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Epidemiology, Classification, Clinical Variations and Laboratory Diagnosis of Dermatophytes: A Narrative Review

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Abstract

Dermatophyte is a keratinophilic fungus which can invade keratinized tissue cause dermatophytosis. It is one of the major superficial fungal infections. According to World Health Organization (WHO), 20-25% world population is affected by dermatophytes. Tinea or ring worm infection may have similar feature with other skin diseases. For this reason, identification is necessary in those cases. The aim of this study was to identify different species of dermatophytes causing dermatophytosis at a tertiary care hospital in Bangladesh by Microscopy, culture and PCR.

Keywords: Epidemiology; classification; clinical variations; laboratory diagnosis; dermatophytes

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Introduction

Dermatophyte is one of the cutaneous fungi. They have both keratinophilic and keratinolytic properties[1,2]. They are capable of invading human and animal keratinized tissue[3]. It belongs to three group named as Trichophyton, Epidermophyton, Microsporum[3-4]. Dermatophytosis lesion is called ring worm lesion. It takes single or multiple ring shape lesions with inflammatory edges[4-5]. Both healthy and immune compromised patients are affected from this infection. The estimated life time risk of acquiring dermatophytic infection is 10.0% to 20.0% cases[6]. Climate, lifestyle, involvement of outdoor activities, disease conditions (diabetes, malnutrition) are responsible for the heterogeneous prevalence[7-8]. Dermatophytic infections have typical features. Sometimes it is confused with other skin disorders[9]. A tinea corporis eruption that is more papulosquamous in presentation may be mistaken for psoriasis, lichen planus, seborrheic dermatitis, pityriasis rosea, or pityriasis rubra pilaris[10]. To avoid a misdiagnosis, identification of dermatophyte infections require prompt and methodical laboratory diagnosis[9-11].

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Epidemiology

According to the World Health Organization, dermatophytes affect about 25.0% of the world population[12]. Majority of the infection by dermatophytes has occurred during the 3rd and 4th decades of their life. Male and female both are affected. It is more frequent in hot and humid climate. The epidemiology of dermatophytoses has changed during last century under the influence of socioeconomic factors, life style, migration of people from the southern to northern hemisphere.

In a large survey performed by the Mycology Reference Laboratory, Bristol, UK, Trichophyton rubrum was the most frequently isolated dermatophyte (70.0% in 2005), followed by Trichophyton interdigitale (20.8%)[13]. A study conducted in the University Hospital of Cadiz from 1998 to 2008 showed a predominance of Trichophyton rubrum (38.2%) with an increasing incidence from 2000, Microsporum canis being only the second most frequently isolated dermatophyte (22.8 %)[14]. The most recent study is that of Foster et al. published in 2004, an epidemiological surveillance study conducted at the

Center for Medical Mycology in Cleveland, Ohio, from 1995 to 2002. In that study, Trichophyton rubrum was the most prevalent fungal pathogen with an increasing incidence observed between 1999 and 2002 from 32% to 47.0% cases[15]. In 1999 to 2001 in Tehran, Epidermophyton floccosum was the main dermatophyte isolated (31.4%), followed by Trichophyton rubrum (18.3%)[16]. In India, a study conducted in a tertiary care centre in a rural area in southern India showed Trichophyton rubrum (58.9%) was the main agent found in the study, followed by Trichophyton mentagrophytes (24.6%)[17].

Pathogenesis

In the infectious process, dermatophytes must overcome the host's innate defence mechanism, so that tissue colonization occurs. The physical and chemical structure of the skin, constant exposure to ultraviolet light, temperature, lack of humidity and the presence of normal microbiota make the growth of pathogenic microorganisms difficult. An important defense mechanism of the body against infectious agents that affect superficial sites is keratinized, the stratum corneum renewal process done by keratinocytes that leads to epithelial sheeding and consequently to the possible removal of the fungus. Therefore, for the pathogen reach to the epidermis, it should adhere to the surface of the tissue. The arthoconidium should germinate and the hypae quickly enter the stratum corneum enter the stratum corneum, preventing that the fungus be eliminated with epithelial shedding. In the pathogenesis of dermatophytosis, the initial interaction between the arthroconidia and the stratum corneum occurs 3 to 4 hours after contact[12,18].

Little is known about the factors that mediate adherence of dermatophytes; however, it has been hypothesised that dermatophytic-secreted proteases could facilitate or even be necessary for efficient adherence. The ability of Trichophyton rubrum to adhere to epithelial cells has been attributed to carbohydrate-specific adhesins, expressed on the surface of microconidia. From a morphological point of view, fibrillar projections have been observed in T. mentagrophytes during the adherence phase[19]. Trichophyton mentagrophytes arthroconidia produce long fibrils when it is on the surface of the stratum corneum, whereas short fibrils are produced inside the dense sublayers. Adherence occurs by the establishment of short and long fibrils that appear to anchor and connect the arthroconidia to the tissue surface and prevent the disconnection of the exposed fungal element under the rough conditions on the skin surface[20].

Dermatophytes are provided with an arsenal of proteases aimed at the digestion of the keratin network into assimilable oligopeptides or amino acids. Once established, the spores must germinate and penetrate the stratum corneum at a rate faster than desquamation. Penetration is accompanied by dermatophytes secreting multiple serine-subtilisins and metallo-endoproteases (fungalysins) formerly called keratinases that are found almost exclusively in the dermatophytes. The mechanism by which mucolytic enzymes, which help in penetration, also provide nutrition to the fungi is unknown[21]. These dermatophytic keratinolytic proteases cannot act before disulfide bridges are reduced within the compact protein network constituting keratinized tissues. Fungal mannans in the dermatophyte cell wall have immuno-inhibitory effects and T. rubrum cell wall mannans (TRM) seem to be involved in an immunosuppression phenomenon, inhibiting lymphoproliferative response of mononuclear leukocytes in response to several antigens (dermatophytic or not) and mitogens[19].

Classification based on Natural Habitat

They have been classified as geophilic, zoophilic and anthropophilic species on the basis of their primary habitat associations. Geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves and horns, as a part of their decomposition process. Zoophilic and anthropophilic dermatophytes are adapted to the animal or human host and are the most frequent agents of superficial mycoses in animals and humans infecting the stratum corneum, hair, claws or nails.

Clinical Types of Dermatophytoses in Human

1. Tinea Corporis: Tinea corporis or ring worm, typically appears as single and multiple,annular, scaly lesion with central clearing.a slightly elevated, redden edge, and sharp margination on the trunk,extrimities or face. The border of the lesion may contan pastule or follicular papules. Itching is variable[23]. Organisms most commonly encountered are Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis. Leisons are commonly found on non-hairy areas of the trunk, although any site of the body may be affected[4].

2 Tinea pedis: Tinea pedis or athlete's foot is the most frequent dermatophytosis around the world. The

incidence of tinea pedis in adult is significantly more than the children. Adult, Immunocompromised patients are more prone to develop tinea pedis[24]. Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton interdigitale, Epidermophyton floccusum are the most common fungi for these lesions[25].

Table 1: Classification of dermatophytes according to morphology[3,22]

Genera and Site of Infection	Name of Species
Epidermophyton (Skin and Nail)	*Epidermophyton floccosum*
	Epidermophyton stockdaleae
Microsporum (Skin and Hair)	*Microsporum audouinii*
	Microsporum boullardii
	Microsporum canis
	Microsporum cookei
	Microsporum duboisii
	Microsporum equinum
	Microsporum ferrugineum
	Microsporum fulvum
	Microsporum gallinae
	Microsporum gypseum
	Microsporum langeronii
	Microsporum nanum
	Microsporum persicolor
	Microsporum praecox
	Microsporum rivalieri
	Microsporum racemosum
	Microsporum vanbreuseghemii
T*richophyton* (Skin, Hair and Nail)	*Trichophyton ajelloi*
	Trichophyton concentricum
	Trichophyton. equinum
	Trichophyton flavescens
	Trichophyton gloriae
	Trichophyton gourvilii
	Trichophyton kanei
	Trichophyton megninii
	Trichophyton mentagrophytes
	Trichophyton onychocola
	Trichophyton phaseoliforme
	Trichophyton redellii
	Trichophyton raubitschekii
	Trichophyton rubrum
	Trichophyton schoenleinii
	Trichophyton simii
	Trichophyton soudanense
	Trichophyton terrestre
	Trichophyton tonsurans
	Trichophyton vanbreuseghemii
	Trichophyton verrucosum
	Trichophyton violaceum
	Trichophyton yaoundei

3. Tinea cruris: Jock itch is another term of tinea cruris[23]. Epidermophyton floccosum, T. rubrum were the most frequently isolated species in the cases of tinea cruris[16].

4. Tinea unguium: Onychomycosis is a fungal infection of the nail plate or nail bed leading to the gradual destruction of the nail plate. Onychomycosis is the most common nail disorder in adults causing about 50 % of all nail diseases. The dermatophytes Trichophyton rubrum and Trichophyton mentagrophytes are the main causative pathogens[26,17].

5. Tinea manuum: This infection usually involves the hands, palms. It may be unilateral or bilateral.it often occurs in patients with tinea pedis. The palmer surface is diffusely dry and hyperkeratotic. These lesions similar to eczema[23].

6. Tinea faciei: This is dermatophytic infection of skin that occurs on non dermatophytic region of face. The causative agents of tinea faciei vary according to geographic region. The patients usually present with the history of photosensitivity. The common clinical feature is similar with other types of dermatophytosis. It may be turn into the form of tinea incognito due to treat with topical steroids.

7. Tinea barbae: This is ringworm infection of beard and moustache areas of face with invasion of coarse hair and it is called tinea sycosis. There are erythematous patches on face which scaling, fragile lusterless hair and tendency to develop folliculitis. Two clinical types: deep type is usually caused by Trichophyton verrocosum and Trichophyton mentagrophytes. Superficial crusted type caused by Trichophyton violaceum and Trichophyton rubrum.

8. Tinea incognito: Tinea incognito is a dermatophytosis of atypical clinical character, usually misdiagnosed and treated with corticosteroids. It is usually caused by Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton flocussums, Microsporum canis, and Microsporum gypseum.The clinical presentation which mimics other skin conditions such as seborrhoeic dermatitis, lichen planus, folliculitis, atopic dermatitis, scleroderma[27].

Laboratory Diagnosis

There are two methods for identification of dermatophytes, the first method which depends on the phenotype differences (Traditional method) and the second method depends on the molecular- based PCR methods[28].

1 **Specimen collection**[29]

• Skin: Collect by scraping the surface of the margin of the lesion using a sterile blunt scalpel.

• Nail: Collect by taking snippings of the infected part of the nail using sterile scissors. Where the nail is thickened, also collect scrapings from beneath the nail.

• Hair: Collect by removing broken hair from the margin of the lesion using sterile tweezers or scraping the scalp with a blunt scalpel.

2 Microscopic Examination of Specimens[23]

• Value: aids in visualizing hyphae and confirming the diagnosis of dermatophyte infection.

• Procedure: Transfer the scale, hair, or debris to a glass slide, and add a few drops of 10.0% to 20.0% KOH. For nail material or hair, gently warm the slide. The wet-mount preparation is then examined under a microscope (X 400) with back-and-forth rotation of the focus knobs. This technique aids the visualization of hyphae (branching, rod-shaped filaments of uniform width with lines of separation [septa]. In tinea capitis, the hair shaft may be uniformly coated with minute dermatophyte spores

2.1 Modification of KOH Preparation for Rapid Detection of Fungus[30]

• Parker's Ink Method: Parker's ink can be added to potassium hydroxide. Parker's ink stains the fungal wall blue and is thus easy to recognize.

• Eosin 1% Method: Eosin 1% can be added to potassium hydroxide to stain the keratin. It lends a pinkish background, while the fungal elements remain unstained.

• Modified Parker's ink and 1% Eosin Method: Add 1% eosin to Parker's ink in 2:1 proportion by volume to prepare modified Parker's ink. The mixture is painted over the affected site and allowed to dry. Then reapply the cellophane tape, gently press it, remove it, stick it over a glass slide and view. The background stains pink due to eosin, while the fungal elements stain blue due to ink. No heating is required. Eosin and Parker's ink can be combined to offer a simple method to demonstrate fungi. The specimen is also easy to transport and does not need warming.

• Calcofluor white stain: This is a colorless dye, a fluorochrome stain, for rapid detection of fungi in wet mounts, smears and tissue sections, especially in scrapings from skin and mucous membrane. Viewed under ultraviolet light, fungal structures display a brilliant apple-green or a ghostly blue-white color.

• Gomori Methenamine Silver Stain (GMS): GMS stain may represent the new gold standard for the diagnosis of onychomycosis31. Fungi are sharply delineated in black with the inner parts of mycelia and hyphae staining an old rose as a result of toning in gold. Mucin also assumes a rose-red color as a result of toning[32].

3 Different Culture Media

3.1. Sabouraud dextrose agar with penicillin and streptomycin: This medium containing procaine penicillin at concentration of 200 units and Streptomycin 0.4g/L to the medium. This medium is use for isolation of fungi from clinical materials.

3.2. Sabouraud dextrose agar with chloramphenicol and cycloheximide: The standard medium for growing dermatophyte is Sabouraud dextrose agar containing chloramphenicol and cycloheximide, which inhibit bacteria and saprophytic fungi respectively. The cycloheximide (Actidione) in a concentration of 0.1 to 0.4 mg per ml suppresses the growth of most saprophytic fungi such as Scytalidium, Hendersonula, Aspergillus, Candida species without deterring the growth of dermatophytes. The various antibiotics like chloramphenicol (0.05 mg/ml) or gentamicin (0.1mg/ml) are used.

3.3 Dermatophyte test medium (DTM): Taplin and coworkers introduced dermatophyte test medium. The incorporation of antibacterial (gentamicin sulfate and chlorotetracycline HCl) and antifungal (cycloheximide) antibiotics in a nutrient agar base provided a selective substratum for the isolation of dermatophytes by suppressing the growth of most fungal and bacterial contaminants. A pH indicator (phenol red solution), which converted the color of the medium from straw yellow to bright red under the alkaline conditions associated with growth of dermatophytes[33].

3.4 Corn meal agar: After inoculation with test fungi, incubate the slants at room temperature. The isolates of Trichophyton rubrum produce dark red vinaceous pigment which appears as red colour on the reverse side. The isolates of Trichophyton mentagrophytes fail to produce this pigment[34].

3.5 Potato dextrose agar: It induces sporulation in dermatophytes and can be used in slide cultures.

3.6 Trichophyton agars: Trichophyton agars (available commercially) are useful for culturing several dermatophyte species,some of which have nutritional requirements (either essential or partial) for growth. For example, T. verrucosum, which requires thiamine and inositol, and Trichophyton tonsurans, which requires thiamine, can be correctly identified with the use of Trichophyton agars[35].

4 Identification of Dermatophytes microscopically from culture

Macroscopically, cultures are evaluated by colour of the colony's top and bottom side, surface characteristics, and margin shape. Also Identification characters include colony pigmentation, texture, and growth rate and distinctive morphological structures,

such as microconidia, macroconidia, spirals, pectinate branches, pedicels, and nodular organs[3,36].

4.1 Tease-mounts (Lactophenol cotton blue): The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls.

4.2 Slide culture[37]: In this technique, a thin square block of a suitable agar is placed on a sterile microscope slide, inoculated with a small amount of the fungal culture, covered with a sterile cover slip, and incubated in a moist environment for up to 2 weeks. The cover slip and agar block are then removed, mounting fluid is added, and a clean cover slip applied to the slide. The fungal growth on the slide is then examined for the presence of spores and other characteristic structures.

4.3. Cellophane Tape Method 30: 5 cm long and 2 cm wide scotch tape (transparent cellophane tape) can be applied over the affected site, pressed firmly and removed. The tape is then stuck on the surface of a glass slide and sent to the laboratory, where it is gently lifted and replaced after placing 3 to 4 drops of 10% potassium hydroxide solution. The undersurface of the slide is warmed gently and examined under microscope.

Species Identification: some physiological criteria are often relied upon for a definite identification. These include-

1. Nutritional requirements: The diagnostic growth requirements of dermatophytes are nicotinic acid for T. equinum, histidine for T megnini, thiamine for T violaceum, and T. tonsurans.

2. Temperature: Although some of the species tolerate a wide range of temperature. most grow best at 25°C to 35°C. T. rubrum was induced to form a red pigment on serum albumin agar at pH 7.0 within 7 days, whereas T.mentagrophytes cannot form it T. verrucosum grows better at 37°C.

3. Urease test by Christensen's urease agar medium: T. mentagrophytes a urease positive organism shows urease activity within 7 days and the colour of medium changed to pink. T.rubrum isolates are urease negative and did not produce urease enzyme[34].

4. Pigment production: Generally, T. rubrum isolates produce a dark cherry red colour under the colony. Confusion exists in differentiation of many slow pigments producing varieties of this species and similar isolates of T.mentagrophytes. For this purpose, stimulation of pigment production has been achieved by growing them on potato-dextrose agar and cornmeal dextrose agar media.

5. Hair perforation Test: Hair perforation test is conducted for differentiating Trichophyton rubrum and Trichophyton mentagrophytes, Trichophyton mentagrophytes produce hair perforating organs that penetrate hair radially and because wedge shaped perforations[34].

PCR (Polymerase Chain Reaction)

Introduction of a PCR-based methodology would increase specificity, simplicity, and speed and potentially even reduce cost[39]. In molecular methods, DNA/RNA probe technology includes southern hybridization, in situ hybridization, fluorescence in situ hybridization, microarray and macroarray. Isothermal amplification technology includes

Table 1: Classification of dermatophytes according to morphology[3,22]

Name of Species	Colony Characteristics	Microscopy Profiles
Trichophyton rubrum	white granule, red pigment	Macroconidia- pencil shaped Microconidia- tear drop
Trichophyton mentagrophytes	white woolly	Microconidia- spherical shaped, cluster of microconidia, Macroconidia- cigar shaped
Trichophyton tonsurans	cream	Microconidia- abundant, Macroconidia- thick wall, irregular
Trichophyton schoenleinii	brown, waxy, colourless	Hyphae-swollen, chlamydospores, favic chendelier
Trichophyton violaceum	slow growing, waxy violet pigment	Hyphae – distorted, conidia- rare
Microsporum audouinii	velvety green to cream, slow growing	Hyphae – comb like with terminal chlamydospores
Microsporum canis	valvety deep yellow pigment	Macroconidia – thick wall Macroconidia (Spindle shaped with 1-5 septa)
Microsporum gypseum	cream, powdery	Macroconidia- thin walled with 4-6 septa
Epidermophyton floccosum	powdery, greenish brown	Macroconidia- club shaped in clusters.

loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA) and nucleic acid sequence-based amplification (NASBA). PCR technology includes multiplex PCR, nested PCR, real-time PCR and reverse transcriptase (RT)-PCR and DNA barcoding has been recently used[40].

Conclusion

Dermatophytes are the most common cutaneous fungi. Tricophyton, Epidermophyton and Micosporum are the species which is responsible for ring worm infection in human and animal. In case of diagnosis microscopic finding is more prior to detect dermatophytosis. On the other hand, culture and PCR can also be done to find out the species of this keratinophilic fungi.

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Conflict Of Interest
The authors have no conflicts of interest to disclose.

Authors' contributions
Samia Afreen Khan involved in conception and design of the study, contributed to the literature search, review and compilation as well as involved in manuscript writing and also reviewed the manuscript and edited.

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