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Antifungal Resistant Pattern of *Tricophyton Rubrum* and *Tricophyton Mentagrophytes* at a Tertiary Care Hospital in Bangladesh

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Abstract | **Original Research Article**

Background: Dermatophytoses are caused by keratinophilic fungi called dermatophytes, which can invade keratinized tissue. Dermatophytoses is a serious health concern in regions with high humidity, dense population density, and poor sanitary conditions. Many antifungal drugs are available to treat this infection but in routine clinical practice, these antifungal drugs are not always effective. For this reason, antifungal susceptibility test is an epidemiological concern to reduce the dermatophytic infection. ***Objective:*** This study was aimed to evaluate the susceptibility patterns of *Tricophyton Rubrum* and *Tricophyton Mentagrophytes* to antifungal drugs. ***Methods:*** To determine the pattern of antifungal susceptibility in dermatophytes, this study comprised 246 patients with a clinical diagnosis of dermophytoses across all age groups. The skin, hair, and nails were sampled in an aseptic manner. These samples were mounted in potassium hydroxide (KOH) and cultured on sabouraud dextrose agar (SDA). Direct microscopy and culture method were used in diagnosis of dermatophytosis. Polymerase chain reaction (PCR) and biochemical tests (urease test and hair perforation test) were used to identify dermatophyte species. An established colony was cultivated on potato dextrose agar (PDA) for sensitivity testing. Following notable sporulation, the spores were collected in normal saline and a standardized inoculum adjusted with 0.5 Mcfarland solution was applied to SDA plates, which were then incubated at room temperature (26-28˚C). After three to seven days, the zone of inhibition was evaluated and the drug's sensitivity, intermediate level, or resistance was determined accordingly. ***Results:*** It was observed that, thirty-one (93.55%) *Trichophyton rubrum* and 45 (91.11%) *Trichophyton mentagrophytes* were resistant to fluconazole. Twenty (64.52%) *Trichophyton rubrum* and 30 (66.67%) *Trichophyton mentagrophyte* were resistant to terbinafine. Resistant to itraconazole and voriconazole were not observed. ***Conclusion:*** It was found that fluconazole was least effective drug followed by terbinafine. Voriconazole and itraconazole were more effective. Alternatively, combination of two drugs could be a better option.

Keywords: Antifungal Drugs, Antifungal Susceptibility Patterns, Dermatophytoses, Direct Microscopy and Culture Method, Disk Diffusion Method.

1. INTRODUCTION

Dermatophytoses or ring worm infection is a major health issue. Healthy and immune compromised patients are affected from this infection [1] The estimated life time risk of acquiring dermatophytic infection is 10-20% [1]. Some investigators reported that the prevalence of dermatophytic infection is 38.75% [2]. Their geographic distribution is extremely variable; climate, lifestyle, involvement of outdoor activities, disease conditions (diabetes, malnutrition) are responsible for the heterogeneous prevalence [3].

Several antifungal agents (Azoles, Allylamines, Morpholines, Oxaborole, Hydroxypyridone) are used to treat dermatophytic infection. All the species does not have similar susceptibility pattern [4]. Both topical and systemic antifungal drugs are used for dermatophytoses. During course of time dermatophytes have evolved drug resistance for single as well as multiple drugs simultaneously [3]. The need for reproducible, clinically relevant antifungal susceptibility testing (AST) has been prompted due to the increasing the number of invasive fungal infections [5].

Typically, broth dilution, agar dilution and disc diffusion tests are used to determine antifungal susceptibility test [5]. The clinical and laboratory standard institute (CLSI) established reproducible standard method M61 for antifungal susceptibility test of filamentous fungi which is either broth micro or macro dilution method [6]. But in many laboratories the equipment like RPMI1640, MOPS, buffering reagent, filter sterilizer, spectrophotometric inoculums determination based on conidial size are not available [6, 7]. Therefore, a simple, inexpensive and reliable method is needed to perform susceptibility testing. Disk diffusion test has the same important advantages. The agar-based disk diffusion (ABDD) susceptibility method for dermatophytes is quick, easy and a good option [8]. Antifungal sensitivity test is significant for the treatment purpose at the current scenario of increasing antifungal resistance [9]. The availability of susceptibility patterns will help in guiding the management and treatment of such infections. It will also provide the opportunity for the clinician to select the best available therapeutic option with maximum efficacy, safety and convenience while minimizing the cost and toxicity [10]. In this background, current study was aimed to evaluate the susceptibility patterns of *Tricophyton Rubrum* and *Tricophyton Mentagrophytes* to antifungal drugs.

2. MATERIAL AND METHODS

Study design

This cross-sectional study was carried out at Dhaka Medical College Hospital (DMCH), Dhaka, Bangladesh, from July 2018 to June 2019. The institutional review board (IRB) of Dhaka Medical College, Dhaka, Bangladesh, was approved this study. This study was intended to determine the pattern of antifungal resistance in dermatophytoses. The study comprised 246 patients with a clinical diagnosis of dermophytoses across all age groups. We collected samples of skin, hair, and nails in an aseptic manner. These samples were mounted in KOH and cultured on sabouraud dextrose agar (SDA). Direct microscopy and culture method were used in diagnosis of dermatophytoses. Polymerase chain reaction (PCR) and biochemical tests (urease test and hair perforation test) were used to identify dermatophyte species. An established colony was cultivated on potato dextrose agar (PDA) for sensitivity testing. Following notable sporulation, the spores were collected in normal saline and a standardized inoculum adjusted with 0.5 Mcfarland solution was applied to SDA plates, which were then incubated at room temperature (26-28˚C). After three to seven days, the zone of inhibition was evaluated and the drug's sensitivity, intermediate level, or resistance was determined accordingly.

Drug susceptibility test of Dermatophytes

Susceptibility of the isolated fungal species were done against Fluconazole, Itraconazole, Ketoconazole, Clotrimazole, Voriconazole, Terbinafine and Griseofulvin by disk diffusion method on Sabouraud Dextrose Agar (SDA) medium. Zone of inhibition were according to table- 1 [11, 12].

Table 1: Inhibition zone diameter criteria for susceptibility and resistance of antifungal discs

Antifungal Drugs	Potency	Zone of Inhibition		
		sensitive	intermediate	resistant
Clotrimazole	10 µg	≥20	19-12	≤11
Fluconazole	25 µg	≥20	19-15	≤14
Griseofulvin	25 µg	≥31	26-30	< 26
Itraconazole	10 µg	≥22	21-15	< 15
Ketoconazole	10 µg	≥30	29-23	≤22
Terbinafine	2 µg	≥26	26-20	≤19
Voriconazole	1 µg	>14	-	<14

Preparation of the disk of antifungal drugs:

All anti-fungal drugs were obtained from commercial sources (Hi media**).** Fluconazole (25µg), Itraconazole (10 µg), Ketoconazole (10µg), Clotrimazole (10µg), Voriconazole (1 µg), Terbinafine (2µg) and Griseofulvin (25 µg). Griseofulvin and Terbinafine were not available commercially and were prepared in laboratory. These two drugs were obtained in powdered form and stock solution of both drugs were prepared by dissolving in dimethyl sulfoxide (DMSO) as follows: Griseofulvin 1.25 mg/ml and Terbinafine 0.1 mg/ml. Blank disks of 6 mm were loaded with 20 µl of prepared stock solution to obtain the desired drug concentration per disk. Then the disks were air dried and were kept at 40˚C until use.

Preparation of the inoculum

The isolates were sub cultured on the Potato dextrose agar (PDA) to enhance sporulation. Suspension was made from seven days old culture with one ml of normal saline and allowed to sediment for 30 minutes and then adjusted with 0.5 McFarland solution.

3. RESULTS

A total 246 samples were included in the present study. Of which, 224 (91%) were skin samples, 16 (7%) were nail samples and 6 (2%) were hair samples (Figure- 1). Direct microscopy and culture method were used in diagnosis of dermatophytoses. Among 246 samples, 92 (37.40%) yielded presence of dermatophytes; 16 (17.40%) cases were detected by direct microscopy, 7 (7.60%) cases were detected by culture method and 69 (75.00%) cases were detected by both microscopy and culture methods (Figure- 2).

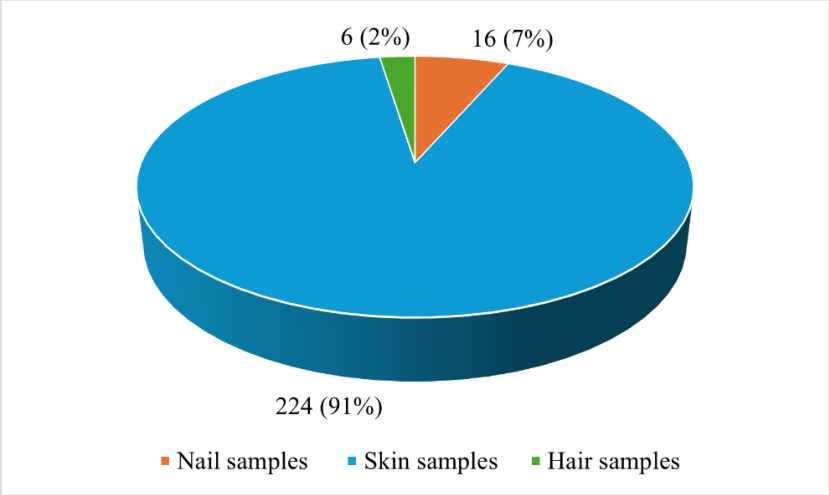

Figure 1: Different samples to detect dermatophytes (N= 246)

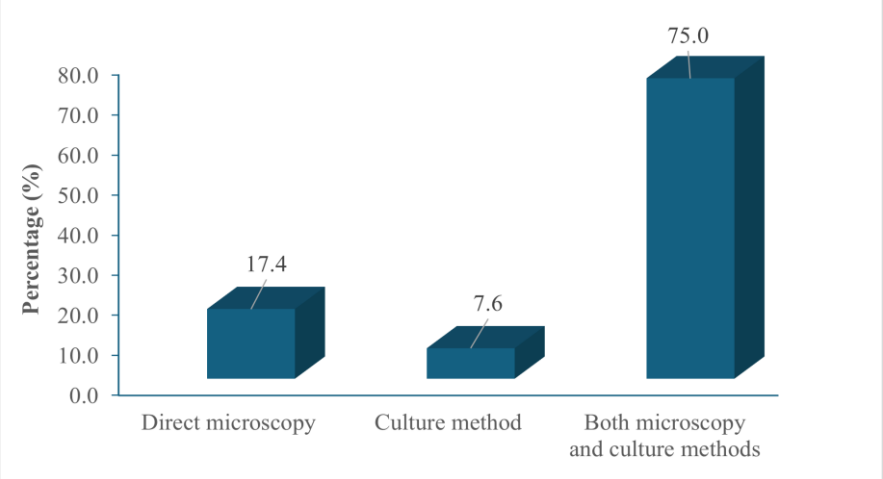

Figure 2: Detection of dermatophytes by microscopy, culture and both methods

Out of 85 microscopy positive cases, 69 (28.05%) were detected by culture; 154 (62.60%) cases were negative by both methods. Sixteen [16 (6.50%)] cases were detected only by microscopy and 7 (2.85%) cases were positive only by culture method (Table- 2).

Table 2: Comparison of microscopy with culture in diagnosis of dermatophytosis (N= 246)

Microscopy	Culture		Total n (%)
	Positive n (%)	Negative n (%)	
Positive	69 (28.05)	16 (6.50)	85(34.55)
Negative	7 (2.85)	154 (62.60)	161(65.45)
Total	**76 (30.90)**	**170 (69.10)**	**246 (100.00)**

Polymerase chain reaction (PCR) and biochemical tests (urease test and hair perforation test) were used to identify dermatophyte species from 76 culture-positive cases. It was found that all Trichophyton mentagrophyte species tested positive for both the urease test and hair perforation test, however none of the Trichophyton rubrum species tested positive by biochemical tests. Although, PCR results for every culture-isolated species were positive (Table- 3).

Table 3: Detection of dermatophytes species by biochemical tests and PCR from culture positive cases (n=76)

Culture positive	Biochemical tests		PCR
	Urease test	Hair perforation test	
Trichophyton mentagrophyte (n= 45)	45 (100.00)	45 (100.00)	45 (100.00)
Trichophyton rubrum (n= 31)	0 (0.00)	0 (0.00)	31 (100.00)

It was observed that, twenty-nine (93.55%) *Trichophyton rubrum* and 41 (91.11%) *Trichophyton mentagrophytes* were resistant to fluconazole. Twenty (64.52%) *Trichophyton rubrum* and 30 (66.67%) *Trichophyton mentagrophyte* were resistant to terbinafine. Seventeen (54.84%) *Trichophyton rubrum* and 25 (55.56%) *Trichophyton mentagrophyte* were resistant to griseofulvin. But, three (9.68%) *Trichophyton rubrum* and 8 (17.78%) *Trichophyton mentagrophyte* were resistant to ketoconazole. Resistant to itraconazole and voriconazole were not observed (Table- 4). It was found that fluconazole was least effective drug followed by terbinafine. Voriconazole and itraconazole were more effective. Alternatively, combination of two drugs may be used.

Table 4: Antifungal susceptibility pattern of the isolated *Trichophyton rubrum* (n= 31) and *Trichophyton mentagrophytes* (n= 45)

Antifungal drugs	*Trichophyton rubrum*		
	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Clotrimazole	28 (90.32)	0 (0.00)	3 (9.68)
Fluconazole	2 (6.45)	0 (0.00)	29 (93.55)
Griseofulvin	6 (19.35)	8(25.81)	17 (54.84)
Itraconazole	31(100.00)	0 (0.00)	0 (0.00)
Ketoconazole	23 (74.19)	16.13)	3 (9.68)
Terbinafine	11(35.48)	0 (0.00)	20 (64.52)
Voriconazole	31(100.00)	0 (0.00)	0 (0.00)
Antifungal drugs	***Trichophyton mentagrophytes***		
	Sensitive n (%)	**Intermediate n (%)**	**Resistant n (%)**
Clotrimazole	38 (84.44)	0 (0.00)	7 (15.56)
Fluconazole	4 (8.89)	0 (0.00)	41 (91.11)
Griseofulvin	20 (44.44)	0 (0.00)	25 (55.56)
Itraconazole	45 (100.00)	0 (0.00)	0 (0.00)
Ketoconazole	37 (82.22)	0 (0.00)	8 (17.78)
Terbinafine	15 (33.33)	0 (0.00)	30 (66.67)
Voriconazole	45 (100.00)	0 (0.00)	0 (0.00)

4. DISCUSSION

Dermatophytes are filamentous fungi, which is mostly found in the skin, hair, and nails that have the ability to break down and draw nutrients from keratin. Dermatophytoses and other fungal infections are more likely to occur in environments with high temperatures and increasing humidity [3]. Drug resistance to the antimycotic medications that are frequently used to treat dermatophytoses and other fungal infections has been reported to rise over time. Due to increasing antifungal resistance pattern among dermatophytes, treatment should be based on antifungal sensitivity testing. This study highlighted that it is necessary to implement culture sensitivity test of fungus on microbiology laboratories so that it could be reduced the resistance of different antifungal drugs against dermatophytic infection.

All specimens were examined by direct microscopy and culture method. In this present study, direct microscopy analysis was positive in 34.55% cases and 65.45% cases was detected by culture method. In accordance, Rahim *et al.*, reported that 32.8% were positive by direct microscopy [13]. Another study by Afshar *et al.*, observed that 36% samples were positive by direct microscopy [14]. These findings were in

agreement with this current study. In this present study culture was positive in 30.89% cases. Similarly, Rahim *et al.*, reported that culture was positive in 30.3% cases [13]. Another study was reported that, out of 80 specimens, 38.75% were positive by culture [2]. These findings were consistent with our study.

In this present study *Trichophyton mentagrophytes* was the predominant species (59.21%) followed by *Trichophyton rubrum* (40.79%). No mixed infection was found. *Trichophyton mentagrophytes* was found to be the main etiological dermatophyte species (23.40%) responsible for dermatophytoses in a related study [9]. In another study, *Trichophyton mentagrophyte* was the most predominant dermatophytes followed by *Trichophyton rubrum* comprising 63.5% and 34.6% respectively [15]. In this context, Nasimuddin *et al.*, reported *Trichophyton mentagrophytes* was isolated as a common species (38.75%) for dermatophytoses followed by *Trichophyton rubrum* (27.31%) [16]. The plausible explanation for this may be *Trichophyton rubrum* is a slow growing organism, and it generally linked to chronic dermatophytoses [15]. In this present study most of the cases were acute dermatophytoses which was the reason behind lower proportion of *Trichophyton rubrum* in the study.

Disk diffusion method was applied to observe the susceptibility pattern of antifungal drugs. Among 31 isolated *Trichophyton rubrum* 93.55% were resistant to fluconazole. It was reported that 94.4% *Trichophyton rubrum* were resistant to fluconazole [17]. Another study documented that the resistance rate for fluconazole among *Trichophyton rubrum* was 93.18% [18]. These findings were similar to the present study. In our study, among 45 isolated *Trichophyton mentagrophytes*, 91.11% was resistant to fluconazole. One previous study reported the resistance rate for fluconazole among *Trichophyton mentagrophytes* was 80% [18]. In accordance, Khatri *et al.*, reported that 80.64% *Trichophyton mentagrophytes* was resistant to fluconazole [3]. These findings were nearly close to this present study. It was documented that; fluconazole resistance is caused by increase drug efflux and stress adaptation [19].

In this present study, among 45 isolated *Trichophyton mentagrophytes*, 66.67% were resistant to terbinafine. Khatri *et al.*, reported that 61.29% *Trichophyton mentagrophytes* was resistant to terbinafine [3]. This finding was similar to our study. Terbinafine resistances are caused by several factors like modification of target enzyme by mutation of ERG1P gene coding the enzyme squalene epoxidase, increase drug efflux and stress adaptation [19, 20].

An appropriate method is necessary to detect antifungal susceptibility test. Disk diffusion method is simple and economical method. It does not require any special equipment to perform this experiment. This test should be done at routine basis in all microbiological laboratory.

5. CONCLUSION

Antifungal resistance causes treatment failure in dermatophytic infection. After survey of the data *Trichophyton mentagrophyte* was the predominant species (59.21%) followed by *Trichophyton rubrum* (40.79%). Among different antifungal drugs, resistance against fluconazole was 93.55% and 91.11% in *Trichophyton rubrum* and *Trichophyton mentagrophytes* respectively. All the isolates were sensitive to itraconazole and voriconazole. So itraconazole and voriconazole are considered to be the most sensitive drug in management of dermatophytic infection. Alternatively, combination of two drugs could be a better option.

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Conflicts of interest: The authors disclose that they have no potential conflicts of interest.

REFERENCES

1. Drake LA, Dinehart SM, Farmer ER, Goltz RW, Graham GF, Hordinsky MK, Lewis CW, Pariser DM, Skouge JW, Webster SB, Whitaker DC. Guidelines of care for superficial mycotic infections of the skin: Tinea corporis, tinea cruris, tinea faciei, tinea manuum, and tinea pedis. Journal of the American Academy of Dermatology. 1996 Feb 1;34(2):282-6.
2. Islam TAB, Majid F, Ahmed M, Afrin S, Jhumky T, Ferdouse F. Prevalence of dermatophytic infection and detection of dermatophytes by microscopic and culture methods. Journal of Enam Medical College. 2018 Feb 7;8(1):11-5.
3. Khatri PK, Kachhawa D, Maurya V, Meena S, Bora A, Rathore L, Seervi KL, Khullar S. Antifungal resistance pattern among dermatophytes in Western Rajasthan. Int J Curr Microbiol App Sci. 2017 Jul 20;6(7):499-509.
4. Fernández-Torres B, Cabanes FJ, Carrillo-Munoz AJ, Esteban A, Inza I, Abarca L, Guarro J. Collaborative evaluation of optimal antifungal susceptibility testing conditions for dermatophytes. Journal of clinical microbiology. 2002 Nov;40(11):3999-4003.
5. Thatai P, Sapra B. Critical review on retrospective and prospective changes in antifungal susceptibility testing for dermatophytes. Mycoses. 2016 Oct;59(10):615-27.
6. Khan S, Singhal S, Mathur T, Upadhyay DJ, Rattan A. Antifungal susceptibility testing method for

resource constrained laboratories. Indian journal of medical microbiology. 2006 Jul 1;24(3):171-6.
7. Razia S, Anwar S, Miah MR, 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.
8. Matar MJ, Ostrosky-Zeichner L, Paetznick VL, Rodriguez JR, Chen E, Rex JH. Correlation between E-test, disk diffusion, and microdilution methods for antifungal susceptibility testing of fluconazole and voriconazole. Antimicrobial agents and chemotherapy. 2003 May;47(5):1647-51.
9. Mathur M, Shrestha S. Clinicomycological Profile and Antifungal Sensitivity Pattern of Commonly Used Azoles in Dermatophytosis. Journal of the Nepal Medical Association. 2015 Apr 1;53(198).
10. Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. In Mayo clinic proceedings, Elsevier. 2011 Feb 1;86(2):156-167.
11. Agarwal RK, Gupta S, Mittal G, Khan F, Roy S, Agarwal A. Antifungal susceptibility testing of dermatophytes by agar-based disk diffusion method. Int J Curr Microbiol Appl Sci. 2015;4(3):430-6.
12. 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 Oct 31;2(4):158-63.
13. Rahim MR, Saleh AA, Miah MR, Anwar S, Rahman MM. Pattern of dermatophyte in Bangabandhu Sheikh Mujib Medical University. Bangladesh Journal of Medical Microbiology. 2012 Jul 1;6(2):11-4.
14. 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 Dec;10(6):433.
15. Bhatia VK, Sharma PC. Epidemiological studies on dermatophytosis in human patients in Himachal Pradesh, India. Springer plus. 2014 Dec;3(1):1-7.
16. Nasimuddin S, Appalaraju B, Surendran P, Srinivas CR. Isolation, identification and comparative analysis of SDA and DTM for dermatophytes from clinical samples in tertiary care hospital. J Appl Dent Med, 2014; 13 (11): 68-73.
17. Hryncewicz-Gwóźdź A, Kalinowska K, Plomer-Niezgoda E, Bielecki J, Jagielski T. Increase in resistance to fluconazole and itraconazole in Trichophyton rubrum clinical isolates by sequential passages in vitro under drug pressure. Mycopathologia. 2013 Aug;176:49-55.
18. Silva LB, De Oliveira DB, Da Silva BV, De Souza RA, Da Silva PR, Ferreira-Paim K, Andrade-Silva LE, Silva-Vergara ML, Andrade AA. Identification and antifungal susceptibility of fungi isolated from dermatomycoses. Journal of the European Academy of Dermatology and Venereology. 2014 May;28(5):633-40.
19. Verma P, Dayal S, Armani A, Sahu P. Antifungal resistance: Where do we stand? European J Biomed Pharm, 2016; 3 (10): 168-174.
20. Yamada T, Maeda M, Alshahni MM, Tanaka R, Yaguchi T, Bontems O, Salamin K, Fratti M, Monod M. Terbinafine resistance of Trichophyton clinical isolates caused by specific point mutations in the squalene epoxidase gene. Antimicrobial agents and chemotherapy. 2017 Jul;61(7):10-128.