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Original Research Article

An Observational Assessment of the Etiological Prevalence, Clinical Types and in Vitro Antifungal Drug Susceptibility Testing Against Dermatophytes in a Tertiary Care Setting

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Abstract

Aim: The aim of the study was to assess the etiological prevalence of pathogenic dermatophytes, clinical types of dermatophyte fungal infections, and in vitro antifungal drug susceptibility testing against dermatophytes to understand the variation in minimum inhibitory concentrations (MICs) levels of antifungals among dermatophytes.

Methods: The present study was conducted in the Department of Skin and VD, Patna medical College and hospital, Patna, Bihar, India. For 1 year. All patients with dermatophyte infections visiting the outpatient department during this period were screened. A total of 300 consecutive patients aged between 18 and 65 years with recurrent cases of tinea and other atypical presentations, receiving antifungal treatment, and willing to have minimum three days washout period before antifungal drug susceptibility testing of the clinical specimen (fungal isolate), were recruited.

Results: In the present study, Itching (93.33%), scaling (88.33%), dryness (83.3%) and inflammation (45%) where the most common clinical presentations Antifungal susceptibility testing was done for all 150 (50%) culture-positive patients. Griseofulvin reported the least mean MIC values, followed by luliconazole, eberconazole, sertaconazole, amorolfine and itraconazole. The mean MIC value of terbinafine (0.05 [0.043] µg/mL) was above the reference range. However, it was noted only in 25 (16.66%) out of total culture-positive patients. The individual high MIC values were reported up to 0.256 µg/ml [range: 0.001–0.03 µg/ml].

Conclusion: Dermatophytosis is a prevalent problem in the Indian scenario due to the hot and humid climate and low socio-economic status. Varied etiological agents, along with regional variations, make the management of this common cutaneous condition challenging. *T. rubrum* was found to be the most common, and *T. mentagrophytes* the emerging/codominant fungal isolate.

Keywords: Antifungal, coastal areas, dermatophytes, tinea infection, trichophyton

Introduction

Dermatophytes are keratinophilic hyaline molds that can cause disease in keratinized tissues like hair, skin, and nail. [1] The members of this dermatophytic group include Trichophyton, Microsporum and Epidermophyton. [2] Based on the reservoir and route of transmission, dermatophytes may be of anthropophilic (human), zoophilic (animals), or geophysics (soil) origin. These organisms are named according to the affected body site: Tinea capitis (head), T. corporis (trunk), T. cruris (perianal area), T. pedis (foot and interdigital area), and T. unguium (nail). [3] The most common etiological agents are Trichophyton rubrum, T. mentagraphytes, T. interdigitale, T. tonsurans, and Microsporum canis. T. rubrum is the most frequently isolated agent in clinics. [1]

Worldwide, several studies have documented a varied prevalence rate of dermatophytosis ranging from 14‑26.8% in North America, East Asia and Europe, and 5‑31.6% in Africa (Ethiopia, Kenya, Nigeria, and Tanzania). [4-6] The regional variations are mainly due to differences in the lifestyle, socioeconomic conditions, underlying risk factors, and environmental factors of different geographic areas. [7] Epidemics of dermatophytosis have also been reported in the area of overcrowding and poor hygienic conditions. [8-10]

The various antifungal agents currently available in clinical use against dermatophytes are terbinafine, itraconazole, fluconazole, luliconazole, etc. Even though antifungal agents' inappropriate use may result in resistant strains, their activity against dermatophytes has not yet been fully explored. The research outlining the antifungal susceptibility of common dermatophyte species in India is inadequate, posing a therapeutic challenge to practitioners. [11] Furthermore, despite the high incidence and clinical relevance, multicentric evidence depicting the present day clinico-epidemiological patterns of dermatophytosis across India is scarce. The magnitude of the concern thus demands studies across different geographic locations within India to increase the generalizability of the data.

Treatment options for dermatophytosis are topical as well as systemic antifungal drugs. But during course of time dermatophytes have also evolved drug resistance for single as well as multiple drug simultaneously. Studies conducted worldwide show that resistance among dermatophytes is not uncommon. [12,13] Due to high temperature and increased humidity, there are increased cases of dermatophytosis and other fungal infections especially in terrain and hilly region of Western Nepal. Since there was increased incidence of drug resistance observed over a period of time to the antimycotic drugs commonly used for the treatment i.e., fluconazole, terbinafine and clotrimazole, the need for testing of antifungal susceptibilities of dermatophytes has become apparent.

The aim of the study was to assess the etiological prevalence of pathogenic dermatophytes, clinical types of dermatophyte fungal infections, and in vitro antifungal drug susceptibility testing against dermatophytes to understand the variation in minimum inhibitory concentrations (MICs) levels of antifungals among dermatophytes.

Methods

The present study was conducted in the Department of Skin and VD, Patna medical College and hospital, Patna, Bihar, India. All patients with dermatophyte infections visiting the outpatient department during this period were screened. A total of 300 consecutive patients aged between 18 and 65 years with recurrent cases of tinea and other

atypical presentations, receiving antifungal treatment, and willing to have minimum three days washout period before antifungal drug susceptibility testing of the clinical specimen (fungal isolate), were recruited.

Patients with a non-mycotic pathology in the area of fungal infection or any condition that, in the investigator's opinion, does not justify the patient's inclusion in the study were excluded from the study. All patients provided written consent in the patient authorization form to participate in the study. A detailed history was obtained from all patients, who were then subjected to clinical examinations and investigations, including a wet preparation for direct microscopic examination, fungal culture and antifungal susceptibility tests.

Sample processing

All the 300 scraping samples were collected, and the specimens were shipped to a central facility. The primary identification of dermatophytes was done using direct microscopy with 10% potassium hydroxide (KOH) mount. Direct microscopic examination of the wet-mount was performed under a microscope, under ×10 and ×40 for fungal hyphae, spores or yeast cells. The Sabouraud dextrose agar (SDA) was used for isolation and identification of fungal isolates. Specimens were cultured on SDA media (Micro Master Laboratories Pvt. Ltd) with 0.05% chloramphenicol alone (Micro Master Laboratories Pvt. Ltd), or with 0.5% cycloheximide (HI Media Laboratories Pvt. Ltd) and 0.05% chloramphenicol (Micro Master Laboratories Pvt. Ltd) and incubated at 30°C for up to four weeks. Cultures were examined once a week and professed negative if no growth was observed until 6 weeks. Identification of dermatophytes to the species level was done by assessing the colony morphology, microscopy (Lactophenol Cotton Blue Mount), and physiological and biochemical tests. Further antifungal drug susceptibility testing was performed, and the minimum inhibitory concentration (MIC) of the drugs was determined.

Antifungal drug susceptibility testing

Antifungal drug susceptibility testing was performed as per the micro broth dilution technique of Clinical and Laboratory Standards Institute Guidelines (CLSI M38-A).6,7 The antifungal drug susceptibility testing was done for seven antifungal agents, namely, luliconazole, sertaconazole, eberconazole, itraconazole, terbinafine, griseofulvin and amorolfine. The MIC for the antifungals was interpreted according to the CLSI M38-A guidelines.

Statistical analysis

All the data from cases was fed in MS Excel (Microsoft office 2018) and then analyzed by Statistical Package for Social Service (SPSS) for window version; SPSS 22, Inc., Chicago, IL). All data were expressed in terms of percentage.

Results

Table 1: Clinical features

Category	Male (n=200) (%)	Female (n=100) (%)	Total (n=300) (%)
Itching	190 (95)	90 (90)	280 (93.33)
Dryness	170 (75)	80 (80)	250 (83.33)
Inflammation	100 (50)	35 (35)	135 (45)
Scaling	180 (90)	85 (85)	265 (88.3)
Pustules	10 (5)	12 (6)	22 (7.33)
Erythema	40 (40)	35 (35)	75 (25)
Alopecia	6 (3)	1 (1)	7 (2.33)
Local hair loss	4 (2)	3 (3)	7 (2.33)

Lesion with central clearing surrounded by an advancing, red, scaly and elevated border (Ring worm lesions)	40 (40)	45 (45)	85 (28.33)
Annular patches of inflammatory or non-inflammatory alopecia	3 (3)	1 (1)	4 (1.33)
Erythema and mild scaling on the dorsal aspect of the hands	1 (1)	1 (1)	2 (0.66)

Itching (93.33%), scaling (88.33%), dryness (83.3%) and inflammation (45%) were the most common clinical presentations [Table 1].

Table 2: Antifungal susceptibility testing among culture positive patients

Category	Culture positive (n = 150)
Terbinafine	
High MIC	25 (16.66)
Susceptible	125 (83.34)
MIC (µg/mL), mean (SD)	0.05 (0.043)
MIC90	0.001–0.03
Griseofulvin	
High MIC	0
Susceptible	150 (100)
MIC (µg/mL), mean (SD)	0.19 (0.082)
MIC90	0.25–3.0
Itraconazole	
High MIC	0
Susceptible	150 (100)
MIC (µg/mL), mean (SD)	0.84 (0.252)
MIC90	0.05-1.0
Luliconazole	
High MIC	0
Susceptible	150 (100)
MIC (µg/mL), mean (SD)	0.29 (0.286)
MIC90	0.05-1.0
Sertaconazole	
High MIC	0
Susceptible	150 (100)
MIC (µg/mL), mean (SD)	0.36 (0.372)
MIC90	0.05-1.0
Amorolfine	
High MIC	0
Susceptible	150 (100)
MIC (µg/mL), mean (SD)	0.60 (0.306)
MIC90	0.05-1.0
Eberconazole	
High MIC	0
Susceptible	150 (100)
MIC (µg/mL), mean (SD)	0.32 (0.251)
MIC90	0.05-1.0

Antifungal susceptibility testing was done for all 150 (50%) culture-positive patients. Griseofulvin reported the least mean MIC values, followed by luliconazole,

eberconazole, sertaconazole, amorolfine and itraconazole. The mean MIC value of terbinafine (0.05 [0.043] µg/mL) was above the reference range. However, it was noted only in 25 (16.66%) out of total culture-positive patients. The individual high MIC values were reported up to 0.256 µg/ml [range: 0.001–0.03 µg/ml]. Higher MIC values were reported for terbinafine for both T. mentagrophytes (0.256 µg/ml) and T. rubrum (0.256 µg/ml). The MIC values for itraconazole were within the range; while griseofulvin had the lowest mean MIC (0.25–3.0 µg/mL). The MICs of itraconazole, luliconazole, amorolfine, sertaconazole and eberconazole were within the reference range.

Discussion

Identification of species responsible for the dermatophytoses and their sensitivity pattern is of great importance not for epidemiology but also for therapeutic point of view. Our study site bears tropical climate where high level of humidity and high temperature favor the growth of fungi causing dermatophytoses.

Most enrolled patients were in the age group of 18–30 years, followed by 31–40 years which agrees with India's reported literature on dermatophytosis-centric studies. [14-16] The higher incidence in young males could be attributed to their increased physical activity, predisposing them to increased sweating. The lower incidence among females seen in this study could be attributed to their hesitation to consult physicians and the financial dependence on males. To prevent the unnecessary usage of toxic drugs, regular surveillance of antifungal susceptibility patterns in patients should be carried out in their long-term interest. [17]

Out of seven antifungal agents tested in this study, high MIC values were reported only for terbinafine. High MIC for terbinafine was reported only in 25 (16.66%) patients out of 150. The overall mean high MIC value reported for terbinafine could be attributed to high individual patients' data of these 20 patients. Higher MIC values were noted in both T. mentagrophytes (0.256 µg/ml) and T. rubrum (0.256 µg/ml), suggesting the virulent nature of T. mentagrophytes and T. rubrum. Furthermore, 88.5% of patients had MIC within range for terbinafine. Hence, the clinician must consider the plausible reasons such as virulence potential of the infecting species, clinical type of dermatophytosis and external factors such as heat, humidity, sweating, type of clothing and the pharmacological factors such as the quality of the drug, compliance, pharmacokinetics and absorption of the drug to understand the recalcitrant infection better. [18]

All 150 (100%) patients were susceptible to griseofulvin, itraconazole, luliconazole, sertaconazole, amorolfine and eberconazole. All the causative agents reported in our study were found to be susceptible to itraconazole. The median MIC (µg/ml) of itraconazole (range 0.05–1 µg/mL) for all 174 patients was 1.0 µg/ml. However, though the MIC of itraconazole was within the range, the upper side of the higher limit was found in the majority of patients. A similar trend

This indicates the need to optimize the use of itraconazole, emphasizing on the right dose and duration of treatment, considering the present effectiveness of oral itraconazole in our routine clinical practice. Moreover, this is the last drug in the current armamentarium, and hence rational use of itraconazole is the need of the hour. Vardai Pai et al. had also reported lower MIC of systemic griseofulvin and topical amorolfine than fluconazole. [19,20]

Conclusion

Dermatophytosis is a prevalent problem in the Indian scenario due to the hot and humid climate and low socio-economic status. Varied etiological agents, along with regional variations, make the

management of this common cutaneous condition challenging. T. rubrum was found to be the most common, and T. mentagrophytes the emerging/codominant fungal isolate. Tinea corporis was the most common clinical type of dermatophytosis. Various techniques are available for antifungal susceptibility testing of dermatophytes but only broth micro dilution technique is currently accepted to determine in‑vitro susceptibility of dermatophytes. As this technique is laborious and need expertise, only few mycology laboratories can perform this test. However, further studies on larger samples are warranted to correlate the MIC values with clinical outcomes to define the MIC breakpoints which will help adapt to therapeutic choices with high chances of success.

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