ARC Journal of Dermatology
Volume 7, Issue 1, 2024, PP 8-15
ISSN No. (Online) 2456-0022
DOI: https://doi.org/10.20431/2456-0022.0701002
www.arcjournals.org

Evaluating Antifungal Drug Susceptibility of Dermatophyte Species in Skin, Hair, and Nails Using the Disc Diffusion Method: Insights from a Tertiary Care Hospital

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Abstract

Background: *Dermatophytes, including Trichophyton, Microsporum, and Epidermophyton, are fungi that cause superficial infections by utilizing keratin from skin, hair, and nails. Affecting about 25% of the global population, dermatophyte infections vary widely by region due to lifestyle, socioeconomic, and environmental factors. Misdiagnosis and antifungal resistance, including multidrug resistance, complicate treatment. Standardized and reproducible in vitro assays, such as disk diffusion, are crucial for assessing antifungal susceptibility. Disk diffusion is a cost-effective, simple method, especially valuable in developing countries for accurate and reliable testing.*

Aim of the study: *This study aims to evaluate the antifungal susceptibility of dermatophyte species isolated from skin, hair, and nail specimens using the disc diffusion method.*

Methods: *This laboratory-based study at the Department of Dermatology and Venereology, BSMMU, Dhaka, Bangladesh, involved 120 participants suspected of dermatophytosis. After applying inclusion and exclusion criteria, 100 positive samples were analyzed. Detailed demographic data were collected, and written informed consent was obtained. Specimens from skin, nails, and hair were examined microscopically and cultured. Antifungal susceptibility was tested using six drugs. Data were analyzed using SPSS, with results presented in tables and graphs, and assessed for susceptibility and resistance based on established criteria. The study adhered to ethical standards and maintained data confidentiality.*

Result: *Out of 120 participants with a mean age of 34.47 years, males were predominant (60.83%). KOH mounts showed 71.67% positivity, and cultures indicated 83.33% positivity. Among dermatophytes, T. rubrum was the most common (54%), followed by T. mentagrophytes (39%). T. rubrum was frequently linked with tinea cruris, while T. mentagrophytes was common in tinea cruris and tinea manuum. Antifungal susceptibility tests revealed high sensitivity of T. rubrum to itraconazole and terbinafine but notable resistance to fluconazole. E. floccosum was resistant to several antifungals but sensitive to itraconazole and terbinafine.*

Conclusion: *This study used the disc diffusion method to assess antifungal susceptibility in dermatophytes from skin, hair, and nails. It found T. rubrum most prevalent and highly sensitive to itraconazole and terbinafine but resistant to fluconazole. T. mentagrophytes showed similar patterns. The study confirms disc diffusion's effectiveness and the need for effective antifungal treatments.*

Keywords: *Antifungal Drug Susceptibility, Dermatophyte Species, and Disc Diffusion Method.*

1. INTRODUCTION

Dermatophytes are among the most frequently encountered fungi in humans and other vertebrates. They spread via direct contact with infected individuals, animals, or contaminated soil [1]. Dermatophytes are mainly a group of closely related filamentous fungi, including Trichophyton, Microsporum and Epidermophyton that are able to damage and utilize keratin found in the skin, hair and nails by obtaining nutrients from keratin [2,3]. They are an important cause of superficial infections (dermatophytosis) [4]. The World Health Organization (WHO) estimates that approximately 25% of the global population is affected by dermatophyte infections [5]. Globally, research has reported a wide range of dermatophytosis prevalence rates, with figures between 14% and 26.8% in North America, East Asia, and Europe, and between 5% and 31.6% in various African countries, including Ethiopia, Kenya, Nigeria, and Tanzania [6-8]. Regional differences in dermatophytosis rates can largely be attributed to variations in lifestyle, socioeconomic conditions, risk factors, and environmental factors across different areas. Additionally, outbreaks of dermatophytosis have been reported in regions characterized by overcrowding and poor hygiene [9]. Dermatophytic infections are primarily diagnosed based on clinical examination; however, they are frequently mistaken for other skin conditions, especially when steroid ointments and creams have been used. This can result in misdiagnosis and inappropriate treatment [10]. Hence, there arises the need for the correct, efficient, and rapid laboratory diagnosis of dermatophytes [11]. Another important point to consider is that resistance to antifungals has started appearing in dermatophytes. Although a range of antifungal drugs is available, they target only a limited number of cellular mechanisms. Some fungi have developed multidrug resistance (MDR) due to the similar action mechanisms of commonly used antifungals, as well as patient-related factors such as neglect, premature discontinuation of long-term treatment, and side effects [5]. Antifungal resistance can be categorized into microbiological and clinical types. Microbiological resistance includes primary (intrinsic) resistance, which is inherent to the organism, and secondary (acquired) resistance, which develops after exposure to antifungal treatments [12]. Clinical resistance refers to therapeutic failure to eradicate a fungal infection by any antifungal agent that is found susceptible in vitro against that organism [13]. Evaluating resistant dermatophytes requires standardized, simple, and reproducible in vitro assays to assess the efficacy of antifungal drugs against these isolates. Various methods for determining antifungal susceptibility in dermatophytes include microdilution, agar dilution, E test, Sensititre, colorimetric dilution, and disc diffusion. While these methods are available worldwide, dilution tests are commonly used in microdilution and macrodilution assays. However, these methods can be challenging to implement in many laboratories [14]. The disk diffusion in vitro assay used to evaluate antifungal susceptibility testing of dermatophytes is a simple, easy to perform and economical method in developing countries, which, in general, shows a good correlation with the reference method for microdilution antifungal susceptibility testing [15]. For evaluating the antifungal susceptibility of dermatophytes, the advantages of a standardized disk diffusion-based assay include ease of use, reproducibility, accuracy, and low cost [4]. This study aims to evaluate the antifungal susceptibility of dermatophyte species isolated from skin, hair, and nail specimens using the disc diffusion method.

2. MATERIALS AND METHODS

This laboratory-based analytical study was conducted with meticulous attention to detail at the Department of Dermatology and Venereology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, over one year, from January 2021 to December 2021. A purposive sampling method was utilized to select 120 participants who were clinically suspected of having dermatophytosis, adhering strictly to predefined inclusion and exclusion criteria. Of these, 100 samples that tested positive in both microscopy and culture were selected for further data analysis. Comprehensive information regarding the study's objectives, aims, and procedures was provided to all participants, with written informed consent obtained prior to their inclusion. Baseline demographic data were collected for each participant, with a strong emphasis on maintaining data confidentiality. The study protocol received approval from the institutional ethics committee.

2.1. Inclusion Criteria

- Clinically suspected cases of dermatophytoses: *Tinea capitis, Tinea cruris, Tinea corporis, Tinea barbae, Tinea faciei, Tinea pedis, Tinea unguium, and Tinea manuum.*

2.2. Exclusion Criteria

- Patients suspected of having Candidiasis.
- Patients suspected of having *Pityriasis versicolor*.
- Intravenous drug users.
- Patients who refused to participate in the study.

Patient samples were collected under aseptic conditions from infected areas, including skin, nails, and hair [16, 17]. Specimens were processed at the Department of Microbiology and Immunology for direct microscopic examination (KOH mount) and fungal culture following standard protocols [18]. Dermatophyte species were identified and isolated by culturing the organisms on Sabouraud Dextrose Agar with supplements.

To prepare the skin lesions for sampling, the area was cleaned with 70% alcohol to remove surface contaminants. Gentle scraping was performed at the erythematous, peripheral, actively growing margins of the lesions (from scale, crust, vesicle, or pustule). Skin scales were collected in an open, sterile, dry Petri dish and placed immediately below the sampling area. For patients with multiple lesions, the most recently affected area was selected for sample collection, and in vesicular lesions, the tops of fresh vesicles were taken as specimens. In cases involving nails, clinically abnormal nails were cleaned with 70% alcohol, and specimens were collected from multiple nails when more than one was affected. In instances involving hair, samples were collected through plucking. Samples were collected in sterile Petri dishes and analyzed under a microscope following the addition of potassium hydroxide. To assess the antifungal susceptibility patterns of dermatophytes, six commonly prescribed antifungal drugs (fluconazole, itraconazole, ketoconazole, miconazole, and terbinafine) were included in this study. The drugs were available in powder form, which facilitated their use in susceptibility testing.

2.3. Preparation of Inocula

Isolates were transferred from distilled water (DW) stocks to potato dextrose agar to enhance sporulation (subcultured). A seven-day-old culture was covered with 1 ml DW, and the colonies were probed with the tip of a sterile wire loop to obtain a mixture of mycelium and conidia. The suspensions were transferred to sterile tubes, allowed to sediment for 30 minutes, and then adjusted with a spectrophotometer set at 65% transmittance and 530 nm.

2.4. Disc Diffusion Assay

The inoculum was evenly spread on the surface of 10 cm Petri dishes containing Sabouraud Dextrose Agar medium and left to air dry. Antifungal disks were then applied to the plates, which were incubated at 25°C for 5-10 days. Once the colonies had grown, the zones of inhibition around the disks were measured and recorded. The criteria for susceptibility and resistance of antifungal disks were assessed according to Pakshir et al. (2009) [19].

2.5. Data Analysis

Data were systematically organized into tables and graphs for clarity, with detailed descriptions provided for each. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software on a Windows platform. Continuous variables are reported as mean ± standard deviation (SD), while categorical variables are presented as frequencies and percentages.

3. RESULTS

The study population (N=120) had a mean age of 34.47 years (SD ± 4.17), with the largest age group being 21-30 years old, comprising 25.83% of the participants. Age distribution showed that 16.67% were 11-20 years old, 20% were 31-40 years old, and smaller percentages fell into other age brackets, including 7.5% above 60 years. Gender distribution was predominantly male, accounting for 60.83% of the sample, while females made up 39.17% (Table 1). In the analysis of KOH mounts, 71.67% tested positive, while 28.33% were negative. Culture findings indicated that 83.33% were positive, with the remaining 16.67% testing negative (Table 2). Figure 1 illustrates the distribution of dermatophytes in a sample of 100 cases. The data shows a significant predominance of *T. rubrum* and *T. mentagrophyte*among the dermatophytes observed. The chart shows that *T. rubrum* is the most prevalent, accounting for 54% of cases. This is followed by *T. mentagrophyte*, which represents 39% of the sample. Less common

dermatophytes include *T. verrucosum*and *E. floccosum*, with 4% and 3% of the cases, respectively. In the analysis of dermatophyte species across various clinical presentations (N=100), *T. rubrum* was most frequently associated with tinea cruris (57.41%) and less so with tinea pedis (1.85%). In comparison, *T. mentagrophytes* predominantly presented with *T. cruris* (53.85%) and *T. manuum* (5.13%). In contrast, *T. verrucosum* and *E. floccosum*were rare and primarily observed in *T. unguium* and *T. cruris*, respectively (Table 3). Regarding antifungal susceptibility (N=100), *T. rubrum* exhibited high sensitivity to itraconazole (88.89%) and terbinafine (77.78%), with significant resistance to fluconazole (14.81%). *T. mentagrophytes* showed similar sensitivity patterns but with slightly lower efficacy, especially against fluconazole and griseofulvin. *T. verrucosum*displayed uniform sensitivity to all tested drugs, while *E. floccosum*was notably resistant to fluconazole, ketoconazole, and griseofulvin, but sensitive to itraconazole and terbinafine (Table 4).

Table 1. *Demographic characteristics of study the population (N=120).*

Variables	Frequency (N)	Percentage (%)
Age (in years)		
1-10	7	5.83
11-20	20	16.67
21-30	31	25.83
31-40	24	20.00
41-50	16	13.33
51-60	13	10.83
> 60	9	7.50
Mean±SD	34.47±4.17	
Gender Distribution		
Male	73	60.83
Female	47	39.17

Table 2. *Sample distribution by KOH mount and culture outcomes (N=120).*

Variables	N	%
KOH Mount (N=120)		
KOH positive	86	71.67
KOH negative	34	28.33
Culture Findings (N=120)		
Culture positive	100	83.33
Culture negative	20	16.67

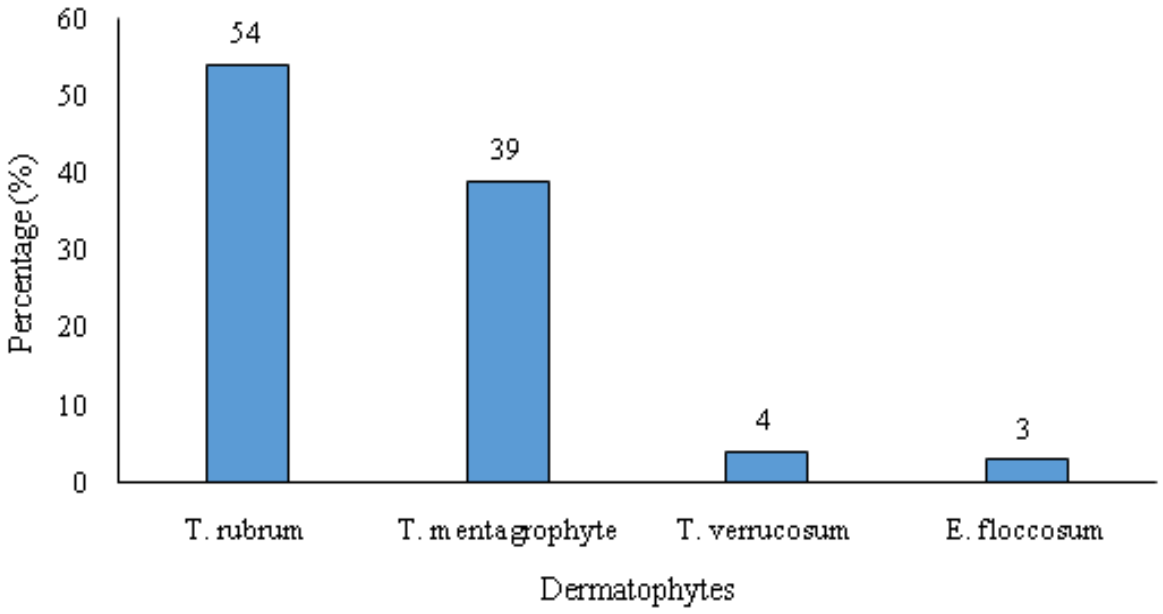

Figure 1. *Distribution of different dermatophytes (N=100).*

Table 3. *Distribution of dermatophyte species across diverse clinical presentations (N=100).*

Clinical types	Dermatophytes								Total	
	T. rubrum (N=54)		T. mentagrophyte (N=39)		T. verrucosum (N=4)		E. floccosum (N=3)			
	N	%	N	%	N	%	N	%	N	%
T. cruris	31	57.41	21	53.85	2	50.00	3	100.00	57	57.00
T. corporis	19	35.19	14	35.90	0	0.00	0	0.00	33	33.00
T. barbae	0	0.00	2	5.13	0	0.00	0	0.00	2	2.00
T. capitis	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
T. pedis	1	1.85	0	0.00	0	0.00	0	0.00	1	1.00
T. manuum	0	0.00	2	5.13	0	0.00	0	0.00	2	2.00
T. unguim	0	0.00	0	0.00	2	50.00	0	0.00	2	2.00
T. facie	3	5.56	0	0.00	0	0.00	0	0.00	3	3.00

Table 4. *Antifungal resistance patterns in isolated dermatophyte species (N=100).*

Antifungal discs	Sensitive	Intermediate sensitive	Resistant
T. rubrum (N=54)			
Itraconazole	48	6	0
Fluconazole	40	6	8
Ketoconazole	35	17	2
Griseofulvin	29	22	3
Terbinafine	42	6	6
T. mentagrophyte (N=39)			
Itraconazole	35	4	0
Fluconazole	28	7	4
Ketoconazole	24	13	2
Griseofulvin	24	11	4
Terbinafine	28	7	4
T. verrucosum (N=4)			
Itraconazole	4	0	0
Fluconazole	4	0	0
Ketoconazole	2	2	0
Griseofulvin	2	2	0
Terbinafine	4	0	0
E. floccosum (N=3)			
Itraconazole	3	0	0
Fluconazole	0	0	3
Ketoconazole	0	3	0
Griseofulvin	0	3	0
Terbinafine	3	0	0

4. DISCUSSION

Among the 120 patients studied, 73 (60.83%) were male and 47 (39.17%) were female, resulting in a male-to-female ratio of 1.55:1. Similar findings of male predominance have been reported in studies by Nagaral et al. (2023), who found 62.3% males and 37.7% females (1.65:1), and Gunasekaran (2017), who observed 62.3% males and 37.7% females (1.5:1) [20, 21]. The highest incidence of dermatophytoses was observed in the 21-30 years age group (25.83%), followed by the 31-40 years group (20.00%). The study showed a low occurrence of the disease in both the younger (1-10 years) and older age groups (over 60 years), with incidences of 5.83% and 7.50%, respectively. These findings are consistent with those reported by Walke et al. (2014) [22]. Most affected patients were male, in their second and third decades of life, engaged in physically demanding occupations such as manual labor, farming, and domestic work. They were from low socioeconomic backgrounds.

The high prevalence of dermatophytoses in these patients may be attributed to factors such as excessive sweating due to strenuous outdoor activity, exposure to infected animals and soil, poor personal hygiene, and lack of awareness about the disease. This observation is supported by studies from Ghosh et al. (2014) and Nagaral et al. (2023) [20, 23]. The lower incidence in females could be due to their reluctance to seek

medical attention, particularly among those from rural areas [24]. In this study, of the 120 clinical samples analyzed, 86 (71.67%) were positive by direct microscopy using KOH mount, and 100 (83.33%) were culture-positive. These findings are in line with those reported by Avinash (2015), who observed a 72.6% KOH positivity rate, and Mahale et al. (2014), who reported a 61.01% culture positivity rate [25, 26]. The discrepancy between KOH positivity and culture positivity, where fungal elements were observed under direct microscopy but failed to grow in culture, could be attributed to factors such as the use of topical corticosteroids or unsatisfactory sample collection resulting in the presence of dead fungal hyphae [27, 28]. Additionally, instances were noted where no fungal elements were seen under direct microscopy. Yet, growth was observed in culture, possibly due to scanty fungal elements missed during microscopic examination or the presence of fungal elements in an inactive sporulating form not visible under direct microscopy [28, 29]. In our study, *T. rubrum* was the most commonly isolated dermatophyte (54.00%), followed by *T. mentagrophytes* (39.00%), *T. verrucosum* (4.00%), and *E. floccosum*(3.00%). These results are consistent with other studies that identified *T. rubrum* as the predominant isolate, including Avinash (2015) (59.6% *T. rubrum*, 26% *T. mentagrophytes*) and Walke et al. (2014) (56.37% *T. rubrum*, 19.39% *T. mentagrophytes*) [22, 25]. However, contrary findings were reported by Gadadavar et al. (2018) and Bhatia et al. (2014), where *T. mentagrophytes* was more common than *T. rubrum* [30, 31]. No isolates of *Epidermophyton* spp. were identified in our study, a result also observed in studies by Bhatia et al. (2014) and Gadadavar et al. (2018) [30, 31]. Clinically, *T. cruris* was the most common presentation (57.00%), followed by *T. corporis* (33.00%). Kucheria et al. (2016) reported *T. corporis* as the most common presentation (31%), followed by *T. unguium* (21%) [32]. The high occurrence of *T. corporis* can be attributed to its symptomatic nature (pruritus), prompting patients to seek medical advice [22]. However, Walke et al. (2014) and Nagaral et al. (2023) found *T. corporis* to be the most common type, followed by *T. cruris*, which contrasts with our findings [20, 22]. Other clinical presentations observed in our study included *T. faciei* (3.00%), *T. manuum* (2.00%), *T. unguium* (2.00%), and *T. pedis* (1.00%). Similar findings were reported by Roopa et al. (2015) and Avinash (2015) [25, 29]. Some studies have compared the disk diffusion method with the reference micro-dilution method, suggesting that disk diffusion is a simple, reproducible, and potentially viable alternative for antifungal susceptibility testing of dermatophytes in routine clinical laboratories [4]. Macura (1993) also reported that the disk diffusion method produces results consistent with those obtained from the dilution method [34]. With the rising incidence of antifungal-resistant strains, early initiation of appropriate antifungal therapy is crucial for effective treatment and prevention of disease spread [24]. In our study, itraconazole demonstrated good antifungal activity against all isolates, while fluconazole showed poor efficacy. These results are consistent with those of Pakshir et al. (2009), where itraconazole was the most effective antifungal drug, and fluconazole was the least effective [19]. Agarwal et al. (2015) similarly indicated that fluconazole had limited activity against dermatophytes, aligning with our findings [35].

5. Limitations of the Study

The study's limitations include the following: the use of the disc diffusion method, which, while cost-effective and easy to implement, may not always provide results as accurate as reference methods like microdilution. Variations in sample collection and preparation techniques could impact the consistency of results. Additionally, the study's single-center design may limit the generalizability of findings to other regions or populations. The study also faced challenges with potential contamination and the variability in antifungal susceptibility testing methods, which may affect the reliability of resistance profiles.

6. Conclusion and Recommendations

This study evaluated the antifungal susceptibility of dermatophytes isolated from skin, hair, and nails using the disc diffusion method. Findings revealed that *T. rubrum* was the most prevalent dermatophyte, followed by *T. mentagrophytes*. The disc diffusion method proved effective for assessing antifungal resistance, showing high sensitivity of *T. rubrum* to itraconazole and terbinafine but notable resistance to fluconazole. *T. mentagrophytes* displayed similar sensitivity patterns with slightly lower efficacy against fluconazole and griseofulvin. The study underscores the utility of disc diffusion for

routine antifungal susceptibility testing and highlights the need for effective antifungal therapy, particularly for resistant strains.

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Citation: *Dr. A K M Rejaul Haque et al. Evaluating Antifungal Drug Susceptibility of Dermatophyte Species in Skin, Hair, and Nails Using the Disc Diffusion Method: Insights from a Tertiary Care Hospital. ARC Journal of Dermatology. 2024; 7(1):8-15. DOI: http://dx.doi.org/ 10.20431/2456-0022.0701002.*