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[Uday Sharma](#)\* and Jagriti Dhungana

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Review

# A Review of the Dermatophytes

Uday Sharma and Jagriti Dhungana

Department of Microbiology, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

\* Correspondence: udysharma1000@gmail.com

## ABSTRACT

Dermatophytes are a group of keratinophilic fungi belonging to either of the genera *Trichophyton*, *Microsporum*, or *Epidermophyton*. These fungi are involved in the superficial fungal infections of skin, hair, and nails in humans as well as animals. Dermatophytosis is one of the most prevalent fungal diseases worldwide, and it does not discriminate any age or sex. The basis of taxonomy and classification of these pathogens has changed many times over time, from a traditional morphological and physiological basis to modern molecular methods. Dermatophytes have an interesting historical perspective, epidemiological trends over time and geography, pathogenesis, different clinical manifestations, and advances in diagnostic technology. This review outlines the modalities of diagnosis and classification of the dermatophytes from culture and microscopy to advanced molecular technology, with highlights on their working principle, advantages, limitations, and disadvantages. This review offers a framework for clinicians, researchers, scientists, and students with insights about the most common yet significant fungal pathogens.

**Keywords:** dermatophytes; phylogeneny; taxonomy of the dermatophytes; molecular diagnosis

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## INTRODUCTION

The word dermatophytes translates to “skin plant” in literal meaning, and they cause the infection of the epidermis of skin, hair, and nail called dermatophytosis (tinea or ringworm). Dermatophytes were previously assigned to the plant kingdom until 1969, when they were reclassified into the Fungi kingdom. (1,5)

Dermatophytes are ascomycetes characterized by hyaline septate hyphae. They prefer warm and moist conditions for growth, which is why their infections are more prevalent in tropical regions worldwide. Additionally, dermatophyte infections of glabrous skin are the most common skin diseases in many tropical countries.(2) They are generally unable to penetrate deeper tissues because they prefer cooler skin temperatures, serum inhibitory factors like beta-globulins, ferritin, and other metal chelators that bind to iron, essential for the growth of dermatophytes. (4) But even though they have been known to cause painless superficial infections, they can also cause widespread lesions and result in significant social, psychological, and occupational health effects, thus compromising the quality of life. Also, these fungi can cause invasive, deeper, and disseminated infections, particularly in immunocompromised patients. (6)

The dermatophytes belong to three anamorphic genera, *Epidermophyton*, *Trichophyton*, and *Microsporum* in the phylum Deuteromycota, which are the forms occurring in the vertebrate hosts, and those capable of sexual reproduction are classified into the teleomorphic genus arthrospora of the phylum Ascomycota; thus, a dermatophyte may have two different names based on their different forms; sexual or asexual. (7)

## HISTORY OF TAXONOMY

In the mid-nineteenth century, when the fungal etiology of flavus was discovered by three European physicians, namely Robert Remak, Johann L. Schonlein, and David Gruby, medical mycology was initiated. Remak, in 1835, made observations on the peculiar structures of rods and

buds in favic lesion crust under the microscope, which he never published but permitted Xavier Hube to cite these observations in a doctoral dissertation in 1837. Remak credited the recognition of the fungal structures to Schönlein, who described their mycotic nature in 1839, and David Gruby, in 1841 to 1844, unaware of the works of Remak and Schönlein, independently described the causative agent of favus. He studied the causative agent both clinically and microscopically. He described the ectothrix invasion of the beard and scalp, naming the causative agent of the latter *Microsporum* (referring to the small spores around the hair shaft) *audouinii*, and described the endothrix hair invasion by *herpes* (*Trichophyton tonsurans*). He also described the clinical and microscopic appearance of thrush in children.

Raimond Sabouraud, by 1890, started his scientific study and published it in his 1910 classic volume *Les Teignes*, the taxonomy, morphology, and methods of culturing the dermatophytes, and also the therapy of the dermatophytes. He described the dermatophytes under four genera, namely: *achorion*, *trichophyton*, *microsporum*, and *Epidermophyton*, on the basis of clinical, cultural, and microscopic characteristics. (2)

The current classification of the dermatophytes was established by Chester Emmons in 1934, on the basis of spore morphology and accessory organs. The myriads of dermatophytes that had created a lot of confusion were critically reviewed based on spore morphology and accessory organs rather than clinical and host factors as well as trivial and highly variable characteristics like colony texture, nodular organs, chlamydospores, pigment production and racquet or spiral mycelium and reduced to only the three genera *Microsporum*, *Trichophyton* and *Epidermophyton* containing overall 19 species. The discovery of new species follows the same classification scheme. (3) According to Ajello L., the species of *Epidermophyton*, *Trichophyton*, and *Microsporum* that are exclusively saprobic or nearly so should not be included under the term dermatophytes, which. (8)

#### ***Epidermophyton* Sabouraud 1907**

*E. floccosum* (Harz) Langeron et Milochevitch 1930

#### ***Microsporum* Gruby 1843**

*M. audouinii* Gruby 1843

*M. canis* Bodin 1902

*M. equinum* (Delacroix et Bodin) Guegue'n 1904

*M. ferrugineum* Ota 1921

*M. fulvum* Uriburu 1909

*M. gallinae* (Megnin) Grigorakis 1929

*M. gypseum* (Bodin) Guiart et Grigorakis 1928

*M. nanum* Fuentes 1956

*M. persicolor* (Sabouraud) Guiart et Grigorakis 1928

*M. praecox* Rivalier, ex Padhye, Ajello et McGinnis 1987

*M. racemosum* Borelli 1965

*M. vanbreuseghemii* Georg, Ajello, Friedman et Brinkman 1962

#### ***Trichophyton* Malmsten 1845**

*T. concentricum* Blanchard 1895

*T. equinum* (Matruchot et Dassonville) Gedoelst 1902

*T. gourvillii* Catanei 1933

*T. kanei* Summerbell 1989a

*T. megninii* Blanchard 1896

*T. mentagrophytes* (Robin) Blanchard 1896

*T. raubitschekii* Kane, Salkin, Weitzman, Smitka 1981a

*T. rubrum* (Castellani) Sabouraud 1911

*T. schoenleinii* (Lebert) Langeron et Milochevitch 1930

*T. simii* (Pinoy) Stockdale, Mackenzie et Austwick 1965

*T. soudanense* Joyeux 1912

*T. tonsurans* Malmsten 1845

*T. verrucosum* Bodin 1902

*T. violaceum* Bodin 1902

*T. yaoundei* Cochet et Doby Dubois 1957 (not validly published) (2, 9, 10)

Emmons' classification, even though it reduced the number of species to only 19 with 35 synonymous names, the synonyms of some entities were later identified as distinct species morphologically, physiologically, or genetically. These organisms were *Microsporum persicolor* (as *Trichophyton persicolor*) and *Microsporum fulvum*; *Trichophyton ewuinum* was excluded without any comment. And, *T. tonsurans sensu stricto* still had been left split into four species. (11)

Further great clarifications were achieved when studies regarding physiological and enzymatic activities of the dermatophytes came forward, like physiological investigations of hair perforation, growth factor response, and other enzymatic reactions such as urease activity and glucose repression of alkalogenic proteolysis on bromocresol purple milk solids glucose agar. (12-16)

Weitzman et al in 1983 introduced the trichophyton agars in which the ability of the strains to assimilate a panel of essential vitamins could be studied. Along with this, the growth temperature, gelatin liquefaction, etc., were included. (17) This method is now referred to as the conventional approach to dermatophyte taxonomy, which combines clinical, cultural, microscopical, and physiological characteristics. (18)

The features and characteristics that had been introduced till now had some drawbacks, for example, cultures were very difficult to maintain because of rapid degeneration and changes after a few transfers on artificial media. The physiological criteria had their drawbacks, with poor discriminatory resolution of the technique. (19)

The discovery of teleomorphs of *Trichophyton (Keratomyces) ajelloi* by Dawson and Gentles in 1959 opened the door to the rapid discovery of teleomorphs of other dermatophytes and other keratinophilic fungi, which again led to the classical genetic study of these fungi. (2)

The dermatophytes capable of reproducing sexually and producing ascospores are classified in the teleomorphic genus called *Arthroderma*, belonging to the family *Arthrodermataceae* of the *Onygenales*, phylum *Ascomycota*. They were previously classified into two different genera, namely *Arthroderma* and *Nannizzia*, which, later on careful morphological study, were considered to have very minor differences and didn't require two different genera to be explained, so the name *Arthroderma* was considered for both. (20)

One anamorphic state of a dermatophyte may exhibit more than one teleomorph state. For example, *Arthroderma benhamiae* is a teleomorph obtained from mating the strains of *Trichophyton mentagrophytes*, while *Arthroderma vanbreuseghemii* is another teleomorph state of the same anamorph. (21, 22)

Similarly, *Microsporum gypseum* exhibits *Arthroderma gypseum* teleomorph along with *Arthroderma incurvatum*. (23, 24)

The course of evolution that led to the dermatophytes' modus vivendi appears to have been highly distinctive. One particularly unusual feature is that crossing over to pathogenesis of hosts lacking regular contact with a particular type of humid soil habitat tends to eliminate the possibility of sexual reproduction, leading to purely asexual evolution. (25, 26)

## CLINICAL SPECTRUM

Over the past decade, a plethora of dermatophyte infections seems to have increased with unusual clinical manifestations. Infections that have an unusual mode of transmission, treatment difficulty, and the number of outbreaks of several zoophilic dermatophytes in humans have been reported in recent times. (27-31)

Although common manifestations of dermatophytic infections are restricted to the stratum corneum of the skin and other keratinized tissues like nail and hair, and for this reason are considered to be trivial, the considerable morbidity/physiological effects can't be overlooked. (32) The name of the types of infection produced by the dermatophytes is based on the site of infection. The classical classification of dermatophytosis includes:

**Tinea corporis:** infection of the body that may mostly involve the trunk, neck, arms, and legs. The most commonly involved etiological agent is *T. rubrum*, followed by *T. mentagrophytes* and *M. audouinii*. Tinea corporis is common worldwide, and high humidity, heat, tight-fitting clothing are correlated with the severity and frequency of the infection. (33, 34)

**Tinea cruris:** Also known by the name jock itch, tinea cruris involves the dermatophytic infection of the genital, pubic, and perineal area. *Trichophyton rubrum* is the most commonly involved agent, while many studies have recognized the increasing prevalence of *T. mentagrophytes* and other dermatophytes, too. The risk factors include excessive sweating, tight-fitting clothes, lack of hygiene, diabetes mellitus, and other immunocompromised states, as well as low socioeconomic status. (35-41)

**Tinea pedis:** Tinea pedis is the infection of the skin of the feet caused by dermatophytes, which is often known as athlete's foot. The common causative agents include *Trichophyton rubrum* (70% of the cases), *Trichophyton interdigitale*, *Epidermophyton floccosum*, *T. mentagrophytes*. (42) About 10% of the world's population is believed to be affected by Tinea pedis, and people who wear occlusive shoes for a long period, who are prone to prolonged exposure to water, walk barefoot in locker rooms, showers, and swimming pools and them with diabetes mellitus are at increased risk of developing tinea pedis. (43)

**Tinea manuum:** It is a dermatophytic infection of the dorsum and/or palm and/or interdigital folds of the hand. (44) Tinea manuum affects adolescents and adults, with the prevalence rates ranging from 0.3 to 13%, with the variability based on geographical location. (45) The most common causative agents include *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Trichophyton verrucosum*, *Trichophyton interdigitale*, and *Microsporum canis* (46, 47)

**Tinea faciei:** Tinea faciei is a dermatophytic skin infection of the face that may affect all age groups, with higher rates in childhood and between 20 and 40 years of age. (48)

**Tinea barbae:** Tinea barbae is a superficial fungal infection of the skin, hair, and hair follicle, which is a relatively rare presentation of dermatophytosis. (49) It occurs in two forms: inflammatory and noninflammatory. The inflammatory form is called kerion, and it is erythematous, boggy, tender, often sterile, weeping nodules or plaques with pustules and draining sinuses.

While non-inflammatory presentation is a diffusely erythematous squamous plaque with perifollicular pustules and papules. (50)

The most commonly involved agents are *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. (50, 51)

**Tinea capitis:** It represents the scalp hair infection caused by the dermatophytes. (52) *Trichophyton soudanense*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Trichophyton rubrum*, and *Microsporum canis* are the most commonly involved organisms. (53)

The presentation could be endothrix, ectothrix, or favus type. (54)

**Tinea unguium:** Onychomycosis, when caused by a dermatophyte, is known as tinea unguium. The most frequently involved dermatophyte is *Trichophyton rubrum*, followed by *Trichophyton* species like *T. Mentagrophytes* and *Epidermophyton floccosum*. (55, 56)

## EPIDEMIOLOGY

About 25% of the world's population is affected by the infection caused by dermatophytes, among which *Trichophyton rubrum* is the commonest causative agent. (57, 58) Based on information in different publications from around the world, *Trichophyton rubrum* complex, *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Trichophyton violaceum*, *Microsporum canis*, etc., are the common dermatophytes causing human infections. (59-64)

Some species, such as *Trichophyton rubrum*, *T. mentagrophytes var. interdigitale*, *Microsporum canis*, and *Epidermophyton floccosum*, have a worldwide distribution, while others have been partially restricted, for example, *T. schoenleinii* (Eurasia, Africa), *T. soudanense* (Africa), *T. violaceum* (Africa, Asia, and Europe), and *T. concentricum* (Pacific Islands, Far East, and India). (65)

The general consideration is that the reason being dermatophytes love warm and humid climates, so they are more prevalent in regions with tropical and subtropical climates. (60)

Moreover, the demographic parameters also play an important role in the pattern of dermatophytosis. For example, old patients are more affected with tinea inguim while children with tinea capitis. (66)

The factors influencing the epidemiology of these fungal infections could be as broad as age and gender, socio-economic scenario, lifestyle of the individuals, type of climate, as well as the pattern of human interaction with animals. One example of so is *Trichophyton tonsurans*, which spread from Latin America to the US and then to Africa, the Middle East, and other areas, causing tinea capitis. (67)

While, based on primary habitat, the dermatophytes can be categorized in to anthropophilic (primarily involved in human infections), zoophilic (primarily involved in the animal. Infections) and the geophilic species (primarily found in the soil that live on decomposing keratinized materials like animal horns, nails feathers and hooves, etc), it does not mean that a particular fungus belonging to zoophilic or geophilic group cannot infect humans for example, being a geophilic fungi but have been isolated from human infections. (68-70) This difference in the host range is believed to be because of the differences in the keratin of the host. (71)

<b>Anthropophilic</b>	<b>Zoophilic</b>	<b>Geophilic</b>
<i>Trichophyton mentagrophytes</i>	<i>Trichophyton mentagrophytes</i> var <i>mentagrophytes</i>	<i>Trichophyton ajelloi</i>
<i>Trichophyton concentricum</i>	<i>Trichophyton equinum</i>	<i>Trichophyton terrestre</i>
<i>Trichophyton megninii</i>	<i>Trichophyton verrucosum</i>	<i>Nannizzia nana</i> (formerly <i>Microsporum nanum</i> )
<i>Trichophyton violaceum</i>	<i>Trichophyton simii</i>	<i>Microsporum cookie</i>
<i>Trichophyton tonsurans</i>	<i>Microsporum canis</i>	<i>Nannizzia gypsea</i> (formerly <i>Microsporum gypseum</i> )
<i>Trichophyton schoenleinii</i>	<i>Microsporum equinum</i>	<i>Microsporum praecox</i>
<i>Trichophyton rubrum</i>	<i>Microsporum gallinae</i>	<i>Epidermophyton stockdaleae</i>
<i>Trichophyton soudanense</i>	( <i>Nannizzia persicolor</i> ) <i>Microsporum persicolor</i>	
<i>Microsporum audouinii</i>		
<i>Microsporum ferrugineum</i>		
<i>Epidermophyton floccosum</i>		

While large-scale studies regarding risk factors of these fungal infections are very scarce, Son H J et al (2022) investigated the risk factors of dermatophytosis using a nationwide study in which 4,532,655 subjects with dermatophytosis were studied. They found out that male sex, higher BMI, heavy drinking habits, and engagement in mild to heavy exercise were more prone to having dermatophytosis. However, there is variation in the results regarding the sex factor associated with dermatophytosis, showing males are more affected by some and females more by others. (72-75)(76-79)

Similarly, diabetes, hypertension, and dyslipidemia also showed a significant correlation. (76) Several studies have pointed out that the rural population is more prone to infection by dermatophytes, indicating lifestyle and socioeconomic status as a probable reason. (77-80) Moreover, the presence of chronic disease and also the poor attitude of individuals towards the prevention of infections were shown to increase the chances of dermatophytosis. (80)

## GENETIC CLASSIFICATION AND IDENTIFICATION:

Introduction of multiple taxa that were synonyms of each other was common before in the time when only the phenotypic system of classification was implied. For example, the species *Keratinomyces longifusus* was considered a different fungus before it was known to be *Microsporum fulvum* with strongly coherent conidia. (83) Similar examples of misjudgment leading to the allocation of a different genus for a mutant can also be found in articles, like confusion between *Bipolaris* and *Dissitimumus*, *Scedosporium* and *Polycytella*, *Exophiala* and *Sarcinomyces*, or *Trichosporon* and *Fissuricella* (81)

The study of dermatophytes with the improved knowledge and the advent of molecular techniques like DNA base composition and DNA hybridization, mitochondrial DNA restriction fragment length polymerase (RFLP), nuclear ribosomal gene sequencing, chitin synthase I gene sequencing, internal transcribed spacer region ribosomal DNA sequencing, fingerprinting techniques have contributed a lot towards the understanding of the phylogeny, taxonomy and biodiversity of the dermatophytes. (82)

### DNA base composition and DNA hybridization

Davidson et al studied the base composition of chromosomal DNA of the dermatophytes to determine if the differences between the species established by traditional criteria was reflected by the differences in their molecular composition of their genetic composition. The species varied in the G+C content of chromosomal DNA, but this difference didn't correlate with the traditional species concept. All the species showed the G + C content to be within a narrow range of 48.7–50.3% contrasting with the marked phenotypic and ecological differences among these keratinophilic fungi. (83) In the same study, *Trichophyton mentagrophytes* showed only 70% homology with strains of *Arthroderma benhamiae*, which was a disagreement with the fact that they were considered to be the anamorphic and teleomorphic states of the same species. (83) In this regard, Graser et al. later in 1999, demonstrated that the anamorphic species created to *A. Benhamiae* was *Trichophyton erinacei* and not *Trichophyton mentagrophytes* (84)

### Mitochondrial DNA restriction fragment length polymorphism

The genes in the mitochondrial genome, when phylogenetically analyzed, reveal that mitochondrial genes can generally be accepted as the descendants of endosymbiotic alpha-proteobacteria and are considered to be of monophyletic origin. Complete sequencing of Mitochondrial DNA (mtDNA) can give an idea about gene content, order, and position, and also the knowledge about introns and intergenic regions. (85-87) mtDNA sequences have become a popular tool for phylogenetic studies over the ribosomal sequences since vital physiological processes and basic adaptive strategies do not always correlate with trees derived from the latter. (88, 89) The smaller size in comparison to nuclear DNA, its presence in abundance and its easier digestion by restriction endonuclease make it an easier and important tool (90) There was no significant intra-species heterogeneity was found in several studies based on mtDNA sequencing (91-95) and *Trichophyton*, *Microsporum* and *Epidermophyton* were congeneric, (95) thus supporting the work of Weitzman et al who had merged *Nannizzia* into *Arthroderma*.

While it is of concern that, although the mtDNA sequencing technique has long been considered a very powerful tool for species-level identification of the dermatophytes, it lacks sensitivity at subspecies identification. (96)

### Nuclear ribosomal RNA gene sequencing

Sequencing of the ribosomal RNA genes has been successful in phylogenetic studies of many fungi. The technique, however, was unable to produce a clear picture to establish the hierarchy of species among the species in the family Arthrodermataceae. Only *Ctenomyces serratus*, *Trichophyton ajelloi*, and *Trichophyton terrestre* were significantly separated, suggesting that the parasitic mode of dermatophytes was only recently adapted. (97) The small RNA subunits (18S rRNA) proved to be useful. Because of its slow evolution, deep evolutionary branches could be studied, and certain regions being variable allow divergences that occur more recently. (82)

### Chitin synthase (CHS) gene sequencing

Unlike other eukaryotic cell walls, the cell wall of fungi is composed of chitin, a polysaccharide, and comparison of the sequence of genes coding for the synthesis of this component, chitin synthase 1 (*CHS1* gene), cloned in *Candida albicans*, *Aspergillus nidulans*, *Histoplasma capsulatum*, and *Sporothrix schenckii* has shown a highly conserved region. This region of the genes has been used to make primers for PCR amplification. (96-98)

Chitin synthase 2 (*CHS2*) has also been detected in dermatophytes, showing lower homology than shown by *CHS1* between *T. mentagrophytes* and *T. Rubrum*. The *CHS3* gene has been cloned for several other fungi but has not been detected in the dermatophytes. (99)

#### **Internal transcribed spacer region ribosomal DNA sequencing**

The nuclear ribosomal internal transcribed regions show a low level of intra-species variation and a high level of interspecies variation, thus making their sequencing technique an important and extensively used tool in fungal phylogenetic as well as ecological studies. (84, 100, 101) These regions are located between the 18S (ITS1) and 5.8S and 26S (ITS2) ribosomal DNAs. The fact that these variable regions are high in number and are flanked by the conserved regions makes PCR-based technology simple and rapid. Internal transcribed spacer regions of rDNA are located between the 18S and 5.8S (ITS1) and the 5.8S and 26S (ITS2) rDNAs. The variable spacer regions are flanked by conserved sequences, and the high number of copies per cell enables simple and rapid analysis by PCR-based technology. (102) Intra-species polymorphism has also been reported by different researchers, but the conclusion is that the level of variation in the ITS region is very low. For this region, these differences can't be considered useful in strain identification, and the ITS spacer rDNA technique remains useful for species-level differentiation only. (103-107)

#### **Non-transcribed Spacer (NTS) Regions of Ribosomal DNA (NTS-rDNA)**

The non-transcribed spacer (NTS) region (AKA intergenic spacer) of the ribosomal RNA (rRNA) genes is the most important region of the rDNA because it contains the nucleotide sequences that trigger and/or terminate transcription. (108) This region is widely used in subspecies typing of the dermatophytes because of a very high degree of sequence variation, which is again due to the number of repeat elements arranged tandemly in a variable internal repeat (VIR), scattered single-nucleotide polymorphisms (SNPs), deletions, and insertions. (109-112) Species-specific PCR primers that target the tenderly repetitive subelements of the intergenic spacer are available for several dermatophytic species. (113, 114)

#### **Random amplification of polymorphic DNA (RAPD) and arbitrarily-primed PCR**

Both of these techniques are designed to generate a complex pattern of PCR products in a single reaction by using arbitrary primers. These primers are not designed to match specific sequences. has greatly enhanced the molecular identification of various dermatophyte anamorphs and teleomorphs. In both methods, the basis concept is the generation of a complex pattern of PCR products in a single reaction by using arbitrary primers that are not designed to match specific known sequences (115) The method have been reported to be rapid and precise as well as results from several isolated can be generated at the same time for distinguishing many fungi including dermatophytes, by several authors. However, at the same time, experimental parameters like primer and template concentration, temperature, magnesium ion concentration, polymerase quality, and electrophoresis conditions have been reported to alter the results. This adds the need for precise optimization of conditions during the study. Moreover, poor reproducibility of the obtained results could be another important factor to reduce the interest of many in this technique. (116-121)

#### **Microsatellite DNA**

Microsatellites, aka sequence repeats (SSRs), short tandem repeats (STRs), inter simple sequence repeats (ISSR), simple sequence tandem repeats (SSTR), variable number tandem repeats (VNTR), SSLP, and STMS, are short (1 to 10 nucleotides) and are a subcategory of tandem repeats (TRs) at a particular genomic location. These are typically non-coding. The mutation rates are between  $10^3$  and  $10^6$  per cell generation, i.e., up to 10 orders of magnitude greater than point mutations (122, 123)

Their dense distribution in the genome, high variability, co-dominant inheritance, and ease of assay make microsatellite DNA an important marker for genetic study. (124) Other molecular tools

mentioned above, like mtDNA-RFLP, NTS-rDNA, RAPD, etc., although they have a remarkable sensitivity and specificity, are sophisticated, expensive, and time-consuming in the case of a large number of clinical samples. (125)

Spesso M F et al reported the application of single microsatellite repetitive oligonucleotides (GACA) 4 and (GTG) 5 to identify *T. rubrum*, *M. canis*, and *M. gypseum*, which are among the most common dermatophytes. Similarly, Zhu et al. also demonstrated similar efficacy of microsatellite PCR and reported (GACA)4 as the most suitable primer to distinguish *T. rubrum*. (126, 127)

## LABORATORY DIAGNOSIS:

The sample collection method and transport of the collected sample play a vital role in laboratory diagnosis of dermatophytosis. Dermatophytes grow radially with young and viable elements at the periphery and old and poorly viable elements at the center. So, the collection should be made from the erythematous, actively growing margins of the lesion. (128) Similarly, for nail samples, the preferred sample is from the debris from beneath the distal end of the nail and scrapings from the nail bed. A sample from the distal part only may lack viable fungal elements. Alternatively, close clipping of the whole nail sample can be used. But before the collection of both skin scraping and nail samples, the collection site should always be first disinfected with 70% alcohol swabs to prevent bacterial contamination. (129, 130) The lesions of the glabrous skin are preferably sampled using cellophane tape or vinyl tape strips to obtain adequate sample material. The cellophane tape sample thus obtained is placed on a drop of 10% KOH or 40% dimethyl sulfide on a glass slide. The sample obtained from this technique is also useful for culture if asepsis has been maintained. (131)

### Direct examination:

The visualization of fungal elements from the sample requires the digestion of the keratin and the dissociation of the collected materials. For this purpose, the simplest, cheapest, and the common reagent used is 10-20% KOH. Other agents like 10% sodium hydroxide (NaOH) and detergents are also some of the proposed reagents, but are not common. (132)

### Staining techniques:

Staining increases the contrast and thus the visualization of the fungal structures with increased sensitivity. There are several stains used in staining the samples from sites suspected of dermatophytic infection.

Calcofluor stain (CFW) is a fluorescent blue dye that binds to the chitin in the Fungal cell walls. Under UV light, it fluoresces brightly, and thus it makes the technique superior for dermatophyte detection. The expensive nature of fluorescent microscopes is a limitation. But, there have been newer methods like those mentioned by Denny G et al., in which a modified version of the KOH microscope was used, in which they suggested the use of the fluorescent compound, CFW, and a 395-nm light-emitting diode flashlight (TaoTronics model TT-FL001, TaoTronics, Sunvalley Group, Shenzhen, China), which is inexpensive as well as easily available and handheld. (133) Other staining techniques include Giemsa stain and Chicago sky blue stain. (134)

For the identification, the sample is inoculated on the media like Sabouraud Dextrose Agar (SDA) or Sabouraud Dextrose Agar with cyclohexamide (a semi-selective agent to reduce the growth of non-dermatophytic fungi). Emmon's modification with the incorporation of chloramphenicol along with cyclohexamide in Sabouraud peptone glucose agar is a better alternative. As the causative agent could also be a non-dermatophyte in a clinical sample, SDA without cyclohexamide should be inoculated side by side, such as SDA with gentamicin and chloramphenicol. Littman Oxgall agar (Difco) is another alternative that restricts the colony diameter of fast-growing contaminant fungi, thus allowing outgrowth of slow-growing etiologic agents. Other media include Lactrimel agar and Trichophyton agar. (135, 136,137)

**Microscopic identification:** For the microscopic identification of the dermatophytes, the fungal growth is subjected to a lactophenol cotton blue mount. The different genus-specific microscopic characteristics are described below.

### *Trichophyton* species

Colony morphology is variable: granular, powdery, persicolor, and downy types. Anthropophilic strains have a downy, powdery, or fluffy texture, while zoophilic strains have more of a granular texture. The reverse shows a variety of yellowish to reddish-brown colors. Most colonies of *T. violaceum* are cream colored, those of *T. soudanense* are yellow-orange, while *T. rubrum* has a brown to reddish tinge. A pH-reversible naphthaquinone pigment, xanthomegnin, has been considered responsible for the color of the *T. rubrum* complex. The color becomes darker at the alkaline pH.

Macroconidia have a cigar-shaped appearance with smooth, thin walls and between 1 and 12 septa; spiral or coiled hyphae may sometimes be present.

Microconidia are more abundant than macroconidia and could have a spherical, globose, pyriform, or clavate shape, which could be borne along the hyaline septate hyphae (*T. rubrum*) or may be clustered (*T. mentagrophytes*). (2, 138, 139)

**Microsporium species:** The colonies of *Microsporium* species may produce an appearance of mostly granular to cottony that is yellow to brownish, while a reverse of cream or brown colored appearance.

The macroconidia have a rough wall that may be asperulate, echinulate, or verrucose with multiple septa (1-15 septa). The shape might vary from spindle (*M. canis*) or fusiform (*M. audouinii*) to obovate (*M. nanum*).

Microconidia are either sessile or stalked and predominantly clavate in shape. The arrangement may be single along the hyphae or in raemes (*M. racemosum*) (2, 140)

**Epidermophyton species:** *E. floccosum* is the only anthropophilic fungus in this genus. It grows as greenish-brown (khaki) colored colonies with a suede-like surface appearance. The older cultures may develop a white pleomorphic mycelium. The reverse usually shows a yellowish-brown pigment. (141, 142)

The hyaline septate hyphae produce abundant macroconidia but are devoid of microconidia. The macroconidia are smooth and thick-walled with 1-9 septa. These macroconidia may occur singly or in a cluster of 2-6, giving a club shape. (2, 140,142)

## PHYSIOLOGICAL TESTS:

### Hair perforation test:

Autoclave-sterilized human hair, ideally from a child under 18 months, is placed in a petri dish. This preparation is inoculated and incubated with several fragments of the pure isolate of the test fungus, along with 25 ml of sterile distilled water and 2-3 drops of 10% sterilized yeast extract, at 25 °C for 21 days with regular examinations. This technique helps to distinguish *T. mentagrophytes* complex, *M. canis*, and *M. gypseum* (show wedge-shaped perforation on the hair shaft), while *T. rubrum*, *M. Audouinii*, and *M. praecox* show a negative result. *M. Canis* is often used as a positive control. (143)

### Polished rice:

The test is performed to distinguish *M. audouinii* from other dermatophytes like *M. canis* that grow and sporulate on rice grain, while the former produces a brownish discoloration of the rice. The pure culture of the fungus is inoculated on the autoclaved rice preparation (1 part rice in 3 parts water, autoclaved) and incubated for 2 weeks at 25-30 °C. (143, 144)

### Urea hydrolysis:

The urea hydrolysis test is performed in Christensen's urea agar, and the incubation period of 2-3 days is considered. *T. mentagrophytes* gives a positive reaction while *T. rubrum* is negative. Other dermatophytes, like *M. canis*, can often give a positive reaction. The limitations of the test are that, on prolonged incubation, about 10 days, generally urease-negative dermatophytes also might produce a positive reaction, and some false-positive reactions might be attributed to some poorly visible bacteria. (145, 146)

**Nutritional requirement test:**

For *Trichophyton* species with poor conidial production and indistinguishable morphological features, this test can be useful. The method uses a vitamin-free base medium (T1) and those that include various vitamins, like inositol (T2), inositol plus thiamine (T3), thiamine (T4), nicotinic acid (T5), and an ammonium nitrate basal medium (T6), and finally, with histidine (T7). A pinhead-sized test fungal inoculum is added to the tubes and incubated at 25 °C for examination on the 7th and 14th day for pH change to alkalinity and growth. (138, 146)

**Growth on BCP-Milk Solids Glucose Medium (BCPMSG):**

This medium is used in the distinction of different *Trichophyton* species. The change in pH (alkalinity) that is detected by the Bromocresol purple (BCP), and the type of growth, which could be either profuse or restricted, allows differentiation between species.

The slants of BCPMSG are inoculated with the test fungus and incubated at 25 °C for 7 days. The growth characteristics and the presence of alkalinity (original pale blue color of BCP to violet-purple) are examined.

*T. rubrum* produces restricted growth without alkalinity, while members of the *T. mentagrophytes* complex grow profusely with an alkaline reaction. *M. persicolor*, although, shows profuse growth, alkaline reaction is absent. (147, 148)

**Temperature Tolerance and Temperature Enhancement:**

The fungi are inoculated on two slants of SDA with an equivalent inoculum size, and one is incubated at 25 °C and the other at 37 °C. As the colonies mature, comparison is made in the growth. At 37 °C, the members of *T. mentagrophytes* complex show good growth, while *T. terrestre* complex (*Arthroderma insingulare*, *A. lenticulare*, *A. quadrifidum*) isolates do not grow. *M. (N.) persicolor* also grows poorly or does not grow at all. The growth of *T. verrucosum* and *T. sudanense* is enhanced, while on the other hand, that of *T. schoenleinii* and *M. Ferrugineum* is not. (149)

**PREVENTION AND TREATMENT:**

The WHO fact sheet on ringworm suggests practicing good hygiene by keeping the skin clean and dry, especially in sweating areas. Close contact as well as sharing of personal items should be avoided with people known to have ringworm. Appropriate footwear, like shoes or sandals, should be worn in the public showers, locker rooms, and pool areas. The surfaces and objects that may have been contaminated with dermatophytes should be properly cleaned and disinfected. Benzalkonium chloride, 1 % sodium hypochlorite, enilconazole (0.2 %), formaldehyde, iodophors, glutaraldehyde, phenolic compounds, benzylammonium bromide, and ethoxylauric alcohol are effective against dermatophyte spores. And finally, the pets should be checked and treated for ringworm by a veterinarian, if necessary, and should be kept healthy. (150, 140)

The treatment of dermatophytes involves topical or oral therapy formulations. The choice between these formulations of antifungal drugs depends upon factors like severity, location, and extent of the infection, as well as patient comorbidities and response to previous treatment, if any. The topical drugs include 1% terbinafine cream for most cases of tinea corporis, tinea cruris, and tinea pedis, which is a first-line topical therapy option.

Oral therapy is considered in scenarios like onychomycosis, tinea capitis, extensive tinea on skin, failed topical treatment, and immunocompromised patients. The drugs include terbinafine, a first-line oral therapy. Terbinafine is superior to fluconazole and itraconazole for the treatment of onychomycosis. Griseofulvin is another first-line therapy for tinea capitis caused by *Microsporum* species, while it is considered a third-line therapy for tinea corporis because of its lesser effectiveness than terbinafine and azoles. And, it is not recommended for the treatment of onychomycosis because of additional limitations like longer treatment duration and higher rates of adverse effects. (151-153)

The readers are suggested to go through different articles for detailed insights about management and drug resistance in dermatophyte infections. (154-160)

### FURTHER WORDS:

The dermatophytes have been confusing and changing their taxonomy since the beginning of time, and there is still a lot left to be understood. Although not an important etiology of mortality, dermatophytosis is a very common cause of morbidity affecting a very large proportion of the population.

The development of technology and molecular advances has changed the scenario of taxonomy, classification, and diagnosis of the dermatophytes and dermatophytosis. However, an incomplete online database hinders the recent advances. More studies regarding the extension of the online database regarding molecular profiles of the pathogenic dermatophyte species would create a clearer understanding and algorithms for rapid consultation.

Furthermore, more studies and awareness seem to be lacking regarding the emergence and dissemination of drug-resistant dermatophytes, along with other fungal pathogens.

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