CHAPTER III

REVIEW OF LITERATURE

3.1 HISTORICAL REVIEW

The three European physicians – Robert Remak, Johann L. Schönlein and David Gruby, inaugurated medical mycology with the discovery of fungal etiology of favus (Weitzman and Summerbell, 1995).

Historically, according to Seeliger, Robert Remak (1835) first observed unique microscopic morphological structures in favic lesions. He did not publish his observations; instead he allowed his findings to be cited in a doctoral dissertation of Xavier Hube (1837). Remak declared that he did not at first identify the etiological agent as a fungus and thus credited this recognition to Johann L. Schönlein, who described their mycotic etiology. Later, Remak cultured the fungus on apple, found it was infectious, and named the causative agent as Achorion schoenleinii, in honor of his mentor’s primary identification (Weitzman and Summerbell, 1995).

The real author of medical mycology was David Gruby based on his mycological findings during 1841-1844. Unaware of Remak and Schönlein observations, Gruby described the clinical entity and microscopic characteristics of the causative agent of favus. He described a fungus causing ectothrix hair invasion of beard and scalp and named the causative agent as Microsporum audouinii and later he also characterized the endothrix hair invasion as Herpes (Trichophyton) tonsurans (Weitzman and Summerbell, 1995; Padhye and Summerbell, 2005).
In 1890, Raimond Sabouraud initiated his work on dermatophytes and published his historic work *Les teignes* in 1910, in which he described the taxonomic classification of dermatophytes, conidial morphology, culture methods and treatment of dermatophytosis. He classified the dermatophytes into four genera – *Achorion*, *Epidermophyton*, *Microsporum* and *Trichophyton*, on the basis of clinical type involved and cultural characteristics (Weitzman and Summerbell, 1995; Padhye and Summerbell, 2005). Meanwhile, in 1925, Baltimore physicist, Robert W. Wood invented Wood lamp, a device used for rapid detection of fungal hair infection (Chander, 2009).

Chester Emmons (1934) re-classified the taxonomic classification of Sabouraud, where he excluded the genus *Achorion* and highlighted the other three anamorphic genera – *Epidermophyton*, *Microsporum* and *Trichophyton* based on conidial morphology and accessory structures. (Weitzman and Summerbell, 1995; Padhye and Summerbell, 2005). In 1984, Punsola and Guarro defined a fourth genus of dermatophytes as *Keratinomyces*, which is a psychrophiles from soil, that grow at 20°C, but the *Keratinomyces ceretanius* has not been identified as a human pathogen. (Chander, 2009).

The identification of teleomorphic state (sexual or perfect) of *Trichophyton ajelloi*, *Trichophyton terrestre*, *Microsporum nanum* by Dawson and Gentles in 1961, using hair bait technique of Vanbreuseghem, led to rapid detection of teleomorphs of other dermatophyte species and related fungi. Griffin and Stockdale independently discovered the production of sexual forms of *Microsporum gypseum* complex. (Weitzman and Summerbell, 1995; Padhye and Summerbell, 2005).
3.1.1 Scientific classification

Kingdom : Fungi
Division : Ascomycota
Class : Eurotiomycetes
Order : Onygenales
Family : Arthrodermataceae
Genus : *Trichophyton, Microsporum, Epidermophyton*

3.2 ETIOLOGICAL AGENTS

3.2.1 Anamorphs

The etiological agents of the dermatophytosis are grouped into three anamorphic genera, *Microsporum, Trichophyton* and *Epidermophyton*, based on the conidial morphology and accessory structures.

3.2.1.1 Microsporum

Colonies (Sabouraud’s dextrose agar (SDA)) are powdery, glabrous or cottony, white to yellowish, reverse cream colored or yellowish. *Microsporum* is characterized by the presence of both macroconidia and microconidia. Macroconidia are thin to thick rough walled, 2–15 septa, spindle or cigar shaped macroconidia borne singly or in clusters. Microconidia are solitary, smooth, thin walled, ovoidal to clavate, arranged along the sides of the hyphae (de Hoog *et al.*, 2009). *Microsporum audouinii* is the first
dermatophyte isolated in favic lesions. The genus *Microsporum* usually affects the skin and hair and occasionally the nails (Padhye and Summerbell, 2005).

### 3.2.1.2 Trichophyton

Colonies (SDA) are waxy, glabrous or cottony, white to cream colored, reverse cream to yellow or reddish brown pigmentation is present. Microscopically, *Trichophyton* is characterized by the development of smooth walled micro and macroconidia. Macroconidia are smooth, mostly thin walled, 2-12 celled, pencil shaped, cylindrical or cigar shaped macroconidia borne laterally or terminally resembling like undifferentiated hyphae or on short pedicels. Microconidia are produced in abundance as compared to the macroconidia, which are smooth, spherical, ovoidal or pyriform to clavate, borne singly along the sides of the hyphae or in grape like clusters (de Hoog et al., 2009). *T. tonsurans* is the first species identified among the genus *Trichophyton*. The genus *Trichophyton* infects the skin, hair and nails (Padhye and Summerbell, 2005).

### 3.2.1.3 Epidermophyton

Colonies (SDA) are slow growing, pale yellow to greenish brown, raised and gently folded, in the reverse yellowish brown pigmentation is usually present. The colonies quickly turn into whitish floccose and sterile. The microscopic morphology is characterized by the production of smooth, thin to thick walled, 2–5 celled, clavate with blunt tip macroconidia borne singly or in clusters. Microconidia are completely absent. Arthroconidia and chlamydospores are usually produced in older cultures (de Hoog et al., 2009). The genus consists of two known species – *Epidermophyton stockdale* and *E.
floccosum, of which the latter is recognized as pathogenic, infecting the skin and nails and rarely affects the hair (Padhye and Summerbell, 2005).

3.2.2 Teleomorphs

Few dermatophytes of the genera Microsporum and Trichophyton are capable of reproducing sexually. Earlier, the perfect state of the Microsporum and Trichophyton species and other keratinophilic fungi are classified in the genera Nannizzia and Arthroderma respectively. Later, Weitzman and Summerbell, 1995 reported that these two teleomorphic genera possessed only minor variations and therefore, based on the taxonomic classification, the species are designated in the teleomorphic genus Arthroderma, of the family Arthrodermatacea.

3.3 ECOLOGY

On evolutionary progression, each dermatophyte species adapted a selective host and led to the development of the three ecological groups - geophiles, zoophiles and anthropophiles which are listed in table 1.

The geophiles are associated with soil, which are rich in keratinous material such as hair, feathers, nails, horns, hooves etc. The geophiles are influenced by the neutral pH of the soil. The geophiles were recognized as ancestral to the pathogenic dermatophytes. Soil exposure is the primary source of infection for the geophilic dermatophytes to infect humans and animals. The zoophiles are associated with animals, which was gradually evolved from soil. Transmission of zoophiles is by direct contact with an infected animal or indirectly by fomites or inanimate objects associated with the keratinous substance
from the infected animal. The zoophiles occasionally infect humans. The anthropophiles are associated with humans, which gradually evolved from zoophilic species. Humans are the natural host for anthropophiles and transmission is by direct contact and indirectly by fomites. The anthropophiles tends to be chronic with mild inflammation, whereas the geophiles and zoophiles cause severe inflammatory reactions (Achterman and White, 2012).

**Table 1 Ecological classification of dermatophytes.** (Chander, 2009)

<table>
<thead>
<tr>
<th>Geophiles</th>
<th>Zoophiles</th>
<th>Anthropophiles</th>
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<tr>
<td><em>E. stockdaleae</em></td>
<td><em>M. canis</em></td>
<td><em>E. floccosum</em></td>
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<tr>
<td><em>M. amazonicum</em></td>
<td><em>M. gallinae</em></td>
<td><em>M. audouinii</em></td>
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<tr>
<td><em>M. cookei</em></td>
<td><em>M. persicolor</em></td>
<td><em>M. ferragineum</em></td>
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<td><em>M. fulvum</em></td>
<td><em>M. equinum</em></td>
<td><em>T. concentricum</em></td>
</tr>
<tr>
<td><em>M. gypseum complex</em></td>
<td><em>T. erinacei</em></td>
<td><em>T. megninii</em></td>
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<tr>
<td><em>M. nanum</em></td>
<td><em>T. mentagrophytes complex</em></td>
<td><em>T. mentagrophytes</em></td>
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<tr>
<td><em>M. praecox</em></td>
<td><em>T. simii</em></td>
<td><em>T. rubrum</em></td>
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<tr>
<td><em>T. ajelloi</em></td>
<td><em>T. verrucosum</em></td>
<td><em>T. schoenleinii</em></td>
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<tr>
<td><em>T. terrestre</em></td>
<td><em>T. equinum</em></td>
<td><em>T. soudanense</em></td>
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<td></td>
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<td><em>T. tonsurans</em></td>
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<td><em>T. violaceum</em></td>
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</table>
3.4 EPIDEMIOLOGY

Epidemiology of superficial infections forms the most predominant outspread of all mycotic infections. It accounts for more than 20-25% of the infections in the world’s population and they are predominantly caused by dermatophytes (Havlickova et al., 2008). The significant clinical risk factors that influence the incidence of dermatophytic infections are the high humid weather, over population and unhygienic conditions. The dissemination of etiological agents causing dermatophytosis and the anatomical location involved vary with the geographical region and most eminently due to tourism. Eventually, the incidence rate is increasing due to several other significant factors like trauma associated with diabetes mellitus, when the CD4 cell count reaches 450cells/µl. There is an increase in the risk of dermatophytosis in HIV patients (Surjushe et al., 2007). Other risk factors are immunosuppressive therapies and extended sports activities using air-tight shoes and communal swimming pools.

Despite the varied clinical risk factors, the spectrum of dermatophyte species is not static. The identification of dermatophyte species and their host preference plays a vital role in epidemiology, community health management and infection control. The epidemiological review of dermatophytes worldwide is discussed briefly.

3.4.1 Asia

In Asia the predominant clinical pattern are varied with the geographical regions. Tinea corporis is the commonest clinical pattern reported in India, likewise in Saudi Arabia the dominant clinical type is tinea capitis. Tinea pedis associated with tinea unguium is frequently reported from China, Japan and Turkey. *T. rubrum* and *T.*
mentagrophytes are the chief dermatophyte species extensive worldwide. The prevalence rate of tinea capitis is increased in children as a result of animal contacts, where the animals act as asymptomatic carriers (Al Samarai, 2007).

The prevalence of the dermatophyte species isolated in the epidemiological studies from different countries is discussed briefly.

1. India

Tinea corporis is the most common clinical type reported in India, followed by tinea cruris, tinea unguium and tinea capitis. T. rubrum followed by T. mentagrophytes or vice versa are the major dermatophyte species frequently isolated in India. Other dermatophyte species included were T. tonsurans, T. violaceum, T. verrucosum, T. schoenleinii, M. gypseum, M. audouinii and E. floccosum. Unlike in European countries, the endemic zoophilic dermatophyte species M. canis is less frequently isolated in India. The incidence of dermatophytosis in male population is four fold increased than the female population; among them male with age group > 60 years were frequently reported (Gupta et al., 2014). M. gypseum was isolated from skin scrapings of an immunocompromised individual with oral candidiasis. The individual is positive for HIV-1 antibody and the CD4 count decreased to 52 cells/mm³ (Bhagra et al., 2013).

Many rare dermatophyte species such as T. terrestr (New Delhi) (Grover et al., 2010), M. nanum (Tiruchirappalli, Jaipur) (Balakumar et al., 2012; Vyas et al., 2013), M. cookie (Rajkot) (Mistry et al., 2014), T. megninii, T. concentricum, T. simii and M. fulvum (Jaipur) (Jain et al., 2014) and M. ferrugineum (Lucknow, Jaipur) (Sahai and Mishra, 2011; Jain et al., 2014) were isolated from different geographical regions in
India. The prevalence of these rare dermatophyte species would no longer be confined to a particular region and in future these rare species would gradually be increased and spread to other regions as a result of travel and migration.

2. Iran

Tinea corporis is the most important clinical pattern in Iran, followed by tinea unguium, tinea mannum, tinea capitis, tinea faciei and tinea pedis (Sepahvand et al., 2009). Unlike India, tinea pedis is more common in Iran (Bassiri-Jahromi and Khaksari, 2009). *E. floccosum* is the chief dermatophyte species isolated in Iran, followed by *T. violaceum*, *T. verrucosum* and *T. tonsurans*. The distribution of anthropophilic dermatophyte species *M. ferrugineum* is emerging in Iran (Rezaei-Matehkolaei et al., 2012).

3. Saudi Arabia

Tinea capitis is the most dominant clinical type in Saudi Arabia, followed by tinea corporis, tinea cruris and tinea pedis. *M. canis* is the most prevalent zoophilic dermatophyte species, which are less frequently isolated from other Asian countries. Other dermatophytes included were *T. mentagrophytes*, *T. violaceum*, *T. rubrum*, *T. verrucosum*, *T. tonsurans*, *T. schoenleinii*, *M. gypseum* and *E. floccosum*. Rare dermatophytes such as *T. concentricum* (Khaled et al., 2015) and *T. soudanense* (Al Sheikh, 2009) were identified, of which the latter species was isolated from scalp infections. The incidence of scalp infections is elevated in female population than male which is uncommon in Saudi Arabia. Especially, children are more susceptible to tinea
capitis which may be as a result of reduced fungistatic fatty acids at the initial age, large family size and sharing of towels, clothing and hair accessories (Al Sheikh, 2009).

4. Nepal

Tinea capitis and tinea unguium are the commonest clinical type reported from Nepal. *T. violaceum* is the most significant pathogen of scalp infections. The dominant clinical form reported is the non-inflammatory gray patch and rarely about 1% of inflammatory kerion type tinea capitis. (Jha et al., 2006). *T. mentagrophytes* and *T. rubrum* are the dominant dermatophyte species isolated from nail infections. Other dermatophyte species included were *T. tonsurans*, *E. floccosum* and *M. canis*. Dermatophytes such as *M. gypseum*, *M. audouinii* and *M. nanum* were less than 3% isolated. Both fingernails and toenails are involved, of which the former are more prone to female with higher frequency of candidial onychomycosis and the latter are male dominant with distal and lateral subungual onychomycosis (Neupane et al., 2011).

5. Japan

Tinea pedis and tinea unguium are the most common clinical pattern in Japan, followed by tinea corporis, tinea cruris, tinea mannum and tinea capitis. Tinea pedis is frequently associated with tinea unguium in summer season (Sei, 2012). *T. rubrum* and *T. mentagrophytes* are the frequently isolated dermatophyte species. Other dermatophyte species included were *M. canis*, *M. gypseum* and *E. floccosum*. *T. tonsurans* is the most predominant dermatophyte species which exist in individuals with high sports activities such as judo (Hirose et al., 2005) and wrestling (Mochizuki et al., 2005) which when compared, the incidence of *T. tonsurans* was less in 2012 (Sei, 2012).
6. **China**

Tinea pedis and tinea unguium are the most common clinical type in China, followed by tinea manuum, tinea cruris and tinea corporis. *T. rubrum* and *T. mentagrophytes* were the most dominant etiological agents of tinea cruris and tinea corporis (Yan *et al.*, 2007). *M. canis* was more common among ectothrix, while *T. violaceum* and *T. tonsurans* were prevalent among endothrix scalp infections. These dermatophytes were responsible for the kerion development (Zhu *et al.*, 2010).

7. **Turkey**

Tinea unguium is the most frequently encountered clinical type in Turkey and particularly, the toenail involvement is commonly associated with the tinea pedis individuals. *T. rubrum* is the most predominant dermatophyte species affecting the skin and nails whereas, *T. mentagrophytes* is more common among scalp infections. A retrospective cohort study was performed for any dominant shift in dermatophyte species isolated, the *T. mentagrophytes* (30.07%) which is common in 1980 was decreased to 5% in 2011 and *T. rubrum* (15.04%) in 1980 was increased to 87.25% in 2011 (Akçaglar *et al.*, 2011).

3.4.2 **Europe**

Tinea unguium (28.2%) is the most significant clinical pattern among Europeans, followed by tinea capitis (27.8%) and tinea pedis (22%) (Simonnet *et al.*, 2011). About 86% of patients, the big toe were infected in at least one foot and distal and lateral subungual onychomycosis were more frequent followed by total dystrophic
onychomycosis. (Dias et al., 2011). In 2010 tinea cruris has six-fold decreased, whereas tinea unguium has increased five-fold in the urban areas of Europe (Vena et al., 2012). The predominance of tinea pedis and tinea unguium clinical pattern are due to varied risk factors such as increased incidence of diabetes, communal swimming pools, occlusive shoes, extended sport activities. T. rubrum, T. mentagrophytes and M. canis are the major dermatophyte species isolated in Europe. T. mentagrophytes var. interdigitale and E. floccosum was less frequently isolated. Domestic animals are one of the major urban lifestyle of many western countries which are the significant source of infection for the predominance of zoophiles. The dermatophytes and the other molds are repeatedly isolated from toenail mycoses, whereas the Candida onychomycosis is more frequently observed in fingernail mycoses (Nazar et al., 2012). Tinea pedis was more frequent and remarkably high prevalence of 55% was observed from healthy carriers (Pérez-González et al., 2009).

The gender predominance of dermatophytic infections was observed, among women there is a combination of tinea unguium and corporis and for men it is tinea pedis and cruris (Vena et al., 2012). A geophilic dermatophyte M. gypseum was reported from a facial tinea incognito in a patient with extreme HIV infection, involved with skin and brain lesions in Italy (Polilli et al., 2011). A zoophilic dermatophyte T. bullosum was isolated from skin lesions of an individual by contact with the infected donkey in France. The dermatophyte species T. bullosum was previously reported in 1933 from cutaneous lesions of horses in Africa and Asia (Sitterle et al., 2012).
3.4.3 Africa

Tinea capitis (26.9%) is the commonest clinical pattern in Africa, particularly among children, whereas tinea unguium (0.8%), tinea corporis (0.6%) and tinea faciei (0.5%) were less frequently reported from Nigeria (Oke et al., 2014). The non-inflammatory grey patch type scalp infection is the commonest clinical pattern among school children in Western Nigeria (Oke et al., 2014). The principal cause of scalp infections is sharing hair accessories at home coupled with large family size. *T. violaceum, T. soudanese, T. mentagrophytes, M. audouinii* are the predominant dermatophyte species isolated among children causing scalp infections in varied regions of Africa. The children with age group between 7-11 years were more susceptible to scalp infections (Adefemi et al., 2011), which may be due to the absence of saturated fatty acids and acts as a natural barrier against fungal infections. Other dermatophyte species included were *T. tonsurans, T. schoenleinii, T. verrucosum, E. floccosum, M. canis* and *M. gypseum*. A rare dermatophyte species *M. langeronii* was isolated from scalp infections (Kechia et al., 2014).

A cross-sectional study on onychomycosis was observed among urban and rural school children in the Central Tunisia where, women were more in number than men with fungal nail infections. Unlike European countries (Simonnet et al., 2011), in both the gender, fingernails were frequently infected than toenails, from which the anthropophilic dermatophyte species *T. rubrum* is frequently isolated from Central Tunisia (Dhib et al., 2013).
3.4.4 America

Tinea unguium (59.9%) is the most significant clinical pattern among Americans, followed by tinea pedis clinical type (24.5%) (López-Martínez et al., 2010). Unlike India, tinea corporis and cruris are less frequently reported in America. In Brazil, higher prevalence of dermatophytosis was reported among females (77.2%) than male population (22.8%) (Costa-Orlandi et al., 2012). *T. rubrum* and *T. mentagrophytes* are the dermatophyte species frequently isolated and *T. tonsurans* is the most predominant pathogen isolated from scalp infections among children (Mirmirani and Tucker, 2013). There was gender predominance for the primary cause of onychomycosis where, in females it is due to yeast infections (*Candida* species) and among males it is the dermatophytic infections (Souza et al., 2010).

3.4.5 Australia

A cross-sectional study was observed for the higher proportions of tinea capitis in school children in Melbourne. Among 153 children screened, dermatophytes were isolated from 32 individuals, among them 23 were symptomatic and 9 were healthy carriers. An outbreak of three anthropophiles *T. soudanense*, *T. violaceum* and *M. audouini* were reported from scalp infections. These dermatophytes are isolated from individuals who originate from Sudan, Africa and Arab countries. These dermatophyte species which are rarely isolated in Australian School children may be gradually increased in future due to continuing migration (McPherson et al., 2008).

Despite of all clinical patterns of dermatophytosis prevalent worldwide, tinea unguium and tinea pedis are more common among developed countries whereas tinea
corporis and tinea cruris are dominant among developing countries. The frequency of clinical pattern of dermatophytosis is varied with seasons. Tinea corporis, tinea cruris, tinea pedis associated with toenail onychomycosis is more common among summer season, whereas fingernail onychomycosis and tinea capitis are predominant in spring and winter seasons. Tinea corporis is common among male and female whereas, tinea cruris is male dominant. Tinea capitis is the principal disease of children and young adolescents, particularly reserved below 10-12 years age group. Fingernail involvement is more common among female population with candidial onychomycosis whereas toenail infections associated with tinea pedis is more common among male population. Exceptionally, discoloration is the most common symptom and the first toenail was more commonly affected with dermatophytes. Since, dermatophytosis is a recurrent superficial fungal infection it should be treated completely to prevent recurrent cutaneous infections. Regular epidemiological surveillance is recommended from many developing and developed countries for the clinical management of the dermatophytosis.

### 3.5 PATHOGENESIS

Dermatophytes can survive solely on the outer epidermal layer of the skin. The infection is acquired by viable arthroconidia (infectious substance) by direct contact with the infected individual or indirectly by fomites. Once the infectious material is adhered to the keratinased host cells, during favorable conditions, the infection progress by germination and spreads radially and penetrates the stratum corneum of the skin. During penetration the dermatophytes utilizes the substrates present on the skin surface for its growth. The matured hyphae break into arthroconidia, which are the infectious
propagules that exist in the environment. These arthroconidia shed in a course of interval, which are partly responsible for the central clearing of the ringworm lesions. The dermatophytes produce virulence enzymes upon substrate specificity \textit{in-vitro} and \textit{in-vivo} and cause mild to severe inflammation with erythema, vesicle or pustules and along with pruritis. The clinical severity of the dermatophytic infections is related to the species and strain of the fungus, the inoculum size, the anatomical site involved and the immune response of the host. Whenever, the host immune response is reduced, re-occurrence of the dermatophytic infection occurs and hence the host immune system is responsible for the clinical course of infection (Chander, 2009).

3.6 IMMUNOLOGY

Dermatophytes are usually restricted to the outer epidermal layer of the skin and cause mild to severe inflammatory reactions. Since the outer layer of the skin lack specific defense mechanism, it is unable to recognize the infection. Hence, both humoral and cell mediated immune system with host specific and non-specific responds to this infection and prevent the fungal invasion into deep tissues. The immune system found to be active against dermatophytes which are comprised of α2-macroglobulin keratinase inhibitor, unsaturated transferrin, epidermal desquamation, lymphocytes, macrophages, neutrophils and mast cells (Weitzman and Summerbell, 1995).

The dermatophyte antigens are of two types, which included glycopeptides and keratinases. The protein part of the glycopeptides stimulates the cell mediated immunity, while the polysaccharide part stimulates the humoral immunity. Immunological studies report that the cell mediated immunity particularly plays a vital role in the defense
mechanism of the dermatophytic infections. Usually the dermatophytic infection responds well to the treatment. When there is a lack of cell mediated immunity, it develops chronic or recurrent infections. This could be due to the fact that the cell mediated immunity probably directs the delayed type hypersensitivity respond to the dermatophyte antigen (trichophytin), which then acts as an important defense mechanism. A variety of antibodies are produced by the host cells in response to the dermatophyte antigens. The immunoglobulin such as IgM, IgG, IgA and IgE are produced and the level of these antibodies thought to be raised high only in chronic infections. The keratinases are produced by the dermatophytes, where the delayed type hypersensitivity responds to this infection when it is administrated intradermal into the animals (Weitzman and Summerbell, 1995).

3.7 CLINICAL MANIFESTATIONS

Dermatophytosis has a variety of clinical presentations which are named according to the anatomical site involved and have similar treatment. It is popularly called as tinea or ringworm. The Latin word tinea means “worm” or “moth”. The infections appear as circular or annular inflammatory lesions on the skin, representing a worm burrowing at the edge of the lesions. The anthropophilic species develop chronicity with mild inflammation, whereas the geophiles and zoophiles cause severe inflammation but they respond well to the treatment. The chronic dermatophytosis denotes the duration and recurrences of dermatophytic infections. The clinical types of dermatophyte infections are described briefly.
3.7.1  Tinea capitis

Tinea capitis is a cutaneous infection of the hair and scalp and almost usually affects the younger children. The infection is characterized by the etiological agents involved and can occur in three varied clinical forms such as grey patch, black dot, favus. The grey patch tinea capitis initially develop a small erythematous papule over a single hair shaft and later stretch centrifugally to other neighboring hairs. Subsequently scaling occurs and hairs turn grey. The black dot tinea capitis is characterized by erythematous scaly lesions invading the hair shaft and breakage of the infected hairs. The detritus present within the hair follicle appear as black dot. The favus (tinea favosa) is an inflammatory type of tinea capitis, usually begins with a erythematous lesions, scaling and later form a thick cup shaped yellow crusts called scutula around the infected hair follicle. The scutula are filled with pathogenic fungi, produces a waxy, honeycomb-like crust on the scalp and therefore named as favus (Latin word for honeycomb). It may lead to alopecia with scarring.

3.7.2  Tinea corporis

Tinea corporis is an infection of the glabrous (non-hairy) skin of body. The infection usually begins with erythematous scaly vesicles which may be single or multiple with sharp edged, raised border and severe itching. The infection slowly spread centrifugally and the lesions appear as a ring with central clearing. Therefore, dermatophyte infections are commonly named as ringworm. The dermatophyte infections are superficial, involving the epidermis of skin; however in some patients the dermatophyte may invade the dermis and hypodermis of skin. This condition is known as
Majocchi’s granuloma, which is induced by trauma of the infected skin or vascular occlusion of hair follicles by which the fungal elements drive into the dermis.

3.7.3 Tinea faciei

Tinea faciei is an infection of the face with exception of beard. The infection is contagious and can easily spread to other regions of the skin.

3.7.4 Tinea barbae

Tinea barbae is an infection of beard and moustache region of the face, invading the hairs. It is also known as barber’s itch. The infection may range from mild superficial to severe inflammatory lesions with folliculitis.

3.7.5 Tinea imbricata

Tinea imbricate is a rare form of tinea corporis caused by *Trichophyton concentricum*. It is a chronic infection which is characterized by concentric rings of scaling dispersed throughout the body and may lead to lichenification.

3.7.6 Tinea gladiatorum

Tinea gladiatorum is an emerging infection in wrestlers and also found in other athletes who have direct skin contact than via fomites. The lesions are often found on the arms, trunk or neck and head.
3.7.7 Tinea incognito

Tinea incognito is an infection produced as a result of inappropriate treatment with topical corticosteroids. It represents a different clinical pattern such as reduced inflammatory reaction from the classic appearance of ringworm infection.

3.7.8 Tinea manuum

Tinea manuum is an infection of the palmar and interdigital spaces of the hands. Hyperkeratosis of the palms and fingers is the most common clinical entity involved in case of tinea manuum.

3.7.9 Tinea cruris (Jock itch)

Tinea cruris is the infection of groin, perianal and perineal regions and progress to the inner thighs and buttocks. The infection is more predominant in adult men. The lesions are erythematous, scaling and sharp margins with raised borders and severe itching. Therefore it is also known as “jock itch”.

3.7.10 Tinea pedis (athlete’s foot)

Tinea pedis is one of the most common dermatophyte infection, involves the interdigital webs and soles. It is most common among athletes who wear shoes for long hours and therefore it is named as athlete’s foot. The infection usually presents with maceration, scaling, fissuring, erythematous vesicles or bullae with itching and burning between the toes or on the soles. It may lead to secondary bacterial infections with lymphangitis and lymphadenitis. When the infection is untreated it persists to chronic
tinea pedis, where the sole becomes hyperkeratotic and may stretch to the sides of the foot and the condition is termed as Moccasin or Sandal ringworm. In many cases it is commonly associated with one hand and two feet of a patient and therefore named as one hand two feet syndrome.

3.7.11 Tinea unguium

Tinea unguium is a fungal nail infection specifically caused by dermatophytes. The infection usually invades the nail plate and nail bed. Onychomycosis is a general term that refers to fungal nail infections caused by dermatophytes, non-dermatophytes or yeasts. Onychomycosis is classified into four clinical types which are described briefly. (i) Distal and lateral subungual onychomycosis (DLSO) infection usually begins at the free edge of the nail plate and progress to the underside of the nail bed. Sometimes, the infection is inflammatory leading to subungual hyperkeratosis, where the nail plate is raised and detached from the nail bed. (ii) Proximal subungual onychomycosis (PSO) is usually restricted to fingernails and mostly caused by Candida species. The fungal hyphae invade the proximal nail fold and it is observed through cuticle as whitish yellow discoloration of the nails. The infection is painful and sometimes with purulent discharge. (iii) White superficial onychomycosis (WSO) usually affects the nail surface, involving the superficial layer of the nail plate and (iv) Total dystrophic onychomycosis (TDO) where the fungal hyphae invade the entire nail plate and results in total destruction of the nails and sometimes combination of lesions of DLSO and PSO may be observed.
3.7.12 ‘Id’ reaction

Dermatophytid or ‘id’ reaction is a secondary infection thought to be found in highly sensitized tinea patients and it is of two types (i) Lichen scrofulosorum-like, which is more common among scalp infections and the infection may spread to the limbs and face and results in kerion development. (ii) Pompholyx-like, this is associated with tinea pedis, involving the sides and flexor of palms and fingers. This is often due to Type III hypersensitivity reaction. The infection normally resolves immediately or response well to the treatment. Sometimes, the infection is increased due to griseofulvin treatment or trichophytin reaction.

3.8 LABORATORY DIAGNOSIS

There are several steps in identification of dermatophytes directly from the clinical specimens which include (i) collection and transport of specimens, (ii) direct microscopic examination, (iii) culture procedures, (iv) Physiologic and nutritional tests.

3.8.1 Collection and transport of specimens

Dermatophytes are the group of filamentous fungi that grow radially at the site of infection. In tinea corporis and tinea cruris, the center region of the infection consists of the poor viable substance and the periphery edge contains the active viable material. Initially, the infection site is cleaned with 70% alcohol to remove the surface contaminants and the topical antifungal cream that might be present. The specimen is collected best by collecting the epidermal scales present at the peripheral active edge of the lesion. The clinical material is collected by using sterile scalpel. If it is inflammatory
with vesicles, then the vesicles are trimmed and included in sampling. In tinea capitis, the hair is first examined with a Wood’s lamp (filtered UV light) at 365nm in a dark room for the presence of fluorescent hairs. The infected hair is plucked with the forceps and included for sampling. If it is a grey patch or black dot tinea capitis type then scalpel can be utilized to scrape the epidermal scales and small portion of the hair root is removed. In tinea unguium, scraping is done to collect the powdery debris from beneath the free edge of the nail plate and close to the nail bed. The clinical specimen is collected directly in a sterile thick black chart paper for clear visibility of the specimen collected and transported to the mycology laboratory.

### 3.8.2 Direct microscopic examination

The direct microscopy with 10% Potassium hydroxide (KOH) is the first screening test performed for the presence of fungal elements in the clinical specimen. Along with KOH, 3-4 drops of Parker ink (black) is added for better appreciation of the fungal elements. The preliminary examination with KOH and instant result will help in treating the patient at the earliest. The specimen is placed in 1-2 drops of 10% KOH on a clean microscopic slide and a coverslip is placed over the sample whereas, for nails, 20% KOH is used. The specimen is gently heated (avoid bubbles by overheating) so that the KOH digest the keratinous material and allows the fungal elements to be visualized. The fungal elements can be made out by using glucan-binding fluorescent brighteners such as calcofluor white and Congo red and visualized under fluorescent microscope. Finally, the preparation is examined under low and high power magnification of the microscope.
3.8.3 Culture procedures

3.8.3.1 Primary isolation media

1. Sabouraud’s dextrose agar (SDA) with chloramphenicol and cycloheximide

   It is a basal medium supplemented with chloramphenicol (to prevent bacterial contamination) and cycloheximide (to prevent saprotrophic growth) which is utilized extensively for the growth of dermatophytes.

2. Dermatophyte test medium (DTM)

   It is a specific medium with dermatophyte supplement (HiMedia), particularly to enhance the growth of dermatophytes. It contains phenol red indicator to differentiate dermatophyte which utilizes the nitrogenous source and produce alkaline by-products which induce the red color to the medium, whereas the saprotrophic fungi utilize carbohydrates and produce acid by-products.

3.8.3.2 Colony characteristics

   The macroscopic morphology of the colony is observed that include (i) color of the colony on the obverse and reverse side, (ii) texture (powdery, cottony, woolly, fluffy, granular, glabrous, floccose, and waxy), (iii) topography (elevation, folding, margins, heaped, button-like, wrinkled etc.) and (iv) rate of growth.
3.8.3.3 Microscopic morphology

1. Lactophenol cotton blue (LPCB) wet mount

Lactophenol cotton blue is used to mount from fungal cultures for the identification of the microscopic morphology of the dermatophytes. The lactic acid preserves the fungal structure, phenol kills the fungus and cotton blue stain is absorbed by the fungal elements (Moore and Jaciow, 1979).

A small portion of the fungal culture is placed in 1-2 drops of lactophenol cotton blue stain on a clean microscopic slide and teased apart using teasing needle. The material is covered by a sterile cover slip and gently heated over the flame. Then the preparation is examined under low and high power magnification of the microscope.

2. Slide culture

The slide culture is performed to observe the undisturbed morphology of the dermatophytes. Enriched media such as Oat meal agar (OMA), Potato dextrose agar (PDA) is utilized especially to increase the sporulation which results in accurate identification of the dermatophyte species.

A sterilized glass petridish with U or V shaped glass rod and glass slide are taken and laid on sterilized glass petridish. Oat meal agar / Potato dextrose agar block is cut little smaller to the breadth of the glass slide and placed on the glass slide. Using the heat sterilized bacteriological needle a small portion of the fungal culture is inoculated onto the four sides of the agar block and a sterile cover slip is placed on it. Two to three ml of sterile distilled water is poured inside the petridish to maintain the
moisture. The lid is closed and incubated at room temperature. The culture in the petridish is examined at regular intervals for the fungal growth. When the sporulation is visible, the cover slip is gently removed from the agar block and placed on few drops of LPCB on a sterile glass slide. The uninterrupted morphology is examined under low and high power magnification of the microscope.

The morphological characteristics like hyphae, different size, shape of microconidia and macroconidia and other specialized structures like spiral hyphae, arthroconidia, chlamydospores, nodular bodies, favic chandeliers etc are observed. The cultural characteristics of dermatophyte species is shown in table 2.
Table 2 IDENTIFICATION CHARACTERS OF DERMATOPHYTES (de Hoog et al., 2009)

<table>
<thead>
<tr>
<th>Dermatophyte species (Inventors)</th>
<th>Colony characteristics</th>
<th>Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Epidermophyton floccosum</em></td>
<td>Colonies (SGA) velvety, sometimes powdery, felty, velvety, becoming woolly, gently folded, pale yellow to ochre or mustard-yellow; reverse yellowish-tan with yellow-brown centre. The colonies quickly become whitish, floccose and sterile.</td>
<td>Macroconidia arranged in clusters, smooth-walled, rather thin-walled, 2-5 celled, 10-40 x 6-12 µm, clavate with blunt tip. Chlamydospores and arthroconidia are common in older cultures.</td>
</tr>
<tr>
<td><em>(Langeron and Milochevitch)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Microsporum amazonicum</em></td>
<td>Colonies (SGA) powdery to fluffy, grey olivaceous buff.</td>
<td>Macroconidia thin-walled, echinulate, spindle-shaped, 4-5 (-8) celled, 13-35 x 3-10 µm, with 1.5 µm thick walls and with narrow scar. Microconidia sessile, clavate.</td>
</tr>
<tr>
<td><em>(Moraes et al.,)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Microsporum audouinii</em></td>
<td>Colonies (SGA) woolly, flat, spreading, with radiating margin, greyish to tannish-white; reverse salmon-pink to rose-brown.</td>
<td>Pectinate hyphae and terminal chlamydospores may be present. Macroconidia rare, when present smooth-walled to sparsely echinulate, thick-walled, irregularly spindle-shaped, frequently somewhat isthmoid and rostrate, with constriction near the middle, of variable size and cell number, 30-82 x 8-34 µm, mostly with slightly bent, verrucose apex. Microconidia rare, ovoidal to clavate.</td>
</tr>
<tr>
<td><em>(Gruby)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsporum ferrugineum (Ota)</td>
<td>Colonies (SGA) glabrous, heaped, wrinkled, sometimes flat, yellow to cream-coloured, reverse cream-coloured to yellow.</td>
<td>Conidia usually absent. Long straight, broad hyphae with predominant cross walls (bamboo hyphae) often present, showing a tendency to disarticulate. Spindle-shaped macroconidia similar to those of <em>M. canis</em> may be produced on dilute Sabouraud dextrose agar. The species is probably a sterile variant of <em>M. canis</em>.</td>
</tr>
<tr>
<td>Microsporum canis (Bodin)</td>
<td>Colonies (SGA) spreading, thin, woolly, strongly radiating, greyish- to tannish-white; reverse deep ochraceous-yellow.</td>
<td>Macroconidia 6-12 cells, rough-walled, with thick cell walls and thinner septa, 35-110 x 12-25 µm, spindle-shaped, with slightly bent, verrucose, rostrate apex. Microconidia clavate to pyriform, sessile alongside undifferentiated hyphae.</td>
</tr>
<tr>
<td>Microsporum cookei (Ajello)</td>
<td>Colonies (SGA) spreading, powdery, becoming yellowish, greenish-buff, or dark brown. A deep grape-red pigment is excluded into the medium.</td>
<td>Macroconidia thick and rough-walled, 6-7 (-10) celled, broadly fusiform with rounded apex, 30-50 x 10-15 µm. Microconidia ovoidal to pyriform.</td>
</tr>
<tr>
<td>Microsporum nanum (Fuentes)</td>
<td>Colonies (SGA) spreading, powdery or cottony, often with some radial, shallow furrows, white, centrally buff; reverse reddish-brown.</td>
<td>Macroconidia mostly 2 celled, rather thin-walled, smooth-walled to verrucose, obovoidal to pyriform, 12-18 x 5.0-7.5 µm, with flat basal clusters. Microconidia sessile alongside undifferentiated hyphae, clavate.</td>
</tr>
</tbody>
</table>
| **Microsporum gypseum**  
| (Guiart and Grigorakis) | Colonies (SGA) growing rapidly, powdery, cinnamon-tan; reverse yellowish-buff, sometimes with pinkish tinges. | Macroconidia in large clusters, rather thin-walled, regularly verrucose, 3-6 (-8) celled, fusiform, 25-60 x 8.5-15.0 µm. Microconidia sessile or stalked, smooth or thin-walled, clavate, 3.5-8.0 x 2-3 µm. |
| **Microsporum fulvum**  
| (Uriburu) | Colonies (SGA) spreading, flat, farinose to floccose, buff to pink buff; reverse red. | Macroconidia thin or rather thick-walled, echinulate, broadly fusiform to clavate, (4) 5-6 (-7) celled, 25-60 x 7-12 µm. Microconidia sessile or short-stalked, clavate. Spiral hyphae often present. Rarely mutants occur with non-maturing conidia in strongly coherent, Christmas tree shaped clusters (‘T. longifusum’). |
| **Microsporum gallinae**  
| (Grigorakis) | Colonies (SGA) moderately fast growing, granular, velvety or satin, more or less wrinkled, white with pinkish or buff tinges. Reverse initially with a yellow, non-diffusible pigment, later a strawberry-red pigment diffuses into the agar. | Macroconidia, when present, arranged in unilateral clusters on pectinate hyphae, 2-12 celled (usually 5 to 6), thin-or thick-walled, smooth-walled to slightly echinulate, cylindrical to clavate with narrow base and blunt tip, sometimes slightly curved, 15-60 x 6-10 µm. Microconidia ovoidal to pyriform. |
| **Microsporum persicolor**  
| (Guiart and Grigorakis) | Colonies (SGA) expanding, powdery to fluffy, pale yellowish-buff to pinkish-buff; reverse ochraceous. On sugar-free media the colonies are rosaceous. | Macroconidia thin-walled, rough-walled at the tip, cigar-shaped, 4-7 celled 40-60 x 4-8 µm. Microconidia in dense clusters, spherical. Spiral hyphae present. |
| **Microsporum racemosum**  
(Borelli) | Colonies (MEA) spreading, powdery, often feathered, cream-coloured, later with reddish staining; reverse grape-red or brown. | Macroconidia stalked, cigar-shaped, thick and rough-walled, 5-10 celled, 55-65 x 12-15 µm. Microconidia stalked, in clusters, clavate. |
| **Micropsorum praecox**  
(Rivalier ex Padhye *et al.*) | Colonies (SGA) moderately expanding, powdery, with concentric, cloudy growth waves, buff; reverse yellow-orange. | Macroconidia moderately thin-walled, echinulate, lanceolate with narrow apex, 6-9 celled, up to 65 x 9 µm. Microconidia, when present, in orthotropic arrangement, pyriform. |
| **Trichophyton ajelloi**  
(Ajello) | Colonies (SGA) expanding, flat, powdery to velvety, cream-coloured to ochraceous-buff; reverse yellowish, a dark purple pigment being exuded into the agar. | Macroconidia hyaline, smooth and thick-walled, cigar-shaped, 8-12 celled, 40-70 x 9-12 µm. Microconidia sparse or absent, ovoidal to pyriform, 3-9 x 2-5 µm. |
| **Trichophyton flavescens**  
(Padhye and Carmichael) | Colonies (SGA) spreading, pale yellow; reverse bright yellow to brown. | Macroconidia 2-6 septate, cylindrical, 25-80 x 8-14 µm abruptly narrowed towards the base. Microconidia ovoidal, 1(-2) celled, 5-16 x 4-8 µm, occasionally absent. |
| **Trichophyton phaseoliforme**  
(Borelli and Feo) | Colonies (SGA) spreading, powdery, white to bright cinnamon, with whitish pycnidium-like bodies. | Macroconidia usually absent; when present, in clusters, cigar-shaped, 2-5 celled. Microconidia curved, cashew nut-shaped, formed laterally on hyphae which locally are wider and densely aggregated in pycnidium-like bodies. |
| **Trichophyton mentagrophytes**  
(Blanchard) | Colonies (SGA) powdery to floccose, cream-coloured to yellowish-buff, powdery colonies frequently somewhat star-shaped; reverse ochre to red-brown, occasionally yellow, or dark brown. | Macroconidia 3-8 celled, smooth and thin-walled, clavate to cigar-shaped, 20-50 x 6-8 µm, usually sparse. Microconidia spherical, 2 µm diameters, sessile, arranged in dense, grape like clusters or alongside the hyphae. Spiral hyphae frequently present. Favic chandelier-like structures and chlamydospores occasionally present. |
|----|----|----|
| **Trichophyton rubrum**  
(Sabouraud) | Colonies (SGA) fluffy to cottony, white, sometimes becoming rose when ageing; reverse wine-red to olive, sometimes yellow. | Macroconidia mostly abundant, when produced thin-walled, poorly differentiated, of variable size, cylindrical to cigar-shaped, 40-55 x 6.0-7.5 µm, with a tendency to disarticulate. Microconidia peg-shaped to pyriform, 3.0-5.5 x 2.0-3.5 µm, sessile alongside undifferentiated hyphae. Occasionally only micro or only macroconidia are present; cultures rarely sterile. |
| **Trichophyton concentricum**  
(Blanchard) | Colonies (SGA) growing slowly, white, becoming cream-coloured, amber, honey-brown to orange, glabrous with a tendency to become slightly velvety, raised and irregularly, deeply folded, often cracking the agar; reverse cream-coloured to brown. | Tangled masses of branching hyphae present; conidia absent or some tear-shaped microconidia formed. |
<table>
<thead>
<tr>
<th><strong>Trichophyton terrestr</strong>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Durie and Frey)</td>
</tr>
<tr>
<td>Colonies (SGA) expanding, felty, fluffy to powdery, whitish to pale cream-coloured or brownish; reverse greyish to yellowish or ochraceous.</td>
</tr>
<tr>
<td>Macroconidia not clearly differentiated from microconidia, 2-6 celled, smooth and thin-walled, cylindrical or slightly clavate, 9-50 x 4-5 µm. Microconidia arising in densely arranged conidiophores with profuse orthotropic branching, short-cylindrical to short-clavate, 4.0-6.5 x 1.5 µm, truncate.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Trichophyton schoenleinii</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Langeron and Milochevitch)</td>
</tr>
<tr>
<td>Colonies (SGA) growing rather slowly, waxy, later becoming velvety, folded, cerebriform and heaped with age, often cracking and splitting the agar, whitish to cream-coloured; margin sometimes feathered due to the presence of favic chandelier; reverse unpigmented or pale yellow.</td>
</tr>
<tr>
<td>Macroconidia and microconidia usually absent. Antler-like hyphae, with dichotomously branched, swollen tips (favic chandeliers), present in submerged margin of fresh cultures. Chlamydospores abundant.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Trichophyton gloriae</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ajello)</td>
</tr>
<tr>
<td>Colonies (SGA) flat, somewhat folded powdery, whitish to cinnamon; reverse yellow.</td>
</tr>
<tr>
<td>Macroconidia in clusters, narrow clavate, mostly 5-11 celled, 9-60 x 3-7 µm, with walls up to 1 µm thick. Microconidia pyriform to clavate, 1.5-6.0 x 1.5-2.5 µm.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Trichophyton vanbreuseghemii</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rioux et al.,)</td>
</tr>
<tr>
<td>Colonies (SGA) velvety, folded, warm buff; reverse cream-coloured.</td>
</tr>
<tr>
<td>Macroconida cylindrical, 4-7 celled, 30-55 x 6-8 µm. Microconida pyriform, 2-7 x 1.5-2.5 µm.</td>
</tr>
</tbody>
</table>
| **Trichophyton erinacei**  
| (Quaife) | Colonies (SGA) expanding, cottony or farinose, white; colony reverse becoming bright citron yellow. | Macroconidia, when present, cylindrical to clavate variable in size, 2-6 celled. Microconidia abundant, slender, clavate up to 6 µm, at right angles alongside hyphae, first widely interspaced, finally close together, liberated by deterioration of supporting hyphae. Arthroconidia common. |
| **Trichophyton interdigitale**  
| (Priestley) | Colonies (SGA) thin, floccose, white or glabrous with deep yellow margin and orange reverse; the latter type of colonies do not produce any conidia but have yellowish hyphae with nodular bodies, and are known as var. nodulare (Kane et al., 1992). | Conidia spherical to tear-shaped, frequently absent. Small nodular clumps of cells, surrounded by yellowish exudate, frequently present, particularly in non-sporulating strains. |
| **Trichophyton tonsurans**  
| (Malmsten) | Colonies (SGA) rather variable; mostly suede-like, radially or irregularly furrowed, white to greyish, yellowish or brownish-buff, sometimes with pinkish or pale olivaceous centre; reverse mahogany red, yellow or brown. | Microconidia of variable size, produced in abundance, formed on loosely clustered branches or thickened terminal hyphae, sessile, clavate to nearly cylindrical, sometimes inflating to ballon-shaped. Macroconidia, when present, variable, often somewhat thick-walled, 2-6 celled, cylindrical to cigar-shaped, 10-65 x 4-12 µm. Terminal and intercalary, swallon chlamydospores are formed in abundance. |
| **Trichophyton simii**  
| (Stockdale et al.) | Colonies (SGA) spreading, evenly granular with fluffy margin, whitish to pale buff; reverse yellowish to salmon, becoming vinaceous. | Macroconidia smooth-walled, fusiform, 30-85 x 6-11 µm, 5-10 celled; individual cells often swelling and becoming liberated as chlamydospires. Microcondia mostly sessile alongside undifferentiated hyphae, clavate to pyriform. |
| **Trichophyton thuringiense**  
| (Koch) | Colonies (SGA) expanding, cottony, white, becoming slightly brownish; reverse reddish brown to buff. | Macroconidia hardly different from microcondia, cylindrical to clavate, 2-5 celled, up to 30 µm in length. Microcondia obovoidal to short clavate with broad base, 3-5 x 2-3 µm, mostly sessile on thin, deteriorating hyphae. Arthroconidia present. |
| **Trichophyton verrucosum**  
| (Bodin) | Colonies (SGA) growing very slowly, heaped or button-like, feathered by a perimeter of submerged hyphae; colonies initially glabrous, later slightly velvety, cream-coloured or greyish-white, sometimes with salmon to yellow tinges; reverse pale cream- or salmon-coloured. | Sporulation absent or reduced. Macrocondia, when present, (on T-3), 4-7 celled, smooth- and thin-walled, stringbean-shaped. Microcondia, when shaped, ovoidal to pyriform. Chlamydospires common in fresh isolates, often arranged in chains, sometimes ending with antler-like hyphal branching without swollen tips. Hyphal tips frequently swallowed in fresh isolates. |
| **Trichophyton violaceum** (Sabouraud and apud Bodin) | Colonies (SGA) growing slowly, glabrous, leathery, wrinkled, yellow, apricot-red or purple-red; reverse dark yellow (formerly *T. soudanense*), red-brown (formerly *T. gourvilii*), purple or violet, or becoming chocolate-brown with age and exuding a diffusible brown pigment into the agar (formerly *T. yaoundei*), or reddish with feathred margin (formerly *T. soudanense*). Strains easily lose their pigmentation by formation of white sectors. Colonies dark brown on Löwenstein’s egg medium. | Hyphae highly distorted; they may have reflexive branching (formerly *T. soudanense*) and are often strongly septate, disarticulating into arthroconidia. Sporulation absent or reduced. Macroconidia very rare. Microconidia, when present, ovoidal, pyriform or clavate. |
3.8.3.4 Preservation of cultures

Pleomorphism is a condition where the culture becomes sterile with white growth that eventually covers the entire dermatophyte culture. If it becomes pleomorphic, then the identification of dermatophytes is impossible. Therefore, to avoid pleomorphism the dermatophytes are to be sub-cultured regularly (every 4 to 5 weeks) and need to be preserved. The preservation techniques included are (i) overlay the culture with sterile mineral oil, (ii) maintain the cultures at 8°C, (iii) sub-culture the fungal spores in sterile distilled water (water culture) and (iv) lyophilization (Moore and Jaciow, 1979).

3.8.4 Physiological and nutritional tests

Since the dermatophytes cannot be differentiated with the morphological characters alone, few nutritional and biochemical tests can be performed to increase the chances of identification of dermatophytes easily by conventional procedures. Georg and Camp (1957) introduced few nutritional substrates for differentiating the Trichophyton species, as they are the predominant genus causing dermatophytosis throughout the world. The medium contains casein basal medium supplemented with thiamine, or inositol, or thiamine and inositol and nicotinic acid (Moore and Jaciow, 1979). These combinations are commercially available under the name Trichophyton agar (Difco).

Moreover, few specific tests such as urease test, in-vitro hair perforation test and pigment production on corn meal agar are available in differentiation of anthropophiles especially, the T. rubrum and T. mentagrophytes. Growth on polished rice grains is another specific test to differentiate M. audouinii from other dermatophyte species.
1. Urea hydrolysis

The fungal culture is inoculated into Christensen’s urea agar for the production of urease. The culture tubes are incubated at 25°C for 5 - 7 days. If the color of the medium turns to pink, then it is urease positive. The special test is performed to differentiate *T. rubrum* and *T. mentagrophytes*, where the latter dermatophyte species produces urease.

2. *In-vitro* hair perforation test

To a sterilized petridish, 10-12 ml of sterile distilled water is poured and enriched with 2 - 3 drops of 10 % yeast extract broth. Three or four small strands of autoclaved pre-pubertal hair are taken and the fungal culture is inoculated along the hair shaft. The lid is closed and incubated at room temperature for 21 days. After sufficient incubation, using sterilized forceps; single strand is picked from the culture plate and placed on few drops of LPCB on a sterile glass slide. The glass slide is gently heated over the bunsen flame. The culture is observed under low and high power magnification of the microscope for the presence of wedge shaped hair perforation.

3. Pigment production on corn meal agar

Corn meal agar supplemented with 1% dextrose is prepared and the fungal culture is inoculated at the center of the petridish. The culture plates are incubated at 25°C for the presence of red pigmentation on the reverse. The specific test is
performed to differentiate *T. mentagrophytes* and *T. rubrum*, where *T. rubrum* produces deep red pigmentation.

4. **Growth on polished rice grains**

This test is specifically performed for the identification of *M. audouinii* where the fungus grows slowly on the rice grains and a brown discoloration is observed on the rice, whereas the other dermatophytes show typical appearance of the colony morphology.

3.8.5 **Immunodiagnosis**

The trichophytin skin test and serological tests (immunodiffusion) are significant in diagnosis of dermatophytosis. The trichophytin is the dermatophyte antigen which is responsible for the delayed type hypersensitivity reaction. A galactomannan peptide is a constituent of dermatophyte antigen, of which the carbohydrate part is associated with immediate response, whereas the peptide part is related to immunity. When the patient lacks delayed type hypersensitivity or with an immediate response, the infection may lead to chronicity. The chronic dermatophytosis is often referred with respect to duration and recurrence of the dermatophytic infections.

3.9 **MOLECULAR BIOLOGY**

Identification of dermatophyte species by phenotypic methods is complicated as on sub-culture they produce different morphological variations among isolates and pleomorphism. Since phenotypic methods have some known drawbacks, molecular
techniques are emerging. Moreover, they are dependent on the genetic makeup which would be more accurate than the conventional identification.

Earlier, a variety of chemotaxonomic methods have been developed to skip the conventional methods in identification of dermatophyte species. The classification was based on the biological compounds such as proteins, nucleic acids, amino acids and peptides present among the dermatophytes. These include zone electrophoresis of culture filtrate proteins, studies on fatty acids by pyrolysis-gas liquid chromatography, total cell protein extracts in polyacrylamide gel electrophoresis and zymogram types and isoelectric focusing of somatic extracts by thin layer polyacrylamide gels (Weitzman and Summerbell, 1995).

3.9.1 Current molecular trends in identification of dermatophytes

3.9.1.1 Polymerase chain reaction (PCR)

Kary Mullis invented Polymerase chain reaction (PCR) in 1983 and awarded Nobel Prize in Chemistry along with Michael Smith (co-worker). PCR is a technology used in molecular biology to amplify a single copy or few copies of the DNA, replicating hundreds to millions copies of the target DNA sequence. The PCR is carried out in a thermal cycler.

The PCR reaction components are

i. DNA template (contains single copy or few copies of the targeted DNA to amplify)
ii. Two primers (complementary to the 3’ end of each sense and antisense strand of the target DNA sequence

iii. Taq polymerase (heat stable DNA polymerase) is an enzyme isolated from the bacterium *Thermus aquaticus* which works on optimum temperature at around 72°C

iv. Deoxynucleoside triphosphates (nucleotides with triphosphates) are the building blocks to synthesize a new strand of DNA

v. Buffers (provide optimum setting for the reaction activity of the Taq enzyme

vi. Ions (potassium ions and Mg$^{2+}$ ions) are used for DNA amplification.

There are several steps in PCR, where the denaturation, annealing and extension are the regular repeated steps called cycles, which undergo a series of 30 - 40 cycles in a PCR reaction, which is discussed briefly.

1. **Initial denaturation**: The DNA is heated to a temperature of 94 - 96°C for 5 - 10 min, where the hydrogen bonds between the double stranded DNA breaks and creates single stranded DNA.

2. **Denaturation**: The DNA is heated to 95°C for 30 sec – 1 min. This is the first regular cycle step in PCR.

3. **Primer annealing**: The reaction mixture is cooled to a temperature between 45 - 60°C for 30 sec – 1 min. This is the second regular cycle step in PCR, where the primers bind to their complementary sequence in the template DNA.
4. **Extension:** The reaction mixture is heated to $72^0$C for 1 min. This is the third regular cycle step in PCR, where the Taq polymerase extends the primer by adding nucleotides in a sequential manner to form a new strand of DNA.

5. **Final elongation:** The reaction mixture is heated to $72^0$C for 5 – 10 min after the last regular cycle in PCR, to guarantee that the single stranded DNA is extended completely.

6. **Final hold:** The reaction mixture can be held at $4^0$C for short term storage.

### 3.9.1.2 Variants of PCR

The restriction patterns of the mitochondrial DNA (mtDNA) of 22 isolates of *T. interdigitale* (*T. mentagrophytes* var. *interdigitale*) using Hae III, Msp I and Hind III were analyzed and the findings were compared with the three telemorphic species which are *Arthroderma simii*, *Arthroderma benhamiae* and *Arthroderma vanbreuseghemii*. They confirmed that the *T. interdigitale* is closely related to *Arthroderma vanbreuseghemii* (Mochizuki *et al.*, 1990). The restriction types of the mitochondrial DNA of 10 dermatophyte (telemorphs) species - *A. benhamiae*, *A. insingulare*, *A. quadrifidum*, *A. simii*, *A. vanbreuseghemii*, *N. fulva*, *N. grubyia*, *N. gypsea*, *N. incurvata* and *N. otae* were studied. They concluded that the *Arthroderma* and *Nannizzia* were identical (Kawasaki *et al.*, 1992).

Arbitrarily primed PCR (AP-PCR) was developed for identification of clinically important pathogens, which differentiated *T. rubrum*, *T. mentagrophytes* complex and *T. tonsurans* with unique band patterns (Liu *et al.*, 1996). PCR fingerprinting was
performed with non-specific primers (AC)10, (GTG)5, M13 core sequence, AP3 were utilized in differentiating the dermatophyte species. They reported that using these primers it was possible to identify the *T. mentagrophytes* variants but unable to detect the intra-species of *T. tonsurans* (Gräser et al., 1998).

Restriction enzyme analysis with number of endonucleases and hybridization with poly (dG - dT) were performed which produced minor differences in identification of dermatophyte species. To overcome these limitations, Random amplified polymorphic DNA (RAPD) based PCR was optimized, which resulted in identification of *T. mentagrophytes* variants, but not the *T. rubrum* species (Howell et al., 1999). RAPD analysis was performed which targeted the non-transcribed spacer (NTS) region of rDNA, resulting in molecular typing of *T. rubrum* isolates (Baeza and Giannini, 2004). Repetitive sequence based PCR (rep-PCR) using DiversiLab system was developed for the identification of dermatophytes. This method was considered to be more rapid within 24 hours in identification of dermatophyte from pure culture (Pounder et al., 2005).

A nested PCR specific to DNA topoisomerase II genes based RFLP in differentiating the dermatophyte species, resulted in identification of dermatophytes upto species level whereas *T. mentagrophytes* var. *interdigitale* and *T. mentagrophytes* var. *quinckeianum* were identical (Kanbe et al., 2003). Pan dermatophyte nested PCR using chitin synthase - I gene was developed in the identification of the etiological agents of onychomycosis (Garg et al., 2007). Semi-nested PCR using pan fungal primers (NS5, ITS 1, ITS 4) based restriction fragment length polymorphism (RFLP) with BciT130 I,
Dde I was performed for identification of dermatophytes, which resulted in 100% coincidence with the culture procedures (Yang et al., 2008).

PCR reverse line blot was considered as a rapid, specific identification tool in detection of dermatophytes directly from the skin, hair and nail specimens (Bergmans et al., 2008). Single strand conformation polymorphism (SSCP) based PCR was developed with the partial chitin synthase - I gene as a biomarker to study the ecology, epidemiology, dermatophytes genetics and most importantly in diagnostic/analytical mutation scanning mechanism (Cafarchia et al., 2009).

The dermatophyte species can be identified instantly from the most distinguished character – macroconidia, where many dermatophyte species produce rarely. They are - Microsporum audouinii, M. ferrugineum, T. concentricum, T. schoenleinii, T. verrucosum and T. violaceum. Along with these species, 29 isolates of T. rubrum, T. mentagrophytes, T. tonsurans, M. canis and T. rubrum var. raubitschekii were analyzed by real time PCR which resulted in 100% sensitivity for the identification, particularly the rare macroconidia forming dermatophyte species (Yüksel and Ilkit, 2012).

3.9.2 Upcoming technology in routine laboratory settings

MALDI-TOF MS is a technology that bypasses the pre-processing steps and directly detects the molecular weight of the proteins from cultures and thus identification of organisms is made simple. MALDI play an important tool in identification of dermatophytes particularly, has they show morphological variations among isolates. In next few years, in microbiology settings, MALDI-TOF would be established as a standard method in identification of clinically important pathogens and start the
appropriate treatment at the earliest. It was noted that the anthropophilic and the zoophilic strains of *T. interdigitale* were unidentified using MALDI (Nenoff *et al.*, 2013).

### 3.9.3 Advances in molecular aspects

Genetic advances have been reported currently which includes targeted gene inactivation, gene silencing and transcriptional profiling methods (Grumbt *et al.*, 2011). Recently, seven clinically important dermatophyte genomes are sequenced completely to analyze the future outbreaks in relation to the biology virulence, pathogenicity and host specificity. Martinez *et al.*, 2012 sequenced five dermatophyte genomes that included *Trichophyton rubrum* an anthropophile which is the commonest species throughout the world, *Trichophyton tonsurans* is found to cause significant scalp infection among children in California (Mirmirani and Tucker, 2013), *Trichophyton equinum* zoophiles causing scalp infections in Germany and the causative agent is associated with dermatophytic infection in horses (Brasch *et al.*, 2008), *Microsporum canis* a zoophile which is the predominant etiological agent causing foot mycosis and onychomycosis in Europe and *Microsporum gypseum* a geophile which is comparatively less frequently isolated throughout the world. Burmester *et al.*, 2011 sequenced the other two dermatophyte genomes which are *Arthroderma benhamiae* (a teleomorph of *Trichophyton mentagrophytes*) which is the principal cause of dermatophytosis next to *T. rubrum* and *Trichophyton verrucosum*, a zoophile which is a predominant pathogen isolated from tinea capitis and tinea unguium clinical pattern.
3.10 VIRULENCE FACTORS OF DERMATOPHYTES

Dermatophytes produce a variety of virulence factors that include both the enzymes and non-enzymes.

3.10.1 Virulence enzymes

Dermatophytes adhere to the keratinized cells of the host such as skin, hair and nails and penetrate the stratum corneum of the epidermis to obtain nutrients as growth substrate. Dermatophytes produce a number of virulence enzymes such as keratinase, protease, phospholipase, lipase and elastase upon different substrate specificities and experimented in-vitro (Muhsin et al., 1997).

The keratinase is produced by bacteria (Bacillus species) (Lakshmi et al., 2012; Kainoor and Naik, 2010), Streptomyces (Syed et al., 2009) and fungi (dermatophytes) (Sharma and Sharma, 2012). Dermatophytes are known to colonize the outer layer of the epidermis, which is rich in keratinous material such as skin, hair and nails. Therefore, the keratinase is the major virulence enzyme produced during infection. The keratinase is produced only in the presence of keratin containing substrate. The keratin is the key structural component of human skin, hair and nails. In addition, the keratin is rich in hair, horns, nails, claws and hooves of mammals, scales and claws of reptiles, feathers, beaks, claws of birds. The keratin contains high disulfide bonds with cysteine groups which gives stability to the protein. The dermatophytes mainly attack the disulfide (d-d-) bond of the keratin substrate and produce keratinase.
Besides keratinase, protease is one of the major virulence enzymes secreted by dermatophytes. The protease is produced when the dermatophytes are cultured in a medium enriched with protein substrate as a sole nitrogen source. The protease secreted by dermatophytes is classified into - endoprotease which cleaves the peptide bonds within a polypeptide and exoprotease which cleaves the peptide bonds particularly at the amino or carboxy terminal site of the polypeptides. The serine protease and metalloprotease are the endoproteases, while aminopeptidase, carboxypeptidase and dipeptidyl-peptidase are the exoproteases produced by dermatophytes (Chinnapun, 2015). *T. rubrum* when cultured on soy protein enriched medium and keratin containing growth medium they produced two leucine aminopeptidases (Lap) (ruLap1 and ruLap2) and two dipeptidyl-peptidases (Dpp) (ruDppIV and ruDppV). The Lap1 and Lap2 are metallopeptidases, while DppIV and DppV are the glycoproteins. Since dermatophytes are keratinophilic, the exo and endoproteases are engaged together on dermatophyte virulence (Monod et al., 2005). Burmester et al., 2011 reported that the completed genome sequences are rich in protease encoding genes, similar to the non-dermatophytes causing dermatophytosis.

The phospholipase activity of dermatophytes is observed upon hydrolysis of phospholipids into fatty acids and lipophilic substances. The phospholipase are classified into four classes – A, B, C, D, among them A class is divided into A1 and A2. Chopra et al., 1981 reported that the phospholipase A activity is high in *T. mentagrophytes* than *M. cookie* and *E. floccosum* and particularly A1 is high in *E. floccosum* and A2 in *M. cookie* whereas both A1 and A2 were equally observed in *T. mentagrophytes*. 
The completed dermatophyte genome sequences are made available to the public, to utilize the opportunity using Bioinformatics tool to identify the virulence genes responsible for their activity and analyze the key components of the dermatophyte species to adapt the selective ecological groups (Achterman and White, 2012).

3.10.2 Non-enzymes virulence factors

*T. rubrum* produces a mycotoxin called xanthomegnin, which is known to be produced by food-borne *Penicillium* and *Aspergillus in-vitro* and *in-vivo* causing nephropathy and death in animals. The xanthomegnin is the major substance that gives red pigmentation on reverse of the *T. rubrum* culture and it was observed in infected skin and nail specimens. The xanthomegnin level is varied among the clinical samples (Gupta et al., 2000). Schaufuss and Steller, 2003 reported that the few dermatophyte species such as *T. rubrum* and *T. equinum* produce complete zone of hemolysis around the colony followed by an incomplete zone of hemolysis, whereas *T. mentagrophytes* complex and *T. verrucosum* produce a zone of complete hemolysis. Thus hemolysis may also act as a virulence marker for dermatophytes.

Recently, Youngchim *et al.*, 2011 reported that dermatophyte species such as *T. rubrum*, *T. mentagrophytes*, *E. floccosum* and *M. gypseum* were known to produce melanin or melanin like compounds *in-vitro* and *in-vivo* and play similar role in the pathogenesis of other pathogenic fungi. Since the dermatophyte genome sequence are available, the sequence identity related to the virulence factors can be analyzed genetically and then experimentally confirm the clinical significance during infection (Achterman and White, 2012).
3.11 TREATMENT AND PROPHYLAXIS

Azole derivatives such as Whitfield’s topical ointment or tolnaftate are used for all types of tinea infections. These are applied twice daily at the site of infection until the lesions completely resolve. The orally active griseofulvin antifungal drug is generally effective against nail and scalp infections. In case of chronic dermatophytosis, the treatment has to be continued for three to six months. Itraconazole, fluconazole and terbinafine are effective against onychomycosis. The clinical course of treatment is about 6 weeks for skin infections, 12 weeks for scalp infections and 12 months for nail infections. The dermatophytosis is said to be recurrent superficial fungal infections, therefore the antifungals are to be continued for another one week to prevent recurrence.

Live spore vaccine, killed hyphal cell wall vaccine and soluble cytoplasmic extract vaccine of *T. mentagrophytes* var. *erinacei* has been experimented in guinea pig *in-vivo*. Among these vaccines, live spore vaccine are found to be effective for dermatophytosis (Chander, 2009).

3.12 FUTURE PROSPECTS

Dermatophytosis remains a major public health problem in both the developed and developing countries worldwide. The completed sequences of the most important pathogenic dermatophytes are available in the public website, which can be utilized in future for molecular identification of specific genes responsible for the virulence activity of dermatophytes. Despite the fact that a variety of dermatophyte species prevalent worldwide, based on some molecular characterization, few dermatophyte species were considered to be grouped under their complexes such as *T. rubrum* complex, *T.*
mentagrophytes complex. Therefore, analyzing the completed sequences genetically, will help in understanding the exact differential morphological characters among the dermatophyte species and their associated genes which can be studied and also analyze the future outbreaks in relation to the biology, virulence, pathogenicity and host specificity.