

Original Article

Detection of Drug Susceptibility to Azoles among *Trichophyton rubrum* Isolates by Disk Diffusion Method

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Abstract

Dermatophytes are filamentous fungi that cause tinea with high prevalence in tropical hot, humid and overcrowded countries like Bangladesh. Antifungal drug resistance among dermatophyte isolates are very common due to irrational and overuse of them. In vitro antifungal susceptibility test may help to select appropriate drug, optimize the therapy and monitor the emergence of drug resistance. This study was undertaken to isolate and identify different dermatophyte species from skin, nail and hair samples and to see the susceptibility pattern of azoles (fluconazole, miconazole and itraconazole) among one of the most common dermatophyte isolates (*Trichophyton rubrum*) by disk diffusion method. A total of two hundred and forty six patients clinically suspected of dermatophytosis attending in the outpatient department (OPD) of Dermatology and Venereology of BSMMU, Dhaka, were studied from September 2018 to August 2019. All the samples of skin, nail and hair were processed for direct microscopic examination and culture. The species of dermatophytes were identified by gross colony morphology, microscopic features and biochemical tests. Antifungal susceptibility was performed by disk diffusion (Neo-Sensitabs susceptibility testing modified for Dermatophytes) method in Sabouraud's dextrose agar (SDA) media. Out of 246 specimens 27.2% were positive by microscopy, 28.9% were positive by culture and 26.0% were positive by both microscopy and culture. *Tinea cruris* (32.4%) was the most prevalent clinical type among all dermatophyte isolates. Among 71 culture positive isolates, *T. rubrum* (71.8%) was most prevalent followed by *T. mentagrophytes* (24.0%) and *E. floccosum* (4.2%). By disk diffusion method fluconazole was found as the most resistant drug (90.2%) against *T. rubrum* isolates and Itraconazole was the most sensitive (80.4%). Miconazole was found as most intermediate sensitive.

Key words: Dermatophytes, Tinea, Antifungal susceptibility test, Disk diffusion.

Introduction

Dermatophytes are pathogenic fungi having a high affinity for keratinized structures like skin, hair or nail causing superficial infections known as dermatophytosis. They belong to three genera- *Trichophyton*, *Epidermophyton* and *Microsporum* and cause clinical lesions named tinea.^{1,2} Dermatophytosis is most common in Tropical and Subtropical countries. In Bangladesh hot and humid climate, overcrowded population, poverty and malnutrition make dermatophytosis a very common superficial infection.³ Study conducted worldwide showed that antifungal resistance of dermatophytosis very common particularly against azoles drug group due to irrational and overuses of them.

In vitro antifungal susceptibility testing could be helpful in the better management of the dermatophytosis.^{4,5} Recently, the Clinical and Laboratory Standard Institute (CLSI) announced the availability of a new standard guideline on antifungal disk diffusion susceptibility testing of yeast, M44-A in 2004, where in vitro testing of molds and dermatophytes was not addressed.⁶ of the several commercial systems now the disk diffusion Neo-Sensitabs method is of potential value for dermatophytes. This method has proven to be useful in testing yeasts and it has been modified to evaluate dermatophyte fungi.⁷

This study was aimed to isolate and identify different dermatophyte species from skin, nail and hair specimen and to determine the susceptibility pattern of azoles (fluconazole, miconazoles and itraconazole) against *Trichophyton rubrum* isolates by disk diffusion method.

Materials and Methods:

The study was carried out from September 2018 to

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August 2019. A total of 246 patients attending in the outpatient department of Dermatology and Venereology of BSMMU, Dhaka, clinically suspected of having dermatophytosis of skin, nail and hair were included in this study. All samples were screened by direct microscopy for presence of fungal elements by treating them with 20% KOH and culture performed in Dermatophyte test media (DTM) with supplements (Cycloheximide, chlortetracycline and gentamicin) (HiMedia, India) at 25-30°C for 21 days. Growth of dermatophytes changed the color of DTM from yellow to red. Species of dermatophytes were identified by observing the colonial morphology in culture by tease mount microscopy test and biochemical tests (Urease test and hair perforation test). The dermatophyte isolates were preserved in screw capped test tube containing 5ml distilled water at 4°C with proper labeling and date of preservation.

Preparation of inocula: The isolates were transferred from distilled water stocks to potato dextrose agar (PDA) (HiMedia, India) media at 25-30°C to enhance conidial growth. Seven days old culture was covered with 10 ml 0.9% normal saline and the colonies were scraped with the tip of a sterile wire loop to obtain a mixture of mycelium and conidia. The suspension was transferred to a sterile test tube and allowed to sediment the heavy particles for 5-10 minutes. The upper homogenous suspension was taken and transferred to another sterile test tube and adjusted to 0.5 McFarland standard visually against a card with white background and contrasting black lines.

Antifungal disk: The antifungal disks were purchased (Biomaxima, Poland) with following potencies: Fluconazole 25µg/disk, miconazole 10µg/disk and itraconazole 8µg/disk.

Disk diffusion assay: The disk diffusion method was done according to Neo-Sensitabs susceptibility testing (2002) method modified for dermatophytes⁷. Within 15 minutes of adjusting the turbidity of inoculum, a sterile cotton swab was dipped into the suspension, rotating and pressing firmly against the inside wall of the tube above the fluid level to remove excess fluid from the swab. The dried surface of a sterile Sabouraud dextrose agar (HiMedia, India) plate was inoculated by evenly streaking the swab over the entire agar surface. The antifungal disks were applied evenly. The plates were incubated at 25°C for 5-10 days. After the colonies being grown the zones of inhibition around the disks were measured and recorded.

Table-I: Zone diameters for antifungal drug susceptibility by disk diffusion method:

Antifungal drugs	Disk Potency	Zone diameter in mm		
		Sensitive	Intermediate	Resistance
Fluconazole	25µg	≥22	21 -15	≤14
Miconazole	10µg	≥20	19 -12	≤11
Itraconazole	8µg	≥15	14 -10	≤10

Ethical consideration: The study was ethically approved by Institutional Review Board (I.R.B), BSMMU (Reference NO. BSMMU/2019/1224 Date: 06/02/2019).

Results:

Out of 246 clinically suspected cases 67 (27.2%) were found as positive by direct microscopy and 71 (28.9%) were culture positive and 64 (26.0%) were both microscopy and culture positive. Three (1.2%) specimens were found as microscopy positive only and 7 (2.9%) were culture positive only (Table II).

Table-II: Distribution of microscopy and culture positivity among all specimens (n=246)

	Culture positive	Culture negative	Total
Microscopy positive	64 (26.0%)	3 (1.2%)	67 (27.2%)
Microscopy negative	7 (2.9%)	172 (69.9%)	179 (72.8%)
Total	71 (28.9%)	175 (71.1%)	246 (100.0%)

Among 71 dermatophyte isolates, 23 *Tinea cruris* (32.4%) was found as most common clinical types followed by *Tinea corporis* 22 (31.0%), *Tinea unguium* 12 (16.9%), *Tinea pedis* 9 (12.7%), *Tinea capitis* 4 (5.6%) and *Tinea mannum* 1(1.4%). Out of 71 culture positive isolates most 51 (71.8%) were *Trichophyton rubrum* followed by *Trichophyton mentagrophytes* 17 (24.0%) and *Epidermophyton floccosum* 3 (4.2%) (Table III).

Table-III: Distribution of dermatophyte isolates from different clinical types of samples (n=71)

Clinical types	Number of culture positive isolates	<i>T. rubrum</i>	<i>T. mentagrophytes</i>	<i>E. floccosum</i>
<i>Tinea capitis</i>	4 (5.6%)	1 (25.0%)	2 (50.0%)	1 (25.0%)
<i>Tinea faciei</i>	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
<i>Tinea corporis</i>	22 (31.0%)	16 (72.7%)	4 (18.2%)	2 (9.1%)
<i>Tinea manuum</i>	1 (1.4%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Tinea cruris</i>	23 (32.4%)	17 (73.9%)	6 (26.1%)	0 (0.0%)
<i>Tinea pedis</i>	9 (12.7%)	8 (88.9%)	1 (11.1%)	0 (0.0%)
<i>Tinea unguium</i>	12 (16.9%)	8 (66.7%)	4 (33.3%)	0 (0.0%)
Total	71 (100.0%)	51 (71.8%)	17 (24.0%)	3 (4.2%)

T=*Trichophyton*, E=*Epidermophyton*

Table IV showed the antifungal susceptibility of *Trichophyton rubrum* isolates against fluconazole, miconazole and itraconazole by disk diffusion method. Among 51 isolates, fluconazole was found as most resistant drug (90.2%) followed by miconazole (13.7%). Itraconazole was found as most sensitive drug (80.4%) followed by miconazole (60.8%). Intermediate sensitivity against miconazole was found as 25.5% followed by itraconazole (11.8%) and fluconazole (7.8%).

Table-IV: Antifungal susceptibility of *Trichophyton rubrum* isolates by disk diffusion method (n=51)

Antifungal drug		<i>Trichophyton rubrum</i>
Fluconazole	Sensitive	1 (2.0%)
	Intermediate	4 (7.8%)
	Resistance	46 (90.2%)
Miconazole	Sensitive	31 (60.8%)
	Intermediate	13 (25.5%)
	Resistance	7 (13.7%)
Itraconazole	Sensitive	41 (80.4%)
	Intermediate	6 (11.8%)
	Resistance	4 (7.8%)

Discussion

Superficial fungal infections are very common in tropical countries like Bangladesh. Among them the most common type is dermatophytosis. Due to irrational and over use, drug resistance is a very common matter. In vitro antifungal susceptibility test may help to select appropriate drug, optimize the therapy and monitor the emergence of drug resistance.³ This study was undertaken to identify different dermatophyte species and to see the susceptibility pattern of azoles (fluconazole, miconazole and itraconazole) among *Trichophyton rubrum* isolates. Among 246 specimens 27.2% were positive by microscopic examination which is comparable with other studies.^{3,8} This result is considered as higher than 14.3%⁹ and lower compared to 45.5%.¹⁰ In present study 28.9% cases were found as culture positive which are comparable with previous studies.^{3,8} This is slightly lower than 43%.¹¹ Higher or lower isolation rates from clinical specimens depends on different factors like collection of specimens, composition and pH of the media, incubation period and temperature, viability of fungal elements and co-existing microbes which may inhibit the growth of pathogenic fungi.

In this study, among all culture positive dermatophyte isolates *Tinea cruris* (32.4%) was found as most common clinical types followed by *Tinea corporis* (31.0%) and *Tinea unguium* (16.9%). These findings were not similar with a previous study from South India where highest incidence of *Tinea corporis* (35.4%) was reported followed by *Tinea cruris* (16.76%) and *Tinea capitis* (16.6%).¹² Another study in North East India reported highest incidence of *Tinea pedis* (29.2%) followed by *Tinea cruris* (26.2%).¹³ These variations in the site of infection between this study and others may be due to geographical variations, nature of the job, the number of study population, cultural variation and hygienic condition. In this study, among 71 culture positive samples *T. rubrum* was found as commonest dermatophyte isolates (71.8%) followed by *T. mentagrophytes* 17 (24.0%) and *E. floccosum* 3 (4.2%). These findings were consistent with previous study³, where *T. rubrum* was found to be 86.6%, *T. mentagrophytes* 8.2% and *E. floccosum* 5.2%. Another study in West Rajasthan, India where *T. mentagrophytes* (41.3%) was most prevalent followed by *T. rubrum* (17.3%), *T. tonsurans* (16%), *T. verrucosum* (12%) and *E. floccosum* (5.3%).¹⁴

By disk diffusion method, among 51 *T. rubrum* isolates 90.2% were resistant to fluconazole followed by miconazole (13.7%). Among them itraconazole (80.39%) was found as most sensitive (92.2%) followed by miconazole (60.78%). Intermediate sensitivity of miconazole was 25.5%. This result was comparable with

a study in Bangladesh where fluconazole was reported as most resistant (95%) and Itraconazole (92.5%) as most sensitive (97.5%) among azole drugs among *T. rubrum* isolates.³ But their study did not include miconazole. A recent study in India reported that *T. rubrum* isolates were 38.46% resistant against fluconazole.¹⁴

Conclusion

In vitro susceptibility showed that among three azoles tested; fluconazole had shown the highest resistance rate. Itraconazole was found to be the most sensitive drug followed by miconazole. Itraconazole can be used to treat most of the *T. rubrum* infections particularly those who show resistance to fluconazole. Antifungal sensitivity should be evaluated before prescribing miconazole.

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Conflict of interest

We do not have any potential conflicts of interest.

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