DISSERTATION ON

A STUDY ON THE PREVALENCE OF

DERMATOPHYTOSIS AND RAPID IDENTIFICATION

OF DERMATOPHYTES IN A TERTIARY CARE HOSPITAL

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Certificate

*Certified that the dissertation entitled "**A STUDY ON THE PREVALENCE OF DERMATOPHYTOSIS AND RAPID IDENTIFICATION OF DERMATOPHYTES IN A TERTIARY CARE HOSPITAL**" is a bonafide work done by **Dr.K.HEMALATHA**, postgraduate Institute of Microbiology, Madras Medical College Chennai, under my guidance and supervision in partial fulfillment of the regulation of The Tamil Nadu Dr. M.G.R. Medical University for the award of M.D. Degree, Branch-4 (Microbiology) during the academic period of August 2003 to September 2006.*

Dr. KALAVATHY PONNIRAIVAN B.Sc., M.D. (Bio)

DEAN
Madras Medical College.
Chennai-600 003.

Prof. A.LALITHA M.D., D.C.P.

Director & Professor
Institute of Microbiology,
Madras Medical College,
Chennai-600 003.

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CONTENTS

INTRODUCTION

Fungal infections are very common in man. Dermatophytosis are the most common types of cutaneous fungal infection seen in man affecting skin, hair, and nail in both developed and developing countries due to advent of immunosuppressive drugs and diseases[84]. Hot and humid climate in the tropical and subtropical countries like India makes dermatophytosis or ringworm as very common superficial fungal infection [84].

The dermatophytes are keratinophilic fungus, which causes dermatophytosis by virtue of their unique ability to degrade keratin and thus colonize and invade the skin and its appendages[89]. The infections caused by dermatophytes are clinically classified on the basis of the location of the lesion on the body. The name 'ringworm' was based on the worm like appearance of lesion with irregular inflammatory border with some clearing of central area of the lesion. The infection are named according to the body site after the Latin word *Tinea* [89]

Dermatophytes are generally classified as anthropophilic, zoophilic and geophilic based on their ecology and host preference[89, 32, 37]. Of which anthropophilic group causes chronic infection, which is difficult to treat[32]. Geophilic fungi such as *M.gypseum* are usually transmitted from soil source and secondarily transmitted by animal. Human infection caused by *M.canis* is an important zoophile can involve variety of animal host, but the principle carrier is cat and dog[88]. *T.verrucosum, T.mentagrophytes* are encountered in rural area and it is mostly through the cattle[37].

The clinical variation of dermatophytes may resemble some other skin diseases such as pityriasis roseae, eczema, lichen planus[37]. Contact dermatitis may resemble like T.corporis[37]. An understanding of predisposing / aggravating factors can give an idea as how to avert the disease. The Pathogenic potential is dependent on variety of local and systemic factors affecting host resistance to dermatophyte infection. Depression of cellular immunity due to various factors such as malignancy, administration of steroids or immunosuppressive drugs, endocrine disorders such as cushing's disease can lead to atypical generalised invasive dermatophyte infection. Early identification and treatment is essential as once infection is established, the individual become carrier and more susceptible to recurrence[89].

Any clinical diagnosis need to be supported by laboratory diagnosis. Culture is a necessary adjunct to direct microscopic examination for definitive identification of etiological agent and in many instances the choice of therapy may be depend upon the specific identification of invasive mould[89]. This is especially important in nail and skin infection, often caused by non-dermatophytic filamentous fungi, which are often resistant to usual dosage of the therapy, used for dermatophytic infection[89]. Identification of fungal hyphae in the macerated skin of the web of toes may be difficult due to superadded bacterial infection. Before starting treatment for dermatophytoses, it is essential to establish the diagnosis of the disease, so that specific therapeutic modalities can be monitored during the course of the treatment.[32]

Rapid identification of dermatophyte species and knowledge of their host preference and ecology play an important role in epidemiology, public health issue and infection control[89]. The varied clinical presentation of tinea, which results in delay in diagnosis, poor compliance in follow up of cases, and consequently spread of infection in the community had rekindled interest in rapid diagnosis method in identification of species[89].

Antifungal susceptibility testing is receiving increased attention with the advent of newer antifungal drugs[53]. Antifungal resistance is important whenever the treatment failure occurs, and need to establish the sensitivity of the causal organism. In such cases antifungal drugs are ideally given on the basis of in vitro sensitivity of the isolates[68]. Choosing the best therapy for each patient has become very important now a days and the day is not so far off when clinicians would expect susceptibility testing for guiding the selection of appropriate drugs.

AIM OF THE STUDY

- To isolate and identify the Dermatophyte species from clinically diagnosed cases of dermatophytosis patients attending the out patient clinic of the Department of Dermatology, Government General Hospital. Chennai.

- To evaluate the incidence of dermatophytosis in the immediate environment and to characterize the dermatophytes isolates from different categeries of patients.

- To compare the sensitivity and specificity of Calcofluor white Staining with 10% KOH wet mount in direct microscopic examination.

- To study the effectiveness and rapid identification using Dermatophyte Identification Medium (DIM) with Sabouraud's Dextrose Medium with antibiotics and cycloheximide in isolation of dermatophytes.

- To perform Antifungal susceptibility testing to find out Minimum Inhibitory Concentration for chronic dermatophyte isolates by Agar Dilution Method using Yeast Nitrogen Dextrose Agar with Fluconzole and Griseofulvin.

REVIEW OF LITERATURE

Historically Agostino Bassi (1835-67) was the first to elucidate the microbial nature of deadly disease of silkworms (*Bombyx mori*). Through meticulous studies and animal experiments carried out over a period of 25 years he established that a mould, now known as *Beauveria bassiana*, was the cause of a devastating disease of the silkworm[89].

The discovery of that fungus could cause a dermatophytosis began when *Robert Remak* observed unusual microscopic structure from favic lesion that he had not recognized as being fungal. Although Remak has priority for the discovery of the first fungus causing human disease, the real founder of medical mycology, was the Parisian Physician, *David Gruby*. Gruby described the clinical and microscopic features of causal agent of *favus* and established the contagious nature of the disease. He also described *Ectothrix* invasion of hair of beard and scalp and named the agent *Microsporum audouni* (1843). Healso described the fungus causing *Endothrix* invasion of hair as *Herpes* (*Trichophyton) tonsurans* (1844).

In the early 1890s, *Raymond Sabouraud*, a French Dermatalogist, Father of Medical Mycology established 'plurality' of ringworm fungi, integrated the mycological and clinical aspects of ringworm. Sabouraud wrote and published his monumental "*Les Teignes*" in 1910. Sabouraud classified dermatophytes into 4 genera Achorion, Epidermophytan, Microsporum, Trichophytan based on the clinical aspects of the disease that they caused, combined with their cultural and microscopic morphology[89].

Emmon modernized the taxonomic scheme of Sabourand, based on highly variable characters such as colony texture, chlamydoconidia, nodular organs, pigmentation, racquet and spiral hyphae he described three anamorphic genera Epidermophytes, Microsporum, and Trichophytes[89].The discovery of teleomorphs (sexual or perfect state) of *T. ajelloe, T. terrestre, M.gypseum, M. manum* (Dawson and gentles 1961) using hair bait technique is led to rapid discoveries of several other telomorphs of several other dermatophyte species and related keratinophillic fungus[89].

The present standard is the modification by *Conant*[37]

I. Trichophyton Malmsten 1845.

Gypseum Group (T.mentagrophytes), *Rubrum Group*(*T. rubrum*). *Crateriform Group (T.tonsurans), Faviform Group (T.schoenleini, T.concentricum*, *T.violaceum, T.verrucosum), Rosaceum group (T. megninii, T. gallenae).*

II. *Microsporum Gruby 1843 -* *M.audouinii, M.canis, M. gypseum*

III. *Epidermophyton Sabouraud 1910 -* *E. floccosum*

EPIDEMIOLOGY:

The prevalence of dermatophytosis is governed by environmental conditions, personal hygiene and individual's susceptibility from place to place[32].The isolation of different species of dermatophytes also varies markedly from one ecological niche to another depending on their primary natural habitat. It is possible that dermatophytosis of some sites, like genitalia is underestimated because of its common and self-healing

nature. As dermatophytosis is prevalent throughout the world it primarily depends on the habits and living conditions of the people as infection is transmitted through fomites[32]. The arthrospores are parasitic propagules and survive in the environment for long time. Some species of the dermatophytes are endemic in certain parts of the world and have a limited geographic distribution. The most common etiologic agents of dermatophytosis in the western countries are *Trichophyton rubrum and Microsporum canis.* In southern and East European countries, the anthropophilic fungi have been replaced by zoonotic species such as *M.canis* and *T.mentagrophytes*. In India *T.rubrum* is the commonest etiological agent for dermatophytosis[32]

SOURCE OF INFECTION

Dermatophyte infection of wild and domestic animal have been recognized for many years. Zoophilic species have gradually evolved from soil to parasitize animals. Animal act as a reservoir of human infection, that is particularly in rural area. Fungi from domestic animal such as dog, cat may initiate an epidemic among children. Human infection are acquired either by direct contact with an infected animal or indirectly by contact with fomites or other inanimate objects associated with keratinous material from animal. Human infection *M.canis* an important zoophile, i.e is usually acquired from cats and dogs[89].

Anthropophilic dermatophytic species are considered to have evolved from zoophilic species (Chemel 1980, Rippon 1988).Human are normal host for their group of species and transmission may occur by direct contact or indirectly by fomites (Weitzman and summer bell 1995). Human to animal transmission of infection by

anthropophilic species is rare, but has been documented in the literature (Kaplan and Gump 1958, Mayer 1989)[89.]

Geophilic species are considered ancestral to the pathogenic dermatophyte (Chemel 1980, Ozegoric 1980, Rippon 1988) The natural habitat of these species in the soil, often associated with the decomposing keratinous material. e.g. hair, feathers, horns, hooves, nails. etc. Exposure to soil is the main source of infection for human and lower animal. Transmission of geophilic species from lower animals to human, or from human-to-human is rare[89].

Classification of Dermatophyte based on ecology and host preference[89]

Geophilic	**Zoophilic**	**Anthropophilic**
M.cookei	*M.canis*	*E.floccosum*
M.gypseum	*M.equinum*	*M.audouinii*
M.nanum	*M.gallinae*	*T.schoenleinii*
M.persicolar	*T.equinum*	*T.mentagrophytes*
M.praecox	*T.mentagrophytes*	*T.rubrum*
T.ajelloi	*T.verrucosum*	*T.tonsurans*
T.simii	*T.simii*	*T.violaceum*

ETIOLOGY

The dermatophytes are hyaline septate molds with more than hundreds species described. Forty-two species are considered valid and less than half are associated with human diseases. These are divided into three main anamorphic genera depending on their morphological characteristics[32]. The anamorph (asexual conidia or imperfect state) of belong to three genera.*Trichophyton, Microsporum, Epidermophyton*[37, 32, 89].

*Epidermophyton Sabouraud (1907)*This genus is characterized by numerous broadly clavate smooth walled macroconidia. Microconidia absent. *E.floccosum* is the only one pathogenic species that attack skin, nail very rarely hair[89].

Microsporum Gruby (1843) Members of this genus produce macroconidia and microconidia. The essential distinguishing feature is the presence of macroconidia that have rough walls ranging from spiny to warty, the shape which vary from egg-shaped to cylindo fusiform. They may have thin to thick cell wall and 1-15 septa depending upon the species. Microconidia are typically clavate (club shaped). Members of this genus attack skin and hair but not nails[89].

Trichophyton Malmsten (1845) Members of this genus produce smooth walled macroconidia and microconidia. The macroconidia may range in shape from elongate to pencil shaped, clavate fusiform to cylindrofusiform, multiseptate, may be thick and thin walled. Microconidia are usually produced in greater abundance than macroconidia along the hyphae singly or clusteras and are sessile or borne on short stalks. Members of this genus attack skin, hair and nail[89].

PATHOGENESIS

The dermatophyte grow only within dead, keratinized tissue i.e the ability of dermatophytes to invade and parasitize the cornified tissues is closely associated with, and dependent upon the utilization of keratin. Keratin is a highly insoluable scleroprotein[32, 37]. The fungal cell produce keratinolytic proteases in *vivo and vitro,* which provide means of entry into living cells. Fungal metabolic products diffuse through the malphigian layer to cause erythema, vesicles and even pustules formation along with pruritis. The hyphae become old, break into arthrospores, which are shed

off. This is partially responsible for the central clearing of the ringworm lesions.Their in vivo activity is restricted to the zone of differentiation, newly differentiated keratin and for infection to persist, the hyphal growth must keep pace with the rate of keratin production.The hyphal tips growing down within the shaft reach to the edges of living keratinizing cells and form Adamson's firinge[32].

COMPLICATION

Chronic dermatophytosis: It is a refractory condition, which runs a course of more than one year with episodes of exacerbations and remissions. Factors responsible for chronicity are the site of infection, poor penetration of the drug in the nail keratin, and drug resistance[59]. Some associated conditions are atopic diathesis, disorders of keratinization, diabetes mellitus, Cushing's syndrome, immunosuppression following renal transplants and AIDS[59, 89.] This work was undertaken to study the clinical and cultural characteristics of patients with chronic dermatophyte infection[59].

CLINICAL FEATURE

Clinical manifestations of dermatophytosis are called tinea or ringworm depending on the anatomical site involved. The clinical condition is Tinea capitis, Tinea corporis, Tinea cruris, Tinea unguium, Tinea pedis, Tinea barbae, Tinea manum[32, 89, 32, 68].

Tinea capitis: Tinea capitis is a dermatophyte infection of the scalp, eyebrows and eyebrushes caused by species of the genera *microsporum* and *Trichophyton*. It is characterized by the production of a scaly erythematous lesion and by alopecia that may produce severe inflammatory suppurative folliculitis with formation of deep

ulcerative Kerion eruption.The commonest types of ringworm are classed according to the site of formation of their arthrospores into *Endothrix* and *Ectothrix*[37].

Kerion: This is severely painful massive acute inflammatory reaction, producing raised circumscribed boggy mass on the scalp, usually suppuratin at multiple points. In severe forms pus oozes from the follicles. These infection are seen in rural area, and the organisms are usually acquired from animal-cattle being the common source. *Tinea profunda*: exaggerated inflammatory response on the glabrous skin and equivalent of kerion of the scalp[37.]

Favus (honeycomb ring worm)**:** Favus is characterised by occurrence of dense masses of mycelium and epithelial debris, which form yellowish cup-shaped crust called scutula. The scutulum develop in hair follicles with the hair shaft in the centre of the raised lesion. Removal of these crust reveal an oozing moist red base. *T. schoenleinii, T.violaceum, M.gypseum* were the causative organisms[37].

*Ectothrix:*Ectothrix is a condition in which the arthrospores i.e Fragmentation of the mycelium into spores appear as a mosaic sheath around the hair or as chains on the surface of the hair shaft.The cuticle of the hair remains intact. *M.audouinii , M.canis, M.gypseum, T.mentagrophytes*, *T.verrucosum, T.rubrum* were the species that causes Ectothrix[32].

Endothrix: Endothrix is a condition, in which the hyphae form arthrospores within the hair shaft, which is severly weakened. The arthospores are observed in chains filling inside shortened hair stubs. *T. tonsurans T. violaceum, T. soundanense*

were the species that causes endothrix. *T.rubrum* is rarely involved in tinea capitis, but cause both endothrix and ectothrix sporulation[32].

Tinea corporis (Ringworm of body, Tinea circinata, Tinea glabrosa)

Tinea corporis is a dermatophyte infection of the glabrous skin. The infection generally restricted to the stratum corneum of the epidermis. The clinical symptoms are result of the fungal metabolites, acting as toxin and allergens.All the species of dermatophyte are able to produce lesion of the glabrous skin. Most universally encountered species are *T.rubrum, and T.mentagrophyte***.** The types of lesion are Dry scaly annular and Vesicular form[37].

Dry scaly lesion: (annular patches) This lesion begin as a small spreading elevated area of inflammation. The margin remains red and sometime slightly swollen, while the central area become covered with small scales and spontaneous healing occurs at the centre as the circulate margin advances. *T.rubrum, E.floccosum* are the organisms involved[37].

Vesicular form (Iris form): In this condition vesicle appears regularly or immediately behind the advancing hyperemic and elevated margin. A crust is formed, their healing follow in this centre of the lesion to leave more or less pigmented area. *T.Mentagrophytes, T.verrucosum* are the organism involved. Variation of the above two types of clinical lesion are psoriasiform lesion, Plaque type lesion, granulomatous lesion[37], which are caused by *T.rubrum* and verrucous form which is caused by *E.floccosum* .*Tinea imbricata* which is a restricted form of tinea corporis caused by *T.concentricum* characterized by polycyclic concentrically arranged rings of papulosquamous patches of scale. [37]

Tinea Cruris : *(Dhobie's itch)*

This condition is a Dermatophyte infection of the groin, perineum, perianal region which is generally severely pruritic. The lesion is sharply demarcated with a raised erythematous margin and their dry epidermal scaling. It tends to occur when conditions of high humidity lead to maceration of the crural region.*T.rubrum* appears to be the predominate species involved**.** *E.floccosum, T.mentagrophytes* may also cause this type of lesion.

In most instances the infection begin on the thigh where it is incontact with the scrotum and spread rapidly. The disease involve inner thigh and spreads downward further on the left because of lower extension of the scrotum that side. *E.floccosum* rarely extend further**.***T.rubrum* infection, the lesion frequently extend over the body particularly waist, gluteal region, thigh. *T.mentagrophyte* may rapidly involve, chest, back, leg, feet, causes severe incapacitating inflammatory disease.In acute lesion there is erythema and intense itching.Older lesions are often lichenified, leathery, plaque like.

Perspiration, humidity, irritation from clothes, maceration of crural skin increases the susceptibility to infection.Disease like Diabetes, neurodermatitis, leucorrhoea, friction from skin fold in obese person are predisposing factors.[32, 37, 68, 89]

Tinea unguium: Tinea unguium is an infection of the nail plates by dermatophytes. This disease is differentiated from onychomycosis which is an infection of nail by non dermatophytic fungi and yeast.Two types of lesion are encountered.Leukonychia mycotica in which, the invasion is restricted to patches or pits on the surface of the nail. The most common isolate is *T.mentagrophytes* and the

invasive type in which lateral or distal edges of the nail are first involved followed by establishment of infection beneath the nail plate. *T.rubrum* is the isolate commonly involved.Tinea unguium with tinea corporis & tinea pedis occurs most commonly with *T.rubrum*, *T.mentagrophyte*, *E.floccosum* and with tinea unguium with tinea capitis *T.tonsurans* is the predominent isolate involved.[32, 37, 89]

Tinea barbae: Tinea barbae which is a dermatophyte infection of the bearded area of the face and neck, and therefore restricted to adult males. *T.mentagrophytes, T.verrucosum* are the isolates commonly involved. The organism are usually acquired from animals. *T.rubrum*, is an infrequent cause of tinea barbae and may represent infection acquired from other part of the body and or transmitted as "barbers itch" from unsanitary barbering practices.[32, 37, 89]

Tinea manum: Tinea manum, which is a dermatophyte infection of the hand, particularly dorsal aspect. Tinea manum refers to that infection where the interdigital area and palmar surface are involved. *T.rubrum, T.mentagrophyte, E.floccosum* are the organisms involved. Tinea manum always associated with tinea pedis.[32, 37, 89]

Tinea pedis: Taenia pedis, which is a dermatophyte infection of the feet involving toe webs and soles. The lesion is of several types varying from mild, chronic and scaling to an acute, exfoliative, pustular and bullous disease. *T.mentagrophyte, T.rubrum, E.floccosum* are the isolates involved.[32, 37, 89]

IMMUNOLOGY

The skin is the primary barrier or defense of the body against invasion by microorganism from the external environment. In dermatophyte infection, this

defense is not abrogated. A peculiar relationship exists between dermatophyte and its host that is unparallel by other microbial agents of disease. The organism is truly a dermato or ectophyte as it does not invade the living cell and its nutritional demands involve no depletion of metabolizable substances from the host. The immune and inflammatory response evoked is the living tissue beneath the site of infection or distal to it are incidental to the diseases[37].

A degree of acquired resistance to the disease has been observed in patients and experimental infection in animal. Clinical records indicate that in a large series of children treated for tinea capitis, none returned with second infection. A similar finding were seen in agricultural workers infected with *T.verrucosum*. These observations are interpreted to mean that there was increased resistance to reinfection as a result of the intital infection. On the other hand multiple episode of tinea pedis occur, and reinfection is common in patients with these diseases[37]. Hypersensitivity can be demonstrated by skin test using trichophytin. Intradermal injection of this substance elicits either delayed (tuberculin) or immediate (urticarial) response. The latter may be passively transferred and is associated with reagenic antibody tentatively identified as an IgE. Purification and chemical analysis of trichophytin shows that it is a galactomannan peptide. Degradation study indicates that immediate reaction is associated with the carbohydrate fraction and delayed reactivity with peptide moiety[37]. The delayed hypersensitivity reaction is found in either experimental or natural infection by *T.mentagrophytes* and several other dermatophytes[37]. Patients with atopy are particularly prone to chronic infection[37]. Trichophytin as presently prepared is not species specific and is common to all dermatophyte from which it is derived its relative antegenicity is influenced by the media in which fungi is grown.

Positive skin test to trichophytin have been elicited in patients with penicillin hypersensitivity and those with cutaneous tuberculosis. The demonstration of delayed or immediate type hypersensitivity to intradermal injection of trichophytin appear to be limited diagnostic and prognostic value[37].

Dermatophytid or "id" reaction are secondary eruption occurring in sensitized patients as result of circulation of allergenic products from primary site of infection. The condition resembled lichen scrofulosorum. It is commonly associated with tinea capitis in children[37].There are two major dermatophyte antigen, glycopeptides and keratinase. The protein portion of the glycopeptide preferentially stimulates cell–mediate immunity, whereas polysaccharide portion preferebly stimulates humoral immunity (Dahl. MV.1993). Keratinases produced by dermatophytes, when injected intradermally into skin of animals elicited delayed type hypersensitivity responses (Grappel SF et al 1972) CMI is the major immunologic defense in clearing dermatophyte infection. Approximately 80-93 % of chronic recurrent dermatophytic infection are estimated to be caused by *T.rubrum*, after these patients fail to express delayed type hypersensivity reaction to trichophytin when injected intradermally (Hay R.J. 1982). Several Trichophytin allergen have been identified based on elicitation of IgE, antibody mediated immediate hypersensivity responses. Evidence of an etiological role for Trichophytin in asthma patients and in some subjects with immmediate hypersensitivity and chronic dermatophytosis is provided by bronchial reactivity to trichophytin. Improvement of asthma after systematic antifungal treatment corroborates this link. Unique features of trichophytin allergens is its ability to elicit delayed type hypersensitivity in individuals who lack immediate hypersensitivity reactivity. The amino acid sequence identity of trichophytin allergens

with diverse enzyme families support a dual role for these protein in fungal pathogenesis and allergic disease[95].

Exoenzyme produced by common dermatophytes, in addition to their ability to cause cutaneous inflammation are thought to contribute to fungal spread. Dermatophytes in all protein media produced high level of alkaline phosphatase, esterases and leucine arylamidase, Brash J. Zaldua M. etall in 1994 concluded that alkaline phosphatases, esterase, leucine arylamidases are important for the parasitic growth of dermatophytes. Enzyme measurement may be helpful for the species identification[9].

Although there are no serological kits commercially available to specifically detect and identify antibodies to dermatophytes, studies of dermatophytes antigen by monoclonal antibodies indicate a potential use of such reagent in the immuno identification of dermatophytes. Occlusion of the infected site appears to increase susceptibility to experimental infections because it increases hydration of the underlying skin and emission of CO_2, helping dermatophyte growth (King et al 1978)[89.] Medical condition such as collagen vascular diseases patients receiving systemic corticosteroid therapy, cushing's disease diabetes mellitus, hematological malignancy atopy, old age and bronchial asthma play a significant role in predisposing patients to chromic dermatophyte infection. Although a host develops a vareity of antibodies as a response to dermatophyte infection namely IgM, IgG, IgA, IgE, it has been accepted IgE plays a role in the suppression of cell mediated immunity[89].

LABORATORY DIAGNOSIS:

The diagnosis of dermatophytosis is based on a combination of clinical observation supplemented by laboratory investigations.

The history of the patient is essential regarding the age, occupation hobbies, living condition with onset duration and course of disease as well as intake of previous treatment.The clinical examination is done in a well-lit room. The physician should observe distribution and type of lesions, concurrent disease and constitutional symptoms of the patient[32].

In the laboratory, diagnosis depends on the demonstration of causative pathogen in tissues by microscopy, isolation of fungus in culture and the serological tests[32]. The skin scrapings should be taken from the active margin of lesion[32, 38, 89].

Wood's Lamp Examination: Wood's lamp is a device that is useful in the diagnosis of superficial cutaneous fungal infections[32]. Wood' s glass consists of barium silicate containing about 9 % nickel oxide. It transmits UV light with peak of 365 nm that shows a characteristic fluorescence. This florescence has been used to demonstrate hair infection mainly by Microsporum species which fluorescence Bright-green, where as Trichophyton fluorescence Dull green and rest of the dermatophytes are nonfluorescence under wood's lamp[32].

Direct microscopic examination: Microscopic examination of properly collected specimen is one of the rapid and effective method of detecting fungi infection[89]. This highly effective screening technique will provide useful information regarding the etiological agents such as Mould, yeast, ectothrix, endothrix, favic hair

invasion[89]. The solutions selected for examination of this specimen are *KOH (10-20%), 20% KOH with 3%DMSO[32, 89] & parker quink ink[83, 89]* for better enhancement of fungus and *calcofluor white[32]*.

Potassium hydroxide wet mount: It is the most widely used method for direct examination of clinical specimen for the presence of fungi. Several modification of 10% KOH. preparations have been made for more rapid detection. This includes incorporating *parker superchrome blue-black ink[89] in the KOH solution* for selective staining of the fungus (Rippon 1988). Modifications of the basic method include *addition of 3% Dimethyl sulpoxide (DMSO) to 20% KOH* to aid in the preparation and cleaning of the specimen without healing. (Rebell & Taplin 1941, Rippon 1988) or *addition of 5-10% glycerin to KOH preparation* (usually 10-25% for nails) *to delay crystallizations of the KOH[88]*, degrading of the fungus and rapid dehydration (Refill and Taplin 1974, weitzman and summerbell 1995). The slides are examined under the microscope with reduced light by lowering the condenser and adjusting the condenser's diaphragms. Fungal hyphae must be differentiated from variety of hyphal like artifacts such as cotton wool or synthetic fibres and from the socalled 'Mosaic Fungus'. The last artificat, which is more difficult to differentiate, consists of cholesterol, deposited around the peripery of the epidermal cell, abrupt change in width and presence of re-entrant angle in the flat crystalline structure and back of internal organelles. (Rippon 1988)[89].

Calcofluor white staining: Calcofluor White Stainng is whitening agent used in the textile and paper industry. It binds chitin and cellulose in fungal cellwall and fluoresces on excitation by longwave UV rays or shortwave visible light. This method

has however, the advantage of allowing easier fungal detection with less search time and technical experience (Elders and Roberts 1986). Calcofluor white can be combined with KOH for rapid clearance of specimen[32].

Savithri sharma et al 1998 from Hydrabad compared the sensitivity and specificity of KOH and CFW staining is Corneal scraping examination for diagnosis of mycotic keratitis. In this study specificity of KOH and CFW were identical (83.8) while the sensitivity were 81.2% and 93.7% respectively, in 16 culture positive mycotic keratitis patients[77].

Jeffrey et al 2001 from New York evaluated 105 patients with suspected Onychomycosis using for diagnostic methods – KOH preparation, Culture biopsy with PAS stain and calcofluor white stain. CFW is highly specific and sensitive technique for detection of dermatophytes. CFW was choosen as good standard for statistical analyser[33].

Culture: Culture is a necessary adjunct to direct microscopic examination[89]. When the suspicion is high, all specimens should be cultured even when the KOH preparation is negative[37]. Because ringworm can appear quite variable and culture is such an easy procedure. It is advised to routine part of dermatologic examination. For mycological examination, cultures are planted by furrowing the specimen into media with the scraping knife. A wide agar slant or specimen jar is recommended[37]. A colony should be examined for the colour of the obverse and reverse, the presence of diffusible pigment, surface texture, topography, and rate of growth. Conidia type, their shape, their size are essential criteria for identification. Presence of other

structure such as pectinate, spiral or antler like hyphae, chlamydocondia or nodular organs may also be helpful for identification[32, 37].

The gold standard media for primary isolation of dermatophytes is **Sabouraud Dextrose Agar – containing cycloheximides and antibacterial** antibiotic[32, 37, 89]. The cycloheximide suppresses the growth of most saprophytic fungi without deterring the growth of dermatophytes. The various antibacterial antibiotics used included chloramphenicol 0.05 mg/ml or Gentamicin 0.02 mg/ml both are satisfactory[32].

Growth is relatively slow usually ten days to three weeks are required at optimum temperature of 25^0C. *T.tonsurans* and T.Verruccosum rare strains that grow better at 37^0C. When growth become evident on the primary isolation media, mycelial stands are transferred to slide culture preparation. For slide culture two media are advised.**Cornmeal Agar with 1% glucose** to stimulate pigment production of *T.rubrum* [37, 89]. SDA with Antibiotics shows normal undisturbed morphology of spore, spore arrangement and mycelial appendages[37].

SLIDE CULTURE: The slide culture in mycology is used to study undisturbed morphological details of fungi, particularly relationship between reproductive structures like conidia coniophore and hyphae[32]. It is indicated when teased mount of LCB is inconclusive in a paricular fungal isolate.

In laboratries without access to mycological consultation or in large public health field studies, the routine isolation and identification of dermatophyte is difficult.Recently two new primary media . **Dermatophyte Test Medium (DTM)[88] & Dermatophyte Identification Medium(DIM)**[70, 71] have been developed, which are helpful in primary isolation of dermatophytes[89]. They both are based on pH changes

caused by proteolytic activity of dermatophytes, which is lacking in saprophytic fungi. Dermatophyte Test Medium, which contains phyton, dextrose, actidione gentamicin, agar and phenol red solution. At 25 degree C, the growth of dermatophytes produce red colour within two weeks. They turned the medium red by raising the pH through metabolic activity while most fungi or bacteria do not.The disadvantages are it does not inhibit the all saprophytic fungi therefore some of them grow in the medium and may induce colour change[32].

Salkin, Arvind, Padhye and Kemna et al 1997 and Sally Gromadzki et al 2003 found a new medium Dermatophyte Identification medium (DIM trade mark pending) was specially developed to eliminate problem of false positive results associated with commercially marketed media, such as DTM. Presumptive identification of an unknown isolate as a dermatophyte required only the transfer of a portion of the suspected colony recovered from the specimen to Dermatophyte Identification Medium. Positive result evidenced by change in the colour of the medium, were observed within 24 to 48 hrs. In their study, they found, false positive result were always due to bacterial growth. When spores are evident, Lacto phenol cotton blue mounting can be made for accurate observation[70, 71].

Dermatophyte Identification Medium contains dextrose, neopeptone, cycloheximide, pencillin, streptomycin, bromocresol purple. At 25 degree C, if there is growth of dermatophytes, the colour of the medium change from pale green to purple within 24 to 48 hrs after growth. The dermatophytes turned the medium to purple by raising the pH through the metabolic activity of dermatophytes and showed less false positive result with fewer fungi than Dermatophyte Test Medium. Most of

the fungi giving false positive reaction are morphologically dissimilar to dermatophytes[32].

Singh & Beena et al 2003, found new medium **Enriched Dermatophyte Medium (EDM),** which contains soytone, carbohydrate, growth stimulants, cycloheximide, gentamicin and agar. They compared the usefulness of this media with Dermatophyte Test Medium & Sabouraud's Dextrose Agar and they evaluated the usefulness of two different microscopic techniques. i.e. Microscopic examinations using 10% KOH and with 40% DMSO with 10% KOH. The results suggest that efficiency of SDA DTM was found equal and slightly better than EDM[84].

Vitamin enriched casein digest media enhance the growth and production of microconidia and macroconidia. A series of vitamin and amino acid test agar is available commercially such as **Trichophyton Agar 1-7** to differentiate some of the *Trichophyton* species by demonstrating their requirement for special growth factors[89].Trichophyton agar no 1is vitamin free casein basal medium. Trichophyton agar no 1 contains Vitamin Casamino acids, Dextrose, Mono potassium Phosphate Magnesium Sulfate, and Agar[90] to which various growth factors have been added namely inositol for no2, inositol and thiamine for no3, thiamine (200µg)[90] for no 4, nicotinic acid for no5 and ammonium nitrate basal medium for no 6 to which histidine has been added in no7[88]. For *T.eqinum* nicotinic acid (no5), for *T.megninii* L-histidine(no7), *T.tonsurans* and *T.rubrum* thiamine (no4) *T.verrucosum* inositol and thiamine (no 3), and for *T.violaceum* thiamine (4) are the requirements[37].

The presence of perforating organisms in vitro hair culture separates *T. rubrum* from *T. mentagrophyte*s. *T. rubrum* produces red pigment on potato dextrose agar or corn meal agar with 1% glucose while *T. mentagrophytes T. tonsurans* do not[3]

TREATMENT

Mild ringworm infection resolve uneventfully with time usually in early adolescence. The more inflammatory the disease the earlier the termination of disease. This is particularly true of zoophilic (*M. Canis, T.verrucosum, T. mentagrophyte*)[37.] However the spread of infection by the orgranism is during infection period, particularly, *T. violaceum* which causes persistent infection in which patients became vector for spreading the disease within family group and the community. Patient should be actively treated to terminate such infection and prevent their spread.Topical treatment of tinea capitis appears to be without benefit. However the addition of topical fungistatic agent, systemic administration of Griseofulvin, is an adjuvant therapy. Griseofulvin is the most effective drug for treating tinea capitis. Dosage schedules vary, but the usual standard treatment is 500 mg for adult and 250 mg for children in four divided doses[37].

In Tinea favosa, *T.schoenleinii* has same sensitivity to Griseofulvin. Resolution of infection has been accomplished with long term use of the drug, the dosage schedule is the same as for Tinea capitis.In Tinea corporis, the normal patient resolves spontaneously after a few months, there are fewer tendencies towards chronicity but reinfection of the same area may occur within few weeks, if the patient is again exposed to infectious material. Drug of Choice at present is tolnafatin, *T.rubrum, T.mentagrophytes, M.canis, M.audounii* and *T.tonsurans* are amenable to

treatment by the drug. Widespread tinea corporis and more severe type of lesion (Granulomatous, Verrucous) may require systemic Griseofulvin therapy. Treatment Schedule is 1 gm in divided doses. Course of therapy is continued for six weeks to eight weeks. Clinical evidence indicates increasing resistance of chronic infection to treatment with Griseofulvin[37].

In Tinea crusis the prognosis is good unless the etiological agent is *T.rubrum,* as chronic disease may ensure invoking the body, feet, hand. Systemic treatment with Griseofulvin 500 mg per day is most useful particularly in *T.rubrum.* The course of treatment should be continued until all clinical, microscopic and cultural sign of the disease disappear[37].

In tinea ungium, systemic griseofulvin therapy has resulted in completed remission of the disease in some patient. The course of therapy is long (a year or more) and good results are not assured. In a study by Russell, 80 percent of patient with fingernail infection were cured after 10 month on a dose of 1 gm per day. Compared with 12 percent of patients with toenail infection. In another study 8 of 14 patients still had infected toenail after 15 months of Griseofulvin Therapy[37].

ANTIFUNGAL RESISTANCE:

This phenomenon is sufficiently uncommon among dermatophytes to make routine testing unnecessary, but in case of chronic dermatophytes or treatment failure occurs without other explanation, it is possible to estimate the sensitivity of the causal organism.

ANTIFUNGAL SUSCEPTIBLITY TESTING

Antifungal susceptibility testing is receiving increased attention with the advent of newer antifungal drugs and it is no longer enough to just diagnose mycoses. Choosing the best therapy for each patient has become very important and the day is not far off when clinicians would come to expect susceptibility testing for guiding the selection of appropriate antifungal drugs. However, susceptibility testing of filamentous fungi is not as advanced as the susceptibility testng of bacteria. Ideally in vitro susceptibliy tests should provide a realiable measure of the relative activities of two or more antifungal drugs, correlate with invivo activity and predict the likely outcome of therapy, and provide a means with which to monitor the development of resistance among normally susceptible organism and predict the therapeutic potentials of new discovered drugs Invitro sensitivity testing of fungi is influenced by a number of technical variables such as inoculum size and preparation, medium composition and pH duration and temperature of incubation and MIC end point deermination. In addition the problem unique to fungi are their slow growth rates and the ability of some of them to grow either as yeast with blastoconidia or as molds with variety of conidia depending on pH, temperature and medium composition.[53]

Several studies have attempted to correlate the Minimum Inhibitory Concentration results with clinical outcome. However, only little evidence is available to support clinical correlation of antifungal susceptibility test results with in vivo outcomes[53].

The most often used method is the macrobroth dilution [M38-P method] by the National Committee for Clinical Laboratory Standards (NCCLS). The Minimum

Inhibitory Concentration is read visually by subjective inspection of growth inbition after incubation.Modification of this method have been proposed which are simpler in technique and at he same reliable[53].

Roberts Cox, Gentles and Babu (1987) observed that mycological cure rate at 4 weeks was 33 for ketoconazole and 29% for griseofulvin; the same at 8 weeks was 53% and 57% respectively in cases of tinea pedis. The efficacy was lower than with topical imidazole preparations where cure rates of over 70% could be expected. The lack of superiority of ketoconazole over griseofulvin combined with the former's hepatoxicitiy indicated that griseofulvin should remain the treatment of choice in symptomatic tinea pedis resistant to topical therapy[37].

Fu, Issacson, Lococo, Foleno, Hilliard et al1992. Found invitro activity of saperconazole against dermatophytes by using agar dilution method in three media Yeast Nitrogen Base Agar, Brain Heart Infusion Agar, Sabouraud DextroseAgar, Minimum Inhibitory Concentration of saperconazole were less than 1μg /ml. Fluconazole ranged from 0.1 to > 128 μg / ml[20].

Yoshida Jono, Okonogi et al1994 found MIC obtained by agar dilution method were similar to those obtained by broth dilution method proposed NCCLS[97].

GohCL, Tay YK, AIiKB, Koh MT and Seow CS etall in 1994 evaluated the invitro susceptibility of griseofulvin, ketoconazole, and itraconazole against various dermatophytes using broth dilution method. The majority of the isolates were sensitive to the three drugs tested. Of the isolates, 82% were sensitive to griseofulvin, 78% to ketoconazole, and 81% to itraconazole all at a concentration of <0.25 μg/ml. Four isolates of *T.rubrum* had Minimumnhibitory Concentration of ≥or64μg/ ml. The

Vitro activity of griseofulvin, ketaconazole, itaconazole are similar against dermatophytes Griseofulvin may be given as first line drug for by such infection[24.]

Pankajalakshmi.V., and Taralakshmi.V. et al (1994) compared in vitro the anti dermatophytic activity of two main compounds, naftifine and terbinafine with those of ketoconazole and intraconazole by agar dilution. Eighty-eight clinical isolates of dermatophytes comprising of *Microsporum canis (50), M audoninii (5), Trichophyton rubrum (6) T mentagrophytes (5), T. violaceum (12), T.Simii (5), T.verrucosum (1), T. Soudanense (1), T. erinacei, and Epidermophyton* floccosum (2) were tested. Terbinafine was found to be most active, inhibiting 68 of the 88 isolates at concentrations of 0.01 μg/mI and (MIC range 0.0001 — 0.1μg/ml]. Naftifine inhibited 84 isolates at a concentration of 0.1 mcg/ml and all at 0.5 mcg/ml (MIC range 0.001-0.5 μg/ml). Itraconazole required 0.1 mcg/ml for inhibiting 50 isolates and 0.5 μg/ml for 85 isolates [range 0.01-1 μg/ml] whereas ketoconazole inhibited. 71 isolates at 1μg/ml and 87 at 2.5 μg/ml [range 0.01 - 5 μg/ml][56.]

Butty, Labecq, Mallie and .Bastide et al1995, studied the comparison of steer agar dilution method and new culture method to evaluate the minimum inhibitory concentration of antifungal compounds on several species of dermatophytes. The new method involves dilution of antifungal drug in solid medium in a Petri dish. Standardized agar cylinders are cut from the plate and filled with inocula of the same size cut from the plate of dermatophyte cultures.Such inocula facilitates analysis of fungus in its natural growth condition invitro. Without being submitted to a disruptive preparative technique. ‘MIC values were similar for the two method of evaluation inspite of important differences between the inocula. The new techniques is reliable,

quick and highly reproducible. It is more efficient than sheet agar diluation method because it avoids labour intensive procedure for the preparation of inocula[11].

Pankajalakshmi.V , Taralakshmi. V. et al(1995) studied the In Vitro Susceptibility testing of 43 isolates of dermatophytes against ketoconazole, miconazole, econozole and griseofulvin by agar dilution and disk diffusion methods. Econazole was the most effective drug inhibiting all the isolates at a concentration of 0.1 μg/ml. The MIC 50s and MIC 90s for ketoconazole and miconazole were 1 and 2.5 μg/mi. Whereas the valves for griseofulvin were 1 and 5 μg/mi. Good correlation was seen between the MIC and size of zones inhibition around the disks[57].

Yoshida, Uchida and Yamaguchi (1997) from Japan developed on ATP bioluminescene assay applicable to rapid fluconazole susceptibility testing of dermatophytes. They evaluated by comparing it with viability, turbidity and fungal protein content-based conventional methods. Fluconazole susceptibility results obtained with strains of candida albicans and dermatophytes by the bioluminescence method in high resolution medium were well correlated with those obtained by conventional methods currently used in clinical microbiology laboratories or reported previously, including a broth dilution method by the National Committee for clinical Laboratory Standards (NCCLS). Thus, ATP bioluminescence assay can be used to monitor fungal growth is liquid culture media. The procedure has considerable potential for the rapid testing of FLCZ susceptibility of dermatophytes and other fungi[97].

Soares Maria Magli Stelato Rocha, et al 2001 defined Minimum Inhibitory Concentration, as lowest concentration or highest dilution of antifungal agent, which

resulted in plates without visible colonies and evaluated invtro activity of antifungal and antiseptic agents by using agar dilution method by using modified yeast nitrogen base[44].

Semra Kustimur, Ayse Kalkanci, Haril Monsuroglu and kadriya senel et al 2003 studied susceptibility testing of antifungal agents. Due to the increasing number of resistant strain, susceptibility testing of antifungal agent. Methodology of macrotube dilution reference method with two different microdilution method, as well as the disc diffusion method by using standard RPMI 1640 (Sigma) medium, Yeast Nitrogen Base for the candida strain Sabouraud Dextrose Agar. Fluconazole overall correlation between microdilution and macrodilution method was 86%. It was 91% between the minimum inhibitory concentrations obtained from macrodilution and disc diffusion zone diameters and concluded that disc diffusion method test was evaluated as low-cast reproducible and efficient very of assesing the invitro susceptibility of candida strains to Fluconazole[80]

Prof.Mostafa chadeganipow et al 2004, invitro evalution of griseofulvin to *T.mentagrophytes, T.verrucosum*, *M.Canis, E.floccosum*, by modified microdilution method. MIC were between 0.43 & 0.95 µg/ ml. *T.verrucosum, M.Canis*, *T.Mentagrophtes* were relatively griseofulvin resistant[48.]

Santos ad Hamdan, et al 2005 found RPMI 1640 appears to be suitabletesting medium for determining Minimum Inhibitory Concentration than that of Sabourand's dextrose broth. All isolates produced clearly detectable growth only after 7 days of incubation. Different incubation period resulted in MIC's that were consistently different for each medium when azoles and Griseofulvin were tested[76]

Belkya, Fermandez, Torres, et al 2005, found significant correlation between disk diffusion method and microdilution method. They evaluated a disk diffusion method to determine the activity of eberconazole against 50 strains of dermatophyte by three culture media RPMI, antibiotic medium 3 and high resolution. No differences were found among the result obtained with three media[8].

A.Esteban, Abarca cabanes et al 2005 found Neo sensitab agar diffusion method, a simple procedure for antifungal susceptibility testing of dermatopbhytes in routine clinical laboratory. Broth micro dilution method Minimum Inhibitory Concentration of Terbinafine was ≤ 0.03 and for Clotrimazole ≤ 0.069 & ≤ 0.919 for Itraconazole[1].

MATERIALS AND METHODS

This study was a discriptive study, where one hundred and five patients, who attended the out patient clinic of the Department of Dermatology, Government General Hospital, Chennai, and clinically diagnosed as dermatophytosis during one year period between August 2004 to August 2005 were the study group.

CLINICAL EXAMINATION

Complete details about data of the patient and history were recorded as shown in the proforma given in the appendix.No:9. These included the age, sex of the patient, duration of the lesion i.e duration less than one year taken as acute and more than one year of lesion were taken as chronic, H/O recurrence, type of the lesion, whether scaly or papular, site of lesion, , any contact with pet animal, house hold contacts and about the details regarding immunosuppressive illness or therapy and treatment taken earlier[32].

COLLECTION OF SAMPLE

One hundred and five cases that were clinically suspected as “ringworm” infection were taken for present study.

Skin: The affected skin lesion was decontaminated with 70% alcohol. This helps in removing the bacterial contamination and topical medication. Dry small scales were scraped off from the margin by flame sterilized rounded scalpel[32].

Hair: Affected hair was collected by plucking them completely, or alternatively the hair was brushed with blunt end of the scalpel[32] .

Nail: Infected nail cleaned with 70% alcohol, then clipped the nail as far back as possible from the edge. Full thickness of the nail was included.[32]

TRANSPORT OF THE SPECIMEN

The samples were collected in sterile black paper, and folded and transported to the laboratory. The use of the paper permits the specimen to dry out which helps to reduce the bacterial contamination. It also provides condition under which specimen can be conveniently stored for longer periods without loss of viability of the fungus[32].

DIRECT MICROSCOPIC EXAMINATION

(a) Direct microscopic examination by 10% KOH:

Principle: 10 to 20% KOH digest protein and mucinous material and dissolve the cement which holds the keratinized cells together. Fungi withstand the digestion due to chitinous cell Wall. KOH provides an advantageous refractive index to reveal the fungal hyphae[89].

Preparation: Appendix 1

Procedure: The material to be examined was placed into a clean glass slide, a drop of 10% KOH was added to the material and mixed, then the coverslip was placed over the preparation, which was kept at room temperature until the material had been cleared. The preparation was observed under lower or high power objective in microscope for the presence of hyphal elements.

(b) KOH-CALCOFLUOR WHITE SOLUTION MIXTURE (KOH-CFW)

Principle: Calcofluor White stain may be used for direct examination using fluorescent Microscopy. The cell wall of the fungi (beta 1, 3 and beta 1, 4 polysacccharide present specifically in the cellulose and chitin of the cell wall in fungi) binds the stain and fluoresce blue-white or apple green depending on the filter combination used. The addition of KOH enhances visualization of fungal element in specimen.KOH-CFW preparation may be preserved for several days at 4°C in a refrigerator[53].

Preparation: Appendix.2.

Procedure: The material to be examined was placed on to clean glass slide and one drop each of KOH –CFW was added or KOH-CFW was mixed equally before processing. A cover slip was placed over the material, and the KOH –CFW preparation was allowed to keep at room temperature (25'C) for few minutes until the material had been cleared. The preparation was observed by fluorescent microscopy equipped with UV or Blue violet filter to achieve radiation on the slide below 450 nm was used.[7]

Quality control:

1. The reagent was checked prior to use, weekly and with each new batch of calcofluor preparation.

2. Aqueous solution of actively growing Candida albicans was used as a positive control[7].

3. The KOH and Calcofluor mixture was used as a negative control[7].

ISOLATION BY CULTURE:

Each of the samples was inoculated into two slopes of Sabouraud's Dextrose Agar (SDA), one SDA with Chloramphenicol and another one SDA with chloramphenicol and cycloheximide and Dermatophyte Identification Medium [DIM] with pencillin and cycloheximide with indicator Bromothymol purple. SDA tubes were incubated at room temperature and DIM at 30°C. Each of the tube was examined for growth in SDA, purple colour change and growth in DIM, daily for the first week, twice a week for subsequent period and the inoculated slopes were retained for atleast four to six weeks before it was reported as negative[32].

Each of the SDA tube was observed for texture, colony morphology, obverse pigmentation and reverse pigmentation. Dermatophyte Identification Medium was observed for colour change of the medium and texture. If growth was present, a Lactophenol Cotton Blue Mount (LPCB) teased mount was prepared and examine under microscopy. Slide culture was put up whenever necessary. Biochemical reaction such as Urease test, hair penetration technique was done whenever necessary for confirmation of the species. Inoculated into Trichophyton agar No1 for confirmation of all Trichophyton isolates and No4 for confirmation of T. tonsurans and T. violaceum and T.rubrum[90].

Preparation of Sabouraud's Dextrose Agar: Appendix:3

Preparation of Dermatophyte Identification Medium: Appendix:4

Preparation of Trichophyton Agar No1 and NO4: Appendix:7.

MICROSCOPIC MORPHOLOGY:

Lactophenol cotton Blue (LPCB) mount was prepared and examined for Hyphal structure, specialized hyphal structure, Microconidia, Macroconidia andtheir arrangement[32].

Principle: Lactophenol cotton blue is both a mounting fluid and staining fluid.

Lactic acid is used to preserve the fungal morphology. Phenol acts as a disinfectant. Cotton blue stains the fungal elements[32].

Procedure: 2-3 drops of LPCB were placed on a clean glass slide, with the help of loop, a small portion of the colony to be examined was removed from the agar surface and was placed on the drop of stain. With the help of two dissecting needles, the hyphal mass was teased, then it was covered with coverslip and teased out hyphal material was observed under low as well as high power objective.

Preparation: Appendix :5.

MICROSLIDE CULTURE:

Whenever it was difficult to establish an accurate identificaion with the teased mounts, micro slide culture technique was put up. Preparation of set: In a 100 mm diameter glass petridish, U-shaped glass rod, a microscopic slide with two cover slips and a metal cap, were placed and which was sterilized by Hot air oven at 160^{o}C for 1 hr.

Procedure: 'U' shaped glass rod was placed into the bottom of a sterile petridish, to serve as support for glass microscopic slide. A SDA block was placed on

the surface of the microscope slide. A small portion of the colony to be studied was inoculated in the margin of agar plug in three or four places with the help of straight inoculating wire. The cover slip was gently heated by passing it quickly through the flame of Bunsen burner and immediately placed over the inoculated

agar block.Sterile distilled water was kept in the metal cap inside the petridih.

The lid was placed on the petridish and incubated at room temperature (25^{o}C) for 3 to 5 days.

When growth visually appeared to be mature, the cover slip was gently lifted from the surface of the agar with pair of forceps, care was taken not to disturb the mycelium adhering to the bottom of the coverslip. Coverslip was placed on the drop of LPCB applied to the surface of the glass slide[39] and observed under low as well as high power objective.

BIOCHEMICAL TEST

UREASE TEST:

Principle: This modified Christensen's Medium detects the utility of various fungi to produce enzyme urease. In the presence of suitable substrate, urease splits urea, and produces ammonia, which raises the pH and colour changes from amber to pinkish red due to phenol red indicator. Modified Christensens Medium was used to distinguish between *T.mentagrophytes* and *T.rubrum*. *T.mentagrophyte* is Urease positive within 7 days, while *T.rubrum* is not Urease positive[32].

Preparation: Appendix: 6.

Procedure: A small portion of the growth to be examined was removed from the SDA agar surface and inoculated into the Modified Christensen Agar Medium and incubated at room temperature for a week and colour change was observed

HAIR PERFORATION TECHNIQUE:

This technique used to distinguish between *T.mentagrophytes, M..canis* which are positive and *T.rubrum, M.equinam* which are negative[32].

Procedure: To the bottom of a sterile petridish a small portion of sterilized hair was placed over moistened filter paper and a portion of the colony to be studied was inoculated directly onto the hair which was incubated at 25^{o}C for 10 to 14 days . Hair was observed regularly under microscope for the presence conical perforation of the hair shaft.

HAIR BAITING TECHNIQUE:

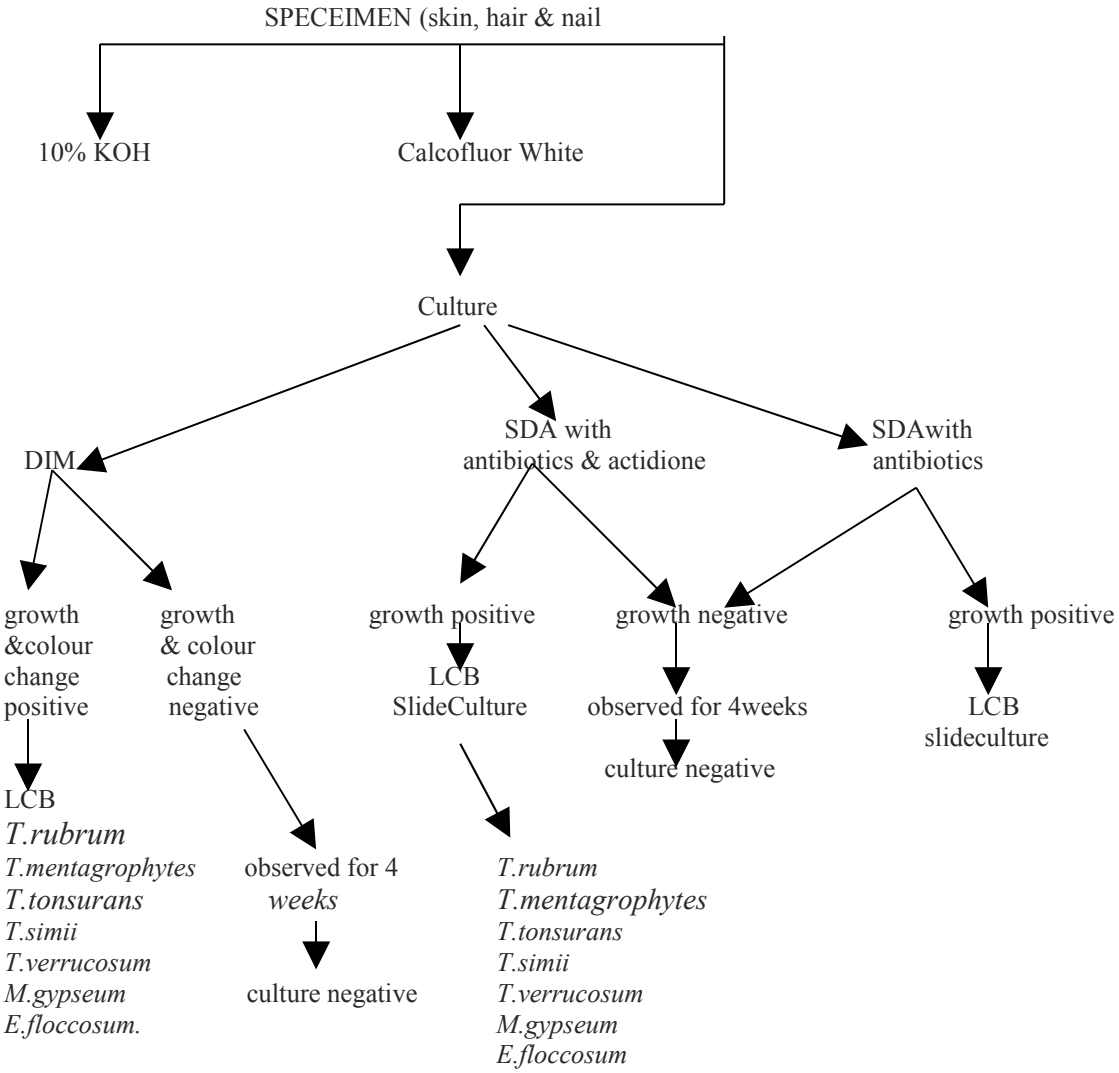
Principle: This technique is used to isolate keratinophilic fungi from soil.

Procedure: Soil sample was placed in a sterile petridish, and sterile hair was placed on the surface of the soil and it was wet with sterile distilled water and was incubated at 30^{o}C. When the growth appeared around the hair which was transfered to a Sabouraud's Dextrose Agar plate and they were examined under microscope for further details for identification[32].

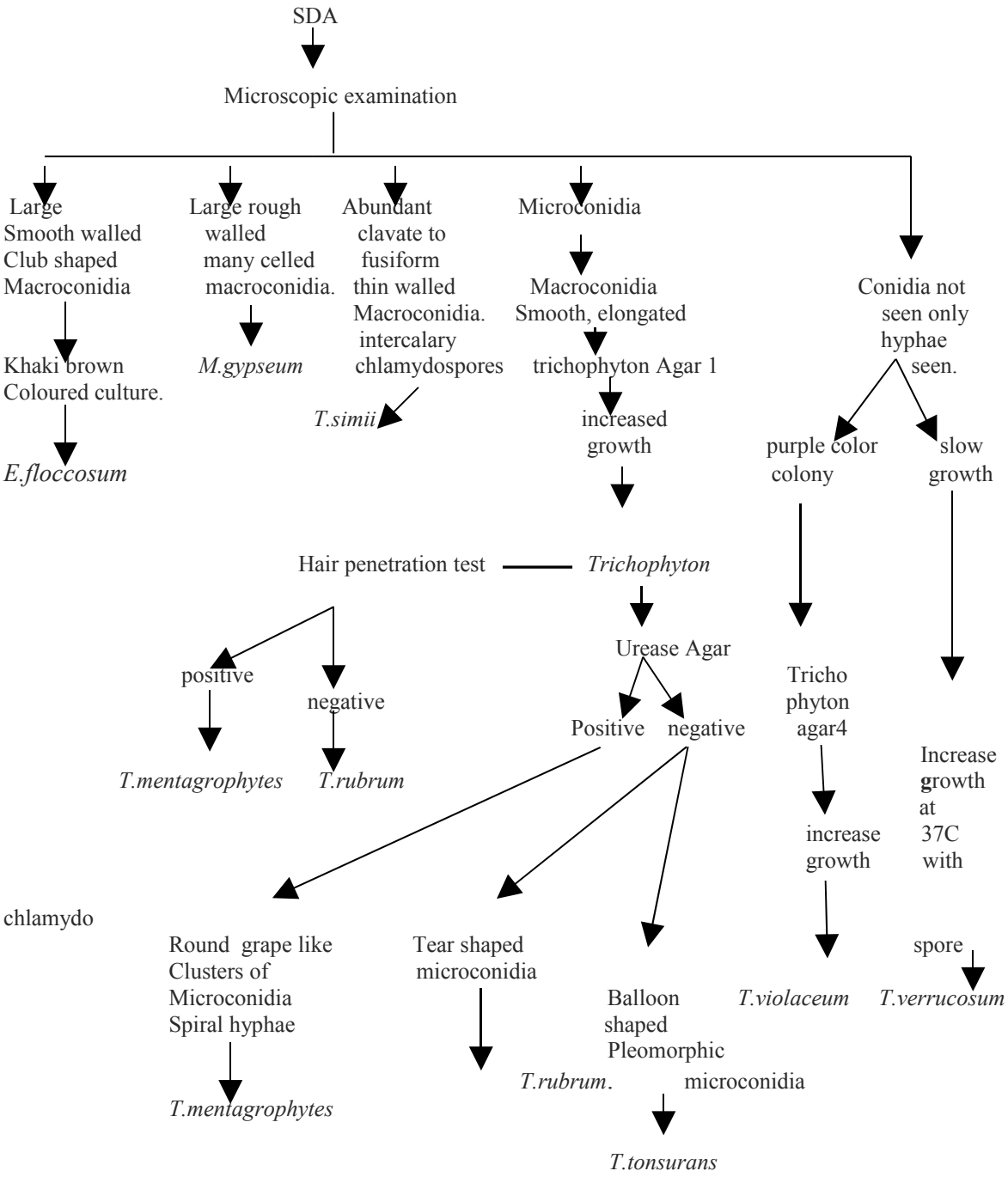
MODE OF IDENTIFICATION OF IMPORTANT PATHOGENIC FUNGI[88]:

Species	Colony on SDA	Microscopic Morphology
T.mentagrophytes	Surface creamy tan to pink powdery pink powdery to granular reverse buff coloured. rapid growth.	Microconidia: Abundant spherical grape like clusters. Macrconidia:smooth walled pencil shaped. Spiral hyphae present.
T.rubrum	Surface typically white fluffy, reverse wine red. Moderate to slow growth.	Microconidia:scanty to Numerous, pyriform Borne along the hyphae '*Bird on the fence'* Appearance. Macroconidia: smooth walled pencil shaped Macroconidia:
T.simii	Surface white, cream or buff. powdery, granular flat; reverse straw to Salmon; rapid growth.	Abundant clavate to fusiform tocylidrical thin walled; fragmenting intercalary chlamydospores
T.tonsurans	Surface white cream powdery, suede like reddish brown reverse slow growth.	Microconidia: pleomorphic along the hyphae.
T.verrucosum	Surface cream to tan, flat Very slow growth. characteristic(*sting of pearl)* appearance.	conidia usually absent. chlamydospores in chain
T.violaceum	Surface lavender to deep purple; glabrous, heaped very slow growth.	Conidia typically absent. irregular hyphae and chlamydospores present.
E.floccosum	surface tan, cinnaman Powdery, reverse buff or reddish. Rapid growth.	Macroconidia: ellipsoidal, thin to rough wall, upto 6 septa. Microconidia abundant.
M.gypseum	Surface olive, khaki yellow brown. Reverse yellow brown.	Macroconidia: numerous, clavate, smooth walled. Microconidia: absent.

MODE OF PROCESSING THE SAMPLE[7]



DERMATOPHYTE IDENTIFICATION SCHEME[7]



ANTIFUNGAL SUSCEPTIBLITY TESTING:

Preparation of Antifungal drugs and dilution scheme (Stock solution)

Minimum inhibitory concentration (MIC) of drug griseofulvin range=0.125µg/ ml to 64µg/ml[53].

MIC of drug Fluconazole =0.125µg/ml to 64µg/ml[53].

Preparation of drug concentration:1mg/ml.

SOLVENT: Sterile distilled water for griseofulvin[32] and Dimethyl sulphoxide (DMSO) for fluconazole[32].

Formulation:

C1V1=C2V2

C1=stock compound concentration

V1=Volume of the test medium

C2=compound concentration to be achieved.

V2=volume of the test medium.

1mg x V1=0.064 x 20

V1=0.064x20=1.28ml.

Dilution of drug used were between 64µg/ml to 0.125 µg/, {64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125}

INOCULUM PREPARATION:

Inoculum suspension was prepared from fresh culture (7 days old)

5 ml of sterile distilled water was poured on young culture which was grown on Sabouraud dextrose agar slant, and shaken well and the distilled water containing conidia was collected in a sterile tube[1].

INOCULUM ADJUSTMENT:

Inoculum size was adjusted to 10^3 to 10^5 spores/ml by microscopic enumeration of conidiophores using cell counting haematocytometer (Neubauer chamber), in WBC counting chamber. 5 ml suspension was taken and centrifuged. Clear suspension was collected in a sterile aliquots[19].

Test: Serial dilutions of stock solution were prepared in tubes.

Yeast Nitrogen Agar[44] was prepared and sterilized in seperate containers (19.5ml) for each drug concentration. The filter sterilized drug solution were added to the agar at 50 degree C and plate were poured. The final concentration of the drug in the YNB were 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 μg/ml for Fluconazole and Griseofulvin.

The plates were divided into 8 divisions and 20 microlitres of suspension of isolates i.e conidia in sterile distilled water were placed one in each division on the plates with different drug concentration of above mentioned drugs and allowed to dry on the agar surface[44].

After incubation at room temperature for 5to 7 days the MIC was read, as the lowest dilution in which no growth was observed.

CONTROL:

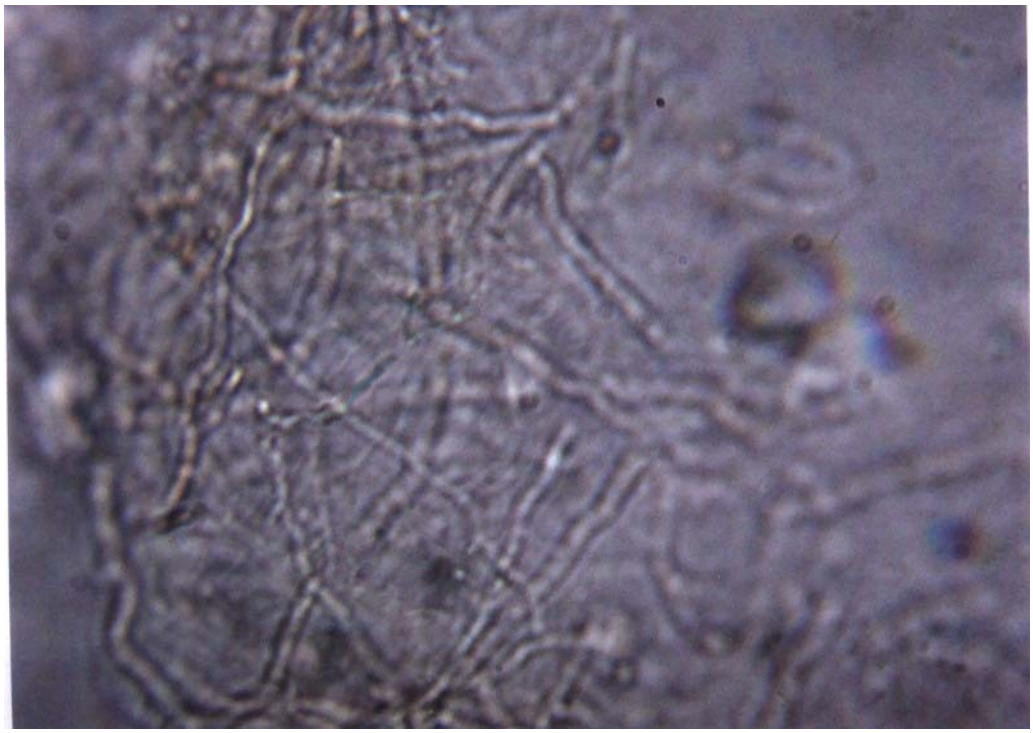
The strain of ATCC Candida albicans sensitive to both drugs was used as control.

Yeast Nitrogen Agar without drug was used as media control.

TAENIA CAPITIS

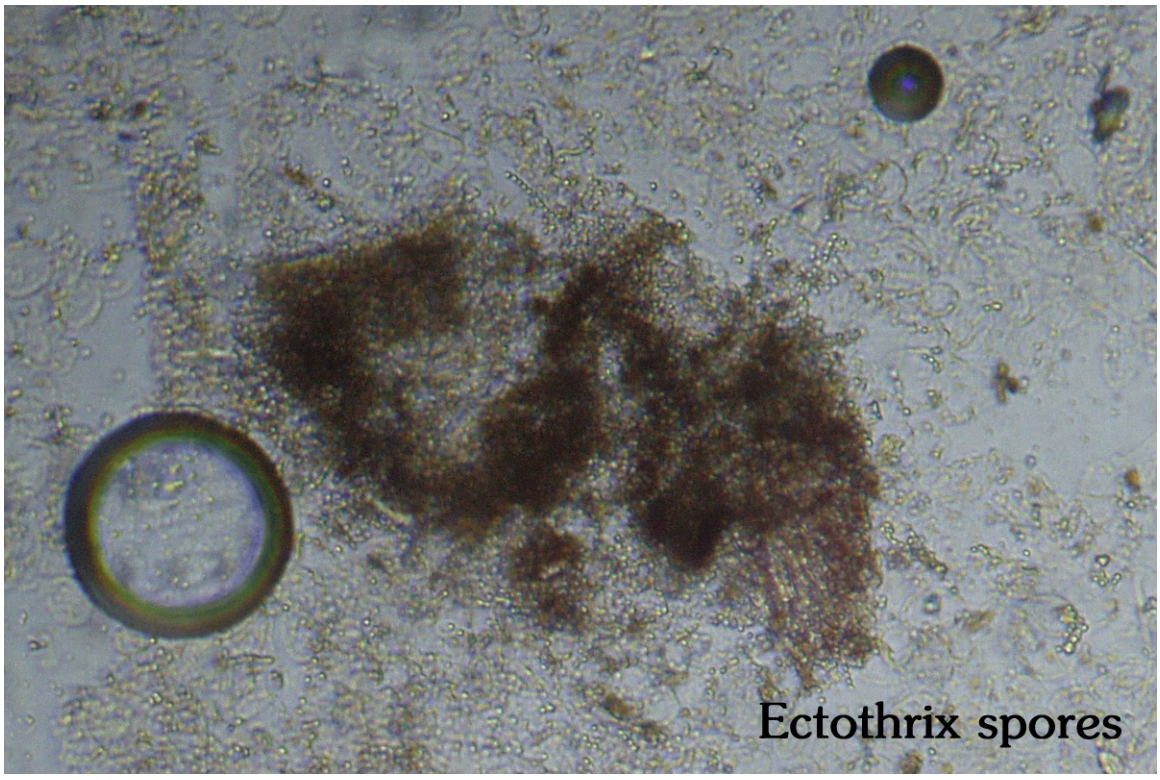
TAENIA UNGUIUM

TAENIA CORPORIS

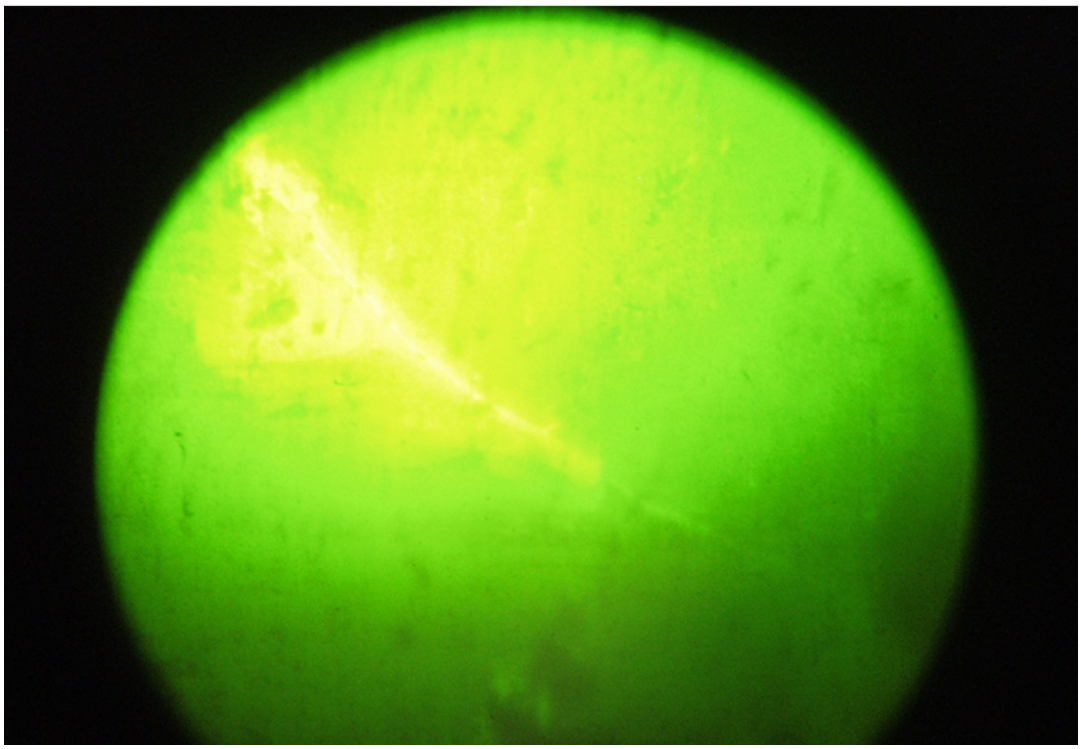


KOH - WET MUNT (40X)

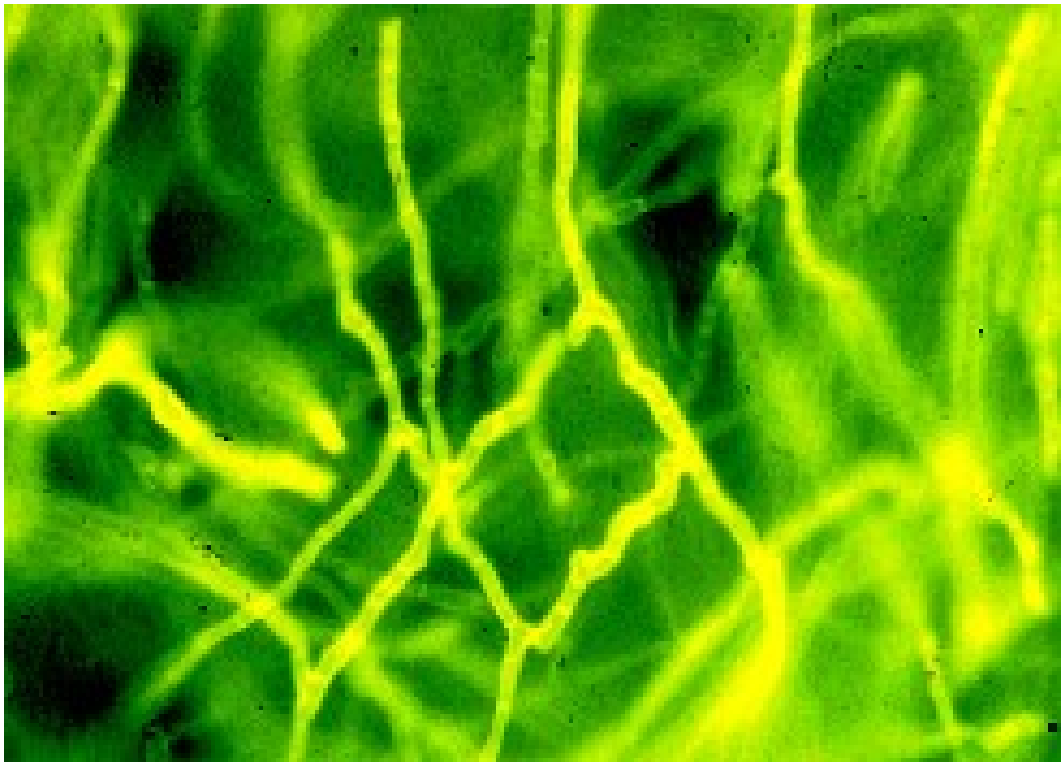
ENDOTHRIX SPORES (40X)

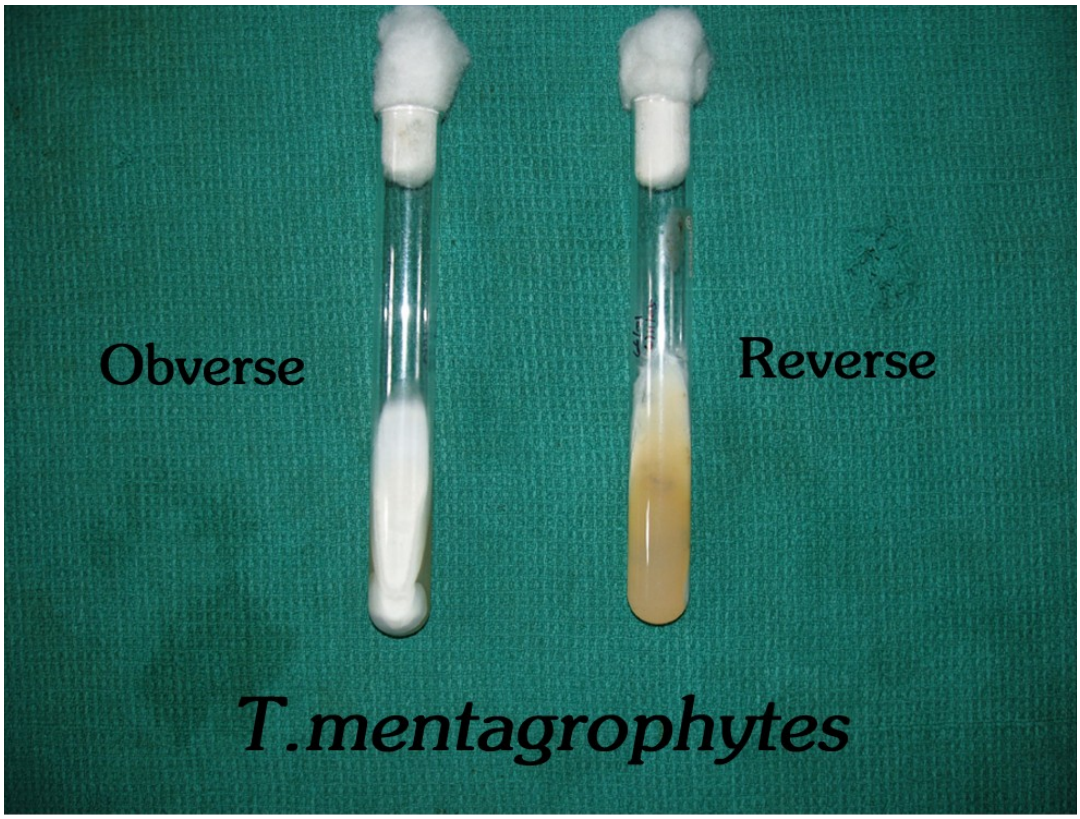


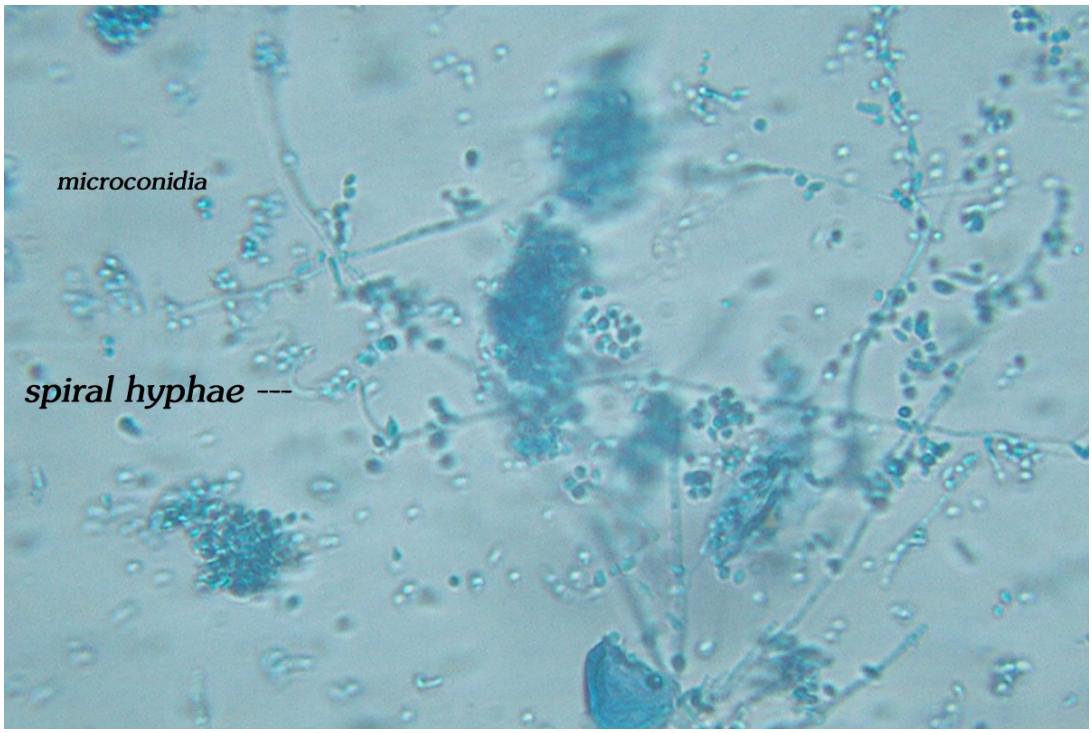
ECTOTHRIX SPORES (40X)



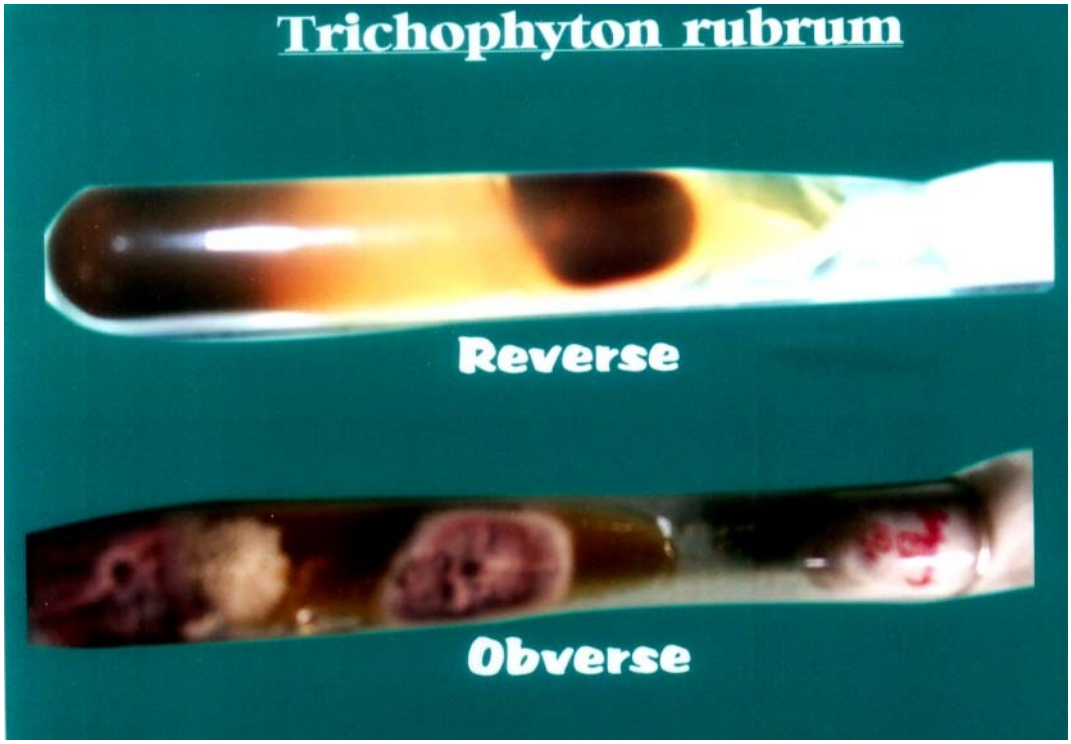
CALCOFLUOR WHITE STAINING

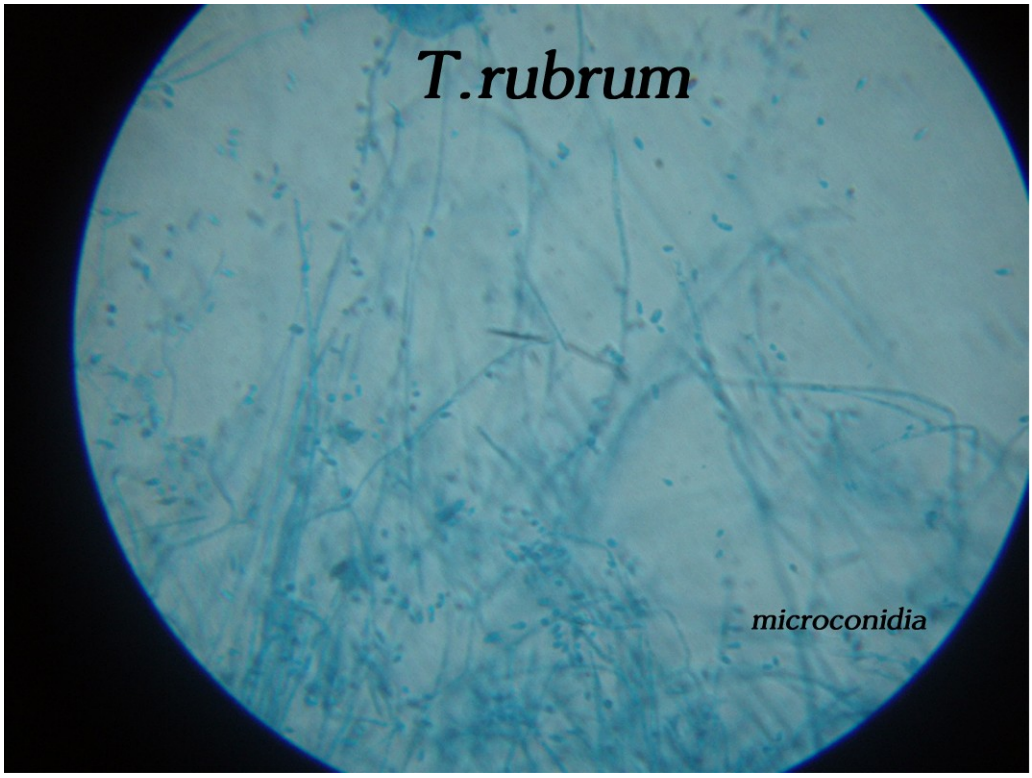




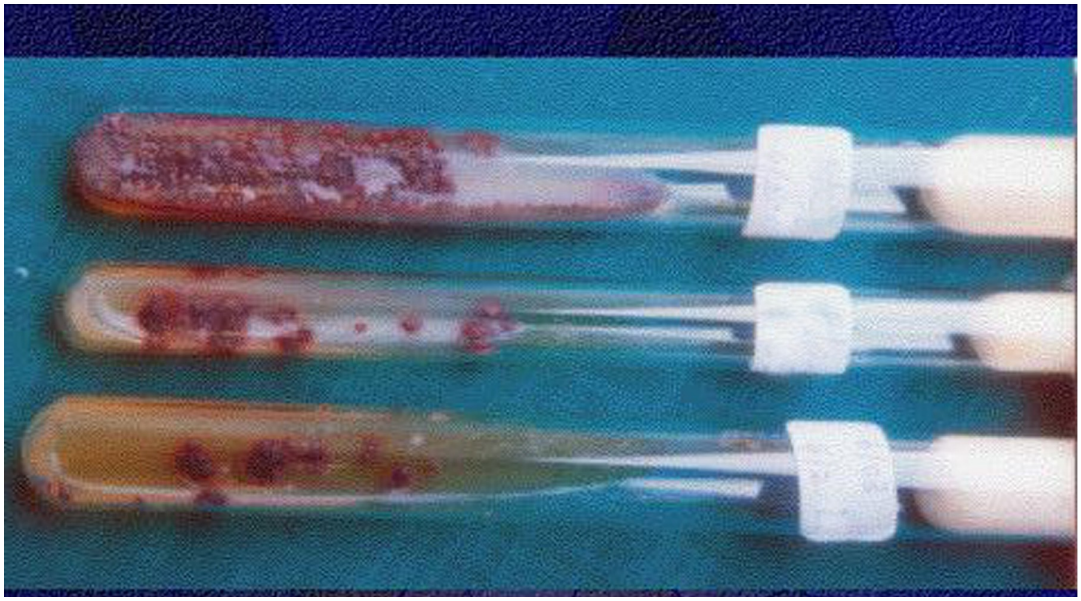


T. mentagrophyte (LCB 40X)

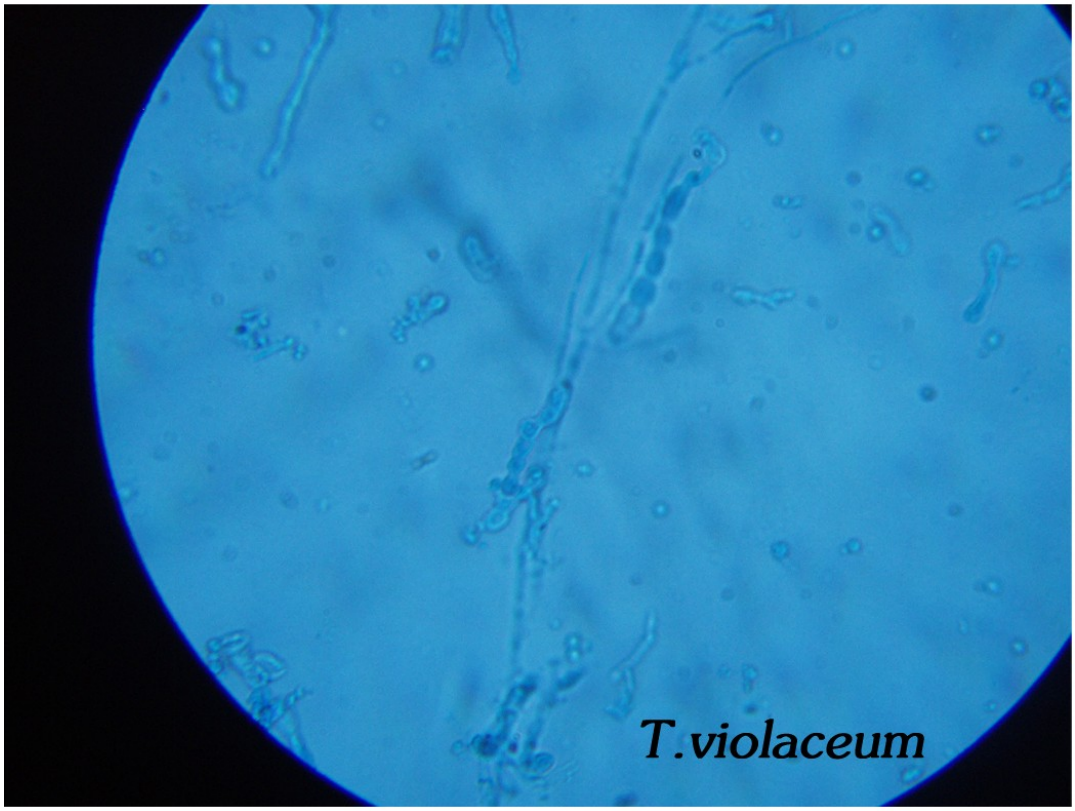




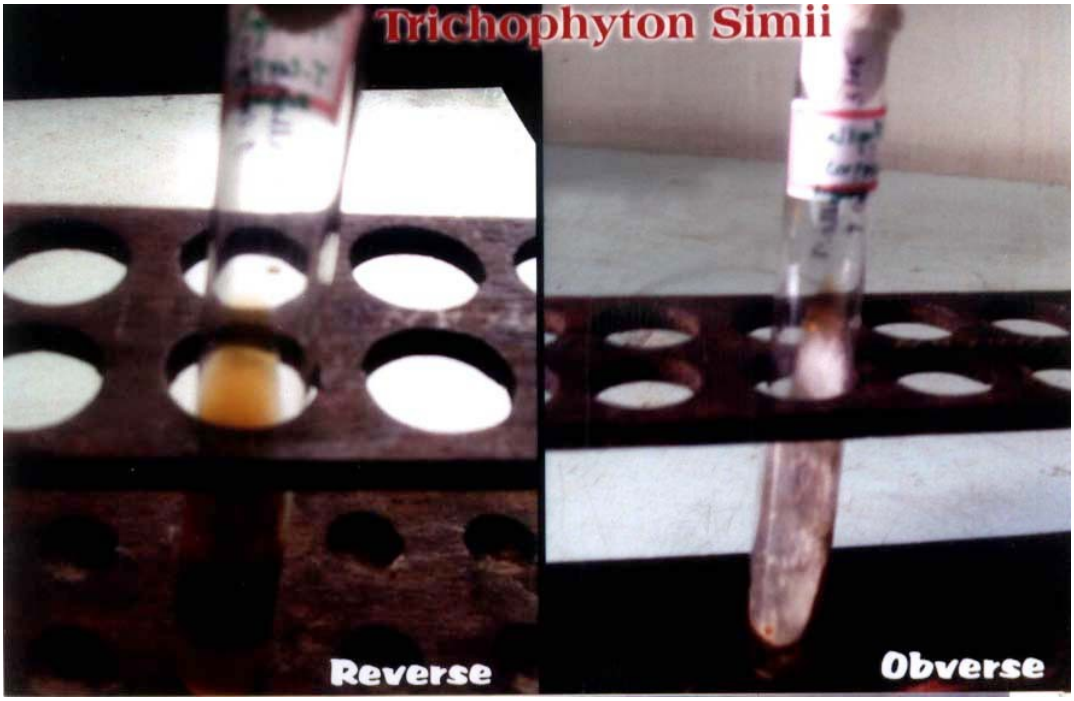
T. rubrum (LCB 40X)



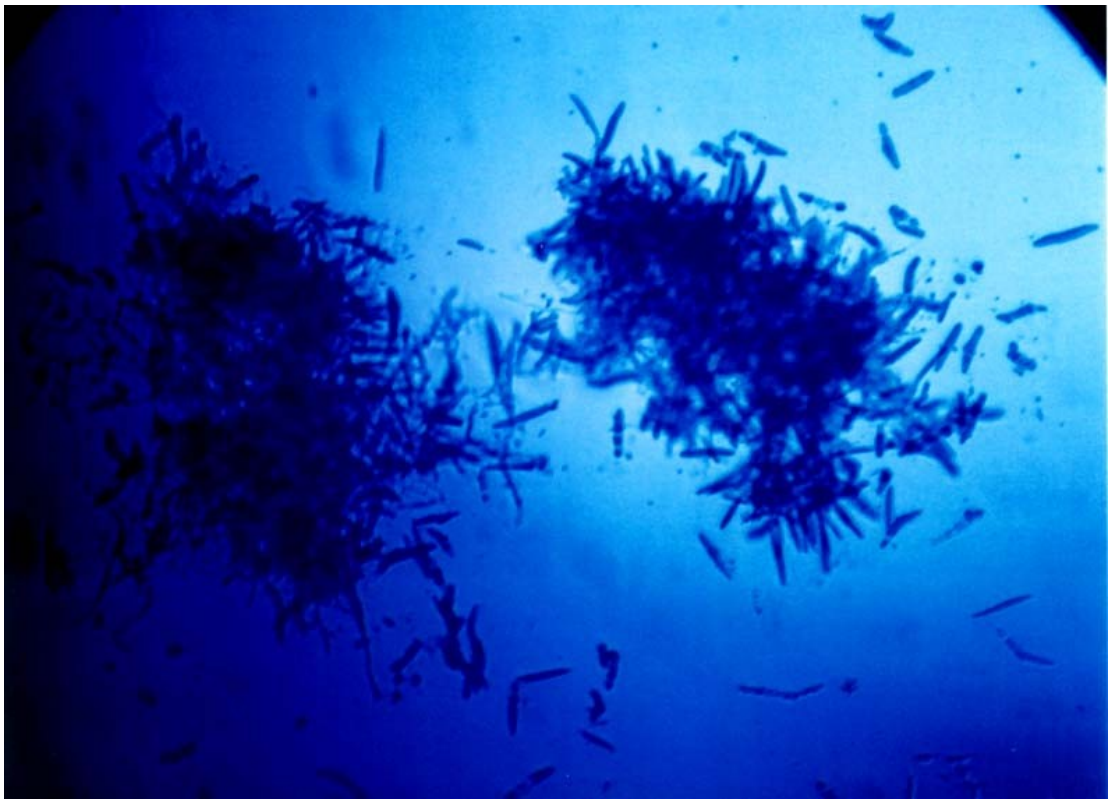
CULTURE



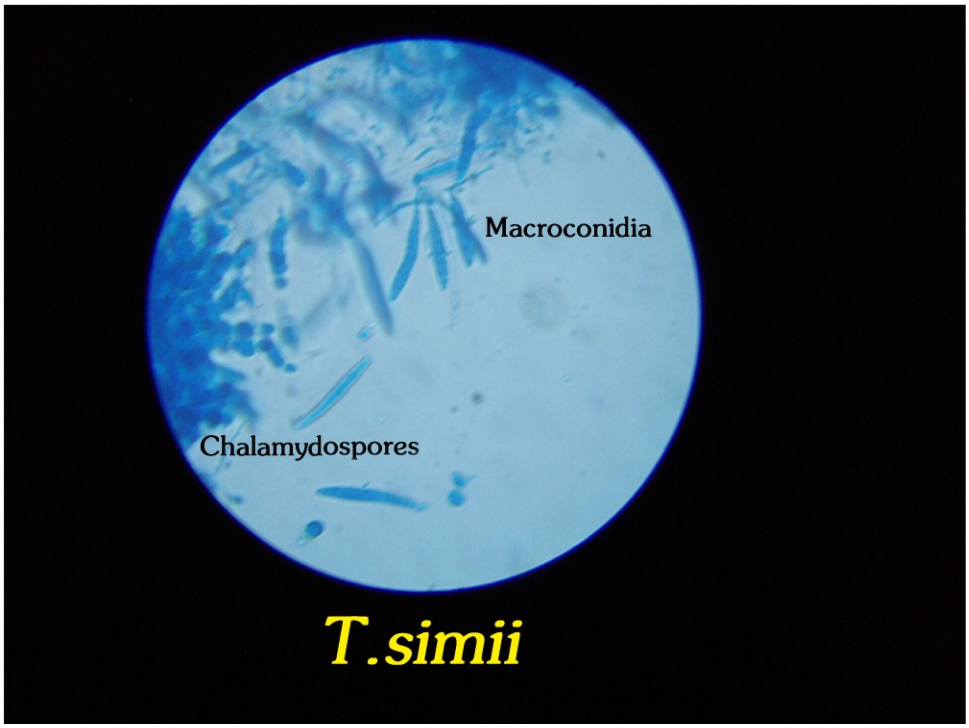
T. violaceum (LCB 40X)



T. simii

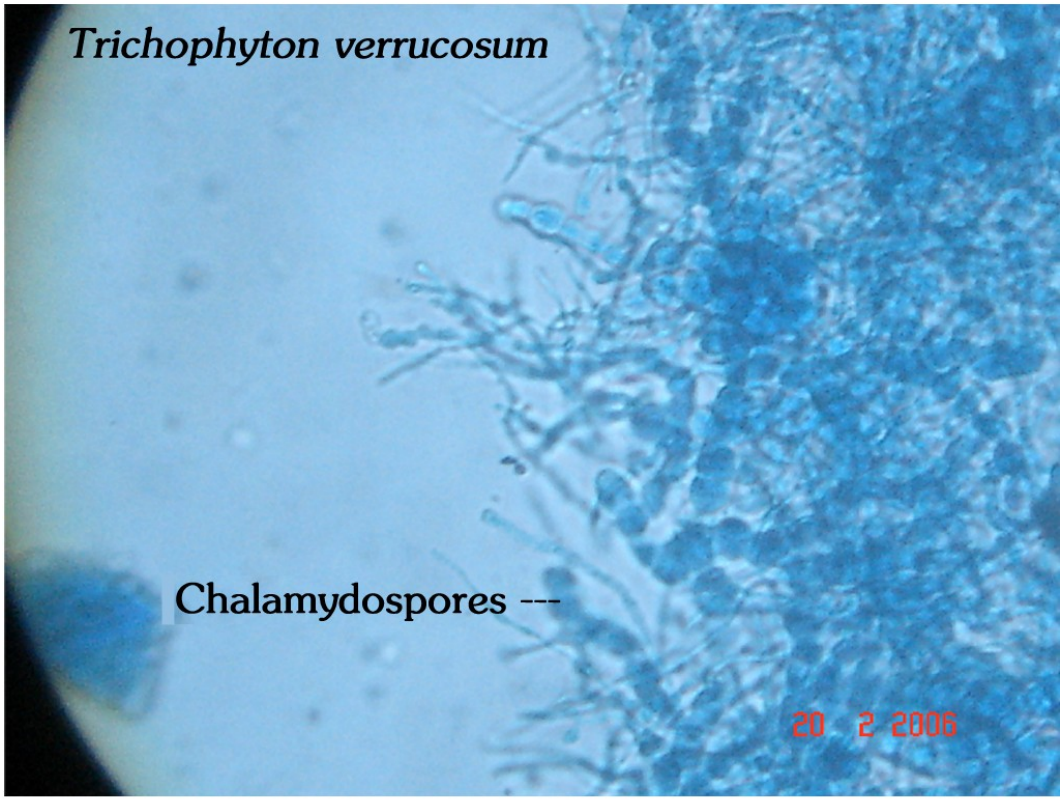


T. simii (LCB 10X)

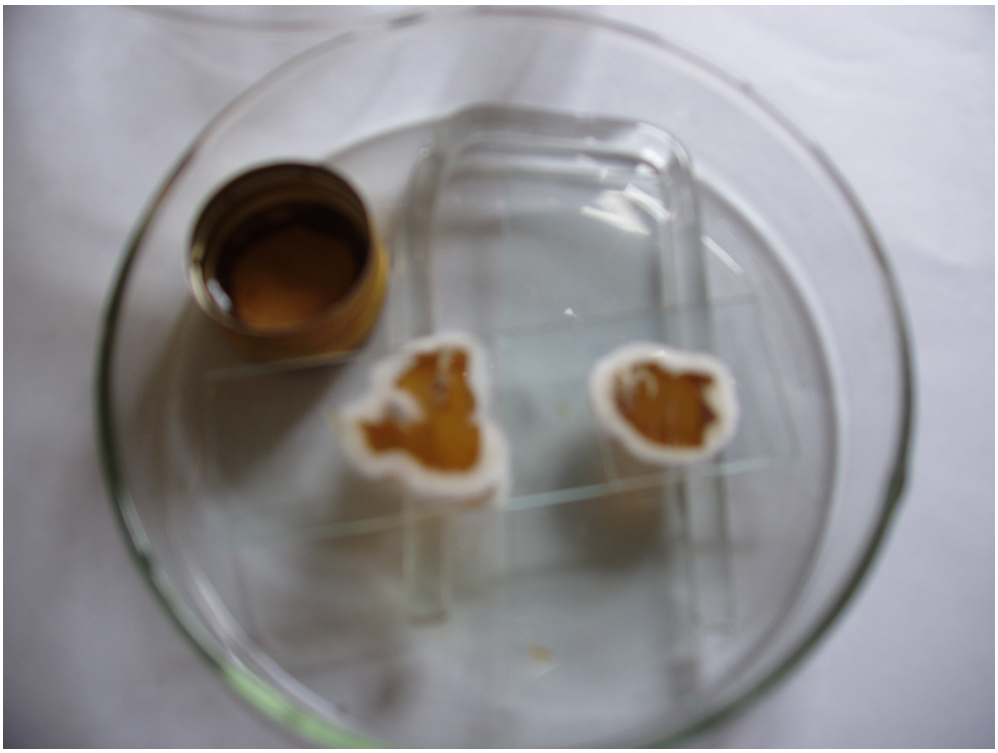


T. simii (LCB 40X)

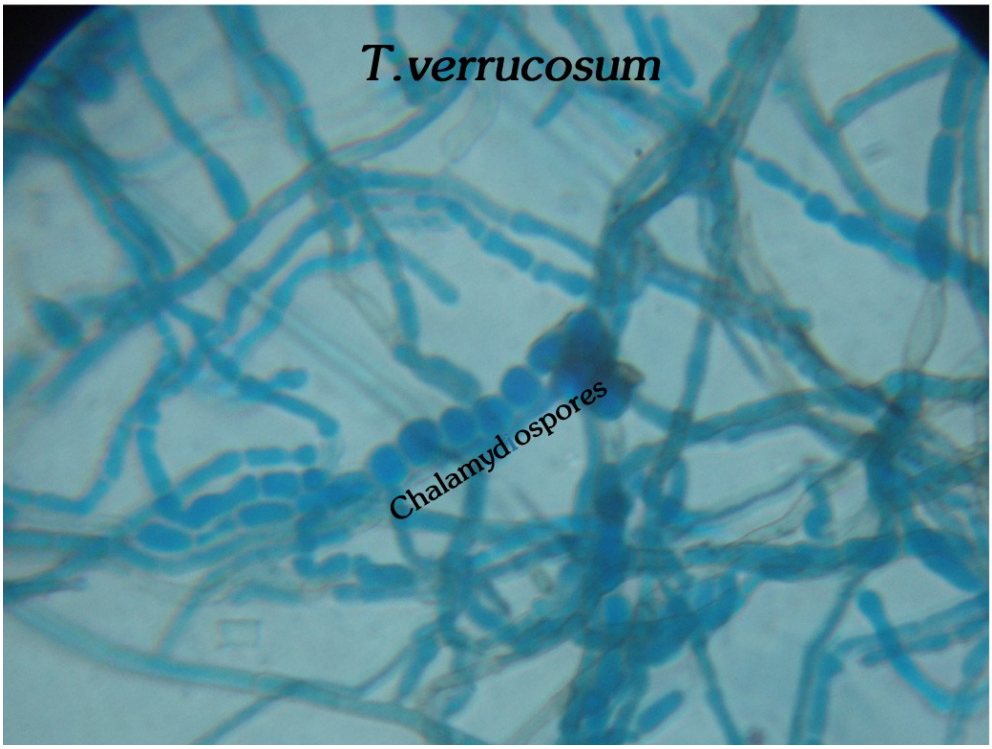
T. verruccosum



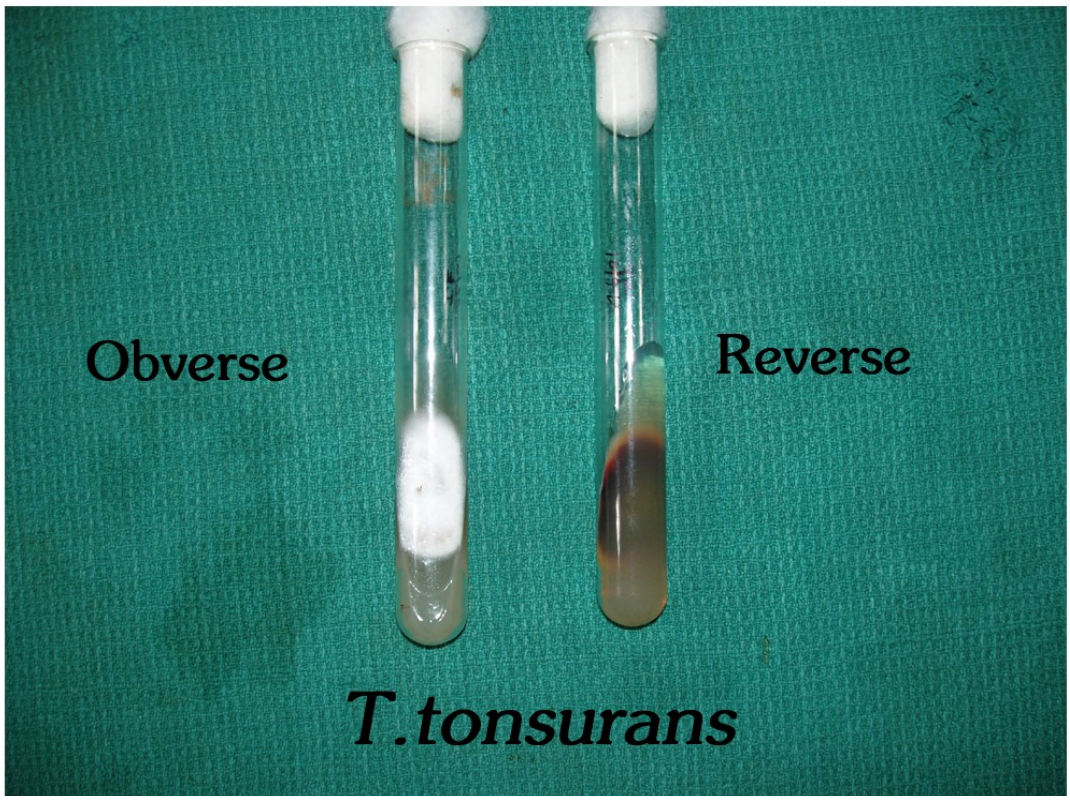
T. verruccosum (LCB 40X)

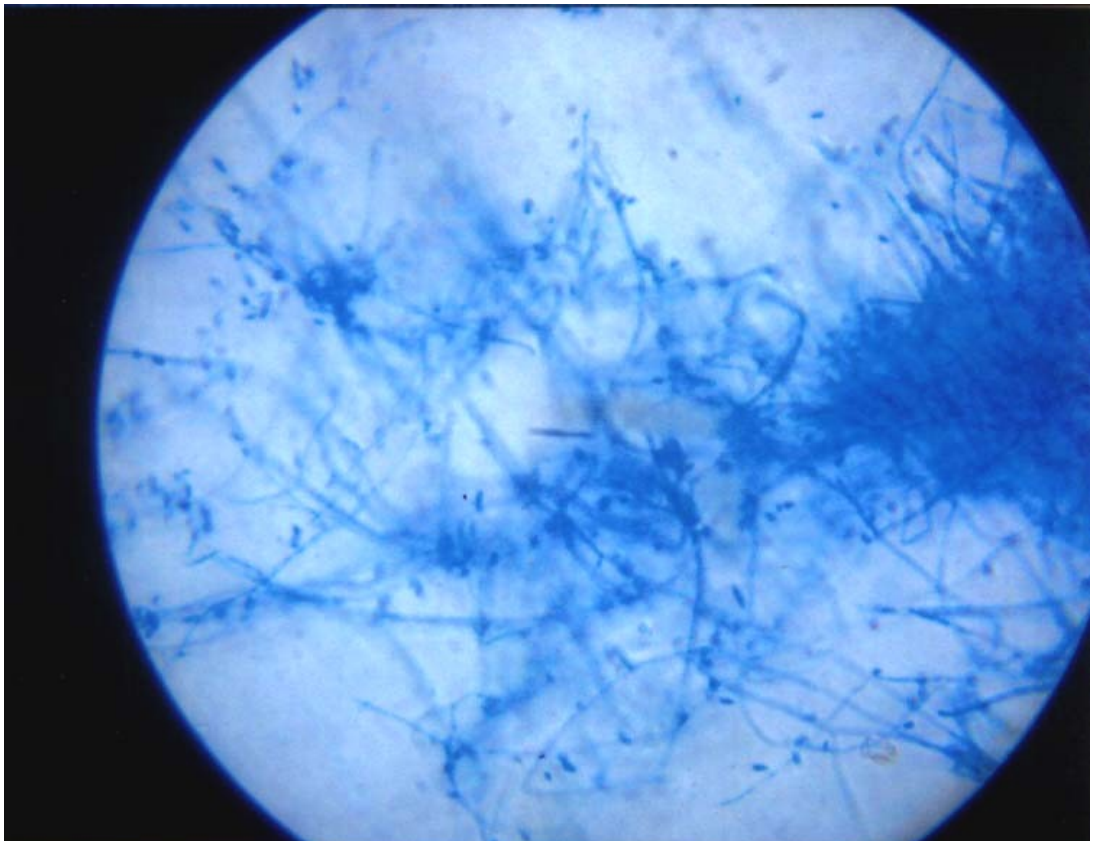


SLIDE CULTURE

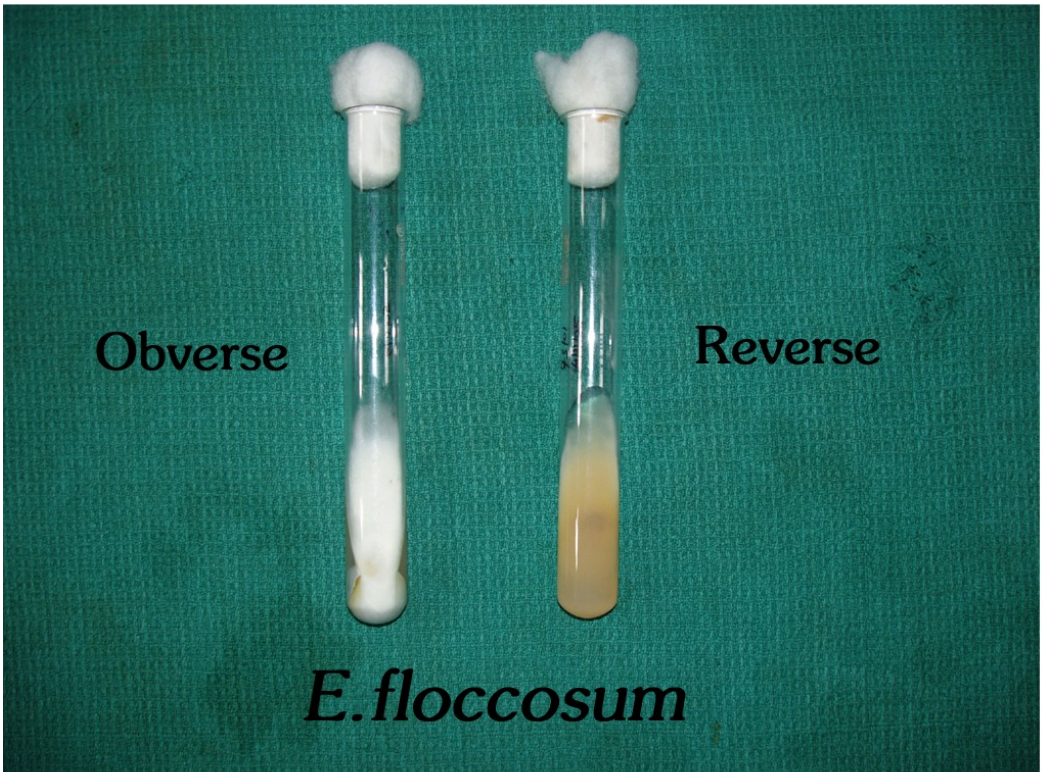


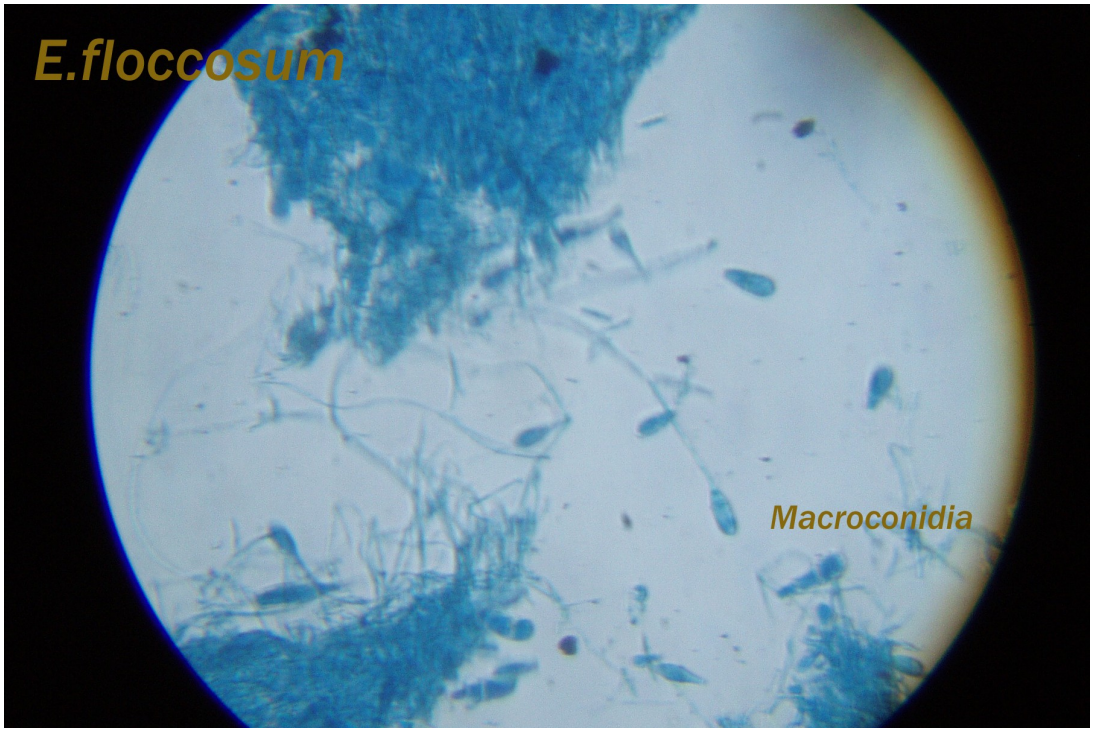
T. verruccosum undisturbed morphology (LCB 40X)



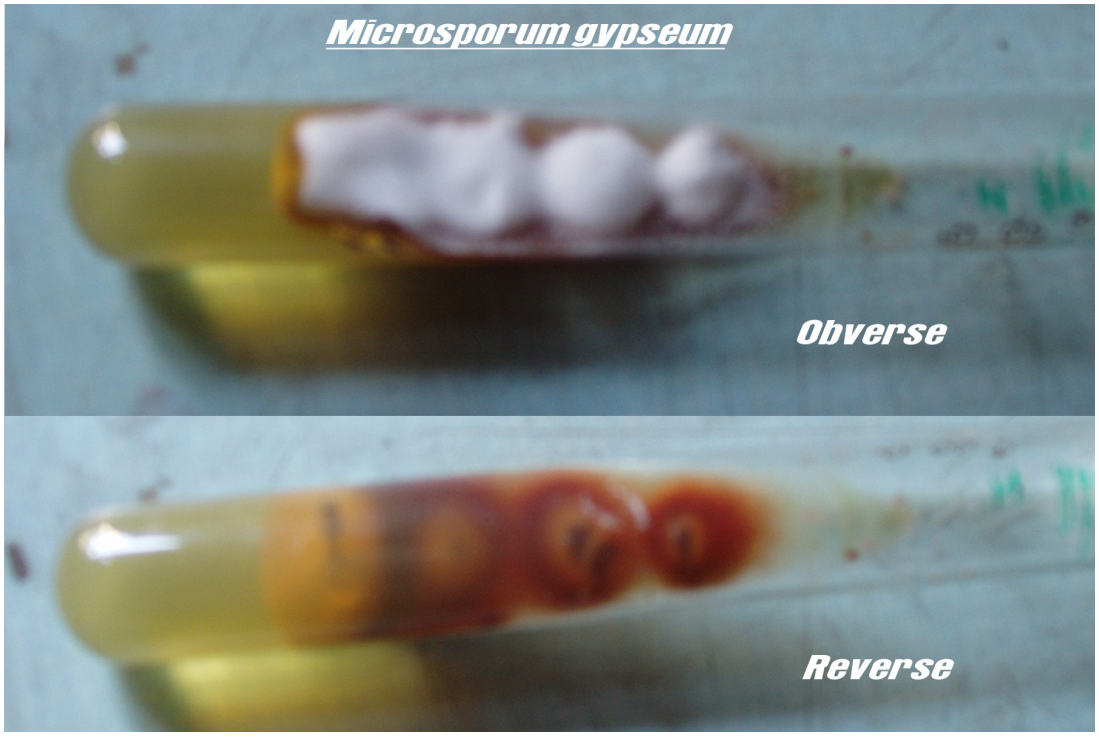


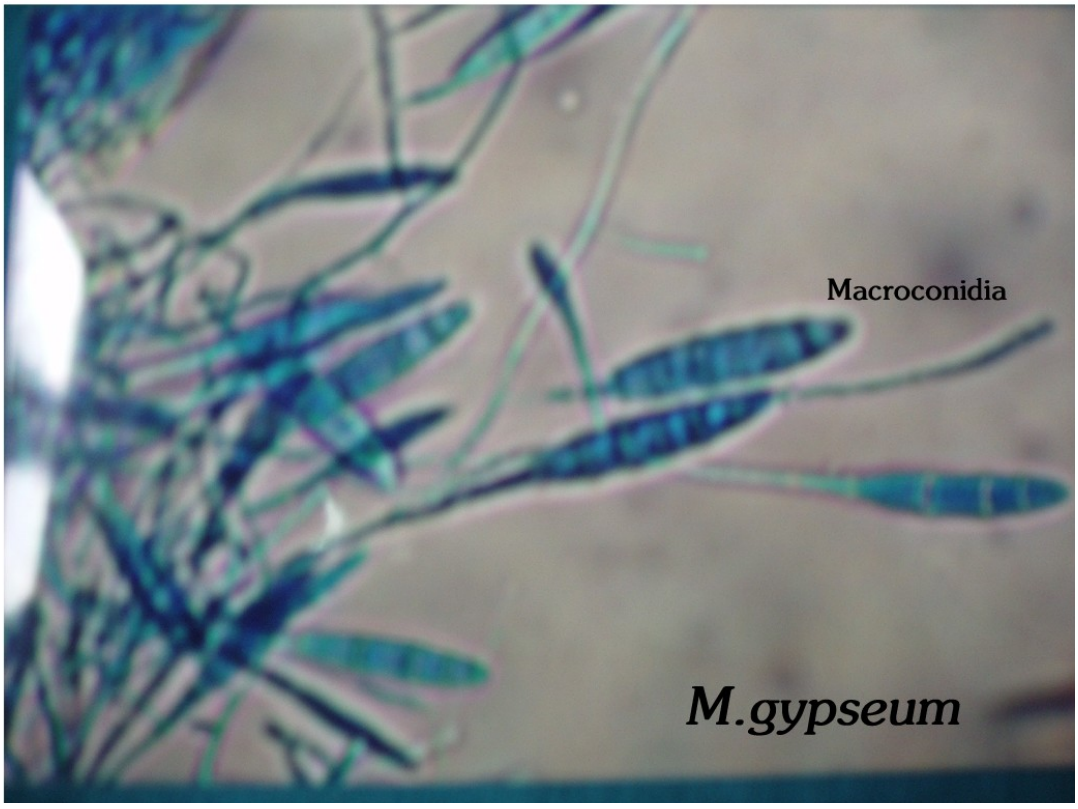
T. tonsurans (LCB 40X)





E. floccosum (LCB 40X)



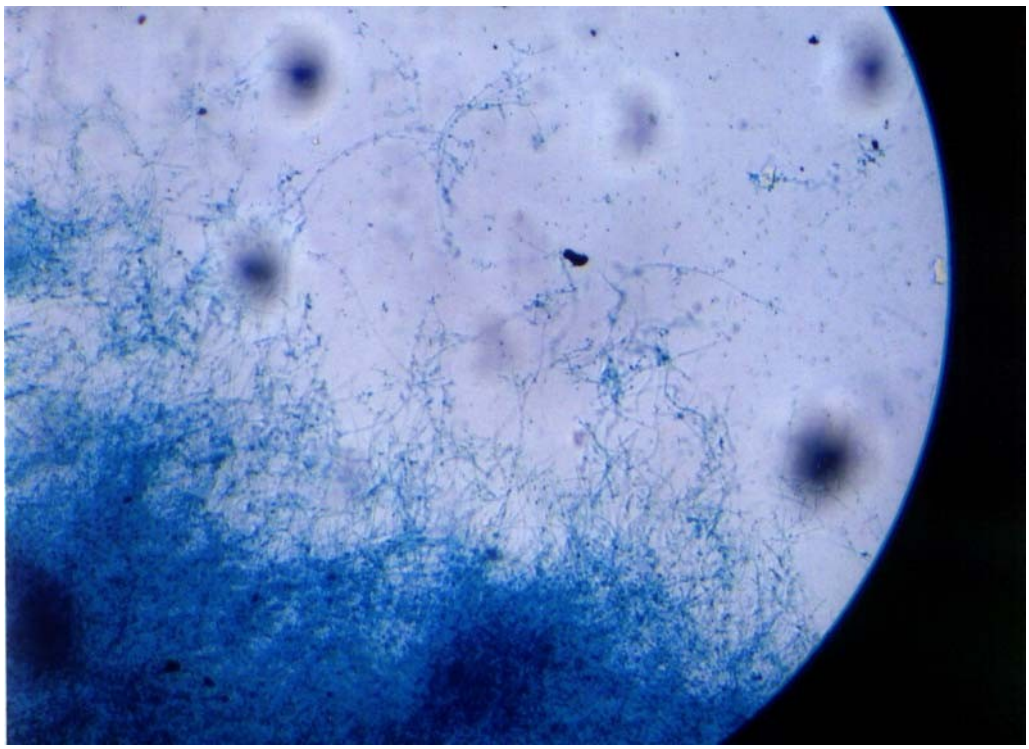


M. gypseum (LCB 40X)


Dermatophyte Identification Medium
Obverse
Reverse

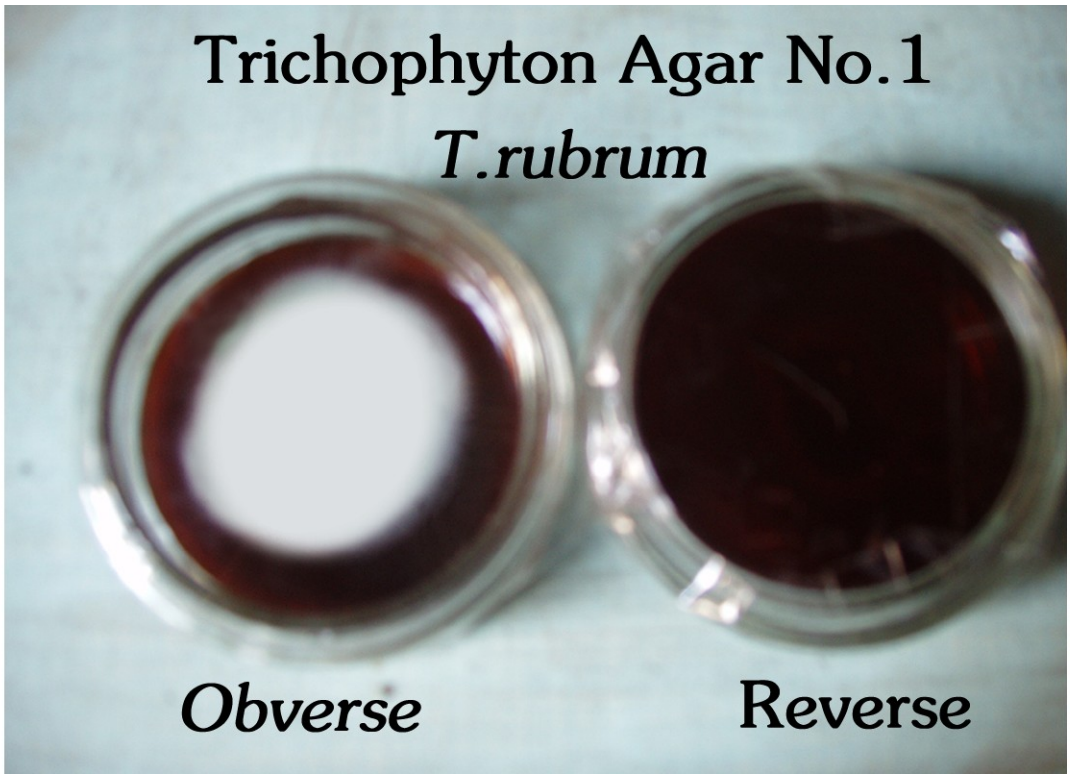

POSITIVE
CONTROL
NEGATIVE

DERMATOPHYTE IDENTIFICATION MEDIUM

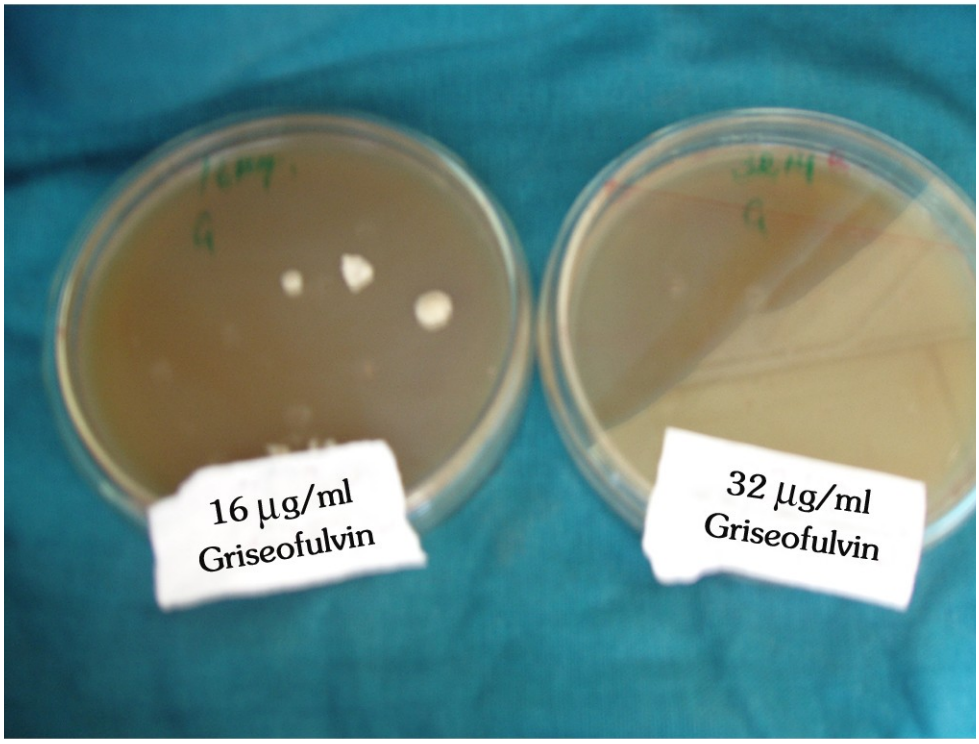


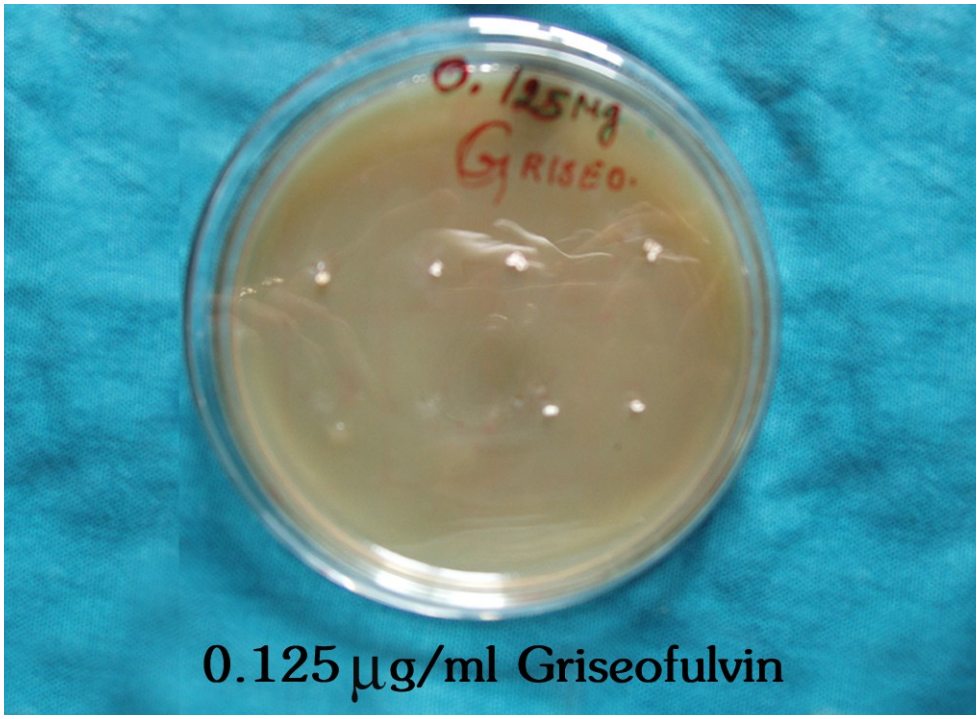
T. rubrum LCB MOUNT 10 X

(FROM DERMATOPHYTE IDENTIFICATION MEDIUM)

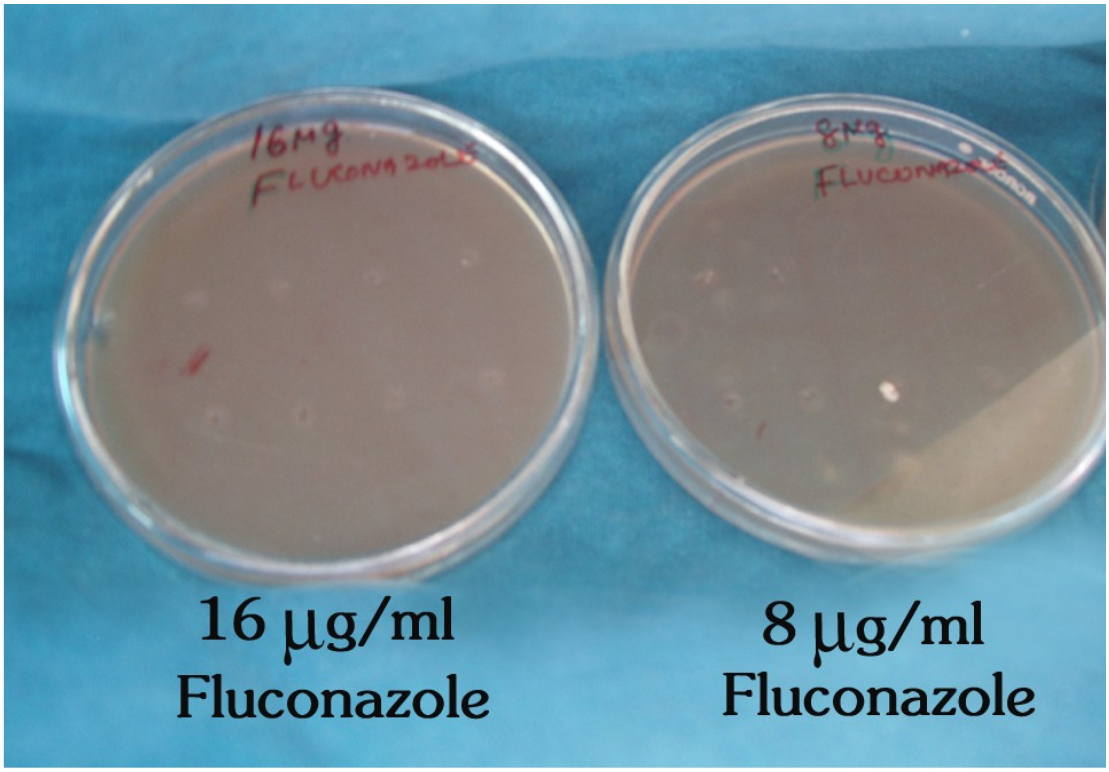


ANTI FUNGAL SUSCEPTIBLITY TESTING





AGAR DILUTION METHOD



FLUCONAZOLE

RESULTS

TABLE – 1
SEX DISTRIBUTION

n=105

SEX	NUMBERS	PERCENTAGE
MALE	60	57.1%
FEMALE	45	42.8%

Out of 105 patients with dermatophyte infection, 60 (57.1%) were males and 45 (42.8%) were females.

TABLE - 2
CLINICAL PATTERN IN DIFFERENT AGE GROUP

n =105

	0-10 years	11-20 years	21-30 years	31-40 years	41-50 years	>50 years	TOTAL
T.corporis	--	10 (13.3%)	27 (36%)	8 (10.6%)	18 (24%)	12 (16%)	75 (71%)
T.unguium	--	2 (25%)	2 (25%)	3 (37.5%)	1 (12.5%)	---	8 (7.6%)
T.cruris	--	4 (57%)	1 (14.2%)	---	1 (14.2%)	1 (14.2%)	7 (6.6%)
T.capitis	7 (100%)	---	----	---	----	---	7 (6.6%)
T.corporis & T.cruris	--	---	----	2 (50%)	2 (50%)	---	4 (3.8%)
T.barbae	---	----	---	1 (50%)	---	1 (50%)	2 (1.9%)
T.manum	--	----	1 (50%)	1 (50%)	----	----	2 (1.9%)
TOTAL	7(6.66%)	16(15.23%)	31(29.52%)	15(14.25%)	22(20.9%)	14(13.3%)	105

The most common affected age group was 21-30 years 31(29.52%), followed by 41-50 years 22 (20.9%), 11-20 years 16 (15.23%), 31-40 years 15 (14.2%), >50 years 14 (13.33%), 0-10 years 7 (6.66%).

TABLE :3

CLINICAL TYPE OF DERMATOPHYTOSIS (SEXWISE DISTRIBUTION)

Clinical Types	Number of case	Male	Female
T.corporis	75 (71%)	42 (56%)	33 (44%)
T.unguium	8 (7.6%)	7 (86%)	1 (12%)
T.cruris	7 (6.6%)	6 (86%)	1 (12%)
T.capitis	7 (6.6%)	1 (15%)	6 (85%)
T.corporis &T.cruris	4 (3.8%)	1 (25%)	3 (75%)
T.barbae	2 (1.9%)	2 (100%)	--
T.manum	2 (1.9%)	1 (50%)	1 (50%)
TOTAL	105	60 (57.1%)	45 (42.8%)

The most common clinical pattern was T.corporis 75 (71%) followed by T.unguium 8 (7.6%), T. capitis 7(6.6%), T.cruris 7 (6.6%), T.corporis & T.cruris 4(3.8%), T.barbae 2 (1.9%), T.mannum 2 (1.9%) Males were predominately affected in T.corporis, T.cruris, T.barbae, T.unguium. Children were only affected in T.capitis.

TABLE:4

PATIENT'S OCCUPATION

n = 105

OCCUPATION	Number (Percentage)
Student	20 (19.4%)
House wife	17 (16.4%)
Security	16 (15.2%)
Agriculturar	11 (10.4%)
Salesman	8 (8.76%)
Coolie	7 (7.66%)
Dobi	6 (5.71%)
Potter	4 (3.8%)
Tailor	4 (3.8%)
Weaver	3 (2.9%)
Mechanic	3 (2.9%)
Horse cleaner	2 (1.9%)
Sweeper	2 (1.9%)
Green Grosser	1 (1%)
TOTAL	105

Among the occupation of the study group, 19.4% were students, followed by House wife 16.4%, Security 15.2%, Agriculturar 10.4%, salesman 8.7%.

TABLE: 5

IMMUNE STATUS OF THE PATIENT

n = 105

Immunocompetent	Immunosuppressed
79 (75.2%)	26 (24.8%)

Out of 105 dermatophytosis patients, 79 (75.2%) were immunocompetent patients and 26 (24.8%) were immunosuppressed patients. (Table 5)

TABLE :6
IMMUNOSUPPRESSIVE ILLNESS IN THE STUDY (105CASES)

Immunosuppressive illness	Number	Acute Dermatophytosis	Chronic Dermatophytosis
Diabetes Mellitus	10 (38.4%)	-	10
Bronchial Asthma	5 (19.2%)	1	4
Renal Transplant with immunosuppressive therapy	3 (11.5%)	1	2
HIV	3 (11.5%)	1	2
Hypothyroidism	2 (7.6%)	1	1
Pulmonary Tuberculosis	1 (3.8%)	-	1
Systemic Lupus Erethematosis on Prednisolone	1 (3.8%)	-	1
Allergic rhinitis on nasal steroid therapy.	1 (3.8%)	1	-
TOTAL	26 (24.8%)	6 (23.07%)	20 (76.9%)

Among the immunosuppressed illness Diabetes Mellitus was the commonest 38.4%, followed by Bronchial Asthma 19.2%, Renal transplant 11.5%. HIV 11.5%, Hypothyroidism 7.6%, Pulmonary Tuberculosis 3.8%, Systemic Lupus Erythematosis 3.8%, Allergic rhinitis (Nasal steroid therapy) 3.8%. All dibetes patients were presented as chronic dermatophytosis and rest of the illness were also predominately presented as chronic dermatophytosis.

TABLE: 7

CLINICAL PATTERN IN ACUTE & CHRONIC DERMATOPHYTOSIS

Clinical Type		Acute Dermatophytosis	Chronic Dermatophytosis
Tinea corporis	75	37 (49.3%)	38 (50.6%)
Tinea unguium	8	4 (50%)	4 (50%)
Tina cruris	7	7 (100%)	--
Tinea capitis	7	7 (100%)	--
Tinea corporis with Tinea cruris	4	2 (50%)	2 (50%)
Tinea manum	2	--	2 (100%)
Tinea barbae	2	2 (100%)	--- --
TOTAL	105	59 (56%)	46 (43%)

In this study acute dermatophytosis were 59(56%) and chronic dermatophytosis were 46(43%). In T.corporis acute and chronic illness was almost equal in ratio. T.cruris and T.capitis presented as acute illness.

TABLE: 8

DETAILS OF DERMATOPHYTE ISOLATES

Dermatophytes	Isolates	No of isolation	Percentage
TRICHOPHYTON	*Trichophyton rubrum*	49	80.3%
	Trichophytonmentagrophytes	4	6.56%
	Trichophyton verrucosum	3	4.99%
	Trichophyton tonsurans	1	1.6%
	Trichophyton simii	1	1.6%
	Trichophyton violaceum	1	1.6%
MICROSPORUM	*Microsporum gypseum*	1	1.6%
EPIDERMOPHYTON	*Epidermophyton floccosum*	1	1.6%
	TOTAL	61	58.1%

Total number of dermatophyte isolates was 61(58.1%). In which *Trichophyton rubrum* was the predominent isolate 49(80.3%) followed by *T.mentrgrophytes* (6.56%), *T.verrucosum* 3 (4.99%), *T.tonsurans* 1(1.6%), *T.simii* 1 (1.6%), *T.violaceum* 1(1.6%) *M.gypseum 1 (1.6%) and E.floccosum 1 (1.6%).*

TABLE:9

DERMATOPHYTES FROM CLINICAL TYPES

Isolates	T.corporis	T.cruris	T.corporis with T.cruris	T.capitis	T.manum	T.ungum	T.barbae	Total
T.rubrum	33 (67.3%)	10 (20.4%)	4 (8%)	1 (2%)	- -	1 (2%)	- - -	49 (76.56%)
T.mentagrophytes	3 (75%)	- -	- -	1 (25%)	- -	- -	- -	4 (6.25%)
T.verrucosum	3 (100%)	- -	- -	- -	- -	- -	- -	3 (4.68%)
T.tonsurans	1 (100%)	- -	- -	- -	- -	- -	- -	1 (1.56%)
T.simii	1 (100%)	- -	- -	- -	- -	- -	- -	1 (1.56%)
T.violaceum	1 (100%)	- -	-- -	1 (100%)	- -		- -	1 (1.56%
M.gypseum	1 (100%)							
E.floccosum	1 (100%)	- -						1(1.56%) 1(1.56%)

The major isolates of *T.rubrum* was from T.corporis 33(67.3%), followed by from cruris 10 (20.4%). *T.mentagrophytes* was mainly isolated from T.corporis 31(75*%). T.verrucosum, T.tonsurans,* and *T.simii, M.gypseum, E.floccosum* were isolatd only from T.corporis*. T.violaceum* was only isolated from T.capitis.(Table 9)

TABLE: 10

DERMATOPHYTE ISOLATES IN ACUTE AND CHRONIC DERMATOPHYTOSIS

Isolates Dermatophytes	Total	Acute Dermatophytosis		Chronic Dermatophytosis	
		Number	Percentage	Number	Percentage
T.rubrum	49	33	67.3%	16	32.6%
T.mentagrophytes	4	2	50%	2	50%
T.verrucosum	3	2	66.6%	1	33.3%
T.tonsurans	1	-		1	100%
T.simii	1	-	-	1	100%
T.violaceum	1	1	100%	-	-
M.gypseum	1			1	100%
E.floccosum	1	1	100%		
	61	39	63.93%	22	36.06%

Dermatophyte Isolation from acute dermatophytosis was 39 (63.93%) compared to chronic dermatophytosis was 22 (36.06%).

TABLE:11

DERMATOPHYTE ISOLATES IN IMMUNOSUPPRESSED ILLNESS

Immunosuppressed illness	***Trichophyton rubrum***	***Trichophyton mentagrophytes***	***Trichophyton tonsurans***	***M.gypseum***
1.Diabetes mellitus 10	6 (C)	2 (C)	1 (C)	1 (C)
2.Renal Transplant 3	3 1(A); 2(C)			
3.Bronchial Asthma 2	2 (C)			
4.SLE 1	1 (C)			
5.Pulmonary TB. 1	1 (C)			
6. Allergic Rhinitis with nasal steroid therapy 1	1 (A)			
TOTAL 18 (29.58%)	14 (77.75%)	2 (11.1%)	1 (3.88%)	1(3.88%)

(A) = = ACUTE ILLNESS (2) (C)==CHRONIC ILLNESS.(16)

Trichophyton rubrum was the commonest isolates in the immunosuppressed illness. In diabetes mellitus, out of 10 isolates, *T.rubrum* was 6, *T.mentagrophytes* was 2; *T.tonsurans*1 and *M.gypseum* 1 were isolated and all were from chronic illness. From rest of the immunosuppressive illness only *T.rubrum* was isolated. The total number of isolates from immunosuppressed illness was 18 (29.58%)

TABLE: 12

COMPARISON OF KOH WET MOUNT, CALCOFLUOR WHITE & CULTURE

n = 60

KOH Positive	Calcofluor White Positive	Culture Positive
41(68%)	34 (56.7%)	34 (56.7%)

Out of 60 suspected cases of dermatophytosis, positivity in KOH was 41(68%), Calcofluor White was 34 (56.7%), and by Culture was 34 (56.7%).

TABLE: 13

CALCULATION OF SENSITIVITY/SPECIFICITY OF KOH WET MOUNT

No=105	Culture Positive 61	Culture Negative 44
KOH Positive 66	51(true positive) (a)	15(false positive) (b)
KOH Negative 39	10(false negative) (c)	39(true negative) (d)

Sensitivity for KOH= (a/a+c) x 100 = (51/51+10) x 100 = 83.6%Specificity for KOH = (d/d+b) x 100 = (39/39+15) x 100 = 72.2%

TABLE: 14

SENSITIVITY AND SPECIFICITY OF CALCOFLUOR WHITE STAINING

	Culture Positive 34	**Culture Negative 26**
CFW Positive 34	34(true positive) (a)	0(false positive) (b)
CFW Negative 26	0(false negative)(c)	26(true negative)(d)

Sensitivity for Calcofluor White Staining = (a/a+c) x 100= 100%.Specificity for Calcofluor Whie Staining = (d/d+b) x 100=100%.

TABLE: 15

COMPARISON OF CULTURE POSITIVITY IN DERMATOPHYTE IDENTIFICATION MEDIUM WITH SABOURAUD'S DEXTROSE AGAR

n=61

Medium	**Culture Positivity**	
	Number	**Percentage**
Sabouraud's Dextrose Agar	61	58.09%
Dermatophyte Identification Medium	51	48.57%%

Out of 105 suspected dermatophytosis patients, Culture positive in Sabouraud's Dextrose Agar was 61 (58.09%); and Dermatophyte Identification Medium culture positive was 51 (48.57%).

TABLE: 16

SENSITIVITY AND SPECIFICITY OF DIM

	Culture Positive 61	**Culture Negative 44**
DIM Positive (Growth & colour change)	51 (true positive) (a)	0 (false positive) (b)
DIM negative	10(false negative) (c)	44 (true negative) (d)

Sensitivity = (a / a +c) x 100 = 83.6%; Specificity = (d / b + d) x 100 = 100%

TABLE: 17

COMPARISON OF GROWTH RATE ON SABOURAUD DEXTROSE AGAR AND DERMATOPHYTE IDENTIFICATION MEDIUM

GROWTH				
Culture positive	**1 st week**	**2 nd week**	**3 rd week**	**4 th week**
SDA 51	1	22	28	-
DIM 51	31	20	--	--

The growth rate of Dermatophyte Identification Medium, after inoculation of the speceimen, 31 isolates were grown with in first week, 20 isolates were grown during second week when compared to Sabouraud Dextrose Agar the growth occur with in first week was one isolate, during the second week 22 isolates were grown and during the third week 28 isolates were grown.

TABLE: 18

MINIMUM INHIBITORY CONCENTRATION OF GRISEOFULVIN

n=20

Isolates	Minimum Inhibitory Concentration µg /ml									
	0.125	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
T.rubrum (16)		14							2	
T.mentagrophytes(2)		1							1	
T.tonsurans (1)	1									
T.simii (1)	1									

The Minimum Inhibitory Concentration of the drug griseofulvin, for two isolates of *T.rubrum* and one isolate of *T.mentagrophytes* the MIC was 32µg/ml. For 14 isolates of *T.rubrum*, one isolate of *T.mentagrophytes the* MIC was 0.25µg/ml, *T.tonsurans, T.simii* it was 0.125µg/ml.

TABLE: 19
MINIMUM INHIBITORY CONCENTRATION OF FLUCONAZOLE

n=20

Isolates	Minimum Inhibitory Concentration µg/ml									
	0.125	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
T.rubrum (16)		15				1				
T.mentagrophytes(2)		1						1		
T.tonsurans(1)		1								
T.simii(1)		1								

The Minimum Inhibitory Concentration of the drug Fluconazole, for one isolate of *T.mentagrophytes*, the MIC was 16µg/ml, one isolate of *T.rubrum* it was 4µg/ml. For 15 isolates of *T, rubrum* and one isolate of *T.mentagrophyte, T.tonsurans*, *T.simii* it was o.125µg/ml. (Table 19)

DISCUSSION

The worldwide prevalence of dermatophytosis is due to advent of Immunosuppressive drugs and diseases, which leads to chronicity, reinfection and spread to the community reinforce the need for regular surveillance of the dermatophytosis and its antifungal susceptibility to antifungal agents commonly used for the therapy.

This study was a descriptive study, one hundred and five cases of clinically suspected/diagnosed Dermatophytosis were subjected to mycological examination. The results obtained are discussed as herewith.

The highest incidence of dermatophytosis was among males 60 (57.1%) when compared to females 45 (42.8%) (Table -1).

This was correlated with the studies of Gupta et al[25] at Ludiana, who reported 70% males, Sumana et al[85] at khammam district found 73%, Mangala et al[42] Davangere, Mohanty et al[47] at Orissa, Peerapur et al[61] at Bijapur , Panda et al[52] at Cuttack, Sen etal[78] at Assam also have shown higher incidence in males.

Higher incidence of the dermatophytosis infection in males are due to increased physical activity and sweating[61].

The commonest clinical pattern of dermatophytosis infection was Tinea corporis 75 (71%) followed by Tinea unguium 8 (7.6%), Tinea cruris 7 (6.6%), Tinea capitis 7 (6.6%) (Table -2).

Sen et al [78](48%) at Assam, Mangala et al[42] (44.5%), Mohanty et al[47] (36.9%) and Gupta et al[25] at Ludiana studied, Tinea corporis to be the commonest clinical presentation. Tinea corporis was most common clinical pattern due to increased itching making them seek medical advice and also trunk including axilla are usually covered with clothes leading to increased sweating, emission of CO_2 (King et al 1978) creating an environment favourable for dermatophytes[89]. Raised carbon dioxide tension is known to facilitate arthroconidial formation and may also aid either adhesion or penetration. Moisture is also important for germination of arthroconidia on keratinocytes[68].

In the present study, the dermatophytosis was the common in the age group of 21-30 years 31(29.52%) followed by 41-50 years 22(20.9%) (Table-2).

Sen et al[78]at Assam 44% and Gupta et al [25]studies at Ludiana, the most common age group was 21-30 years 39%, Mohanty et al[47] studies at Orissa and Sumana et al[85] studies found the commonest age group was 21-30 years.

Tinea corporis incidence was more common in males 42 (56%) compared to female 33 (44%). Tinea corporis, Tinea cruris, Tinea facei, and Tinea unguium infection, males were predominately affected. Tinea capitis was seen only in the age group of 0-10 years children, in which female children were predominately affected (Table 3).

Sen et al[78]studied Tinea corporis was common in males and Gururaj Kumar et al[26] found Tinea capitis was common in children.

The highest incidence of Tinea capitis in children before puberty due to fungistatic effect of long fatty acid in sebum after puberty[26]. This activity appears to reside in saturated fatty acid with chain length of 7, 9, 11, 13 Carbon residue. It has been postulated that their presence on the skin in the post pubertal children may account for the spontaneous resolution of Tinea capitis after this age and rarity of infection in adult[68].

The occupation status of the study group were analysed, of these students were predominent group, then house wife , security agriculturer, potter, dhobi, horse cleaner . Sumana et al studies indicates, prevalence in agriculturer 65% (Table -4)

Lack of hygiene and over crowding are some of the factors responsible for higher incidence of dermatophytosis among the house wife and students[85]. High humidity and increased sweating are factors which causes dermatophytosis among others[85].

Out of 105 dermatophyte infected patients, 79 (75.2%) were immunocompetent 6 (24.8%) were immunosuppressed patients (Table -5).

Among the immunosuppressed illness associated with dermatophytosis, the Diabetes mellitus 10 (38.4%) was predominent followed by Bronchial asthma 5 (19.2%), Renal Transplant 3 (11.5%), HIV 3 (11.5%), Hypothyroidism 2 (7.6%), Pulmonary Tuberculosis1 (3.8%), Systemic Lupus Erythematosis 1 (3.8%), Adenoid polyp1(3.8%). Of these diabetes with dermatophytosis presented as chronic dermatophytosis and rest of the illnesses were also presented predominately as chronic dermatophytois (Table -6).

Depression of cellular immunity may play an important role in Chronicity.[89] Medical condition such as collagen vascular diseases, patients receiving systemic corticosteroid therapy, Cushing's disease, diabetic mellitus, hematological malignancy, atopy, old age HIV positive patients may play a significant roll in predisposing patients to chronic dermatophytic infection[89].

Prasad P VS et al found at Annamalai nagar the factors responsible for chronicity were onychomycosis, body surface area of involvement, prolonged sun exposure and diabetes mellitus[59].

Patients with acute dermatophytosis was 59(56%) and chronic dermatophytosis was 46 (43%). In T.corporis there was almost equal ratio of acute 37 (49.3%) and chronic 38 (50.6%) dermatophytosis.. Tinea cruris, Tinea capitis, Tinea facei were presented as acute dermatophytosis (Table 7).

Overcrowding and poor socio economic status was correlated with chronicity. Factors responsible for chronicity are the site of infection, poor penetration of the drug in the nail keratin, and drug resistance. Some associated conditions are atopic diathesis, disorders of keratinization, diabetes mellitus, Cushing's syndrome, immunosuppression following renal transplants and AIDS[59].

Out of 105 dermatophytosis patients, total number of dermatophyte isolates were 61 (58.09%). Culture negativity could be due to bacterial contamination.[59]. In this study three non dermatophyte isolates Fusarium was identified from T.unguium (Table - 8).

All three species, *Trichophyton, Microsporon, Epidermophyton* were isolated. Of these majority of belong to Trichophyton species. *Trichophyton rubrum* was the predominent isolate 49 (80.3%) followed by *Trichophyton mentagrophytes* 4 (6.55%), *Trichophyton verrucosum 3* (4.99%), *Trichophyton tonsurans* 1 (1.6%), *Trichophyton simii* 1 (1.6%) *Trichophyton violaceum* 1(1.6%). *Microsporum gypseum* 1(1.6%), *Epidermophyton floccosum* 1(1.6%) (Table 8).

The results correlated with that of Sen et al[78] at Assam *T.rubrum* was Commonest 68.63% followed by *T.mentagrophytes* 23.5%. *T.violaceum* 1.96%, *T.tonsurans* 1.96%. Mohanty et al[47] studies at Orissa indicated *T.rubrum* was the commonest 68.34% followed by *T.mentagrophytes* 17.1%. Suruchi Bhagra et al[85] at Shimla studied *T.rubrum* 66.17% was the predominent isolate followed by *T.mentagrophytes* 19.11%, Mangala et al[42] studied *T.rubrum* 65.2%, was the predominent isolate followed by *T.mentagrophytes* 21.2%, a similar studies were found by V.Sumana et al[85] at Khammam district Andhra *T.rubrum* 60%.

The Major Dermatophyte isolates from clinical types, the *T.rubrum* was mainly isolated from Tinea corporis 67.3% and then from Tinea cruris 20.4%. *T.mentagrophytes* mainly isolated from Tinea corporis 75% and then from Tinea capitis 25%. *T.verrucosum, T.tonsurans T. simii M.gypseum, E.floccosum* were isolated from Tinea corporis . *Trichophyton violaceum* which was isolated from Tinea capitis (Table 9).

Sumana et al [85]studied majority of *T. rubrum* isolates were from Tinea corporis 29 (48.3%).

The isolation from acute dermatophytosis was 39(60.93%) compared to chronic dermatophytosis 25 (39.7%). *T. rubrum* isolates from acute dermatophytosis was 33 (67.3%) and from chronic dermatophytosis was 16 (32.6%). *T. tonsurans* and *T.simii*, *M.gypseum* were from chronic dermatophytosis. *T.violaceum and E.floccosum* isolated from acute dermatophytosis (Table 10).

Among these *T.simii*, which was zoophillic, the source of infection was from patient's pet animal dog, from which the same isolate was identified appropriate measure was taken against source of infection and was treated.

T.verrucosum, which was also zoophilic, the source of infection was from patient's working place (race course) that is from horse, appropriate measure was taken and the source of infection was cured.

Among the Dermatophyte isolates from immunosuppressed patients, *T.rubrum* was the predominent isolate (Table - 11).

From Diabetes mellitus out of 10 isolates, *T.rubrum 6, T.mentagrophytes2, T.tonsurans* 1, *M.gypseum* 1 were isolated and all isolates were from chronic dermatophytosis.

Prasad et al[59] observed *Trichophyton rubrum* was the commonest organism in chronic dermatophytosis.

Out of 60 suspected case of dermatophytosis, positivity in KOH wet mount was 68%, in calcofluor white staining was 56.7% and by culture was 56.7%. The sensitivity and specificity of KOH mount was 83.6% and 72.2% respectively (Table - 12).

Haldane et al[28] showed the sensitivity of 88% and the specificity of 95% for KOH wet mount . In KOH wet mount false positive may occur due to fibres of cotton wool, synthetic material starch grain fat droplet vegetable debris, mosaic fungus (network of material including cholestral crystals), which was deposited around the periphery of the keratinised epidermal cells[89].

Both the sensitivity and specificity of Calcofluor white staining was 100% and 100% (Table -14).

Haldane et al[28] showed the sensitivity of Calcofluor white staining was 92% and specificity was 95%.

It is difficult to differentiate hyphae from collagen fibres and other artifacts by conventional KOH wet mount. Therefore Calcoflour White Stain was far superior to the conventional staining techniques for detection fungi in clinical speceimen[32].

Calcofluor binds with chitin and cellulose in fungal cell wall and fluoresces on excitation by longwave UV rays or shortwave visible light[7]. This method has however, the advantage of allowing easier fungal detection with less search time and technical experience (Elders and Roberts). It is technically simple quick and highly reliable to identify fungi even if the observer is relatively inexperienced[32].

Out of 105 suspected cases of dermatophytosis infection, culture positive in Sabouraud Dextrose Agar was 61(58.09%) and in Dermatophyte Identification Medium positive was 51 (48.57%) (Table -15).

The sensitivity and specificity of Dermatophyte Identification Medium were 83.6% and 100% respectively (Table-16).

Salkin et al[70] found sensitivity of dermatophyte Identification Medium was 99% and the specificity was 100%.

In this study, on Dermatophyte Identification Medium, all three isolates of *T.verrucosum* and seven isolates of *T.rubrum* gave false negative as it did not grow on this medium..

The sensitivity of the Dermatophyte Identification Medium indicating the development of dermatophyte through the shift in its colour from greenish yellow to purple. The high specificity of Dermatophyte Identification Medium is due to colony morphology and purple colour change of Dermatophyte Identification Medium, so the colony morphology and colour change of DIM were considered together as criteria in identification process, so the specificity of Dermatophyte Identification Medium rose to 100%. [70]

Dermatophyte Identification Medium uses bromocresol purple as pH indicator, and it has higher concentration of Cycloheximide it has substrate neopeptone, comes from animal protein. All this factors may eliminate the problem of false positive results[23].

The growth rate on Sabouraud Dextrose Agar and Dermatophyte Identification Medium was compared. In Dermatophyte Identification Medium out of 51 isolates grown 31 were grown within first week, 20 were grown within 2nd week compared to Sabouraud Dextrose Agar 1 isolate was grown within first week, 22 isolates were grown during second week, 28 isolates were grown during third week . Growth can be identified earlier in the Dermatophyte Identification Medium (Table -17).

Antifungal susceptibility testing was done for 20 isolates and it was a sample study. The Minimum Inhibitory Concentration (MIC) value of griseofulvin was determined.16 isolates of *T.rubrum* were tested , the MIC was 0.25µg/ml for 14 isolates, and 32µg/ml for 2 isolates In case of *T.mentagrophytes* for 1 isolate MIC was 0 .25µg/ml.and for another isolate MIC was 32µg/ml. *T.tonsurans* MIC of griseofulvin was 0.125µg/ml and for *T .simii* the MIC was 0.125µg/ml (Table -18).

The Minimum Inhibitory Concentration of Fluconazole for 15 isolates of *T. rubrum* was 0.25µg/ml and for one isolate it was 4 µg/ml. For *T.mentagrophytes* one isolate the MIC was 0.25µg/ml and for another one it was16µg/ml. *T.tonsurans* and *T.simii* the MIC was 0.25µg/ml (Table -19).

In this study, one isolate of *T.mentagrophytes* showed higher MIC for both the drugs. 32µg for Griseofulvin and for Fluconazole 16µg/ml, the isolate was from Diabetic Mellitus patient with extensive lesion, and two isolates of T.rubrum had high MIC for griseofulvin(32µg/ml). Rest of the isolates MIC was within limits.

This correlates with Goh cl et al[24] studies, the MIC of griseofulvin were less than 0.25µg/ml. Venugopal et al[91] studies MIC value for griseofulvin were 1 and 5µg/ml. for dermatophytes.

Samina Sayeed et al[73] studied, MIC value of Fluconazole were lower as compared to those for griseofulvin.

SUMMARY

This study was a descriptive study, where 105 samples were processed from clinically suspected dermatophytosis cases. Male patients were 57.1% and female patients were 42.8%.More than 71% of samples were from Tinea corporis. The most common affected age group was 21-30 years 29.5%. In Tinea capitis Children were only affected Patient with acute dermatophytosis were 56% and with chronic dermatophytosis were 43%. 75% were immunocompetent patient and 25% were immunosuppressed patients. Of which Diabetes mellitus was predominent illness 36.4% and presented as chronic dermatophytosis. *Trichophyton rubrum* was the commonest isolate 80%, *T.mentagrophytes* 6.5%, *T.verrucosum* 4.9%, *T.simii*, *T.violaceum, M.gypseum*, *E.floccosum* 1.6% each. *Trichophyton rubrum* mostly isolated from Tinea corporis and both in acute and chronic dermatophytosis. From the acute dermatophytosis of T.capitis *T.violaceum* was isolated. In diabetes *T.rubrum* was the commonest isolates followed by *T.mentagrophytes T.tonsurans* and *M. gypseum*, predominately from chronic dermatophytosis. From the rest of the immunosuppressed patients *T.rubrum* was the commonest isolate. *T.simii,* and *T.verrucossum* (2) isolates from Tinea corporis also were predominately from chronic dermatophytosis. One isolate of *T.mentagrophytes* was from Diabetes Mellitus, had high Minimum Inhibitory Concentration for Griseofulvin 32µg/ml and Fluconazole 16µg/ml and two isolates of T.rubrum had high MIC for griseofulvin 32µg/ml. Rest of the isolates had MIC within limits.

CONCLUSION

- Dermatophtes isolated included predominately *Trichophyton* species, of which *T.rubrum* was the commonest in both acute and chronic dermatophytosis.

- *T.mentagrophytes, T.rubrum, T.tonsurans, T.simii, T.verrucosum*, *M.gypseum* were other isolates from chronic illness.

- Source of infection of *T.simii*, *T.verrucosum* was from patient's pet animal. Source of infection to be monitored especially in zoophilic isolates.

- *T.violaceum* was isolated from T.capitis and *E.floccosum* from T.corporis.
- Calcofluor white is ideal for microscopic examination for detection of fungi in clinical sample even though it is cost effective, accurate diagnosis can be obtained without false positive results and it does not require experienced person for interpretation.

- Dermatophyte Identification Medium is an inexpensive rapid means of presumptively identifying dermatophyte from clinical samples. It is useful in laboratories which has no access to mycological consultation and in large public screening. It is particularly useful in rapidly identifying the dermatophytes, so the antifungal drugs can be started earlier in order to prevent its complication.

- Majority of the dermatophyte isolate had MIC within normal range. Periodic evaluation of Antifungal Susceptiblity Testing is neccessary especially in immuno suppressive illness and in chronic dermatophytosis to find out antifungal resistance.

APPENDIX

1) Preparation of KOH mount:

1.	Potassium hydroxide	10gm
2.	Glycerol	10ml
3.	Distilled water	80ml

KOH crystals were dissolved in distilled water and then glycerol was added to prevent drying and was stored in room temperature.

2) Preparation of Calcofluor White Staining:

1.	Calcoflour White	0.05gm
2.	Evan's Blue	0.02gm
3.	Distilled water	50ml.

All the ingredients were dissolved and mixed well and stored at room temperature at dark place.

3) Preparation of Sabouraud's Dextrose Agar

1.Peptone	10gm.
2.Dextrose	40gm.
3.Agar	20gm.
4.Distilled water	1000ml.

All the ingredients were dissolved by boiling. Cycloheximide 500mg in 10ml of acetone, and gentamicin 20mg in 10 ml of 95% alcohol were added to the boiling medium and mixed well and pH was adjusted to 5.6. Dispensed the medium in the tubes and autoclaved at 121C for 15 minutes.

4) Preparation of Dermatophyte Identification Medium

1.Dextrose	20mg/ml
2.Neopeptone	10mg/ml
3.Agar	20mg/ml

The ingredients were dissolved by boiling. Bromocresol purple was made up as 0.2% Solution 2.5ml of this solution was added to each 100ml of the medium as final concentration of 0.005%, pH was then adjusted to within a range of 5.5to 5.7.Cycloheximide was added 4mg/ml which was dissolved in 10ml acetone and which was then autoclaved at 121*C for 15 minutes. After autoclaving penicillin20units/ml, streptomycin 40units/ml were added and the medium was aseptically dispensed into small tubes. The medium was light green when uninoculated and it was stored at 4 to 8*C .

5) Preparation of Lactophenol Cotton Blue

1.Lactic acid	20ml
2.Phenol crystals	20gm
3.Glycerol	40ml
4.Cotton blue	0.05gm
5.Distilled water	20ml

The phenol crystals were dissolved in the liquids (lactic acid, glycerol, and distilled water) by gentle warming and then cotton blue was added and stored.

6) Preparation of Christensen's Urea Agar

1.	Glucose	5g
2.	Sodium chloride	5g
3.	Potassium dihydrogen phosphate	2g
4.	Peptone	1g
5.	Agar	20g
6.	Distilled water	1lit

The ingredients were dissolved in 1 lit of water by steaming, 6ml of phenol red (0.2% in 50% alcohol) was added. Autoclaved at 115*C for 30 minutes and allowed to cool 60*C and 5ml of urea (40% aqueous filter-sterilized solution) was added to every 100ml of medium. 5ml medium was dispensed aseptically into test tubes and slant was made.

7) Preparation of Trichophyton Agar No 1&4:

59 grams of dehydrated powder was suspended in 1 litre of purified water and was mixed thoroughly, and was heated with frequent agitation and boiled for 1 minute to dissolve the powder completely and then autoclaved for 121^{o}C for 12 minutes and was poured in the plates.

59 grams of dehydrated powder was suspended along with 200µg Thiamine in 1 litre of purified water and was mixed thoroughly, and was heated with frequent agitation and boiled for 1 minute to dissolve the powder completely and then autoclaved for 121°C for 12 minutes and was poured in the plates.

8) Preparation of Yeast Nitrogen Base Agar Medium

1. Yeast Nitrogen Base 6.7gm
2. Glucose 10gm
3. Distilled water 100ml

Dissolved the ingredients and was filter sterilized. Appropriate drug concentration were added and poured in petri dishes.

9) PROFORMA:

Name, Age, Sex, Op No, Lab No,

Occupation

Duration of the illness < Acute (less than one year duration)
Chronic (more than one year duration)

H/O Recurrence

Family History

Previous same illness & Treatment

H/O Contact with pet animal.

Immunosuppressive illness.

Diabetes mellitus

Bronchial Asthma

Renal Transplant

HIV

Hypothyroidism

Others

Site of the lesion

Type and percentage of lesion

Erythematous Lesion

Non-Erythematous

Scaly lesion

ID reaction.

Clinical Diagnosis

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