation and identification of dermatophytes. M.D. thesis in microbiology. Rajib Gandhi Univarsity of Health Science, Karataka, Bangalore, India. . Indian Journal of Dermatology.2015; 6(2): 1-7.

20. Dass SM, Vinayaraj EV, Pavavni K, Pallam A, Rao MS. Comparison of KOH, calcofluor white and fungal culture for diagnosing fungal onycomycosis in an urban teaching hospital, Hyderabad. Indian J Microbiol Rec. 2015; 2(3): 148-53.

21. Afshar P, Larijani LV, Rouhanizadeh H. A comparison of conventional rapid methods in diagnosis of superficial and cutaneous mycoses based on KOH, Chicago sky blue 6B and calcofluor white stains. Iranian journal of microbiology. 2018; 10(6) : 433.

22. Rahman MA, Chowdhury OA, Debnath MR, Ahmed SM, Das S, Choudhury R, Akter R, Zaman S, Mahamud F. Comparison among Different Culture Media for the Detection of Dermatophytes. Mymensingh Med J. 2018;27(3):626-30.

23. Siddique MA, Alam MS, Ahmed AA, Haque JA, Naz,S, Khan AH, Rahman MI. Antifungal susceptibility test of Ketoconazole, Itraconazole and Fluconazole against Dermatophytes on Rpmi-1640 medium. TAJ. 2009; 22(1): 73-7.

24. Sharma KH, Vyas N, Mishra KR, Sharma B. In vitro antifungal Susceptibility testing of Dermatophytes isolated from clinical samples in tertiary care hospital. International Journal of Medical and Health Research.2019; 5: 40-5.

25. Khatri KP, Kachhawa D, Maurya V, Meena S. Antifungal Resistance Pattern among Dermatophytes in Western Rajasthan. Int J Curr Microbiol App Sci. 2017; 6(7): 499-509.

26. Budhiraja KR, Sharma S, Sharma S, Kaur J, Roopam B. Antifungal susceptibility pattern of dermatomycosis in a tertiary care hospital of North India. Int J Res Dermatol. 2018; 4(2): 240-5.

27. Alim EAM, Halim AMR, Habib AS. Comparison of Broth Micro Dilution and Disc Diffusion Methods for Susceptibility Testing of Dermatophytes. The Egyptian Journal of Hospital Medicine. 2017; 69 (2): 1923-30.

28. Rahim MR, Saleh AA, Miah RA, Anwar S, Rahman MM. Dermatophytes causing skin, nail and hair infections and sensitivity pattern of Trichophytonrubrum against common antifungal drugs. Bangladesh J Med Microbiol. 2011; 10(06): 28-34.

29. Sabtharishi V, Katragadda R, Ravinder T. A study on the antifungal Susceptibility pattern of Dermatophytes isolated in a tertiary care hospital. Int J of Bioassay. 2017; 6(5): 5379-82.

30. Khadka S, SherchandBJ, Pokhre MB, Dhita S, Rija MR. Antifungal Susceptibility Testing of Dermatophytes by Agar Based Disk Diffusion Assay in Tertiary Care Hospital, Nepal. MRJI. 2017; 19(2): 1-5.