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Original Research Article

Assessing Different Species of Dermatophytes Causing Dermatophytosis at a Tertiary Care Setting: An Observational Study

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Abstract

Aim: The aim of this study was to identify different species of dermatophytes causing dermatophytosis at a tertiary care hospital.

Material & Methods: This cross-sectional study was conducted in the Department of Microbiology, in between the duration of 2 years. A total of 200 patients were selected following selection criteria. Patients at any age of either sex, who attended the outpatient department of Microbiology, during the study period, were enrolled.

Results: Among total number of 200 cases most common age group affected with dermatophytosis was 21-30 years age group with 70 cases and least common age group affected 51 and above years age group with 5 cases (2.5%). In the study subjects dermatophytosis was predominant in males compared to females. Tinea corporis was more common (45%) followed by Tinea cruris (27.5%). Out of 200 clinical samples collected 168 were skin scraping, 20 were hair stubs and other 12 were nail scrapings and clippings. Fungi were demonstrated in 135 cases by direct microscopy. 70 cases were positive by both microscopy and culture. 65 cases were positive by microscopy and negative by culture. 20 cases were negative by microscopy but culture positive. Fungal culture was positive in 45% of cases in SDA medium whereas it was positive in 40% of cases on DTM medium.

Conclusion: Dermatophytosis is a common infection in young adults specially females. Trichophyton mentagrophyte is more frequent type of dermatophyte. Majority of dermatophytes could be identified by both direct microscopy and culture methods.

Keywords: Dermatophyte; Dermatophytosis; Teniasis; Trichophyton; Polymerase Chain Reaction (PCR).

Introduction

Superficial mycoses refer to the diseases of the skin, nail, and hair caused by fungal agents. Over the past decades, the prevalence of these infections has been on a rising trend; accordingly, they have affected 20-25% of the world's population. These diseases are more common in the tropical countries due to humidity, elevated temperature, and sweating. The major examples of superficial mycoses include dermatophytosis, pityriasis versicolor, and candidiasis. [1] Superficial mycosis is more prevalent in tropical and subtropical countries, such as India. Among all fungus, dermatophyte is one of the cutaneous fungi. They have both keratinophilic and keratinolytic properties. [2,3] They are capable of invading human and animal keratinized tissue causing dermatophytosis. [4]

Dermatophytosis has several distinct cutaneous manifestations. [3,4] The severity of the disease depends on various factors including- strain or species of infecting dermatophyte, the sensitivity of the host and the site of infection. [5] Dermatophyte belongs to three group named as Trichophyton, Epidermophyton and Microsporum. [4] According to WHO, the prevalence rate of superficial mycotic infection worldwide has been found to be 20%–25%. Dermatophytic infections are commonly encountered in more than 50% of patients attending dermatology outpatient departments in South India. [6] Dermatophytes are hyaline septate moulds with more than 100 species. Nearly 40% of dermatophytes account for the incidence of other medical illnesses. The severity of dermatophytic infections depends on a variety of factors, such as host reactions to the metabolic products of the fungi, virulence of pathogenic species or particular strain, anatomical site of the infection, and local environmental risk factors. [7] Dermatophytes are mainly aerobic fungi producing enzyme-like proteases that digest keratin and permit

colonization, invasion, and infection of the skin, hair shaft, and nails. The dermatophytic infection spreads easily by direct contact with the infected humans and animals or through fomites. Although the infection is non-invasive and curable, its widespread nature and therapeutic costs are major public health problems, imposing a high economic burden on society, especially in the developing tropical countries like India [8] due to high morbidity as well as cosmetic damage. [9]

Dermatophytic infections have some typical features. [9] Sometimes it is confused with other skin disorders.9 A tinea corporis eruption that is more papulosquamous in presentation may be mistaken for psoriasis, nummular eczema, seborrheic dermatitis or pityriasis rosea. [10] Inverse psoriasis, seborrheic dermatitis, candidiasis, erythrasma, lichen simplex chronicus, darier's disease, and pemphigus vegetans may be mistaken for tinea cruris. [11] Vaginal candidiasis, which often affects women, may be distinguished from tinea cruris of males; satellite lesions and white pustules of candida may be seen, whereas dermatophytes do not. [12] Therefore, to avoid a misdiagnosis, identification of dermatophyte infections requires prompt and methodical laboratory diagnosis. [9,10]

Fungal culture and light microscopic mycological examination are required for identification of dermatophyte infections phenotypically. [10] Microscopic examination is easy, rapid and inexpensive diagnostic test; but it may show false negative results up to 15% cases. [13] While culture methods are specific diagnostic tests for dermatophyte identification, although culture of species takes approximately 4 weeks for the growth of the fungus. [14] Molecular approaches have been developed to provide more accurate alternatives for dermatophyte identification. Sequencing methods targeting the ITS region are the most popular techniques used for definitive identification of a fungal strain. [14,15] The accuracy in diagnostic methods is important to provide definitive treatment of dermatophytosis. [14,15]

This study was aimed to identify different dermatophytes species causing dermatophytosis at a tertiary care hospital (DMCH) in Bangladesh by microscopy, culture, biochemical tests, and PCR.

Material & Methods

This cross-sectional study was conducted in the Department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India in between the duration of 2 years. A total of 200 patients were selected following selection criteria. Patients at any age of either sex, who attended the outpatient department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India during the study period, were enrolled.

Inclusion Criteria: Patients with clinically suspected cases of ring worm infection, without using any antifungal drugs for last one month.

Exclusion Criteria: Patients having concomitant other skin infections like- pityriasis versicolor, seborrheic dermatitis, eczema, lichen planus, psoriasis vulgaris etc.

Methodology

Specimen collection

After a detailed clinical history along with all aseptic precaution lesions of the study patients were examined carefully under proper light. The affected area was cleaned properly (with 70% ethyl alcohol), then following standard procedure skin scales/crusts and pieces of nails were collected by gentle scrapping across the inflamed margin of the lesions. Hairs were epilated aseptically with sterilized tweezers.

Microbiological methods

KOH wet mount

The specimens collected were subjected to KOH wet mount preparation. 10% KOH used for skin and hair samples and kept for 10-15 minutes and 40% KOH used for nail samples, kept overnight. A small amount of sample is taken and added to the drop of KOH placed on a glass slide and a cover slip is placed on it. On microscopy, branching hyaline mycelia which frequently show arthrospores production were seen.

Culture

Irrespective of demonstration of fungal elements on microscopy, the specimen was inoculated onto Sabouraud's dextrose agar with 0.05% Chloramphenicol and 0.5% Cycloheximide and incubated at 28°C for up to four weeks, and was observed periodically for growth. If no growth was found after four weeks, it was taken as negative for fungal growth.

Macroscopic examination of culture

The growth on Sabouraud's dextrose agar was examined to study the colony morphology based on following characteristics. On obverse for colour and consistency and on reverse for the presence or absence of pigment, whether diffusing or not.

Tease mount

By lactophenol cotton blue

A small portion of a colony was picked and suspended in two drops of lactophenol cotton blue placed on a clean slide.

The mycelial mat was teased apart with dissecting needles, covered with cover-slip and observed under microscope for presence of aseptate slender hyphae, macro and microconidia and their arrangement.

Dermatophyte Test Media (DTM)

This medium was used to confirm whether the fungus grown was dermatophyte. All isolated dermatophytes were inoculated onto DTM and incubated at 28°C for 7 days and observed for color change. Color change of the medium from yellow to red indicated growth of dermatophytes. All species of dermatophytes showed this color change. Dermatophyte species were further confirmed based on urease test

Urease test

This test is to differentiate between T. mentagrophytes and T. rubrum. Christensen's urea agar slant was inoculated with the test fungus. T. mentagrophytes hydrolyses urea usually within seven days and colour of the medium changes to pink. T. rubrum isolates were negative for urease test.

Results

Table 1: Age and gender wise distribution of clinically diagnosed dermatophytosis

Age in years	N	%
≤ 10	15	7.5
11-20	20	10
21-30	70	35
31-40	50	25
41-50	40	20
>51	5	2.5
Gender		
Male	128	64
Female	72	36

Among total number of 200 cases most common age group affected with dermatophytosis was 21-30 years age group with 70 cases and least common age group affected 51 and above years age group with 5 cases (2.5%). In the study subjects dermatophytosis was predominant in males compared to females.

Table 2: Clinical types of dermatophytosis and clinical samples collected

Clinical types	N	%
Tinea corporis	90	45
Tinea cruris	55	27.5
Tineafacei	10	5
Tinea pedis	10	5
Tinea barbae	8	4
Tinea manuum	3	1.5
Tinea capitis	12	6
Tinea unguium	12	6
Clinical samples collected		
Skin scrapings	168	84
Hair stubs	20	10
Nail scraping and clipping	12	6

Tinea corporis was more common (45%) followed by Tinea cruris (27.5%). Out of 200 clinical samples collected 168 were skin scraping, 20 were hair stubs and other 12 were nail scrapings and clippings.

Table 3: Comparison of results obtained in the direct microscopic examination and culture

Culture	Number of cases		Total
	KOH+ve	KOH-ve	
Culture positive	70	20	90
Culture negative	65	45	110
Total	135	65	200

Fungi were demonstrated in 135 cases by direct microscopy. 70 cases were positive by both microscopy and culture. 65 cases were positive by microscopy and negative by culture. 20 cases were negative by microscopy but culture positive.

Table 4: Comparison of culture positivity in Dermatophyte Test Medium with Sabouraud's Dextrose Agar

Fungal Culture medium	Culture positivity Number
Sabouraud's Dextrose Agar (with cycloheximide and chloramphenicol)	90
Dermatophyte Test Medium	80

Fungal culture was positive in 45% of cases in SDA medium whereas it was positive in 40% of cases on DTM medium.

Discussion

The dermatophytes are a group of closely related fungi that have the capacity to invade the keratinized tissues of skin, hair and nails and cause an infection, dermatophytosis, commonly referred to as ringworm or tinea. Earth has been documented as a natural territory for fungi which cover individual kingdom with evolution.[16] Among all fungus, dermatophyte is one of the cutaneous fungi. They have both keratinophilic and keratinolytic properties. [2,3] They are capable of invading human and animal keratinized tissue causing dermatophytosis. [4] Dermatophytosis has several distinct cutaneous manifestations. [3,4] The severity of the disease depends on various factors including- strain or species of infecting dermatophyte, the sensitivity of the host and the site of infection. [5] Dermatophyte belongs to three group named as Trichophyton, Epidermophyton and Microsporum. [4] Further they are divided into anthopophilic, zoophilic and geophilic according to their natural habitat. [4,5]

Among total number of 200 cases most common age group affected with dermatophytosis was 21-30 years age group with 70 cases and least common age group affected 51 and above years age group with 5 cases (2.5%). Madhavi et al [17] found that tinea infections were more common in the 16‑year‑old to 45‑year‑old age group. In our study, a higher incidence of tinea infection of the capitis was noted. A higher incidence of dermatophytosis was seen in males than in females, which supports Sumathi et al [18] findings. Male predominance may be due to increased outdoor physical activities and increased sweating, which create a favorable environment for fungal infections, as well as a greater opportunity for exposure to infection than females. In the study subjects dermatophytosis was predominant in males compared to females. Tinea corporis was more common (45%) followed by Tinea cruris (27.5%). Jha et al [19] found that the most common genus was Trichophyton, followed by Epidermophyton.

Out of 200 clinical samples collected 168 were skin scraping, 20 were hair stubs and other 12 were nail scrapings and clippings. In the present study, aqueous KOH was used as a clearing agent for the direct demonstration of fungi in skin or hair scrapings, [20] but the addition of dimethyl sulfoxide (DMSO), as described by Rebell and Taplin [21] was found to be a better preparation over plain KOH. The addition of DMSO allows for the rapid clearing of keratin and almost immediate examination of the sample without a warming of the slide. [22] On the other hand, Tampieri [23] reported that it seems difficult to rely on results of direct microscopy with KOH to establish the diagnosis of fungal infection as it could not detect the characteristic morphology of the three genera and it lacks sufficient sensitivity, although it is highly efficient as a screening technique before therapy is initiated because of the expense, duration, and potential adverse effects of the treatment.

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Conclusion

Dermatophytosis is a common infection in young adults specially females. Trichophyton mentagrophyte is more frequent type of dermatophyte. Majority of dermatophytes could be identified by both direct microscopy and culture methods.

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