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Original Research Article

A Study on Resistance to Systemic Antifungal Drugs in Dermatophytosis

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Conflict of interest: Nil

Abstract:

Dermatophytosis is a common fungal infection of the skin caused by dermatophytes. This study aimed to identify the species of organisms causing dermatophytosis and evaluate their susceptibility to commonly used antifungal medications. A cross-sectional descriptive study was conducted on 100 patients diagnosed with superficial dermatophytosis. Data on demographics, disease history, and clinical manifestations were collected. Samples were obtained from lesions and hair, and microscopic examination and culture were performed. Trichophyton, Mentagrophytes and Trichophyton rubrum were the most commonly isolated species. Tinea corporis was the predominant clinical type. Antifungal susceptibility testing revealed Terbinafine to have the lowest minimum inhibitory concentration (MIC) among the tested drugs. The study emphasizes the importance of accurate identification and susceptibility testing to guide effective treatment of dermatophytosis.

Keywords: MIC, CLSI

Introduction

Dermatophytosis, a prevalent fungal infection affecting the skin, is primarily caused by dermatophytes. There are three main genera of dermatophytes responsible for causing dermatophytosis: Trichophyton, Epidermophyton, and Microsporum. It is common worldwide, with a 20% incidence in the USA.[1] Dermatophyte infection can lead to a range of clinical manifestations, from mild to severe, resulting from their attack of keratinized tissues. Five dermatophyte infections are recognized clinically: Tinea capitis, Tinea corporis, Tinea pedis, Tinea cruris, and Tinea unguium. Dermatophytosis is reported to be more prevalent in India, likely attributed to its tropical climate and the associated poor socioeconomic conditions which tend to result in inadequate personal hygiene. In

India, Tinea cruris and Tinea corporis are the commonly observed forms of dermatophytosis.[2,3] Antifungal drugs with less toxicity and better pharmacokinetics are now available to treat dermatophytosis. With some exceptions (e.g. Griseofulvin), the common antifungal drugs (Fluconazole, Itraconazole, and Terbinafine) act against the ergosterol synthesis pathway.

However, the emerging resistance to the common antifungal agents poses difficulty in treating these conditions. Though topical antifungal therapy effectively treats most superficial fungal infections, severe infections, and clinical types like Tinea unguium, Tinea capitis requires systemic treatment.[4] Testing the susceptibility in a controlled laboratory setting can be used to evaluate the effectiveness of a particular antifungal agent for the treatment of dermatophytosis; ascertaining the in vitro susceptibility of the drugs could be beneficial. Studies have demonstrated a significant correlation between the in vitro results obtained through the microdilution test and disc diffusion method and the therapeutic response in cases of dermatophyte infection.[5] Inappropriate antifungal use, including inadequate dosing or failure to complete the full course of treatment, has been associated with the development of resistance in clinical settings. The emergence and progression of antifungal resistance due to the misuse, inadequate, and irregular treatment of fungal infections is becoming increasingly common, leading to increased difficulty in treating these infections. Therefore, evaluation of the antimicrobial resistance patterns of drugs is essential for optimal clinical outcomes. Due to the socioeconomic realities of our nation, The establishment of a dependable and consistent approach for conducting in vitro antifungal susceptibility testing is crucial.[6]

The primary objective of this study was to identify the species of organisms isolated from individuals with dermatophytosis and assess the susceptibility of these isolates to four frequently employed antifungal medications.: Griseofulvin, Terbinafine, Itraconazole, and Fluconazole.

Materials And Methods

The cross-sectional descriptive study took place at the Department of Pathology, and Microbiology serving as the setting for the research, NRI Institute of Medical Sciences, Sangivalasa, from March 2022 to June 2022, with a sample size of 100 patients (male and female) diagnosed with superficial dermatophytosis.

The present study included all cases of superficial dermatophytosis in individuals aged 10 years and above. Participants with a history of allergy to the study drugs, as well as those who had received treatment (either topical or systemic) in the previous six months, were excluded from the study. For ethical reasons, pregnant and lactating women were also excluded.

Prior to their inclusion in the study, all participants provided informed written consent, demonstrating their voluntary agreement to participate. Data collected included demographic information (name, age, sex, weight, residential address, and occupation) as well as disease-related information (duration of the disease, site involved, and previous history of treatment, both topical and systemic). In addition, past medical and family histories were also recorded.

To obtain a sample from the active edge of the lesion, a sterile disposable scalpel blade was utilized for scraping purposes without causing any injury to the skin surface. To obtain scalp samples, the root of the hair was taken using autoclaved sterile tweezers. Following the collection of the scrapings, they underwent direct microscopic examination and culture to facilitate subsequent antifungal susceptibility testing.

The collected material was placed on a glass slide, and a drop of 10-20% potassium hydroxide (KOH) was added for examination, while hair specimens required the use of 40% KOH. Subsequently, a cover slip was placed over the material, and it was left undisturbed for a duration of twenty minutes to facilitate the digestion of keratin. The dermatophytes were then observed and identified under the light microscope as translucent, non-pigmented, septate mycelia, or arthrospores. In the case of infected hair, ectothrix, and endothrix infections were identified, as arthrospores were observed as either being outside the hair shaft as a sheath or within the hair shaft.[7]

The samples were carefully collected on sterile chart paper, followed by autoclaving at a temperature of 121°C for a duration of 15 minutes. Subsequently, the samples were safely transported to the laboratory for further analysis cultivation process was performed using Sabouraud's Dextrose agar (Emmon's modification), which consisted of 40g of 2% dextrose, 10g/L of peptone, 15g/L of agar, 0.2mg/100mL of gentamycin, and 50mg/100mL of cycloheximide.

The pH of the medium was maintained at 5.6 ± 0.2 (HIMEDIA ref: M063-500G). (8). Additionally, Dermatophyte Test Medium (DTM) was used, which contains 10g/l of glucose, 10g/l of papaic digest soybean meal, 0.2 of phenol red, and 20g/l of agar, with a pH of 5.5 ± 0.2 (HIMEDIA ref: M188-500G). Due to the production of alkaline metabolites by dermatophytes, the presence of these fungal organisms in the medium is indicated by a colour change from yellow to red in the pH indicator, phenol red.

The identification of fungal taxa was determined using a combination of colony morphology and biochemical tests The plates were incubated at ambient room temperature and periodically observed for any signs of growth. Plates that did not exhibit visible growth within a duration of four weeks were subsequently discarded. To observe the hyphal structure as well as microconidia and macroconidia, a lactophenol cotton blue mount was employed.. Hair perforation and urease tests were used to differentiate between Trichophyton mentagrophytes/Trichophyton rubrum and Microsporumcanis/Microsporum equinum, with the former testing positive. Urease test in Christensen's medium was used to detect hydrolysis of urea by Trichophyton mentagrophytes, indicated by the medium becoming deep red-white.

Antifungal susceptibility testing of dermatophytes was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) M38-A guidelines, utilizing the broth microdilution method..(9)

Preparation of antifungal stock solution:

A microdilution method was employed to assess the susceptibility of dermatophyte species to fluconazole, griseofulvin, itraconazole, and terbinafine. Seven- to fifteen-day-old growth of dermatophyte species was transferred to Sabouraud's dextrose broth and mixed with a vortex mixer to form a suspension. The turbidity of the suspension was adjusted with 1% McFarland standard. Solutions of the antifungal drugs were prepared at an initial concentration of 1000mg/L and further diluted in distilled water or organic solvents. 100µL of the inoculum was added to each row of the first ten wells and 100µL of each dilution of the antifungal drug was added to each row of the first ten wells. 200µL of the inoculum was added to each row of the eleventh well, with 200µL of Sabouraud's dextrose broth added to the twelfth well as a control. The microdilution trays were placed in an incubator at a temperature of 28°C for a period of four days. The minimum

inhibitory concentration (MIC) was determined as the lowest concentration ofthe antifungal drug that effectively inhibited the growth of dermatophytes.

Results:

The study findings revealed a higher prevalence of the disease among individuals aged 15 to 25 years compared to other age groups. The study population had 67 female patients and 33 male patients (Figure – 1). Among the diverse clinical types of dermatophytosis, Tinea corporis was identified as the most prevalent, constituting 60% of the cases. This was followed by a combination infection of Tinea corporis and Tinea cruris, which accounted for 22% of the cases. (Figure – 2). It was further revealed that 63 were positive for potassium hydroxide (KOH) and 27 had a positive culture result. (Table – 1). The primary organism isolated in the study was Trichophyton mentagrophytes, accounting for 48.14% of the cases. Trichophyton rubrum was the second most commonly identified species, comprising 33.3% of the cases. Additionally, Microsporumgypseum was found in 14.8% of the cases, while Microsporumcanis was isolated in 3.7% of the cases. (Figure – 3). The antifungal drug Terbinafine had the lowest minimum inhibitory concentration (MIC) among the four antifungal drugs examined, namely Terbinafine, Itraconazole, Fluconazole, and Griseofulvin, against the four predominant species. Itraconazole was the next lowest MIC, followed by Fluconazole and Griseofulvin. (Figure – 4) (Table – 2).

Images:

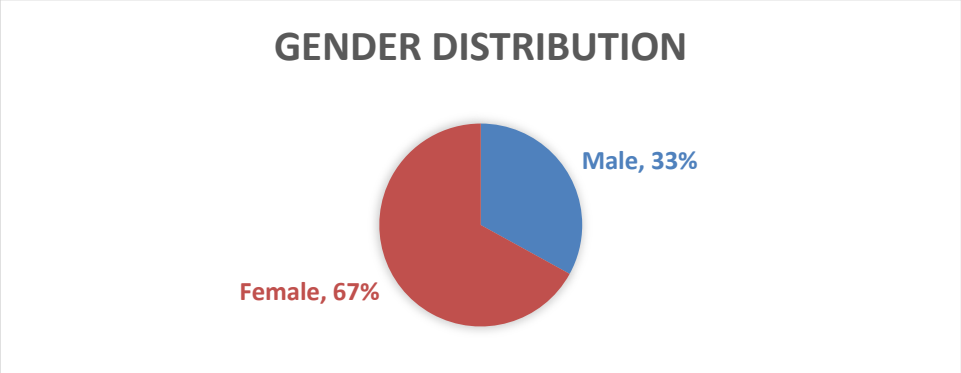

Figure 1: Gender distribution

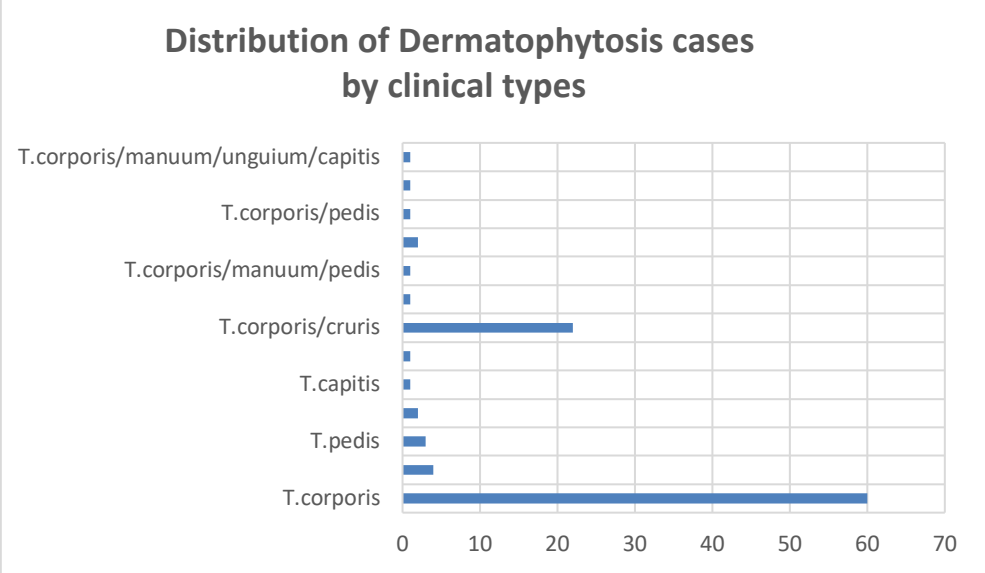

Figure 2: Distribution of Dermatophytosis cases by clinical types

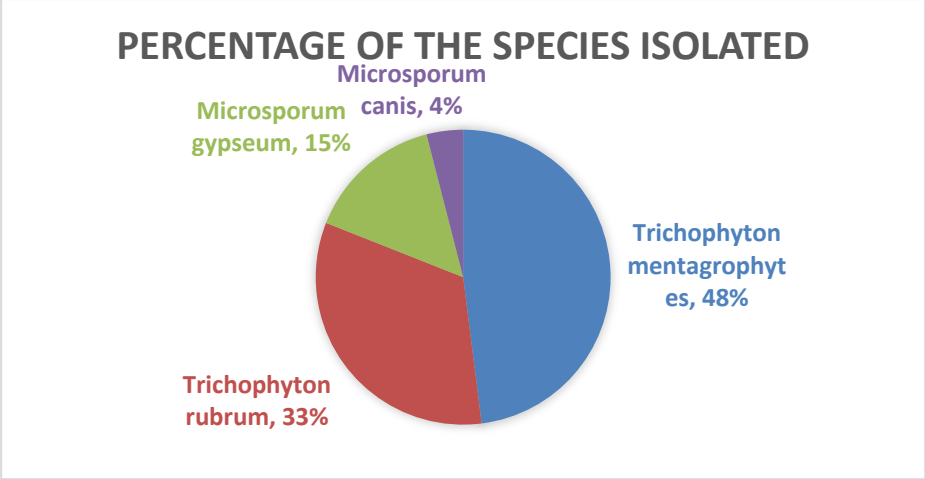

Figure 3: Percentage of the species isolated

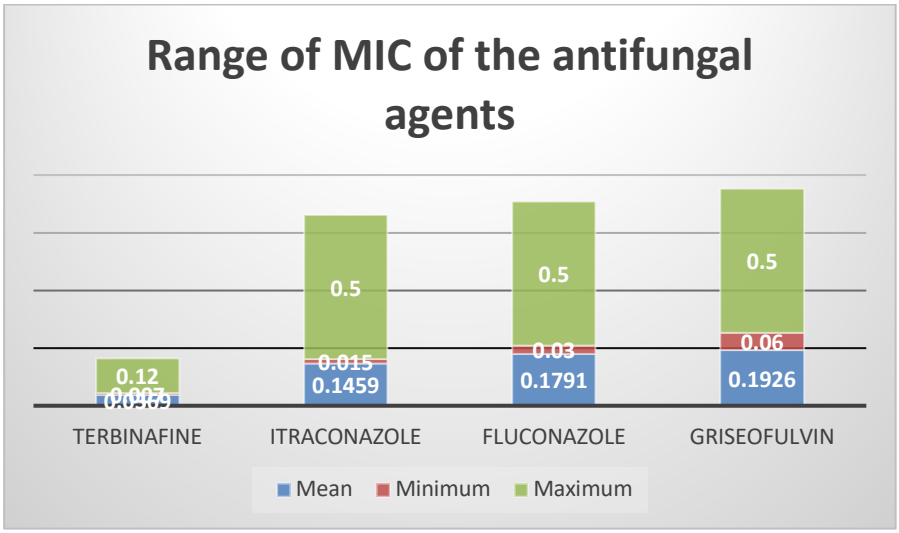

Figure 4: Range of MIC of the antifungal agents

Table 1: Clinical types and culture positivity

Diagnosis	Skin Scraping (KOH)		Culture	
	Positive	Negative	Positive	Negative
T.corporis	39	21	14	46
T.cruris	2	2	2	2
T.pedis	2	1	1	2
T.faceii	1	1	1	1
T.capitis	1	0	0	1
T.manuum	0	1	0	1
T.corporis/cruris	14	8	7	15
T.corporis/manuum	1	0	1	0
T.corporis/manuum/pedis	1	0	0	1
T.corporis/faceii	1	1	1	1
T.corporis/pedis	0	1	0	1
T.corporis/cruris/faceii/manuum	1	0	0	1
T.corporis/manuum/unguium/capitis	0	1	0	1
Total	63	37	27	73

Table2: Comparison of MIC for each species

Drug	SPECIES				
	Trichophytonmentagrophytes (n=9)	Trichophytonrubrum (n=13)	Microsporumgypseum (n=4)	Microsporumcanis (n=1)	Total (n=27)
Terbinafine	0.0267	0.0427	0.043	0.03	0.369
Itraconazole	0.1056	0.1854	1150	0.12	0.1459
Fluconazole	0.1444	0.1992	0.1113	0.5	0.1791
Griseofulvin	0.1856	0.2192	0.1057	0.25	0.1926

Discussion

This study included a total of 100 patients who were clinically suspected of having dermatophytosis. The findings of our study indicated that the highest frequency of cases was observed among individuals aged 15-25 years (31%), followed by the age groups of 25-35 years (28%) and 35-45 years (24%). The incidence of the condition was found to be low in the age group over 45 years, with a reported 17% prevalence. A similar concurrence was noted in most studies conducted by other workers, with the peak incidence in the third decade. [1, 2]

According to a study by KAK Surendran et al., the 16 to 30 age range had the highest incidence.(10) According to a recent study conducted by Suruchi Bhagra et al., the third decade of life exhibited a higher prevalence among the age groups studied.[11] In the majority of studies conducted by other workers, a male predominance was seen.[10 -12] In our study the number of males was 33% and the number of females was about 67%.

Mycological examination and culture of the study group revealed 63% positive results in direct microscopy for the presence of fungal elements and 27% positive results in culture.

The genus isolated from the culture were Trichophyton and Microsporum of which the commonest genus isolated was Trichophyton. The species Trichophyton mentagrophytes (48.14%) was predominant among them followed by Trichophyton rubrum (33.33%), Microsporumgypseum(14.81%), and Microsporumcanis (3.70On the contrary, several studies conducted by different researchers such as Kanwar et al., Bindhu et al., Singh et al., and Grover et al., have consistently reported Trichophyton rubrum as the most predominant species of dermatophytes, closely followed by Trichophyton mentagrophytes. [3, 13 – 15]

The most commonly observed clinical presentations were Tinea corporis (60%), followed by mixed infections of Tinea corporis and Tinea cruris (22%), Tinea cruris (4%), and Tinea pedis (1%). These findings align with the research conducted by SS Sen et al., DD Belurkar et al., and V Bindu et al., who similarly identified Tinea corporis as the most prevalent type of lesion..[13, 16, 17] We observed one case of Tinea capitis in an adult female. In the evaluation of antifungal susceptibility among dermatophyte isolates, Terbinafine exhibited the narrowest range of Minimum Inhibitory Concentration (MIC) values, ranging from 0.007 to 0.12 µg/mL, whereas Itraconazole demonstrated a slightly broader MIC range of 0.015 to 0.5 µg/mL. Gupta AK et al. in their study, concluded that terbinafine is a highly effective antifungal agent against dermatophytes.[18] The study conducted by Favre et al. provided evidence of the superior efficacy of allylamine terbinafine as an antifungal agent against certain species of dermatophytes..[19] Other authors have verified similar results, indicating that Terbinafine possessed the most effective minimum inhibitory

concentrations (MICs) against dermatophytes.[6]

Fluconazole exhibited the highest minimum inhibitory concentrations (MICs) within the range of 0.03-0.50 µg/mL, while Griseofulvin demonstrated MICs ranging from 0.06 to 0.50 µg/mL. Consistent findings were reported in other studies conducted by Favre et al. and Fernandez et al., corroborating similar results.[19, 20] Through our investigation, it was observed that Terbinafine exhibited the highest in vitro antifungal activity against the isolated species of Dermatophytes, as evidenced by the lowest Minimum Inhibitory Concentration (MIC) values. Following Terbinafine, Itraconazole, Fluconazole, and Griseofulvin demonstrated progressively higher MIC values in that order.

Conclusion

The predominant presentation observed in our study was Tinea corporis, followed by a mixed infection of Tinea corporis and Tinea cruris as the second most frequently encountered infection. The genus isolated were Trichophyton and Microsporum of which Trichophyton mentagrophytes were predominant followed by Trichophyton rubrum, Microsporumgypseum, and Microsporumcanis.

The lowest range of MIC in in-vitro antifungal susceptibility testing was found with Terbinafine followed by Itraconazole, Fluconazole, and Griseofulvin. Terbinafine had the least MIC of all four species isolated.

The assessment of in-vitro susceptibility demonstrated favourable activity of the tested antifungal drugs against the dermatophytes. Notably, Terbinafine exhibited the lowest Minimum Inhibitory Concentration (MIC) value among the tested agents. Based on the results of in vitro susceptibility testing, Terbinafine emerged as the most effective drug for the treatment of dermatophytosis.

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