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Original Article

Prevalence of Dermatophytic Infections and Their Antifungal Susceptibility Pattern in the Rajshahi Region

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Abstract

Background: Dermatophytes are the most significant fungi because of their widespread involvement in the population and their prevalence worldwide. Bangladesh's hot and humid climate, overcrowded population, poverty, malnutrition, and ignorance make dermatophytosis a common cutaneous infection. These infections occur in both healthy and immunocompromised patients. Dermatophytes are responsible for most cutaneous fungal infections, and the estimated lifetime risk of acquiring a dermatophyte infection is 10-20%.

Objective: To isolate and identify different dermatophytes and their antifungal susceptibility pattern in the Rajshahi region.

Materials and Methods: Different clinical samples (e.g., skin scrapings, nail clipping, and hair plucking) were collected under aseptic precautions. The isolation and identification of dermatophytes were performed through a microscopic examination using 10% KOH mount, mycological culture, and species identification by lactophenol cotton blue mount from positive culture. In addition, all dermatophytes isolates were subjected to antifungal susceptibility testing using the agar-based disk diffusion method in Mueller Hinton agar media.

Results: Out of 171 samples, *Trichophyton rubrum* was the predominant dermatophyte species with 76(71.7%), followed by *T.mentagrophyte* were 15(14.2%), *E. floccosum* were 12(11.3%), and *M. canis* were 03(2.8%). voriconazole, clotrimazole, and itraconazole were more effective drugs. Griseofulvin was the least effective drug, followed by fluconazole.

Conclusion: This study indicates dermatophytosis is a common skin disease in northern Bangladesh. Due to the increasing trend of antifungal drug resistance among dermatophytes, treatment should be based on antifungal sensitivity testing.

Keywords: Dermatophytes, antifungal agents, antifungal susceptibility test.

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Introduction

Fungal infections are a common health problem in Bangladesh. The prevalence of these diseases in a community usually depends on age, sex, occupation, personal hygiene, educational status, and economic condition of the patients. Some investigators reported that the disease pattern of fungal infections varies among the countries and regions within the same country.[1] The incidence of dermatophytosis has been increasing recently,

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especially in active workers, geriatric and pediatric populations, and immunocompromised patients.[2] Although dermatophytes are not a life-threatening fungus, it becomes a major public health problem due to high morbidity and cosmetic damage.[3]

According to World Health Organization, the 20-25%world population is affected by dermatophytes. The prevalence of dermatophyte infection in Bangladesh is 37.4%, in India at 69.8%, and in North America, East Asia, and Europe prevalence rate ranges from 14-26.8%.[3,4]

Dermatophytes consist of three genera. *Trichophyton*, *Microsporum*, and *Epidermophyton.* Worldwide the most common dermatophyte *is Trichophyton rubrum*. Other frequently implicated agents include *Trichophyton mentagrophytes*, *Microsporum canis*, *Microsporum gyps*um, and *Epidermophyton floccosum*.[5]

The laboratory diagnosis of dermatophytosis routinely involves direct microscopic examination of clinical specimens followed by in vitro culture techniques. The microscopic examination is usually done by KOH and Lactophenol cotton blue mount. Microscopic identification of fungal elements directly from clinical specimens is a rapid diagnostic method, but it lacks specificity and sensitivity. 6 Culture is taken as the gold standard because of its high specificity. High false negative results were reported in various studies, and this may vary depending on the experience of the mycology laboratories and their isolation methods.[7]

In the last two decades, the incidence of dermatophytes and other fungi increased with an increasing variety of drug resistance. Therefore it is essential to evaluate the antifungal susceptibility test of dermatophytes using a standardized, simple, and reproducible in vitro assay. In vitro susceptibility test is helpful in selecting an effective antifungal agent to treat dermatophytes. Susceptibility tests can also help to distinguish relapse or reinfection.[8]

So, the aim of this study was to detect dermatophytes by direct microscopic examination with KOH mount and Lactophenol cotton blue mount, to isolate and identify the different species of dermatophytes by mycological culture from skin, hair and nail specimens and in vitro susceptibility of antifungal drugs by agar based disc diffusion method. In addition, the present study is also helpful in providing locally applicable data and guiding the selection of appropriate antifungal drugs for empirical therapy and preventing antifungal resistance.

Materials and Methods

A cross-sectional descriptive study was conducted from January 2019 to December 2019 at the Department of Microbiology, Rajshahi Medical College, and the outpatient Department of Dermatology and Venereology. A questionnaire and a checklist were the tools for data collection. Before collecting specimens, each patient was interviewed, and relevant information was recorded systematically in a pre-designed standard data sheet. The samples from the patients were collected in aseptic precautions from infected areas such as skin, nails, and hair. Specimens were processed at the Department of Microbiology for direct microscopic examination and fungal culture as per standard protocol. Culturing of organisms from skin, nails, and hair was done on a selective medium such as Sabouraud's chloramphenicol agar with supplements for the 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 were made based on macroscopic observation of fungal colonies and lactophenol cotton blue mount microscopic examination. Antifungal susceptibility testing was performed after identifying dermatophytes using Mueller Hinton agar media. Antifungal disks of Clotrimazole, Fluconazole, Miconazole, Itraconazole, Ketoconazole, Voriconazole, Terbinafine, and Griseofulvin were evenly distributed on the inoculated plate. Within 30 minutes of applying the disks, the plate was inverted and incubated aerobically at 25°C for five days. After the colonies grew, the inhibition zones around the disks were measured and recorded. Criteria of susceptibility of antifungal disks were measured.[8,9,10,11]

Results

The present study was conducted on a total of 171 samples of skin, nails, and hair from the outpatient department of Dermatology and Venereology, Rajshahi Medical College Hospital, Rajshahi, from January 2019 to December 2019. Among 171 specimens, 112 were skin, 39 were nails, and 20 were hair and scalp.

Table 1: Detection of dermatophytes by culture from different sites of the lesion (N=171):

Site of lesion	Culture-positive (%)	Culture negative (%)
Skin n=112(65.5)	73(65.2)	39(34.8)
Nail n=39(22.8)	23(58.9)	16(41.1)
Hair n=20(11.7)	10(50)	10(50)
Total N=171(100)	106(62.1)	65(38.1)

Table I shows the culture positivity of dermatophytes from different sites of the lesion. Out of 112 skin, 39 nail, and 20 hair specimens, culture positive were 73(65.2%), 23(58.9%), and 10(50%), respectively.

Table 2: Distribution of gender according to the site involved (N=171).

Site of lesion	Name of the clinical lesion (n)	Percentage %	Gender	
			Male (%)	Female (%)
	T. corporis **(n=62)**	36.3	24(38.7)	38(61.3)
	T. cruris **(n=18**)	10.5	16(88.9)	02(11.1)
	T. pedis **(n=22)**	12.9	14(63.6)	8(34.4)
	T. faciei **(n=4)**	2.3	02(50)	02(50)
Skin (n=112)	T. manuum**(n=4)**	2.3	01(25)	03(75)
	T. barbae **(n=2)**	1.2	02(100)	00
Nail (n=39)	T. unguium**(n=39)**	22.8	13(33.3)	26(66.7)
Hair (n=20)	T. capitis **(n=20)**	11.7	6(30)	14(70)
Total (N=171)		100	78(45.6)	93(54.4)

Table II: shows the gender-wise distribution of the study population according to clinical diagnosis. In the current study, among 171 study population, 93(54.4%) were female, and 78(45.6%) were male, with male and female ratio were 1:1.2. Tinea corporis 62(36.3%) was the most common type of dermatophytosis; where male were 24(38.7%) and female were 38(61.3%).

Table III: Results of KOH mount microscopy and culture in the diagnosis of dermatophytosis (N=171):

Test result	Number of cases	Percentage (%)
Total microscopy positive	92	53.8%
Total culture positive	106	62.1%
Only microscopy positive	13	7.6%
Only culture positive	27	15.8%
Both microscopy and culture positive	79	46.2%
Total positive cases	119	69.6%
Total negative cases	52	30.4%

N.B.:

- Total positive cases = Only microscopy positive + Only culture positive + Both microscopy and culture positive
- Only microscopy positive= Total microscopy positive- Both microscopy and culture positive
- Only culture positive= Total culture positive- Both microscopy and culture positive

Table-3 shows the results of KOH mount microscopy and culture in diagnosing dermatophytosis. Of 171 cases, 92 (53.8%) were positive by KOH mount microscopy, and 106 (62.1%) were culture positive. 27(15.8%) cases were positive by culture but negative by KOH mount microscopy. 13(7.6%) cases were positive by KOH mount microscopy but negative by culture. Both microscopy and culture-positive cases were 79 (46.2%). Out of 171 cases, total positive cases were 119 (69.6%), and total negative cases were 52 (30.4%).

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:

	FLU		ITC		KCA		MCL		VOR		AGE		TRB		CLO	
Dermatophytes	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 the susceptibility pattern of antifungal drugs against different species of dermatophytes. The highest sensitivity was shown to Itraconazole (84%), clotrimazole (83%), and voriconazole (83%), followed by Miconazole (73.6%) and Ketoconazole (72.6%). The 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 to the studies done by Rao et al. in India and Kakande et al. in Uganda.[12,13] But dissimilar to the study of Islam et al. and Rahim et al. in Bangladesh.[14,15] This variation may be due to the non-viability of fungal elements in some cases, and/or other reasons may be co-existing microbes that may inhibit the growth of pathogenic fungi.

Among 171 study population, 78(45.6%) were male, and 93(54.4%) were female, with female predominance. This study is nearly similar to the study of Ghosh et al. and Nahar et al. in Bangladesh.[16,17] The highest incidence in females may be prolonged exposure to water during household work, such as exposure to detergents while cooking and cleaning. The body of the females remains covered by wet clothes, which may help to keep the body moist and provide a favorable environment for the growth of fungus. Nevertheless, the study of Islam et al. in Bangladesh and Dabas et al. in India showed that the prevalence of dermatophytosis was high among males.[14,18] The higher prevalence amongst males may be due to increased outdoor physical activity, increased sweating, and increased opportunity for exposure.

In the present study, Tinea corporis was the most common dermatophytosis encountered (36.3%), followed by Tinea unguium (22.8%). A similar study was observed by Niranjan et al. and Janardgan et al. in India.[19,20] Tinea corporis is the commonest clinical type of dermatophytosis in female due to patterns of clothing worn by the women in the catchment area- saree, salwar suits- acts as precipitating factors due to friction, maceration, high rate of sweating in the waist region, and collection of dust particles at belt line make this site more vulnerable to fungal growth. Tinea unguium(66.3%) and Tinea capitis (70%) are predominant in females due to prolonged contact with water and detergents; work associated with constant trauma to the nails, use of occlusive footwear resulting in hyperhidrosis, poor scalp hygienic condition, relative negligence in

hairdressing and sharing of fomites like towels, combs, Etc.[21]

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. The direct microscopic finding is similar to the other studies by Niranjan et al. and Dass et al. in India.[19,22]. However, dissimilar to the study of Afshar et al. and Rahim et al.[23,24]The negative results of direct microscopic examination may be associated with an inadequate amount and preparation of specimens, skills of the observer, and a non-suitable temperature of the specimens.

Among the 106 culture-positive dermatophytes, Trichophyton rubrum was the commonest isolate 76(71.7%), followed by Trichophyton mentagrophyte 15(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 by Santosh et al. and Singh et al.in India.[25,26] But dissimilar to the study of Sharma et al. and Sowmya et al. in India.' [27,28]. This variation may vary depending on the geographical area and social, cultural, environmental, and occupational factors.

In this study, 08 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 are the least effective antifungal drugs. This result was comparable to the study done by Sharma et al. Khatri et al. and Budhiraja et al. in India.[27,29,30]. According to the study of Alim et al. Rahim et al. and Sabtharishi et al. Griseofulvin and Fluconazole were shown to be the highest-resistance antifungal drugs.[31,15,32] griseofulvin and fluconazole showed the highest resistance because of universal usage due to their low cost and dosage and their widespread availability in all levels of healthcare centers, which in turn has turned up to increased resistance profile for that drugs.[31] The problem of dermatophytic infection is increasing daily, as demonstrated by various studies. From the data analysis, the detection of fungus by microscopic examination is less sensitive compared to the culture method. So, culture can be used as a definitive procedure for screening and 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.[14] 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 the assessment of dermatophyte resistance against antifungal drugs.[33]

Conclusion

Dermatophytosis is a common skin disease in northern Bangladesh. Due to the increasing trend of antifungal drug resistance among dermatophytes, treatment should be based on antifungal sensitivity testing.

Conflict of interest: None declared

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