
**“ISOLATION, IDENTIFICATION AND ANTIFUNGAL
SUSCEPTIBILITY OF DERMATOPHYTES FROM
CLINICAL SAMPLES – ONE YEAR STUDY”**

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**KLE UNIVERSITY BELGAUM,
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LIST OF ABBREVIATION

ATCC	American Type Culture Collection
NCCLS	National Committee for Clinical Laboratory Standards
CLSI	Clinical and Laboratory Standards Institute
Ti	Tinea
Spp	Species
KOH	Potassium hydroxide
PDA	Potato dextrose agar
SDA	Sabouraud Dextrose Agar
LCB	Lactophenol cotton blue
KET	Ketoconazole
SER	Sertaconazole
CLO	Clotrimazole
TER	Terbinafine
MIC	Minimum inhibitory concentration

ABSTRACT

INTRODUCTION:

Infection of the keratinized tissues (hair, skin & nail) in man and animals by keratinophilic fungi (dermatophytes) results in dermatophytosis. Dermatophytes are a group of closely related keratinophilic fungi that can invade keratinized tissue of humans and animals such as skin, hair and nails causing dermatophytosis. Dermatophytes consist of three genera namely *Trichophyton*, *Microsporum*, and *Epidermophyton*. Worldwide the most common cause of tinea pedis, tinea unguium (onychomycosis), tinea cruris, tinea manuum, tinea corporis, and tinea faciei is *Trichophyton rubrum*.

AIMS AND OBJECTIVES:

- To isolate and identify the fungi causing dermatophytosis.
- To perform antifungal susceptibility testing of the fungal isolates by microbroth dilution method.

MATERIAL AND METHODS:

The study was a cross sectional study. All clinically diagnosed cases of Dermatophytosis attending Out Patient department of Dermatology, Venerology and Leprosy of Dr. Prabhakar Kore Charitable hospital was included in the study and those who were already on treatment for Dermatophytosis were excluded from the study.

100 samples of skin hair and nail were collected and subjected to KOH. Culture was done on SDA with and without antibiotics. Isolates were identified by

morphology, LCB preparation and slide culture. Antifungal susceptibility by microbroth dilution method was carried out to determine the MIC values for Ketoconazole, Sertaconazole, Terbinafine and Clotrimazole.

RESULTS:

In our study 100 (skin-77, nail-20 and hair-3) samples were included out of which 63 samples were KOH positive and 37 were KOH negative. 88 samples were culture positive and 22 were culture negative. *T. rubrum* was the commonest fungus isolated (33) followed by *T. mentagrophytes* (20), *T. tonsurans* (8) and *M. gypseum* (2). Other isolates included *Acremonium* species, *Curvularia* species and *Penicillium* species (17). MIC of *T. rubrum* against Terbinafine was (0.03-0.5µg/ml), Clorimazole (0.03-0.62µg/ml), Sertaconazole (0.03-0.62µg/ml), Ketoconazole (0.03-4µg/ml).

CONCLUSION

In our study *T. rubrum* was the commonest dermatophyte isolated.

MIC of *T. rubrum* against Terbinafine was (0.03-0.5µg/ml), Clorimazole (0.03-0.62µg/ml), Sertaconazole (0.03-0.62µg/ml), Ketoconazole (0.03-4µg/ml). The other isolated species had comparable MIC. Susceptibility of *T. mentagrophytes* and *T. tonsurans* against Ketoconazole was in the higher range.

Periodic evaluation of Antifungal Susceptibility Testing is necessary to know the MIC range of prevailing strains against commonly used drugs so as to guide the empirical treatment of dermatophytosis.

KEY WORDS : Dermatophytosis, KOH, *T. rubrum*, MIC

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INTRODUCTION

Dermatophytes are keratinophilic and keratinolytic fungi. They are responsible for dermatophytosis which are superficial mycosis affecting skin (examples for dermatophytosis include, *Ti. corporis*, *Ti. cruris*, *Ti. pedis*). Hair (*Ti. capitis*), beard (*Ti. barbae*); nails (onychomycosis or *Ti. unguium*). Dermatophytosis is a nonfatal disease except in extremely rare cases of Hadida and Schousboe's dermatophytic disease.¹

Infection generally involves skin and is restricted to the cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts.² Reactions to a dermatophyte infection may be a consequence of the host's reaction to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and due to local environmental factors.

Dermatophytes are a group of closely related organisms that can use keratin as a nitrogen source. On the basis of clinical, morphologic and microscopic characteristics three genera of Dermatophytes are recognized as *Trichophyton*, *Microsporum* and *Epidermophyton*.

Dermatophytes and their congeners have long been divided into anthropophilic, zoophilic, and geophilic species on the basis of their primary habitat associations³. Anthropophilic Dermatophytes are primarily associated with humans and rarely infect other animals⁴. Zoophilic Dermatophytes usually infect animals or are associated with animals but can occasionally infect humans. Geophilic Dermatophytes are primarily associated with keratinous materials such as hair,

feathers, hooves, and horns after these materials have been dissociated from living animals and are in the process of decomposition. These species may cause human and animal infection. Geophilic species are thought to have been ancestral to the pathogenic Dermatophytes, preadapted to cutaneous pathogenesis by their ability to decompose keratin and their consequent close association with animals living in hair and feather-lined nests in contact with soil⁵.

The clinical manifestations of Dermatophytes may resemble some other skin diseases such as Pityriasis roseae, Eczema, Lichen planus.⁶ Contact dermatitis may resemble T.corporis⁶. An understanding of predisposing / aggravating factors can give an idea as to how to avert the disease. The pathogenic potential is dependent on a 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, and endocrine disorders such as Cushing's disease can lead to atypical generalized invasive dermatophyte infection. Early identification and treatment is essential, as once infection is established, the individual becomes a carrier and is more susceptible to recurrence⁷.

Because of difficulty in clinically differentiating dermatophytosis from other non-mycotic dermatosis, particularly in the dystrophic nails, it is important to establish biologically an accurate diagnosis. A definitive diagnosis of dermatophyte infection needs to be done before the initiation of antifungal therapy because of the long duration of the treatment, its high cost, and the potential side effects of the drugs. Knowledge of the zoophilic or anthropophilic origin of the dermatophyte may allow setting up prophylactic measures¹.

Any clinical diagnosis needs 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 depends upon the specific identification of the invasive mold⁸. 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 infections. 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 dermatophytosis, it is essential to establish the diagnosis of the disease, so that specific therapeutic modalities can be monitored during the course of the treatment.⁸

Rapid identification of dermatophyte species and knowledge of their host preference and ecology play an important role in epidemiology, public health and infection control. The varied clinical presentation of Ti., which results in delay in diagnosis, poor compliance in follow up of cases, and consequently spread of infection in the community, has rekindled interest in identification of Dermatophytes⁷.

Now effective drugs are available for chemotherapy of fungal infections and further modalities are being developed for management of the same. The development of the National Committee for Clinical Laboratory Standards (NCCLS) reference method M38A in the 1990s has improved the reproducibility of in vitro anti-fungal susceptibility test data and facilitated the establishment of interpretive break points for the azoles.

Standardization of in vitro susceptibility testing provides consistent and reproducible data that may predict clinical response when used in conjunction with individual patient risk factors. An ideal antifungal drug should have broad spectrum

activity, should be effective in vivo, minimum side effects and there should be no drug resistance and minimum side effects.

There is always need to know the epidemiology of prevailing species in an area, as it would guide clinicians in treating dermatophytoses. Dermatophytoses are recurrent infections, and patients are not compliant to the long term treatment. This would present as a resistant infection. Clinician usually changes the drug therapy, without knowing the susceptibility pattern of the isolate. Dilemma of whether the infection encountered is resistant infection or recurrent infection is to be resolved through laboratory investigation. Thus the present study was undertaken to know the prevailing Dermatophytes in our area and their antifungal susceptibility pattern.

AIMS AND OBJECTIVES

1. To isolate and identify the fungi causing dermatophytosis.
2. To perform antifungal susceptibility testing of the fungal isolates by microbroth dilution method.

REVIEW OF LITERATURE

HISTORY

Historically, medical mycology, began with the discovery of the fungal etiology of favus and centered around three European physicians in the mid-19th century: Robert Remak, Johann L. Schonlein, and David Gruby.

Remak in 1835 first observed peculiar microscopic structures appearing as rods and buds in crusts from favic lesions. He never published his observations, but he permitted those observations to be cited in a doctoral dissertation by Xavier Hube in 1837. Remak claimed that he did not recognize the structures as fungal and credited this recognition to Schonlein, who described their mycotic nature in 1839. However, Remak established that the etiologic agent of favus was infectious, cultured it on potato slices, and validly described it as *Achorion schoenleinii*, in honor of his mentor and his initial discovery⁸.

The real founder of dermatomycology was David Gruby on the basis of his discoveries during 1841 to 1844, his communications to the French Academy of Science, and his publications during this period¹⁰. Independently, and unaware of the work of Remak and Schonlein, he described the causative agent of favus, both clinically and in microscopic details of the crusts, and established the contagious nature of the disease¹⁰.

Raymond Sabouraud, one of the best known and most influential of the early medical mycologists, began his scientific studies of the Dermatophytes around 1890, culminating in the publication of his classic volume, *Les Teignes*, in 1910¹¹. Sabouraud's contributions included his studies on the taxonomy, morphology, and methods of culturing the Dermatophytes and the therapy of the dermatophytoses. He

classified the Dermatophytes into four genera, *Achorion*, *Epidermophyton*, *Microsporum*, and *Trichophyton*, primarily on the basis of the clinical aspects of the disease, combined with cultural and microscopic observations. The medium that he developed, is in use today for culturing fungi (although the ingredients are modified) and is named in his honor, Sabouraud glucose (dextrose) agar¹². Sabouraud's treatment of *Ti. capitis* by one-dose, single-point roentgenologic epilation achieved cure in 3 months as opposed to the then current therapy of manual epilation and topical application of medications.

In 1934, Chester Emmons¹³ modernized the taxonomic scheme of Sabouraud and others and established the current classification of the Dermatophytes on the bases of spore morphology and accessory organs. He eliminated the genus *Achorion* and recognized only three genera *Microsporum*, *Trichophyton*, and *Epidermophyton*.

Nutritional and physiological studies of the Dermatophytes pioneered at Columbia University by Rhoda Benham and Margarita Silva and at the Center for Disease Control, in Georgia, by Libero Ajello, Lucille K. Georg, and coworkers simplified the identification of Dermatophytes and led to reduction of the number of species and varieties.

The discovery of sexual reproduction in the Dermatophytes opened the door to classical genetic studies with these fungi, e.g., determining the cause of pleomorphism and clarifying the taxonomy and understanding of the incompatibility systems operating in these fungi.

The successful oral therapy with griseofulvin of experimental dermatophytosis in guinea pigs reported by Gentles in 1958 revolutionized the therapy of

dermatophytosis and initiated the first major change in the therapy of *Ti. capitis* since the work of Sabouraud.

In 1980's azole derivatives were discovered which further revolutionized treatment of dermatophytosis.

ANATOMY OF SKIN

The skin covers the entire external surface of the body.¹⁴ Skin is the largest organ of the body and is composed of three layers

- 1) Epidermis
- 2) Dermis
- 3) Subcutaneous tissue

Epidermis:

The epidermis, the outermost layer is formed by an ordered arrangement of cells called keratinocytes which synthesize keratin.¹⁴

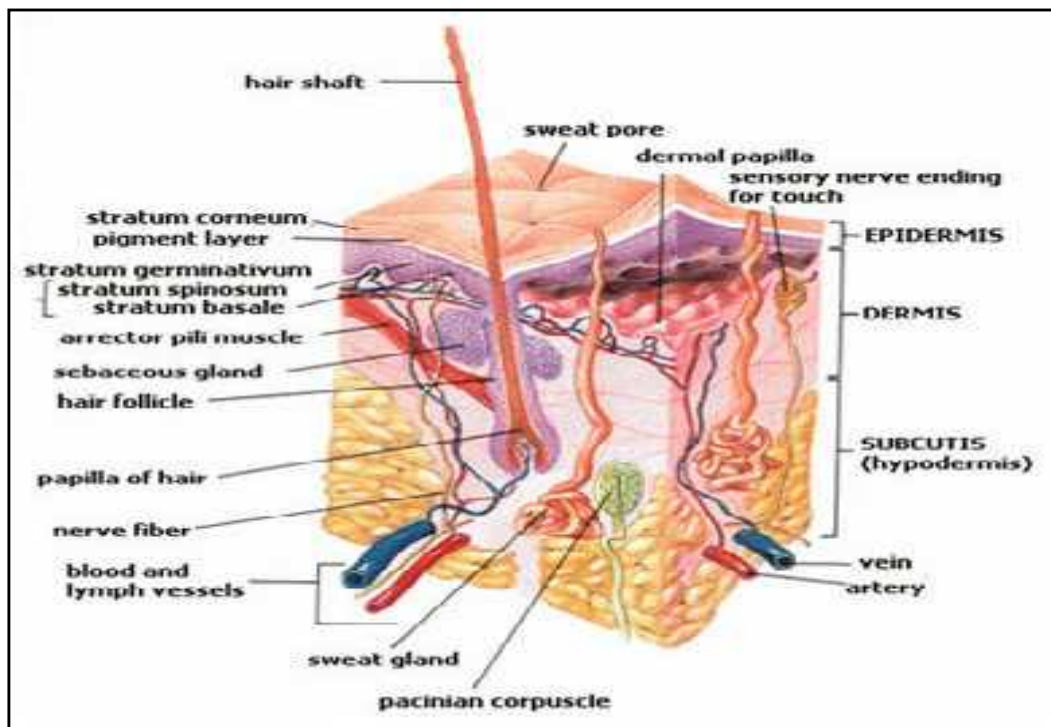


Figure No.1: Skin anatomy

Dermis:

This layer consists of irregular, moderately dense connective tissue. It has a matrix composed of an interwoven, collagenous and elastic network in an amorphous ground substance of glycosaminoglycans, glycoproteins and bound water which accommodates nerves, blood vessels, lymphatics, epidermal appendages and a changing population of cells.¹⁴

Appendages of the skin:

Hair:

Hairs develop from cells lining the deep invaginations of the epidermis called hair follicles. An active hair follicle has a terminal expansion called the hair bulb which has a deep recess on its underside occupied by a papilla of dermal connective tissue. A mass of cell in the interior of the bulb around the papilla constitutes the cell matrix whose proliferation and differentiation gives rise to the hair.¹⁵

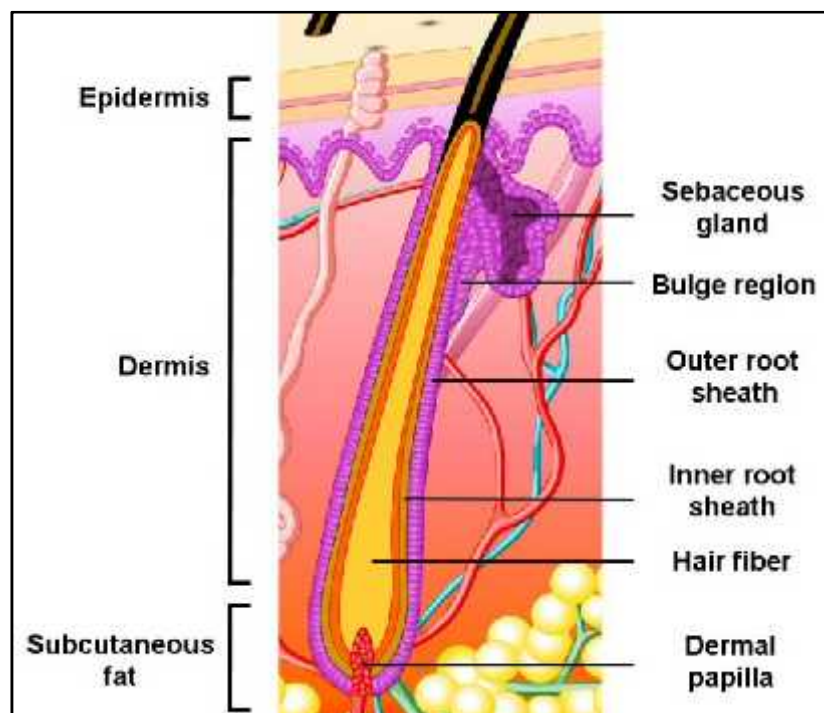


Figure No. 2: Pilosebaceous unit

Nail:

Nails are homologous with the cornified layer of the epidermis. They contain anucleated, keratin filled squames in 2 or 3 horizontal rows. Nail plate originates from nail matrix. The matrix epithelium consists of basal and prickle cell layer keratinocytes along with few melanocytes and langerhan cells.¹⁵

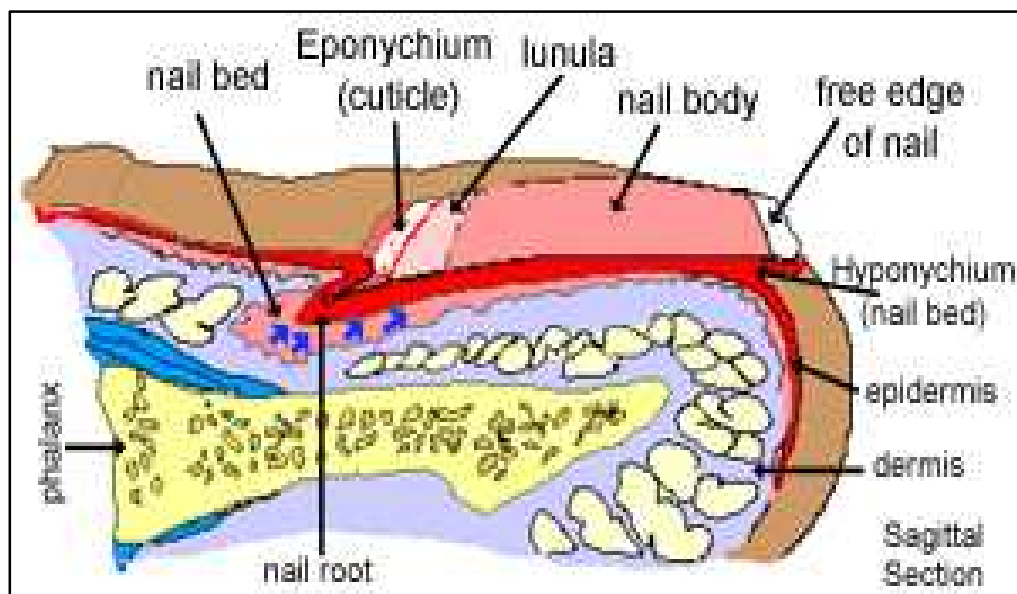


Figure No. 3: Anatomy of nail

Taxonomy and classification¹⁶:

The Dermatophytes are hyaline septate molds that infect the keratinized tissue with more than hundred species described. These are divided into three main anamorphic genera depending on their mycological characteristics:

- 1) *Trichophyton*
- 2) *Microsporum*
- 3) *Epidermophyton*

Table 1: Classification of Dermatophyte based on ecology and host preference

Geophilic	Zoophilic	Anthropophilic
<i>M. cookei</i>	<i>M. canis</i>	<i>E. floccosum</i>
<i>M. gypseum</i>	<i>M. equinum</i>	<i>M. audouinii</i>
<i>M. nanum</i>	<i>M. gallinae</i>	<i>T. schoenleinii</i>
<i>M. persicolor</i>	<i>T. equinum</i>	<i>T. mentagrophytes</i>
<i>M. praecox</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
<i>T. ajelloi</i>	<i>T. verrucosum</i>	<i>T. tonsurans</i>
<i>T. simii</i>		<i>T. violaceum</i>
		<i>M. ferrugineum</i>
		<i>T. concentricum</i>
		<i>T. gourvillii</i>
		<i>T. soudanense</i>
		<i>T. megninii</i>
		<i>T. yaoundei</i>

The *Trichophyton species* usually infects skin, nails and hairs. *Microsporum* species infect skin and hair but not the nails and *Epidermophyton* species infect skin as well as nails but not hair.

Trichophyton sp.

The genus *Trichophyton* is characterized by the development of smooth-walled micro- as well as macroconidia. Macroconidia are mostly borne directly on the hyphae or on pedicels, and are thin- or thick-walled, ranging from 4-8 × 20-50 µm in size. They may be few or absent in many species. Microconidia are pyriform, spherical, clavate or of irregular shape and range from 2 - 3 by 2 - 4 µm in size. The different species are named below

- *Trichophyton ajello,*
- *Trichophyton concentricum*
- *Trichophyton equinum*
- *Trichophyton mentagrophytes var. nodular*
- *Trichophyton mentagrophytes var. erinacei*
- *Trichophyton mentagrophytes var. interdigitale*
- *Trichophyton mentagrophytes var. mentagrophytes*
- *Trichophyton mentagrophytes var. quinckeanum*
- *Trichophyton rubrum, Trichophyton rubrum downy strain*
- *Trichophyton rubrum granular strain*
- *Trichophyton schoenleinii*
- *Trichophyton Soudanense*¹⁷

Trichophyton mentagrophytes

On Sabouraud's dextrose agar, surface may be buffy and powdery or white and downy. It may become pinkish or yellowish. Powdery form may have concentric and radial folds. Colonies rapidly develop a dense fluff. There may be little or no conidiation Reverse is usually brownish tan but sometimes may be colorless, red or yellow.

Microscopy: Coiled, septate spiral hyphae are seen. Microconidia in powdery cultures are very round and clustered on branched conidiophores. In fluffy cultures microconidia are tear shaped, smaller and fewer. Macroconidia (4-8×20-50µm) cigar shaped and smooth thin walled with narrow attachments to hyphae contain 1 to 6 cells more commonly seen in young cultures.

Hair Perforation Test: Positive within 14 days¹⁸.

Clinical significance:

T.mentagrophytes var. *mentagrophytes* is the zoophilic form of *T. mentagrophytes* which is distributed worldwide and a wide range of animal hosts including mice, kangaroos, cats, horses, rabbits and sheeps. It may produce inflammatory skin or scalp lesions in humans, particularly in rural workers. Kerion of the scalp may occur. Invaded hairs show an ectothrix infection but they do not fluoresce under Wood's light.

Trichophyton rubrum

Surface is granular or fluffy, buff to white. Reverse is deep red or purplish, sometimes it may be brown, yellow orange or even colorless.

Microscopy: Septate hyphae, tear shaped microconidia (2-3.5×3-5.5µm) usually form singly all along the sides of the hyphae. Macroconidia (4-8×40-60µm) are long thin walled with parallel sides and have 4-10 cells. The macroconidia may or may not have terminal appendages.

Clinical significance:

Trichophyton rubrum is an anthropophilic dermatophyte. The downy strain known to be most widely distributed dermatophyte of man. It commonly causes chronic infections of skin, nails and sometimes of scalp. The granular strain is a common cause of *Ti. corporis*.

Trichophyton schoenleinii

On Sabouraud's dextrose agar, colonies are slow growing, waxy with a deeply folded honey-comb-like thallus and some sub-surface growth. The thallus is cream colored to yellow to orange brown. Cultures are difficult to maintain in their typical convoluted form, and rapidly become flat and downy. Reverse is colorless or pale yellowish orange to tan¹⁹.

Microscopy: Hyphae are septate highly irregular and knobby. The subsurface hyphae usually form characteristic antler like branching structures commonly called "favic chandeliers" they have swollen tips that resemble nail heads. Chlamydoconidia are numerous. Microconidia and macroconidia are absent. A few distorted clavate microconidia may be formed by some isolates when grown on polished rice grains¹⁹.

Clinical significance:

Trichophyton schoenleinii is an anthropophilic fungus causing favus in humans. Favus is a chronic, scarring form of *Ti. capitis* characterized by saucer-shaped crusted lesions or scutula and permanent hair loss.

Trichophyton tonsurans

In Sabouraud's dextrose agar, colonies show considerable variation in texture and colour. They may be suede-like to powdery, flat with a raised center or folded, often with radial grooves. The colour may vary from pale-buff to yellow, the reverse colour varies from yellow-brown to reddish-brown to deep mahogany.

Microscopy: Hyphae are relatively broad, irregular, with numerous septa. Numerous characteristic microconidia varying in size and shape from long clavate to broad pyriform, are borne at right angles to the hyphae, which often remain unstained by lactophenol cotton blue. Numerous swollen giant forms of microconidia and intercalary chlamydoconidia are produced in older cultures. Macroconidia are rare irregular in form and bit thick walled.

Hydrolysis of Urea: positive at 5 days.

Hair Perforation Test: positive within 14 days¹⁹.

Clinical significance:

Trichophyton tonsurans is an anthropophilic fungus with a worldwide distribution which causes inflammatory or chronic non-inflammatory scaling lesions of skin, nails and scalp. Invaded hairs show an endothrix infection and do not show fluorescence under Wood's light.

Trichophyton verrucosum

On Sabouraud's dextrose agar, colonies are slow growing, small, button-or-disk shaped, white to cream coloured, with a suede-like to velvety surface, a raised center, and flat periphery with some submerged growth. Reverse pigment may vary from non-pigmented to yellow.

Microscopy: Broad, irregular hyphae with many terminal as well as intercalary chlamydospores. The tips of some hyphae are broad and club-shaped, and occasionally divided, giving "antler" effect. When grown on thiamine-enriched media, occasional strains produce clavate to pyriform microconidia borne singly along the hyphae.

Growth at 37⁰C: Unlike other Dermatophytes growth is enhanced at 37⁰C

Clinical significance:

Trichophyton verrucosum is a zoophilic fungus causing ringworm in cattle. Infections in humans result from direct contact with diseased cattle or fomites and are usually highly inflammatory involving the scalp, beard or exposed areas of the body (nails, skin). Invaded hairs show fluorescence under Wood's ultraviolet light in cattle but not in humans.

***Trichophyton violaceum*¹⁹**

On Sabouraud's dextrose agar, colonies are very slow growing, heaped and folded and a deep violet in colour. Cultures may become pleomorphic, forming white sectors and at times non-pigmented strains may occur.

Microscopy: Hyphae are relatively broad, much branched and distorted. Young hyphae usually stain well in lactophenol cotton blue, whereas older hyphae stain

poorly and show small central fat globules and granules. Conidia are usually not seen, Numerous chlamydoconidia are usually present.

Clinical significance:

Trichophyton violaceum is an anthropophilic fungus causing inflammatory or chronic non-inflammatory scaling lesions of skin, nails, beard and scalp, producing the so called "black dot" *Ti. capitis*. Invaded hairs show an endothrix infection.

Microsporum sp.

Microsporum species form both macro- and microconidia on short conidiophores. Macroconidia are hyaline, multiseptate, variable in form, fusiform, spindle-shaped to obovate, ranging from 7-20 × 30-160 µm in size, with thin- or thick- echinulate to verrucose cell walls. Their shape, size and cell wall features are important characteristics for species identification. Microconidia are hyaline, single-celled, and pyriform to clavate, smooth walled, 2.5-3.5 × 4-7 µm in size and are not diagnostic for any one species. Seventeen species of *Microsporum* have been described, however only the more common species are included in these descriptions. *Microsporum audouinii*, *Microsporum canis*, *Microsorumcanis var. distortum*, *Microsporum cookei*, *Microsporum equinum*, *Microsporum ferrugineum*, *Microsporum fulvum*, *Microsporum gallinae*, *Microsporum gypseum*, *Microsporumnanum*, *Microsporum persicolor*.

Clinical significance:

The genus *Microsporum* contains a number of important species that are the principle causative agents of animal and human dermatophytoses [*Ti.* and ringworm].¹³

Microsporum gypseum

On Sabouraud's dextrose agar, colonies are usually flat, spreading, suede-like to granular, with a deep cream to tawny-buff to pale cinnamon coloured red surface. Many cultures develop a central white downy umbo (dome) or a fluffy white tuft of mycelium and some also have a narrow white peripheral boarder. A yellow-brown pigment, often with a central darker brown spot, is usually produced on the reverse, however a reddish-brown reverse pigment may be present in some strains.

Microscopy: Cultures produce abundant, symmetrical, ellipsoidal, thin-walled, verrucose, 4-6 celled macroconidia. The terminal or distal ends of most macroconidia are slightly rounded, while the proximal ends (point of attachment to hyphae) are truncate. Numerous clavate shaped microconidia are also present, but these are not diagnostic.

Clinical significance:

Microsporum gypseum is a geophilic fungus with a world-wide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather. Usually produces a single inflammatory skin or scalp lesion. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light.

Microsporum canis

On Sabouraud's dextrose agar, colonies are flat, spreading, and white to cream-coloured, with a dense cottony surface which may show some radial grooves. Colonies usually have a bright golden yellow to brownish yellow reverse pigment, but non-pigmented strains may also occur.

Microscopy: Macroconidia are typically spindle-shaped with 5-15 cells, verrucose, thick walled and often have a terminal knob. A few pyriform to clavate microconidia are also present. Macroconidia and/or microconidia are often not produced on primary isolation media and it is recommended that sub-cultures be made onto boiled polished rice grains to stimulate sporulation. Hair Perforation Test: Positive at 14 days.

Clinical significance:

Microsporum canis is a zoophilic dermatophyte of world-wide distribution which is a frequent cause of ringworm in humans, especially children. Invades hair, skin and rarely nails. Cats and dogs are the main sources of infection. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light¹⁴.

Microsporum nanum

Colonies are flat, cream to buff in colour with a suede-like to powdery surface texture. Young colonies have a brownish-orange pigment which deepens into a dark reddish-brown with age.

Microscopy: Cultures produce numerous small ovoid to pyriform macroconidia with 1-3 cells, but mostly 2 cells, with relatively thin, finely echinulate (rough) walls, and broad truncate bases. Many macroconidia are borne on conidiophores (stalks) which do not stain readily. Occasional clavate microconidia are present, which distinguishes *M.nanum* from some species of *Chrysosporium*.

Clinical significance:

Microsporum nanum is a geophilic and zoophilic fungus frequently causing chronic non-inflammatory lesions in pigs and a rare cause of T. in humans. Also

present in soil of pig-yards. Infections in man usually contacted directly from pig or fomites. Invaded hairs may show a sparse ectothrix or endothrix infection but do not fluoresce under Wood's ultraviolet light.

Epidermophyton floccosum

On Sabouraud's dextrose agar colonies are usually slow growing, greenish-brown or khaki coloured with a suede-like surface, raised and folded in the center, with a flat periphery and submerged fringe of growth. Older cultures may develop white pleomorphic tufts of mycelium. A deep yellowish-brown reverse pigment is usually present.

Microscopy: Smooth, thin-walled macroconidia which are often produced in clusters growing directly from the hyphae. Numerous chlamydoconidia are formed in older cultures. No microconidia are formed²⁰.

Clinical significance:

Epidermophyton floccosum is an anthropophilic dermatophyte with a world-wide distribution which often causes wide range of dermatophytosis. It is not known to invade hair. *E. floccosum* infections may become epidemic among personnel using common shower or gym facilities, e.g. athletic teams, troops, ship crews and inmates of institutions.

EPIDEMIOLOGY AND ECOLOGY

Ecological Differentiation:

Dermatophytes are broadly classified into three groups on the basis of their natural habitats and host preferences.²¹

1. Anthrophilic species almost exclusively infect humans; animals are rarely infected. They are usually transmitted either directly through close human contact or indirectly through sharing of clothes, towels or combs. E.g., *T.mentagrophytes*, *T.rubrum*, *M.audouinii*.
2. Geophilic species are soil associated organisms and soil per se or soil borne keratinaceous debris is a source of infection for humans as well as other animals. E.g., *M.gypseum*, *M.racemosum*, *T.vanbreuseghemii*.
3. Zoophilic species are essentially pathogens of nonhuman mammals or rarely birds; however, animal to human transmission is not uncommon. E.g., *M.canis*, *M. gallinae*, *T.equinum*.

Epidemiology:

According to the World Health Organization (WHO), Dermatophytes affect about 25% of the world population.²² *Ti. capitis* is a condition most commonly seen in children.²³ Contact sports may distribute the disease among adolescents and young adults. It is estimated that 30 to 70% of adults are asymptomatic hosts of these pathogens and that the incidence of the disease increases with age. Risk factors associated with onychomycosis are age, abnormalities in nail morphology, genetic factors, poor hygienic conditions, diabetes mellitus, and immunodeficiency²⁴.

Source of Infection²²:

Ringworm is transmitted indirectly via loose hairs and desquamated epithelium. Use of contaminated barber shop instruments, combs, hair brushes and hats is a common method of transmitting *Ti. capitis*.

The major source of *Ti. pedis* is the contaminated floors of homes or public places such as bathing facilities. *T.concentricum* is transmitted from diseased mother to child soon after birth.

Humans are infected by zoophilic Dermatophytes by coming in direct or indirect contact with various domesticated animals. Rats or field mice were known to be source of outbreak of dermatophytosis. Source of human infection due to *M.gallinae* and *M.nanum* have been shown to be chickens and pigs respectively. Soil is the source of various *Microsporum* and *Trichophyton* species²⁴.

PATHOGENESIS

The exclusive localization of Dermatophytes is in the keratinized tissues. They have the ability to secrete keratinolytic agent which provides means of entry into living cells²¹.

Transfer of infecting organisms from soil, animals or humans is accomplished by means of arthrospores, which are shed from the primary host with skin scales or hair²⁵. The ability of *T.rubrum* to adhere to epithelial cells has been attributed to carbohydrate specific adhesins expressed on surface of microconidia²⁶.

Adherence

On the stratum corneum, the first phase of dermatophyte invasion involves the adherence of infectious arthroconidia to keratinocytes. *In vitro*, this process is completed after about 2 h of contact, at which stage germination and penetration of the keratinocyte occurs. Different Dermatophytes show similar kinetics, which are also unaffected by the source of the keratinocytes. The germination of arthroconidia and hyphal prolongation which follows adherence proceeds radially, and *in vitro* there is evidence of indentation of keratinocyte layers beneath the growing hyphae, possibly resulting from enzymic action²⁸

After adherence fungal metabolic products diffuse through malphigian layer to the epidermis to cause erythema, vesicles and even pustule formation along with pruritis. There *in vivo* activity is restricted to the zone of differentiation, newly differentiated keratin and for infection to persist, hyphal growth must keep pace with the rate of keratin production. The hyphal tips growing down within hair shaft reach to the edges of living keratinizing cells and form Adamson's fringe. The infective process ceases and healing occurs when the balance of fungus and host is tilted in favour of host thereby the upward movement of keratin carries active hyphae away from keratinous zone²¹.

Variations in the composition of inhibitory fatty acids in sebum determine the susceptibility to dermatophytosis. Other skin factors thought to be important include local CO₂ tension and the presence of surface moisture as well as unsaturated transferrin.^{25, 28}

IMMUNOLOGY

Dermatophyte colonization is characteristically limited to the dead keratinized tissue of the stratum corneum and results in either a mild or intense inflammatory reaction. Although the cornified layers of the skin lack a specific immune system to recognize this infection and get rid itself of it, nevertheless, both humoral and cell mediated reactions, specific and nonspecific host defence mechanisms respond and eventually limit the fungus to prevent invasion of the deeper viable tissue. This array of defense mechanisms thought to be active against Dermatophytes consists of N-2 macroglobulin keratinase inhibitor, unsaturated transferrin, epidermal desquamation, lymphocytes, macrophages, neutrophils and mast cells²⁸. There are two major classes of dermatophyte antigens: glycopeptides and keratinases. The protein portion of the glycopeptide preferentially stimulates cell mediated immunity (CMI), whereas the polysaccharide portion stimulates humoral immunity²⁹.

Although the host develops a variety of antibodies to dermatophyte infection i.e., IgM, IgG, IgA and IgE, they apparently do not help to eliminate the infection³⁰. Approximately 80-93% of chronic or recurrent infections are estimated to be caused by *T.rubrum*. Enhanced proliferation of the skin in response to inflammation may be the final mechanism that removes the fungus from the skin by epidermal desquamation. An inherited autosomal recessive trait has shown to predispose to infection by *T.concentricum*.

HLA-DR6 gene confers protection against the development of onychomycosis³¹.

CLINICAL MANIFESTATIONS

Traditionally, infections caused by Dermatophytes (ringworm) have been named according to the anatomic locations involved by appending the Latin term designating the body site after the word *Ti.*, e.g., *Ti. capitis* for ringworm of the scalp. The clinical manifestations are as follows:

- (i) *Ti. barbae* (ringworm of the beard and mustache)
- (ii) *Ti. capitis* (scalp, eyebrows, and eyelashes)
- (iii) *Ti. corporis* (glabrous skin)
- (iv) *Ti. cruris* (groin)
- (v) *Ti. favosa* (favus)
- (vi) *Ti. imbricata* (ringworm caused by *T. concentricum*)
- (vii) *Ti. manuum* (hand)
- (viii) *Ti. pedis* (feet)
- (ix) *Ti. unguium* (nails)

Several anatomic sites may be infected by a single dermatophyte species, and different species may produce clinically identical lesions. The major etiological agents may be global, such as *T. rubrum*, while the distribution of others may vary geographically.

Ti. barbae

Ti. barbae, an infection of the bearded area, may be mild and superficial or a severe inflammatory pustular folliculitis, the latter form more commonly caused by

the zoophilic Dermatophytes *T.verrucosum*, *T.mentagrophytes* var. *mentagrophytes*, and *T.mentagrophytes* var. *erinacei*.

Ti. capitis

Ti. capitis, an infection commonly involving the scalp, is usually caused by members of the genera *Microsporum* and *Trichophyton*. The infection may range from mild, almost subclinical, with slight erythema and a few patchy areas of scaling with dull gray hair stumps to a highly inflammatory reaction with folliculitis, kerion formation, and extensive areas of scarring and alopecia, sometimes accompanied by fever, malaise, and regional lymphadenopathy. Both the skin surface and hairs are involved. Infection of the hair may be described as ectothrix (sheath of arthroconidia formed on the outside of the hair shaft) or endothrix (arthroconidia formed within the hair shaft). The current predominant cause of Ti. capitis in most of North, Central, and South America is *T. tonsurans* (endothrix) replacing *M. audouinii*(ectothrix).

Ti. corporis

Ringworm of the body, usually involving the trunk, shoulders, or limbs, and occasionally the face (excluding the bearded area), may be caused by any dermatophyte. The infection may range from mild to severe, commonly appearing as annular, scaly patches with sharply marginated, raised erythematous vesicular borders.

Ti. cruris (“Dhobie Itch”)

Infection of the groin, perianal, and perineal areas, and occasionally the upper thighs, is usually seen in adult men. *T.rubrum* and *E. floccosum* are the most frequent etiologic agents. Lesions are erythematous to tawny brown and covered with thin, dry

scales. They are usually bilateral and often asymmetric, extending down the sides of the inner thigh and exhibiting a raised, sharply margined border that is frequently studded with small vesicles.

Ti. Favosa

Ti. favosa, usually caused by *Trichophyton schoenleinii*, is severe and chronic, characterized by the presence on the scalp and glabrous skin of yellowish, cup-shaped crusts called scutula, which is composed of epithelial debris and dense masses of mycelium. The disease is most common in Eurasia and Africa.

Ti. imbricata

Ti. imbricata, the chronic infection which is a specialized manifestation of Ti. corporis, is characterized by concentric rings of overlapping scales scattered throughout the body. It is geographically restricted to certain of the Pacific islands of Oceania, Southeast Asia, Mexico, and Central and South America. *T. concentricum*, a strictly anthropophilic dermatophyte, is the only etiologic agent.

Ti. manuum

The palmar and interdigital areas of the hand are usually involved in Ti. manuum, most frequently presenting as unilateral diffuse hyperkeratosis with accentuation of the flexural creases. Most infections are caused by *T. rubrum*.

Ti. pedis (“Athlete’s Foot”)

The feet, especially the soles and toe webs, are most frequently involved in Ti. pedis. The most common clinical manifestation is the intertriginous form, which presents with maceration, peeling, and fissuring, mainly in the spaces between the fourth and fifth toes. Another common presentation is the chronic, squamous,

hyperkeratotic type in which fine silvery scales cover pinkish skin of the soles, heels, and sides of the foot (moccasin foot). An acute inflammatory condition, characterized by the formation of vesicles, pustules, and sometimes bullae, is most frequently caused by *T. mentagrophytes*. The more chronic agents of Ti. pedis are *T. rubrum*, *T. mentagrophytes* var. *interdigitale*, and *E. floccosum*.

Ti. unguium

Invasion of the nail plate by a dermatophyte is referred to as Ti. unguium; infection of the nail by non dermatophytic fungi is called onychomycosis. The latter word is often used as a general term for a nail infection. There are two main types of nail involvement: invasive subungual (distal and proximal) and superficial white mycotic infection (leukonychiatrachophytica). *T. rubrum* and *T. mentagrophytes*, respectively, are the most common Dermatophytes of this infection.

LABORATORY DIAGNOSIS

Collection and Transport of Specimens

Dermatophytes, as filamentous fungi, undergo radial growth. The centers of infected skin patches may consist of the older and poorly viable material, as may portions of older nail plate in onychomycosis. In Ti. corporis, where the “rings” of ringworm are well defined, collection is best made by collecting epidermal scales from near the advancing edges of the rings. The lesion is lightly disinfected with alcohol in gauze and then scraped from center to edge, crossing the lesion margin, using a sterile blunt scalpel blade or equivalent. If the lesions have vesicles or bullae, the tops of the vesicles or bullae should be clipped and included in the sample. Suppurating lesions may be sampled with a swab when it is impractical to obtain

scrapings. Other skin dermatophytoses, such as *Ti. pedis* and *Ti. manuum*, are scraped in such a way that the whole infected area is represented, since an advancing margin is often not evident.

In *Ti. capitis* and *Ti. barbae*, the basal root portion of the hair is best for direct microscopy and culture. In prospective *Microsporum* infections, a Wood's light may be used to allow detection of the most heavily infected hairs. Hairs are best sampled by plucking so that the root is included. If this is not possible due to hair fragility, as in "black dot" *Ti. capitis*, a scalpel may be used to scrape scales and excavate small portions of the hair root. Brushes with stiff bristles, run firmly across the lesion, have also been used successfully to sample *Ti. capitis*³². Similar techniques may be used to sample animal dermatophytoses³³.

The common distal-subungual type of *Ti. unguium* is traditionally sampled, after light alcohol disinfection, by scraping the debris from beneath the distal end of the nail with a scalpel and collecting scrapings from near the nail bed, where viable inoculum is most likely to be encountered³³. Close clipping of the whole nail end is an alternative to this procedure, as is nail drilling. In difficult to sample, degraded nails, specialists may use a Skele curette, a surgical instrument with a small, spoon-like end with a sharpened edge. Superficial white onychomycosis is sampled by scraping material from the white spots on the surface of the nail. Discarding the uppermost layer of material is recommended in order to reduce the presence of contaminant inoculum.

Sample materials are best transported in dry, strong black paper folded in the manner of a herbarium packet. Bacteriological transport media should not be used as they may allow growth of contaminants and their viscosity may result in substantial

loss of the available specimen. Moisture of any kind is to be avoided. Black paper allows easy visualization of small skin squames; it should be thin enough to fold tightly at the corners and not ‘leak’ specimen.

LABORATORY TESTING OF SPECIMENS

Direct Microscopic Examination:

Direct microscopy provides an early and reasonably reliable method of diagnosing or excluding fungal infections.

1) Potassium hydroxide (KOH) preparation:

Direct mounts are made by mixing a small portion of the material in 2-3 drops of 10% KOH on a microscope slide. A cover slip is placed over KOH specimen and the slide is gently heated. The slide is allowed to cool and ‘ripen’ for few minutes before examination. Alternatively the can also be placed in a tube containing few drops of KOH. The KOH ‘cleans’ the specimen by digesting proteinaceous debris, bleaching pigments and loosening sclerotic material without damaging fungus, making hyphal forms easier to see. The slides are examined under bright field microscope, first under 10x and then under 40x. The hyphae stand out as highly refractile long branched septate threads. At times these hyphae fragment into rounded or barrel shaped arthrospores. The arthrospores are outside the hair shaft in chains in a mosaic pattern depending on the species involved and whether it is endothrix or ectothrix. 20% KOH are used for nail samples. In case the nails do not soften satisfactorily, the tube may be kept in an incubator at 37⁰C for 1 hour. Hair should be examined as soon as possible after mounting.

Some modifications of KOH preparation³⁴:

- Addition of 5% glycerin to 25% KOH or NaOH prevents desiccation.
- 20% KOH dissolved in 40% DMSO helps in rapid penetration and maceration of tissue without resorting to heating³⁵.
- Addition of Parker Superchrome Blue-black ink to KOH solution selectively colors the hyphae making them more prominent.
- Sodium sulphide may also be used as a clearing agent.
- Eosin 1% may be added to KOH to stain the keratin. It lends a pinkish background while fungal elements remain unstained³⁶.
- Modified Parker's ink and 1% Eosin method: Eosin 1% is added to Parker's ink in 2:1 proportion. The mixture is painted over the affected site and allowed to dry. Apply cellophane tape, gently press, remove it, stick over glass slide and observe under microscope. Background stains pink and fungal elements stain blue.

2) Calcofluor white stain:

Calcofluor white is a fluorescent brightener which selectively binds to chitin and cellulose in the fungal cell wall. It fluoresces light blue color when exposed to ultraviolet light (346-365nm).

3) Acridine Orange:²¹

A drop of 0.01% acridine orange may be added to KOH and observed under fluorescent microscope.

4) PAS (Periodic Acid Schiff): ²¹

Nail clipping stained with PAS is more rewarding as compared to KOH wet mount. The polysaccharides of fungi are oxidized by periodic acid to form aldehyde groups that yield magenta coloured compound with Schiff's fuchsin sulfide.

5) Grocott-Gomori's Methenamine Silver Stain: ²¹

This stain works on the principle of liberation of aldehyde groups and their subsequent identification by reduced silver method. The aldehyde reduces methenamine silver nitrate complex resulting in brown black staining fungal cell wall due to deposition of reduced silver wherever aldehydes are located.

Culture:

The most common media used for the isolation of Dermatophytes is Sabourauds Dextrose agar with chloramphenicol and cycloheximide to inhibit bacterial and saprobic fungal contamination, incubated at three temperatures i.e., 25⁰C, 30⁰C and 37⁰C³⁴. Dermatophyte test medium (DTM) is used for the presumptive identification of Dermatophytes. On incubation at 25⁰C, the dermatophyte test media turns red due to change in color of the indicator phenol red by increased pH through their metabolic activity while most fungi do not. Potato flakes agar amended with cycloheximide and chloramphenicol is available as Rapid Sporulating Medium to promote rapid conidiation and colony pigmentation³⁵.

SDA with 1% thiamine can be used for sporulation. The media should be inoculated and kept at room temperature for minimum of 4 weeks. Sporulation usually occurs in 7 – 10 days. Some stains like *T.verrucosum* may take longer and some stains of *T.tonsurans* grow better when incubated at 37⁰C.

Identification:

Identification is based on

- 1) Colony characteristics in pure culture on SDA
- 2) Microscopic morphology

1) **Colony characteristics:** ³⁶

In observing gross colony morphology, note the color of the surface and the reverse of the colony, the texture of the surface (powdery, granular, wooly, cottony, velvety or glabrous) the topography (elevation, folding, margins, etc.,) and the rate of growth.

2) **Microscopic morphology:**

The appearance and arrangement of the conidia and other structures may be determined by tease mounts or slide culture preparation mounted with lactophenol cotton blue. Sometimes special media like corn meal agar, potato glucose agar, lactrimel agar, rapid sporulation medium may be required to stimulate sporulation.

a. **Tease mount with Lactophenol Cotton Blue (LCB)**

For preparing a mount, a portion of fungal fragment is removed with a spud and is teased on a glass slide in a drop of LCB stain using 2 teasing needles. A cover slip is placed and examined under the microscope.

b. **Slide culture:**

Microscopic structures are beautifully preserved for study in fine details. A microscopic slide is placed on a bent glass rod at the bottom of a petri dish along with 1-2 cover slips and a filter paper. Petri dishes are closed with their lid, wrapped with

craft paper and sterilized using hot air oven. Block of 1 x 2cm of Sabourauds agar poured into petri dishes up to a depth of 4mm is cut using sterile scalpel blade. The block is transferred to the surface of the glass slide. The agar block is inoculated at four sides with spud using the fungal strain to be identified. The inoculated block is covered with sterile cover slip and incubated at room temperature. A little sterile distilled water is added on the filter paper to avoid drying of agar. When growth appears, usually 48-72hrs a drop of LCB is placed on a slide and cover slip from block is placed over it. Likewise drop of stain is placed on glass slide of the slide culture after removing agar block; fresh cover slip is applied over it and is examined under the microscope.²¹

c. Scotch Tape Technique:

A 4cm strip of scotch tape No. 800 is looped back on itself with the adhesive side out and held between the thumb and index finger. The adhesive side is pressed firmly to the surface of the fungal colony. It is gently pulled and is placed in a small drop of LCB on a microscopic slide.

PHYSIOLOGICAL TESTS:

1) In vitro hair perforation test:

This is performed to differentiate between *T.mentagrophytes* and *T.rubrum* as well as *M.canis* and *M.equinum*. This test is taken positive when dermatophyte species show wedge shaped perforation in the affected hair. It is positive in *T.mentagrophytes* and *M.canis*.

2) Urease Test:

Is done on Christensen's medium. *T.mentagrophytes* hydrolyze urea thereby turns medium red while *T.rubrum* does not hydrolyze.

3) Special nutritional requirements:

The method employs a casamino acids basal medium i.e., vitamin free [*Trichophyton* agar, (T1)] and to which various vitamins are added i.e., inositol (T2), thiamine + inositol (T3), thiamine (T4), and nicotinic acid (T5). In addition, the series includes an ammonium nitrate basal medium (T6) to which histidine is added (T7).

After inoculation, incubated at room temperature or 37⁰C (if *T.verrucosum* is suspected) and read after 7 and 14 days. The amount of growth is graded from 0-4+

4) Growth on Polished Rice Grains:

This is a useful test for differentiating *M.audouinii* from *M.canis* and from other Dermatophytes that typically grow and sporulate on rice grains.

5) Temperature tolerance and temperance enhancement:

Used for distinguishing *T.mentagrophytes* complex from *T.terrestre*, *T.mentagrophytes* from *M.persicolor* and *T.verrucosum* from *T.schoenleinii*. At 37⁰C, members of *T.mentagrophytes* complex show good result whereas *T.terrestre* does not grow and *M.persicolor* generally grows poorly or not at all; growth of *T.verrucosum* and *T.soudanense* is enhanced but that of *T.schoenleinii* and *M.ferruginum* is not.

6) Hair bait technique:

This technique is used for the isolation of geophilic species like *M.gypseum* from soil.

Immunodiagnosis²¹:

- Skin tests with dermatophyte antigen trichophytin are used for the diagnosis of dermatophytosis.
- Trichophytin is a crude extract from Dermatophytes producing positive delayed type hypersensitivity in most of the adults.
- The patients with delayed type of reaction are more susceptible to chronic dermatophytosis.

Serological tests:

Various serological tests like immunodiffusion are done to establish the diagnosis of dermatophytosis

Animal pathogenicity:

It is done on guinea pigs. *M.canis*, *M.gypseum* and *T.mentagrophytes* may be established more readily in laboratory animals as compared to other species. It is done for studying nature of lesions and immunity produced by the fungus.

Molecular Identification Techniques:

Methods used are largely based on arbitrarily primed PCR or restriction fragment length polymorphism generated from PCR products. An oligonucleotide probe for *T.rubrum* has been developed. Nested PCR targeting Chitin Synthase I (CHSI) gene in skin and hair specimen of patients clinically suspected with dermatophytosis is used³⁷.

Treatment:

Both local and systemic treatment is used for dermatophytosis.

Polyene:

Are naturally occurring compounds exhibiting a complex structure. All act by binding to sterols in the fungal cell membrane. Griseofulvin was the first antifungal agent to be described. The activity appears to be directed against the process of mitosis.³⁸ Oral Griseofulvin is the drug of choice for nails and scalp infections.

Allylamine: They act by the inhibition of squalene epoxide in the formation of cell membrane. Two main compounds are Naftifine and Terbinafine. Terbinafine used extensively, is active against most of the agents of *Ti. capitis*.

Azoles:

Many imidazole and triazole derivatives display antifungal activity. They act by interfering with the demethylation of lanosterol during the synthesis of ergosterol. Most widely used as bifonazole, clotrimazole, econazole, fenticonazole, isoconazole, miconazole, sulconazole and terconazole.

The triazoles commonly used are fluconazole, itraconazole and voriconazole. Ketoconazole has been used to treat severe superficial dermatophyte infections. It may be more effective in *T.rubrum* infection.

Topical antifungal agents for treatment of dermatophytosis:

- Morpholine derivatives: Amorolfine used as treatment for onychomycosis in the form 5% nail lacquer.

- Allylamine: Topical terbinafine has been shown to produce response in some dermatophyte infections.
- Benzoic acid compound ointment.
- Azole derivatives: Clotrimazole, ketoconazole

INVITRO SUSCEPTIBILITY TESTING OF DERMATOPHYTES

The National Committee for Clinical Laboratory Standards (NCCLS) M-38-A 2007 which describes the standard parameters for testing MIC of established agents against filamentous fungi. This has been latter modified for testing Dermatophytes M-38-P with several important factors, such as temperature (28 versus 35°C) and time of incubation (4 to 10 days versus 21 to 72 hours)³⁹

Antifungal susceptibility testing is receiving increased attention with the advent of newer anti-fungal drugs. However susceptibility testing of filamentous fungi is not as advanced as susceptibility testing. In vitro susceptibility tests should provide a reliable measure of relative activity of the anti-fungal agent, correlate with in vivo activity and predict the likely outcome of the therapy, provide a means with which to monitor the development of resistance and predict the therapeutic potentials of newer drugs⁴⁰.

In vitro susceptibility 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 determination. In addition, there are problems unique to fungi like their slow growth rates and the ability of some of them to grow either as yeasts with blastoconidia or as molds with a variety of conidia depending on pH, temperature and medium composition. The trailing end

point observed with the azoles is another major problem encountered in the susceptibility testing of the fungi. Several studies have attempted to correlate the MIC results with outcome. However, only little evidence is available test results with in vitro outcomes. The retrospective nature of the studies, the documented variability of the non-standardized in vitro methods and the difficulty in defining mycoses and their responses to therapy are responsible for this status ⁴¹. The methods that have been most frequently applied to antifungal susceptibility testing are –Disc diffusion method, Episilometer test, agar dilution test, micro and macrobroth dilution test, spectrophotometric methods and flowcytometry.

MATERIAL AND METHODS

The present study of dermatophytosis was carried out in the Department of Microbiology, KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre Belgaum, over a Period of one year from January 2012 to December 2012. All clinically diagnosed cases of dermatophytosis in all age groups and of both sexes, attending the Outpatient Department of Dermatology and Venereology were taken for the study.

Inclusion Criteria:

All skin, hair and nail samples from clinically suspected cases of dermatophytosis.

Exclusion Criteria:

Patients who are already on treatment for dermatophytosis.

Statistical Analysis:

Will be done using percentage.

Sample calculation: $4pq/d^2$

$p=50.4\%$ (percentage of cases who were positive for Dermatophytes on culture) ⁴²

$$q= 49.6 \quad (100-p)$$

$d= 10\%$ absolute error.

$$4 * 50.4 * 49.6 / 10^2$$

$$=99.99 \sim 100$$

Specimen Collection:

From the Skin:

The affected area was first thoroughly swabbed with 70% alcohol to remove surface contaminants. After the alcohol dries, the skin scrapings were collected from the border of the active lesions with a beard parker blade in a sterile black paper envelop.

From the scalp:

Hairs from the scalp were epilated with a flame sterilized forceps and the active border area was scraped with a scalpel to collect epidermal scales on a sterile black paper envelop.

From the nail:

The affected nail was first cleaned with 70% alcohol. The upper portion of the infected nail was scraped away and material were collected from the deeper part of the distal end of the nail on to sterile black paper envelop.

Direct Examination:

Potassium Hydroxide (KOH) Preparation:

The samples of skin and hair were placed in few drops of 10% KOH and 20% KOH for nail samples on a clean glass slide, then cover slip was placed over the preparation and the slide was gently heated by passing over the flame 3-4 times. After 15-20 minutes, the specimen was examined first under the low power of microscope and then under the high power with low condenser to look for the presence of hyphae

or arthrospores. The hyphal forms were differentiated from epidermal cell outlines, cotton and vegetable fibers.

Culture:

The specimens collected were inoculated on to Sabourauds Dextrose agar containing Chloramphenicol (50mg/l) and Cycloheximide (500mg/l); irrespective of demonstration of fungal elements on KOH mount. Each sample was inoculated into a pair of tubes. One tube with antibiotic and other without antibiotic and were incubated at 27⁰C. The cultures were examined daily for a period of 4 weeks. Slopes showing no growth for 4 weeks were discarded. If growth was obtained on Sabourauds Dextrose agar, identification was made based on colony morphology, microscopic appearance and other relevant tests. The isolates were inoculated on potato dextrose agar for better conidiation.

Macroscopic Examination of Culture:

The growth on Sabourauds dextrose agar was observed to study the colony morphology, the color of the surface, the reverse of the colony, the texture of the surface, the topography and the rate of growth.

Microscopic Examination of Culture:

- a) **Tease Mount:** For preparing a mount, a portion of fungal fragment was removed with a spud and was teased on a glass slide in a drop of LPCB stain using 2 teasing needles. A cover slip was placed and examined under the microscope.

- b) **Slide culture:** A microscopic slide was placed on a bent glass rod at the bottom of a petri dish along with 1-2 cover slips and a filter paper. Petri dishes

were closed with their lid, wrapped with craft paper and sterilized using hot air oven. Block of 1x1cm of Sabourauds Dextrose agar with a depth of 4mm was cut using sterile scalpel blade. The block was transferred to the surface of the glass slide. The fungal strain to be identified was inoculated at four sides of the agar block with the spud. The inoculated block was covered with sterile cover slip and incubated at 25°C. A little sterile distilled water was added on the filter paper to avoid drying of agar. When growth appears, a drop of LPCB was placed on a slide and cover slip from block was placed over it. Likewise drop of stain was placed on glass slide of the slide culture after removing agar block; fresh cover slip was applied over it and examined under the microscope

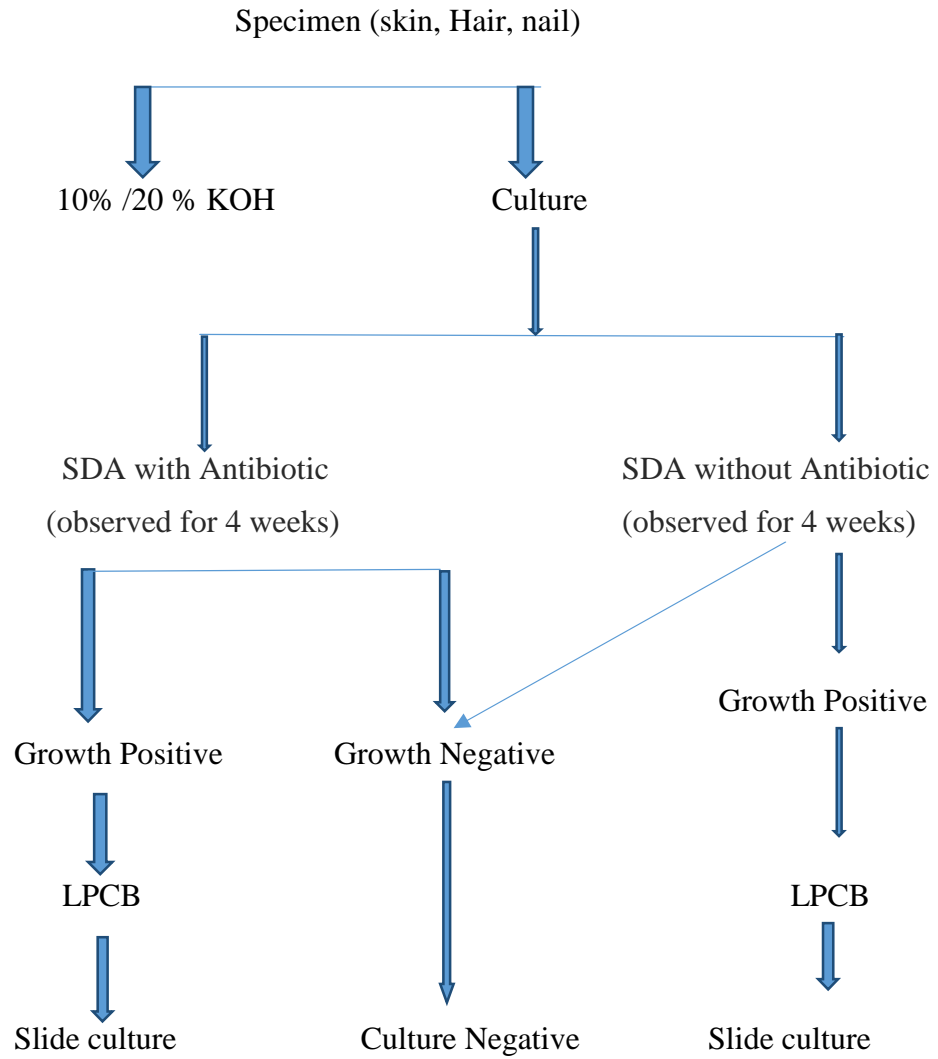
BIOCHEMICAL TEST

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.

This test is done on Christensen's medium. This is done to differentiate *T. mentagrophytes* from *T. rubrum*. *T. mentagrophytes*, hydrolyse urea and the medium becomes deep red while *T. rubrum* does not hydrolyse urea. Urea broth may also be used which is more sensitive.

MODE OF PROCESSING THE SAMPLE



ANTIFUNGAL SUSCEPTIBILITY TESTING FOR DERMATOPHYTES

MICROBROTH DILUTION METHOD

REQUIREMENTS:

- a) Sterile test tubes for drug dilution / inoculum preparation
- b) Sterile disposable microtitre plates
- c) Sterile Micro pipette / sterile tips /Gloves / disposable face masks
- d) Whatmann filter paper no 40.
- e) Antifungals (Ketoconazole, Clotrimazole, Terbinafine, Sertaconazole)
- f) Solvents (DMSO, Ethanol,Methanol)

MEDIUM

RPMI 1640 with glutamine, without bicarbonate in MOPS (3N-Morpholino propanesulphonic acid), buffer sterilized by membrane filtration.

ANTI FUNGAL STOCK SOLUTION

10ml stock solution was prepared for each drug with concentration of 1600 μ g/ml.

For water insoluble drugs-diluent DMSO.

Solvents

- DMSO (Ketoconazole)
- Methanol (Sertaconazole)
- Ethanol (Terbinafine, Clotrimazole)

Drug dilutions

a. Antifungal stock solution preparation

$$\text{Weight (mg)} = \frac{\text{volume (mL)} \times \text{desired concentration (mg/mL)}}{\text{Antifungal potency}}$$

For example, to prepare for a broth microdilution test series containing a water insoluble

Drug that can be dissolved in respective solvents, for which the highest desired test concentration is 1600 μ g/ml, first weigh 16.0 mg (assuming 100% potency) of antifungal powder and dissolve in 10ml of solvent. This will provide a stock solution at 1,600 μ g/ml.

Label 9 tubes as 3–11.

Add appropriate amounts of DMSO to each tube as follows:

- Add 0.5 mL of DMSO to tubes 3, 6, and 9
- Add 0.75 mL of DMSO to tubes 4, 7, and 10
- Add 1.75 mL of DMSO to tubes 5, 8, and 11
 - a) Label the stock solution tube (1,600 μ g/mL) as tube 2
 - b) Transfer from tube 2, 0.5 mL to tube 3 and 0.25 mL to tubes 4 and 5
 - c) Transfer from tube 5, 0.5 mL to tube 6 and 0.25 mL to tubes 7 and 8
 - d) Transfer from tube 8, 0.5 mL to tube 9 and 0.25 mL to tubes 10 and 11
 - e) Finally, discard 1 mL from tube 11
- Notice that when dilutions have been prepared, all the tubes contain 1 mL

Dilution of drug used were between 32 μ g/ml to 0.0625 μ g/ml, {32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 }

DRUG DILUTION

To prepare 5ml volume of antifungal agent first pipette 4.9 ml volumes of RPMI1640 medium into each of 10 sterile test tubes. Now, add 0.1ml solvent in tubes (sterility control and growth control) .Add 0.1ml of the corresponding drug dilution to each of the tubes. These volumes were adjusted according to the total No. of test required. Because there will be 1:2 dilution of the drug when combined with the inoculum, the working antifungal solutions are 2 fold more concentrated than the final concentration.

Final concentration after inoculation is {16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 0.031 μ g/ml }

INOCULUM PREPARATION

7-15 days old cultures grown on PDA at 25° C was used. Mature colonies were covered with 10ml of sterile saline (0.85 %).Growth scraped by sterile Pasteur pipette. Heavy particles were allowed to settle for 15-20minutes at room temperature. Supernatant was mixed with a vortex for 15 seconds. Then it was filtered through Whatmann filter paper no 40.Turbidity was adjusted spectrophotometrically to 530nm 65 -70%absorbance. Each suspension was diluted 1: 50 in RPMI 1640 medium.

The growth control wells contains 0.1ml of the corresponding diluted inoculum suspension and 0.1ml of the drug diluent without antifungal agents.

TEST PROCEDURE

Test was performed in sterile microtitre plates. Aliquots of 100µls of drug dilutions were inoculated in 1-10 microtitre wells. Add 100 µl of inoculum into each well from 1 to 12. Growth control - tube 12 with inoculum and without antifungal drug.

INCUBATION

All microdilution trays were incubated at 28°C without agitation.

READING RESULTS

Lowest concentration of the drug which permitted no macroscopically visible growth after 7 days was taken as MIC (Minimum Inhibitory Concentration).

MIC results recorded in µg/ml. (NCCLS M-38A)³⁹

QUALITY CONTROL

Trichophyton mentagrophytes ATCC 9533 was procured from V.M. Medical College Sholapur.

Antifungals were obtained from

Setraconazole, Clotrimazole, Ketoconazole – GLENMARK Mumbai

Terbinafine - MICROLABS Bangalore

Clotrimazole - FDC Ltd Mumbai



Photograph 1: *Ti. unguium*



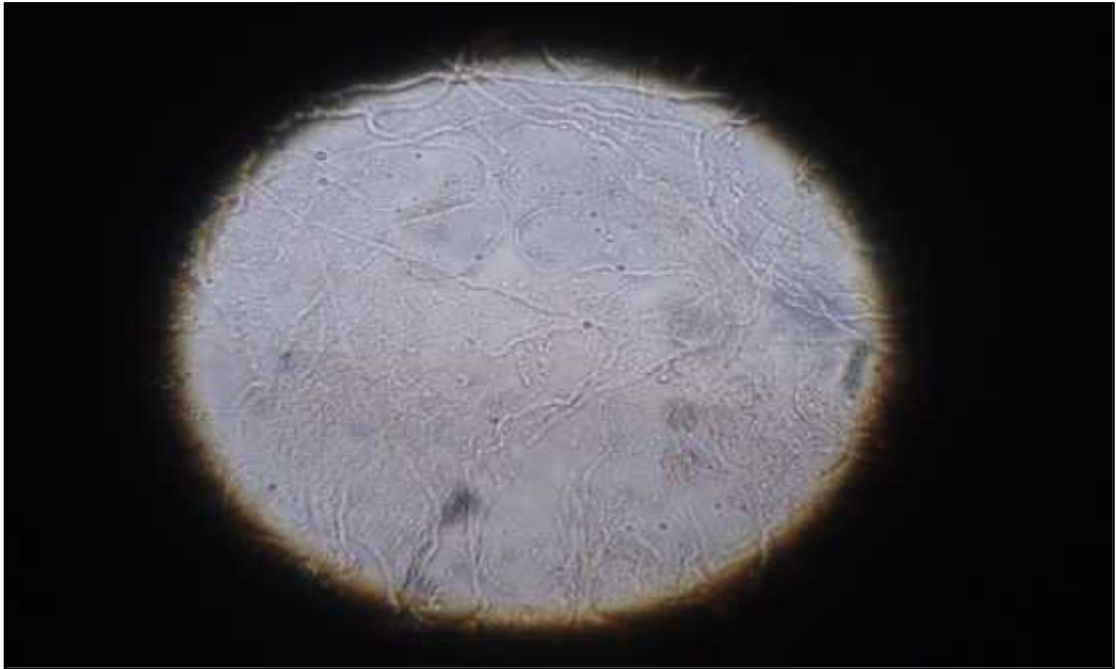
Photograph 2: Ti. pedis



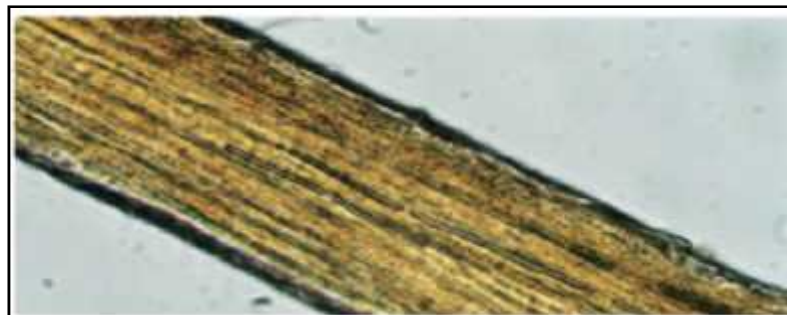
Photograph 3: Ti. corporis



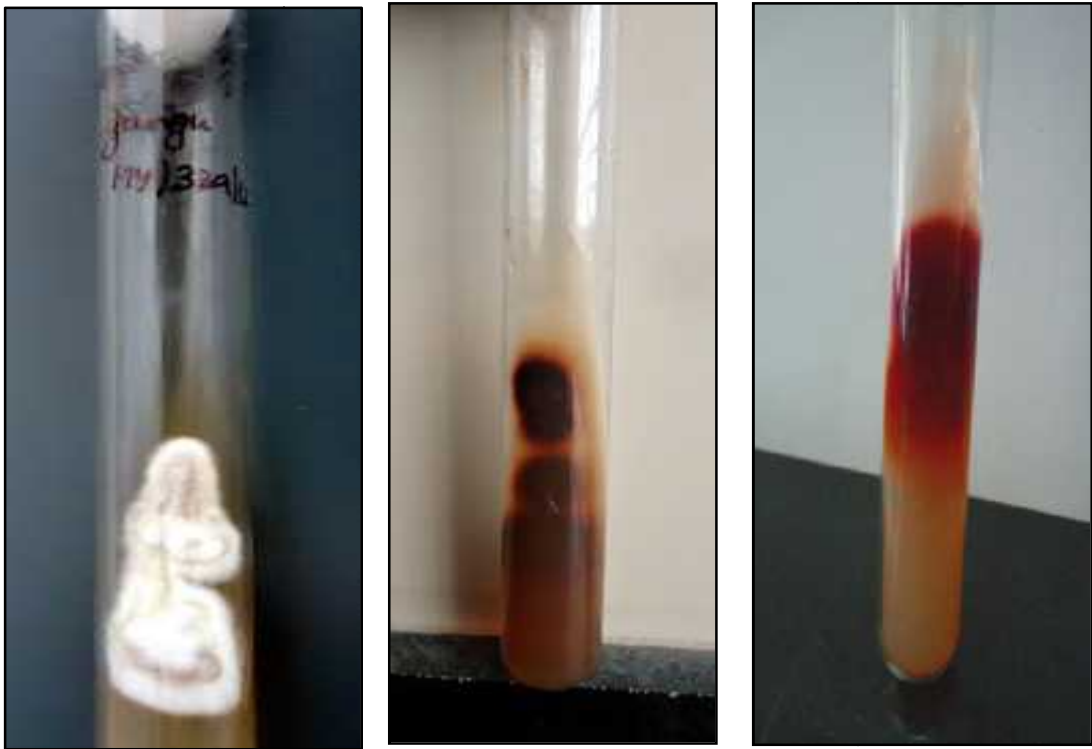
Photograph 4: Ti. capitis



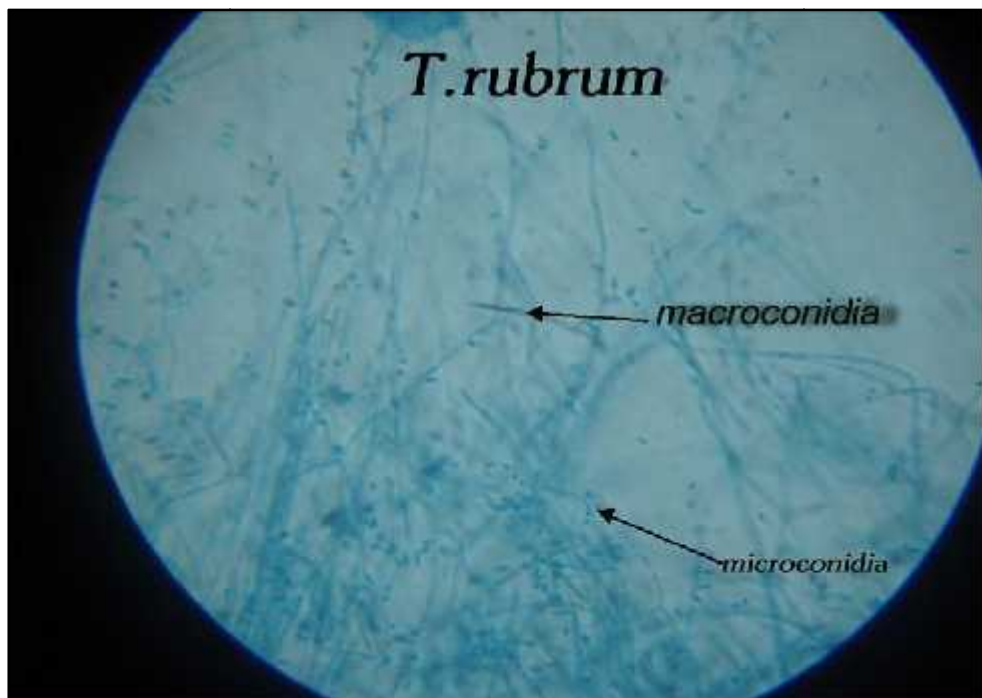
Photograph 5: KOH Preparation



Photograph 6: KOH preparation for hair shaft



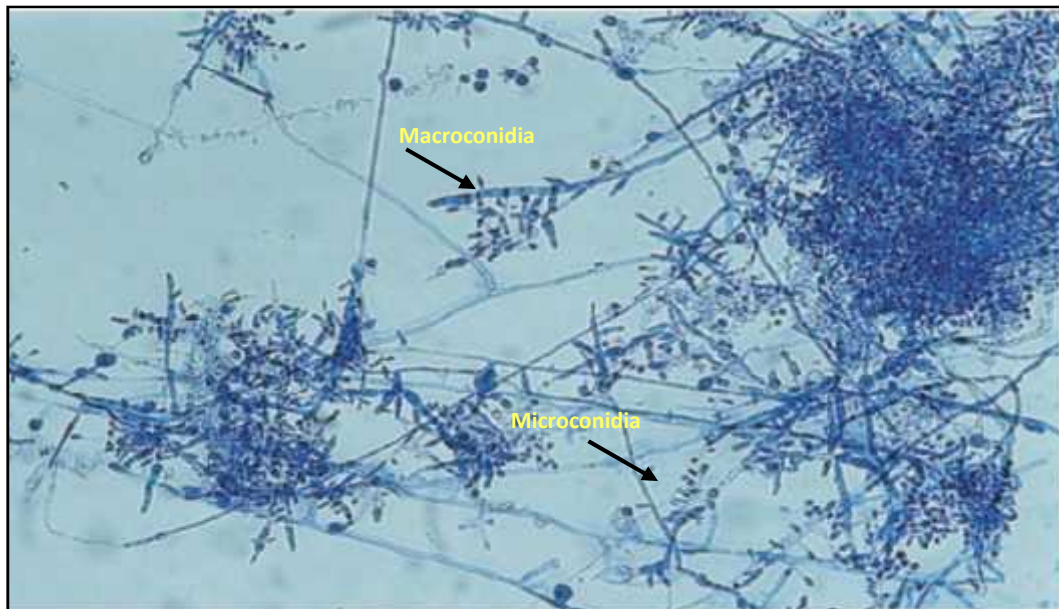
Photograph 7: *T. rubrum*



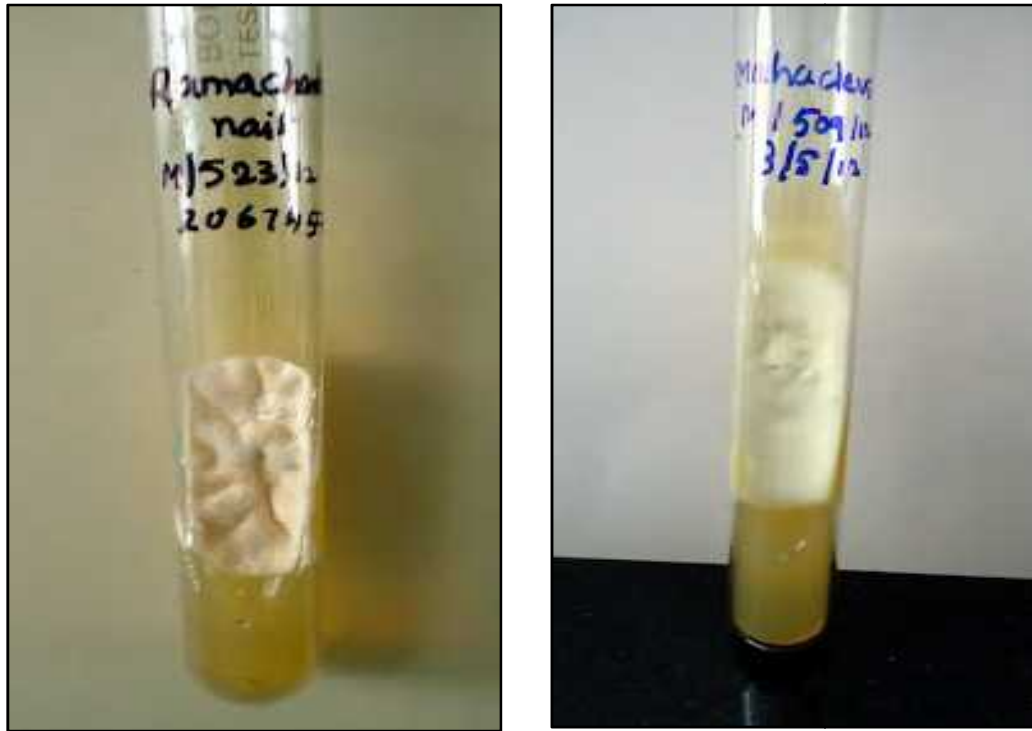
Photograph 8: LPCB Preparation of *T. rubrum*



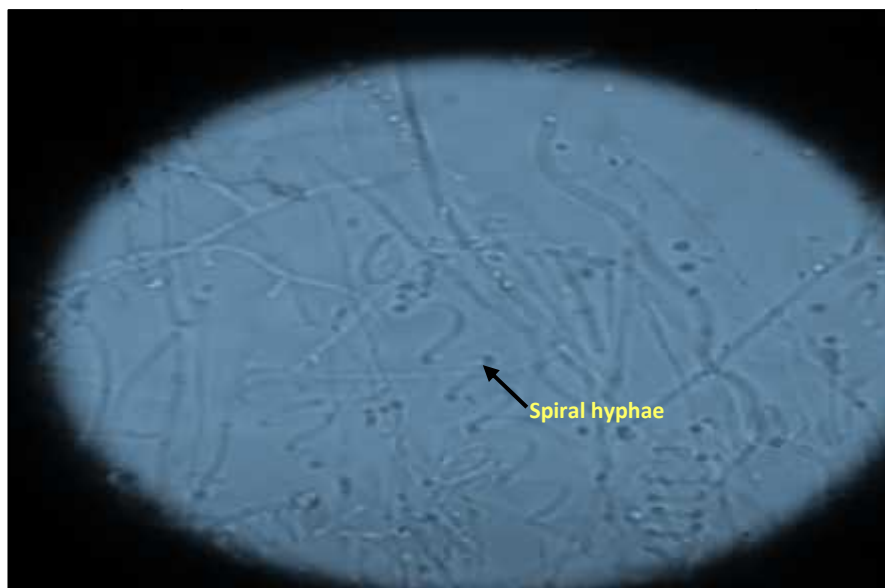
Photograph 9: *T. tonsurans*



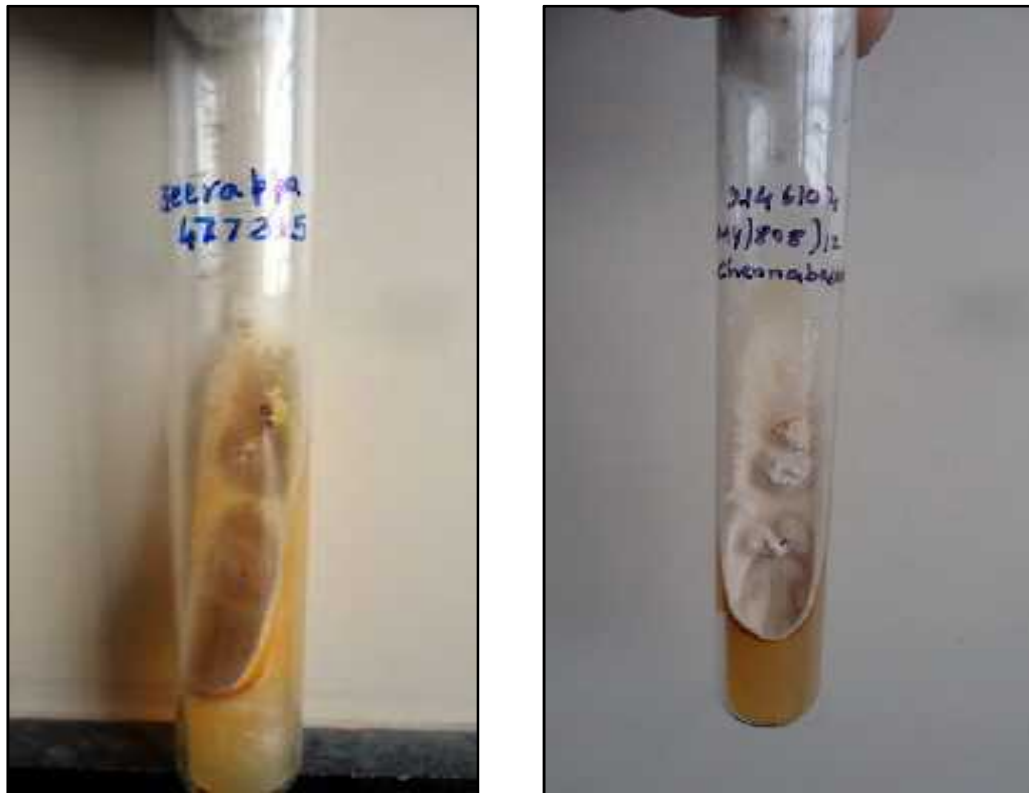
Photograph 10: LPCB Preparation of *T. tonsurans*



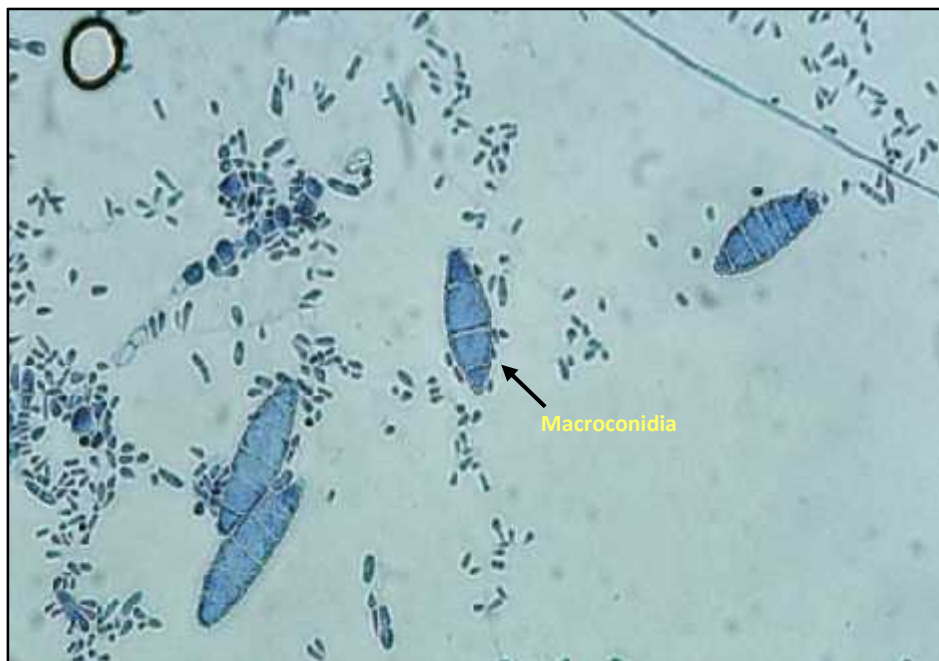
Photograph 11: *T.mentagrophytes*



Photograph 12: LPCB Preparation of *T.mentagrophyte*

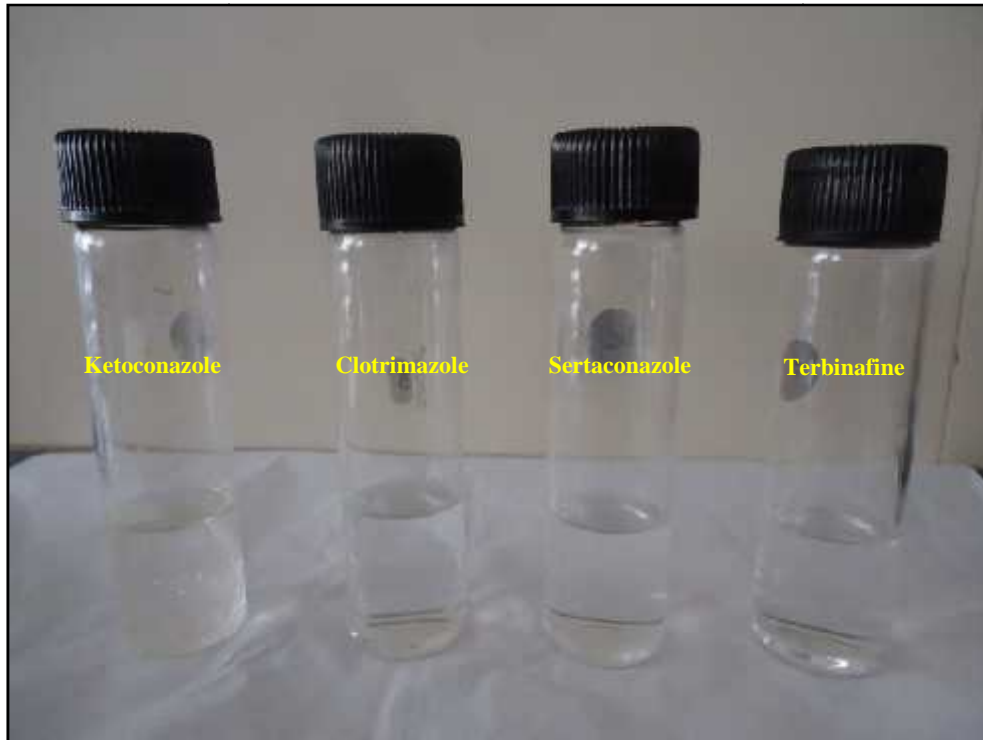


Photograph 13: *M.gypseum*



Photograph 14: LPCB Preparation of *M.gypseum*

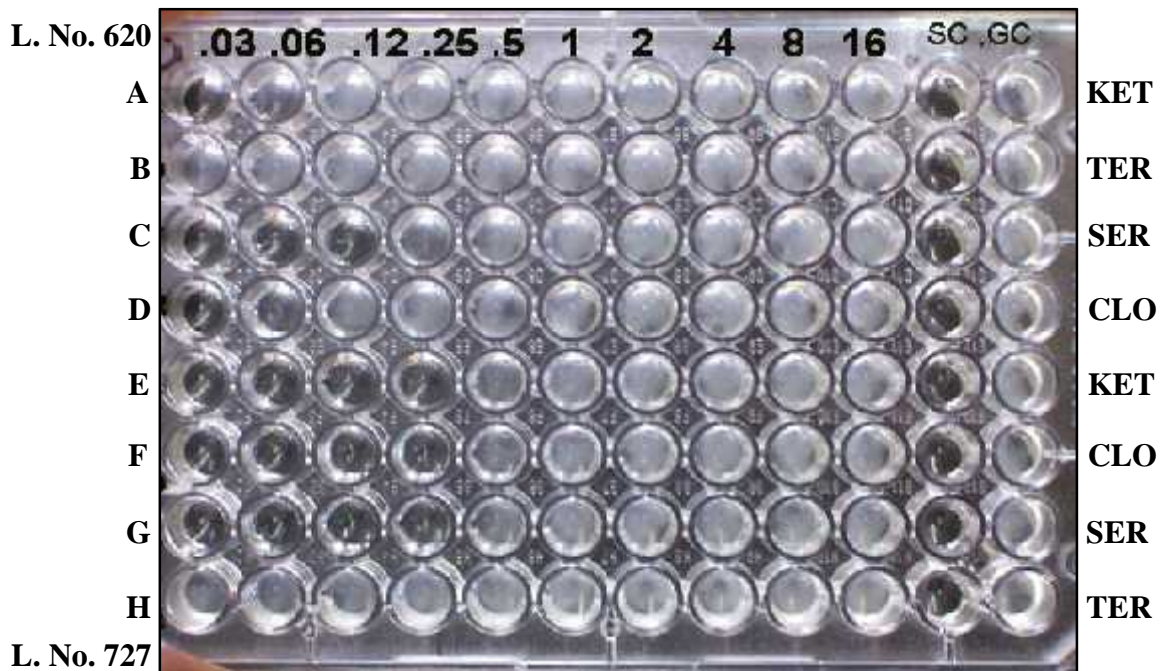
Photograph 15: Antifungal Susceptibility Microbroth Dilution



Stock solution kept at 4⁰-8⁰ C.



Dilutions of antifungal agents.



L. No.	-	Lab Number
A – KETO	-	0.06µg/ml
B – TER	-	<0.03µg/ml
C – SER	-	0.12µg/ml
D – CLO	-	0.06µg/ml
E – KETO	-	0.5µg/ml
F – CLO	-	0.12µg/ml
G – SER	-	0.12µg/ml
H – TER	-	<0.03µg/ml

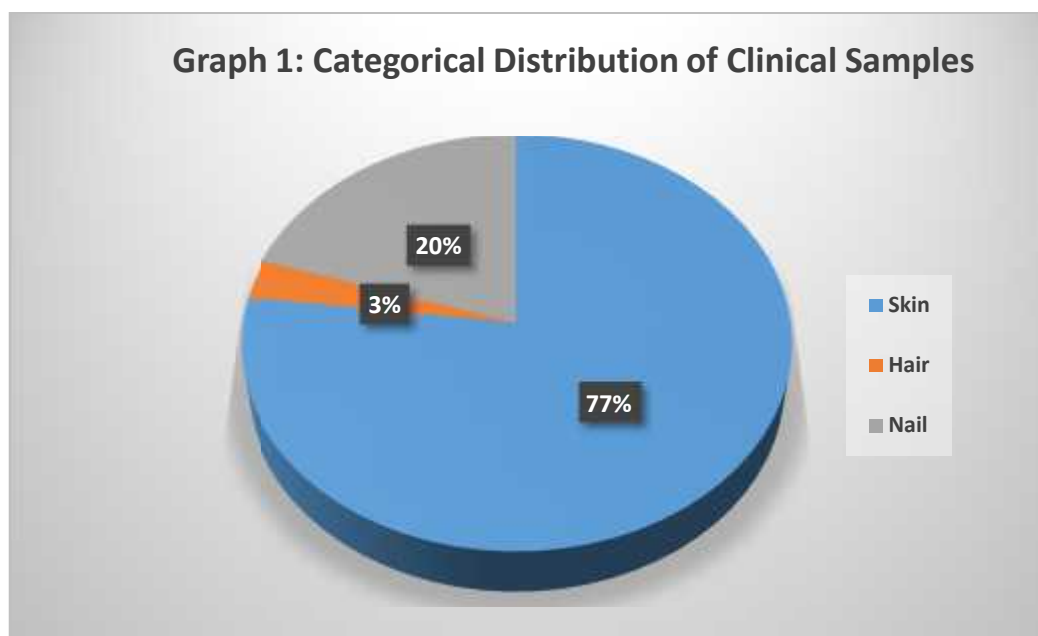
RESULTS

The present study of dermatophytosis was carried out in the Department of Microbiology, KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre Belgaum, over a Period of one year from January 2012 to December 2012. All clinically diagnosed cases of dermatophytosis in all age groups and of both sexes, attending the outpatient department of Dermatology and Venereology were taken for the study and patients who were already on treatment for dermatophytosis were excluded from the study.

In our study *T.cruis* (32) was the most common and *T.manuum* (2) was the least common clinical presentation with *T.rubrum* as the most common isolate.

Table 2: Categorical Distribution of Clinical Samples

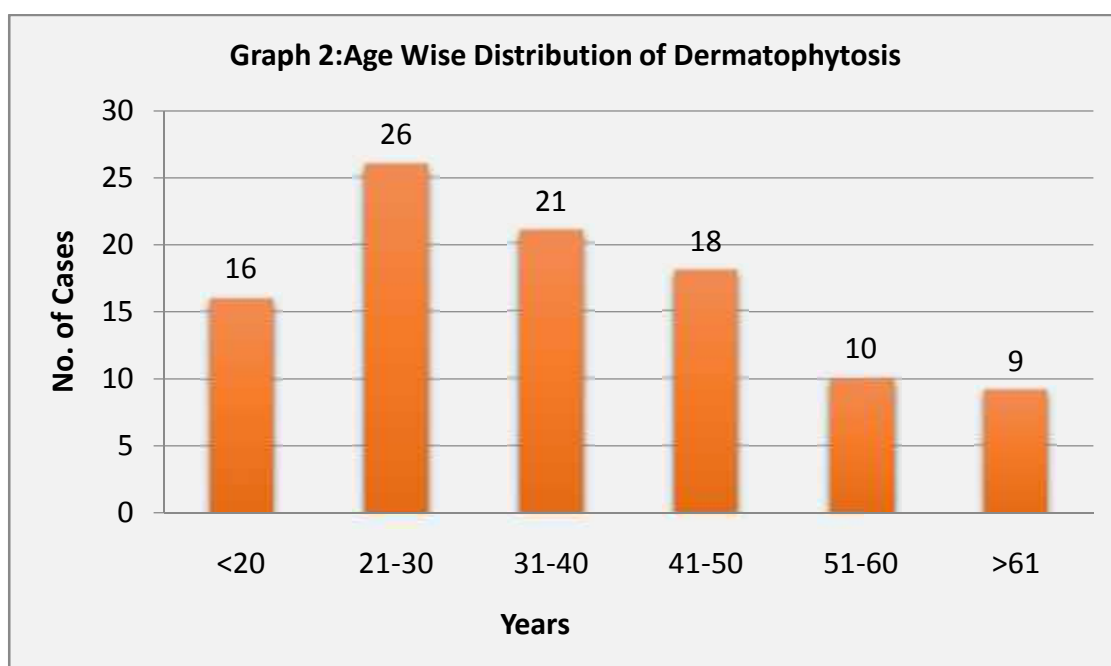
Samples	No of Samples	Percentage
Skin	77	77%
Hair	3	3%
Nail	20	20%
Total	100	100



In our study Dermatophytosis commonly involved skin followed by nail and hair.

Table 3: Age Wise Distribution of Dermatophytosis

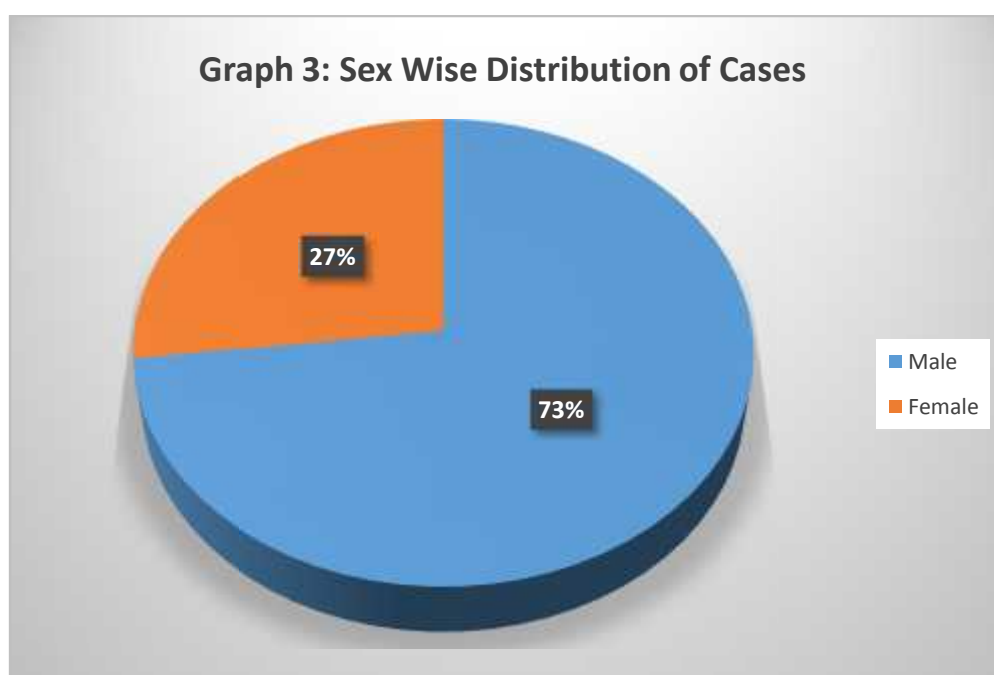
Age Group (Yrs)	No of Cases	Percentage
<20	16	16%
21-30	26	26%
31-40	21	21%
41-50	18	18%
51-60	10	10%
>61	9	9%
Total	100	100



Our study showed more cases of dermatophytosis in the age group of 21-30 years (26 cases) followed by 31-40 years (21 cases). Least Incidence was seen in age group above 61 years.

Table 4: Distribution of Males and Female Patients among Cases of Dermatophytosis

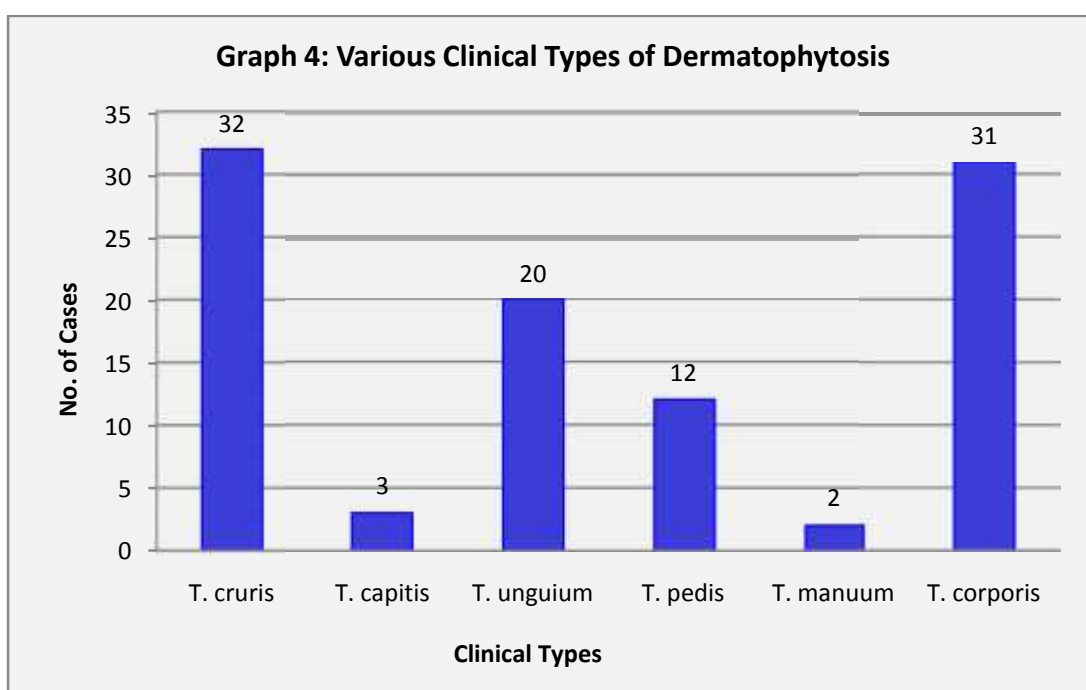
	Male	Female	Total	M:F
No of Cases	73	27	100	2.7:1
Percentage	73%	27%	100	



Incidence of Dermatophytosis was high in males and male to female ratio of 2.7:1.

Table 5: Various Clinical Types of Dermatophytosis

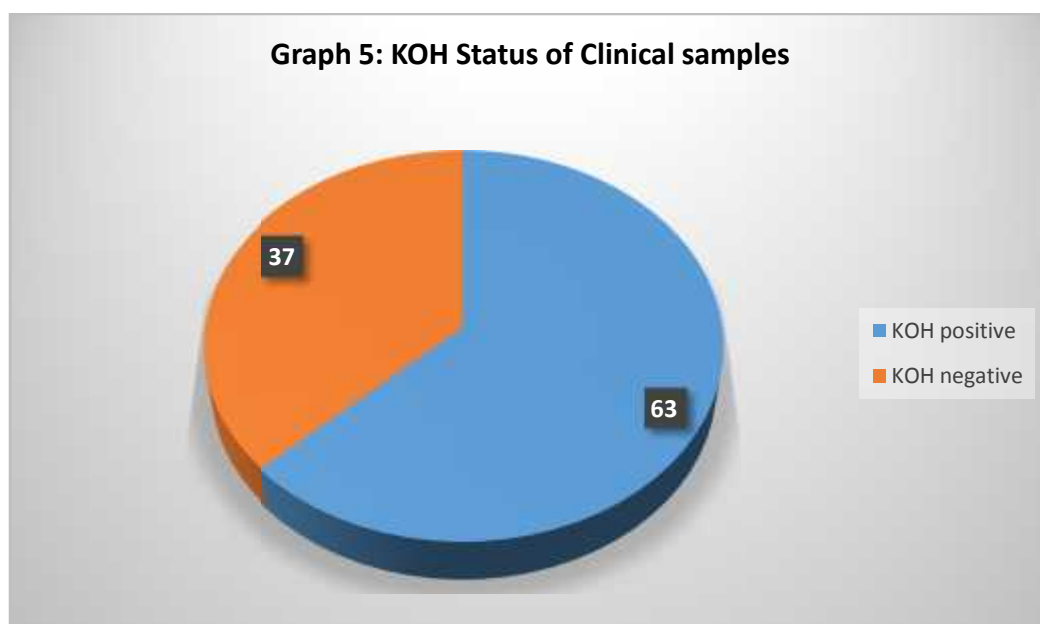
Dermatophytosis	Number of Cases
Ti. cruris	32
Ti. capitis	3
Ti. unguium	20
Ti. pedis	12
Ti. manuum	2
Ti. corporis	31



Ti. cruris was the most common and Ti. manuum was the least common clinical type of dermatophytosis encountered in our study.

Table 6: KOH Status of Clinical samples

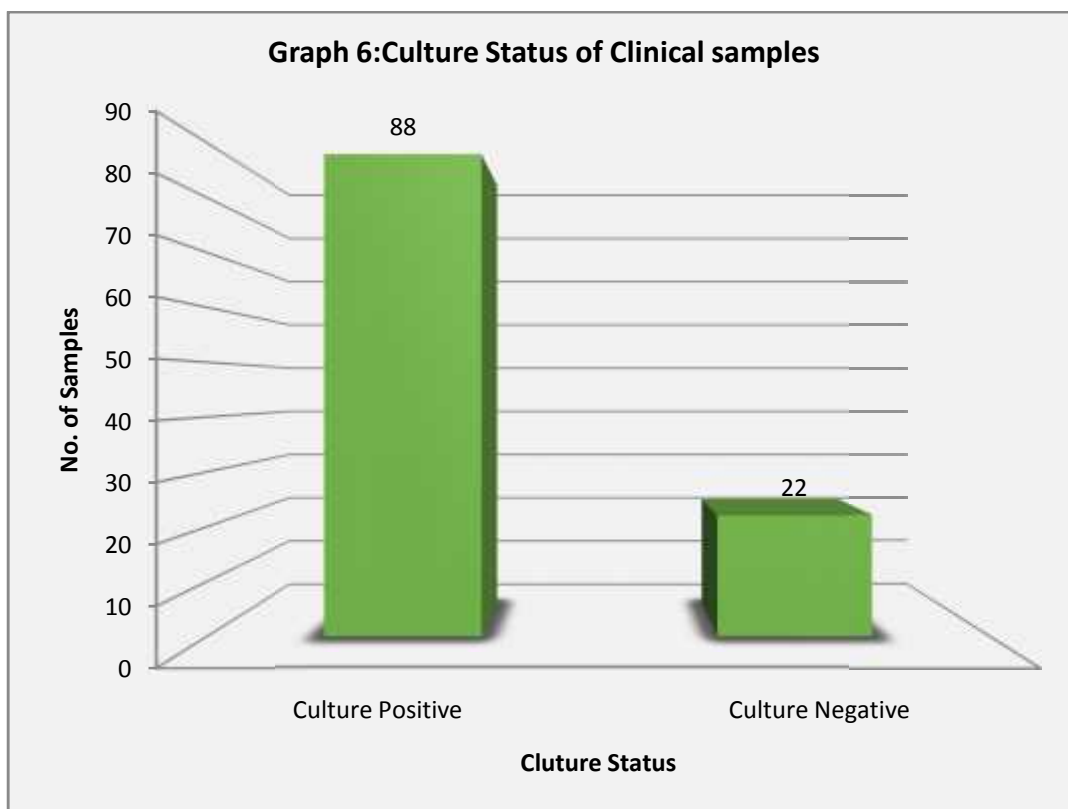
KOH Status	No of samples
KOH positive	63
KOH negative	37



Out of 100 clinical samples 37 samples were KOH negative and 63 samples were KOH positive

Table 7: Culture Status of Clinical samples

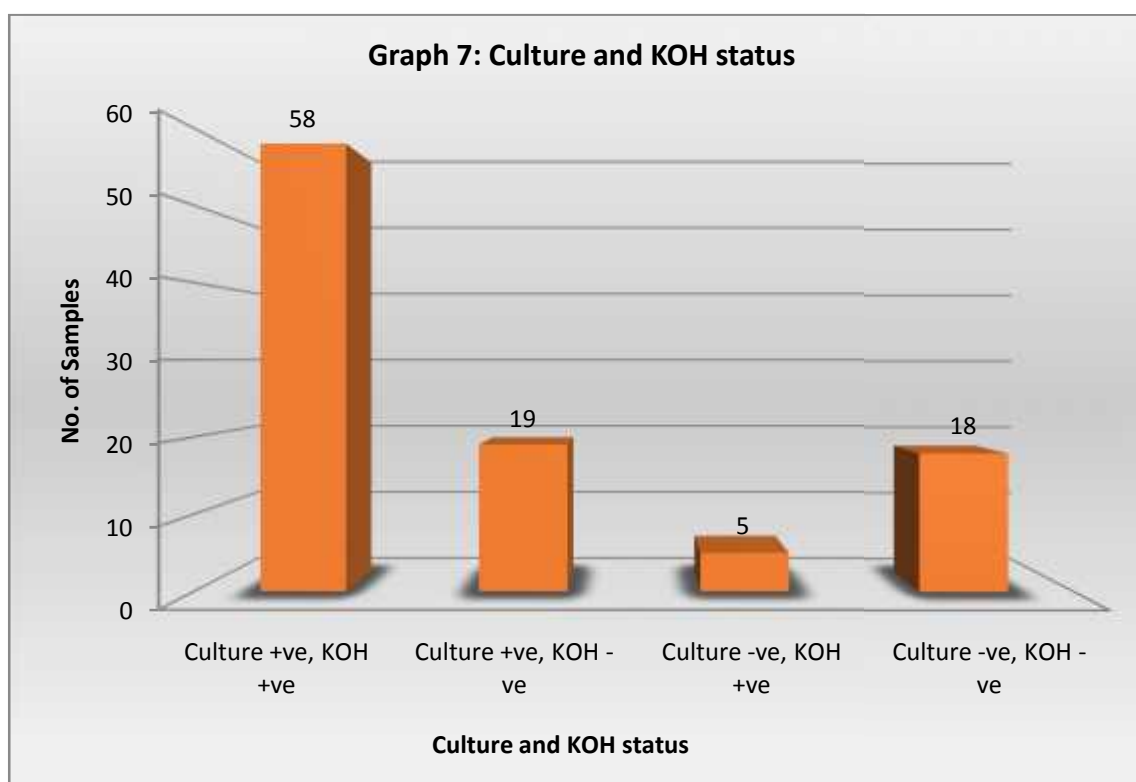
Culture Status	No.of samples
Culture Positive	88
Culture Negative	22



Out of 100 clinical samples 88 were culture positive and 22 were culture negative.

Table 8: Culture and KOH status

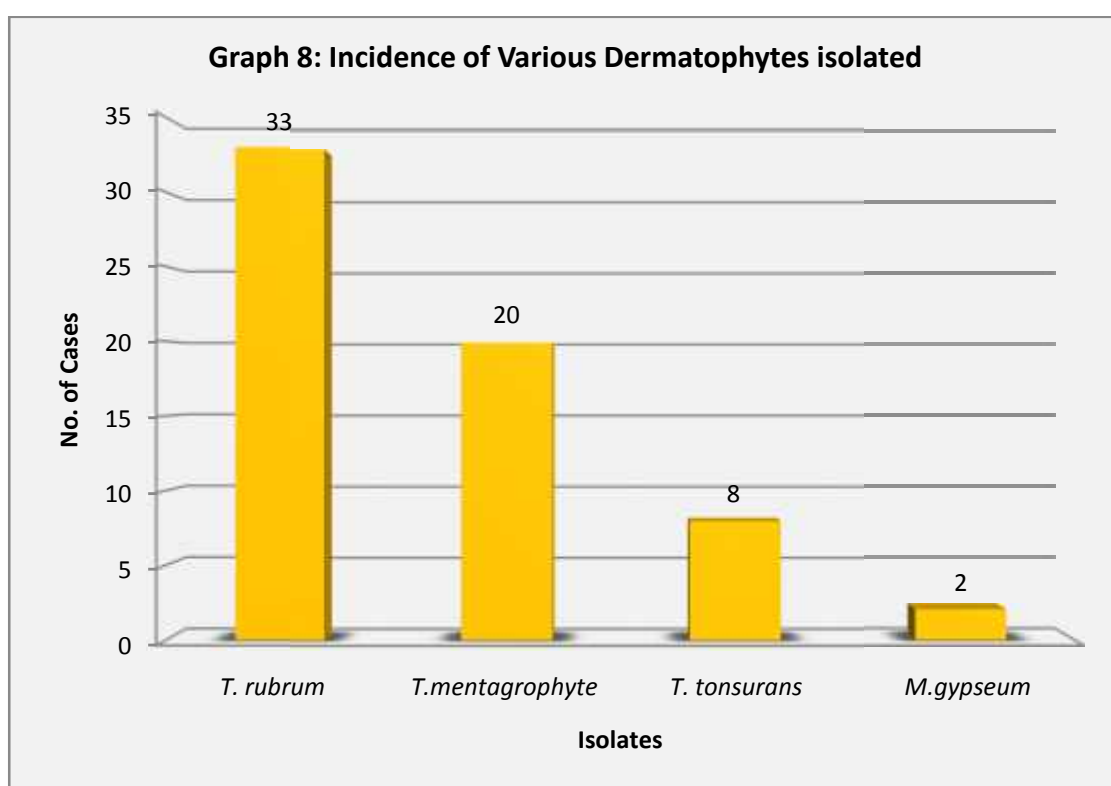
Culture and KOH status	No. of samples
Culture +ve, KOH +ve	58
Culture +ve, KOH -ve	19
Culture -ve, KOH +ve	5
Culture -ve, KOH -ve	18



Out of 100 clinical samples 58 samples were culture positive and KOH positive. 19 samples were Culture positive and KOH negative. 18 samples were Culture as well as KOH negative. 5 samples were KOH positive and culture negative.

Table 9: Incidence of Various Dermatophytes isolated

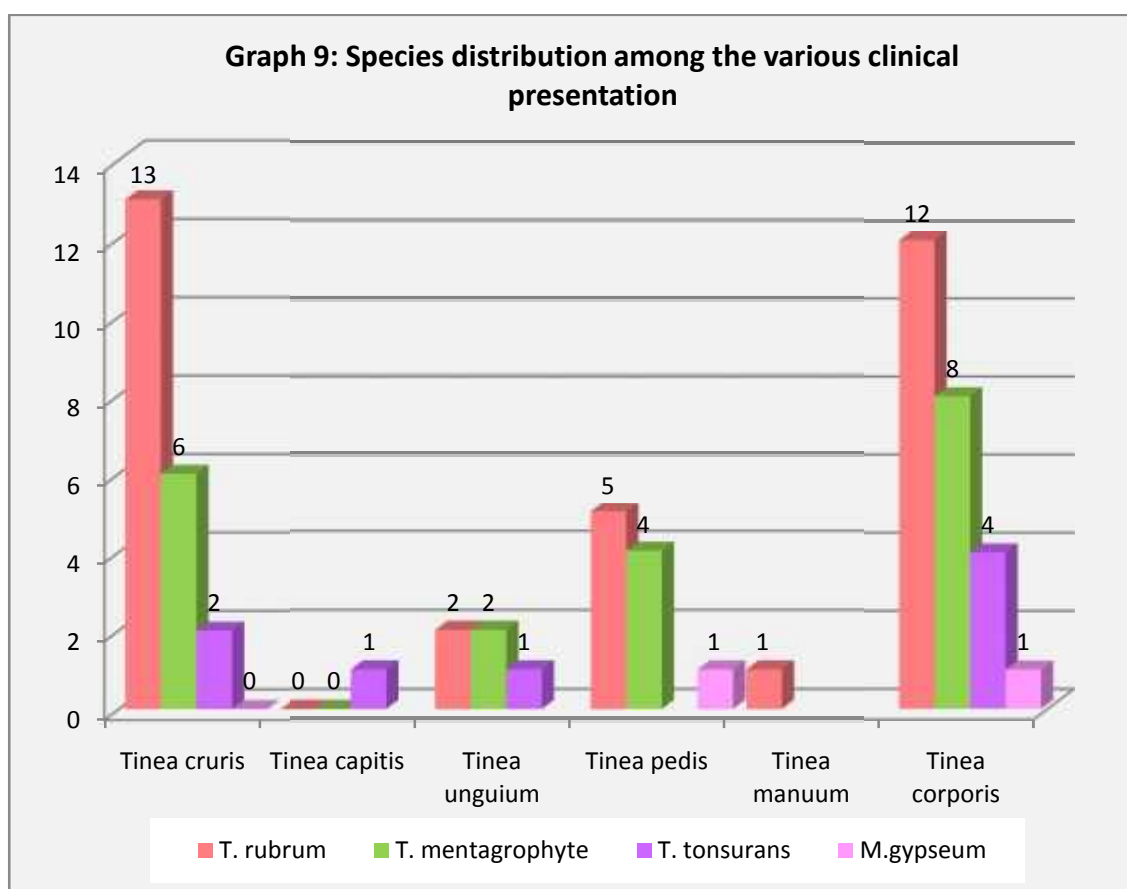
Isolates	Number of Cases
<i>Trichophyton rubrum</i>	33
<i>Trichophyton mentagrophytes</i>	20
<i>Trichophyton tonsurans</i>	8
<i>Microsporum gypseum</i>	2



Out of 63 isolates of Dermatophytes in our study *T. rubrum* was the most common (33), followed by *T. mentagrophyte* (20), *T. tonsurans* (8) and *M. gypseum* (2).

Table 10: Species distribution among the various clinical presentation

<i>Dermatophytosis</i>	<i>Trichophyton rubrum</i>	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton tonsurans</i>	<i>Microsporum gypseum</i>	<i>Total</i>
Ti. cruris	13	6	2	0	21
Ti. capitis	0	0	1	0	01
Ti. unguium	2	2	1	0	05
Ti. pedis	5	4	0	1	10
Ti. manuum	1	0	0	0	01
Ti. corporis	12	8	4	1	25
Total	33	20	08	02	63



Out of 32 clinical cases of *Ti. cruris*, 21 Dermatophytes were isolated. *T. rubrum* was isolated from 13 cases, *T. mentagrophytes* was isolated from 6 samples, and *T. tonsurans* was isolated from 2 samples.

Our study included 3 cases from *Ti. capitis* cases, out of which *T. tonsurans* was isolated from 1 sample.

Out of 20 cases of *Ti. unguium*, *T. rubrum*, *T. mentagrophytes*, and *T. tonsurans*, were isolated from 2, 2, and 1 clinical samples respectively.

Out of 12 cases from *T. pedis*, *T. rubrum* was isolated from 5, *T. mentagrophytes* from 4, and *M. gypseum* from 1 clinical sample.

Ti. manuum was the least common clinical type of dermatophytosis in our study with an incidence of 2 cases. From 1 sample *T. rubrum* was isolated.

Out of 31 clinical cases from *Ti. corporis*, *T. rubrum* was the commonest isolate with an incidence of 12. *T. mentagrophyte* was isolated from 8 clinical samples. *T. tonsurans* was isolated from 4 samples. *M. gypseum* from 1 clinical sample.

Table 11: Minimum Inhibitory Concentration for various antifungal agents

Dermatophyte	MIC Value Range in µg/ml			
	Ketaconazole	Terbinafine	Clotrimazole	Sertaconazole
<i>T. rubrum</i>	0.03-4	0.03-0.5	0.031-0.5	0.03-0.62
<i>T. mentagrophytes</i>	0.03-1	0.03-1	0.031-0.5	0.03-0.125
<i>T. tonsurans</i>	0.125-1	0.03-0.06	0.031-0.5	0.03-0.125
<i>M. gypseum</i>	0.12-0.5	0.15	0.031-0.5	0.03-0.125

In present study antifungal sensitivity was determined for *T. rubrum*, *T. mentagrophytes*,

T. tonsurans and *M. gypseum*. Antifungals tested were Ketaconazole, Terbinafine, Clotrimazole and Sertaconazole.

Range of MIC values of ketoconazole for *T. rubrum*(33), *T. mentagrophytes* (14), *T. tonsurans* (7)and *M. gypseum* were 0.03-4µg/ml, 0.03-1µg/ml, 0.125-1µg/ml and 0.12-0.5µg/ml respectively.

In remaining 6 isolates of *T. mentagrophytes* 3 had MIC of 2µg/ml and other 3 isolates had MIC of 3µg/ml for ketoconazole, which is above the normal range (0.03-1µg/ml) and 1 isolate of *T. tonsurans* had MIC of 2µg/ml.

Range of MIC values of Terbinafine for *T. rubrum*, *T. mentagrophytes*, *T. tonsurans* and *M. gypseum* were 0.03-0.5µg/ml, 0.03-1µg/ml, 0.03-0.06µg/ml and 0.15µg/ml respectively.

Range of MIC value of Clotrimazole for *T. rubrum*, *T. mentagrophytes*, *T. tonsurans* and *M. gypseum* was 0.031-0.5µg/ml.

Range of MIC value of Sertaconazole for *T. rubrum* was 0.03-0.62µg/ml and for *T. mentagrophytes*, *T. tonsurans* and *M. gypseum* was 0.03-0.125µg/ml.

All the above mentioned MIC values of Ketoconazole, Terbinafine, Clotrimazole and Sertaconazole was compared with the standard *T. mentagrophytes* ATCC 9533

MIC value of *T. mentagrophytes* ATCC 9533:

Ketoconazole – 0.06µg/ml

Terbinafine - <0.03µg/ml

Clotrimazole – 0.03µg/ml

Sertaconazole – 0.06µg/ml

DISCUSSION

In present study 100 clinically diagnosed cases of Dermatophytosis were studied. Of them 77 were skin scrapings, 20 were nail clippings and 3 were hair stubs. Out of these samples, Dermatophytes were isolated in 63 cases. Among 63 Dermatophytes isolated, *T.rubrum* was the commonest species (33) followed by *T.mentagrophytes* (20). *T.tonsurans* was isolated from (8), *M.gypseum* in (2), other than Dermatophytes *Penicillium spp* was isolated in 3 cases, *A.niger* in 7, *Acremonium* in 3 and *Curvilaria* in 4. No fungal growth was seen in 22 clinical samples. In 2 cases along with the Dermatophyte *Penicillium spp* was also isolated but their pathogenicity could not be proved by repeated isolation.

The overall isolation rate of Dermatophytes was 63%. *Ti. cruris* accounted for maximum number of cases.

Table 12: Isolation rate in various studies

Studies	Sample size	Isolation rate
Abu Elteen et al (1999) ⁴²	350	56.8%
Agarwalla et al (2001) ⁴³	100	94%
Seema Bose et al(2013) ⁴⁴	150	60.67%
Sanchita Karmakar et al (1995) ⁴⁵	250	8.6%
Asticcioli et al (2008) ⁴⁶	100	97%
Bindu V et al (2002) ⁴⁷	150	45.3%
Sumana MN et al (2002) ⁴⁸	150	24%
Present Study	100	63%

Present study shows that out of 100 clinically diagnosed cases of dermatophytosis 63 were affected by Dermatophytes. Our results are comparable to studies conducted by Abu Elteen et al (1999)⁴² where in isolation rate was 56.8%. In a study conducted by Bindu V et al (2002)⁴⁷ and Seema Bose et al (2013)⁴⁴ isolation rate was 45% and 60.67% respectively. Which is again comparable to results of present study. Extreme variation in isolation rate in few other studies may be due to differences in techniques used for sample collection, culture and other methods of identification.^{48,45}

Table 13: Commonly involved age group in various studies

Study	Age group in years
Khosravi AR et al (1994) ⁴⁹	20-29
Bindu V et al (2002) ⁴⁷	11-20
Sumana MN et al (2002) ⁴⁸	11-20
Agarwalla et al (2001) ⁴³	11-20
Sanchita Karmakar et al (1995) ⁴⁵	0-30
Abu Elteen et al (1999) ⁴²	30-39
Seema Bose et al(2013) ⁴⁴	21-30
Present Study	21-30

In present study patients with age group 21-30 were commonly involved by dermatophytosis. Our results are comparable to results from study conducted by Seema Bose et al (2013)⁴⁴ where in commonly involved age group was 21-30 years. Most of the other studies also showed high incidence of dermatophytosis in young patients. This may be due to higher incidence of health education, self-care and exposure in young patients.

Table 14: Sex ratio in various studies

Studies	Sex Ratio
Singh KA et al(1994) ⁵⁰	4:1
Sanchita Karmakar et al (1995) ⁴⁵	2:1
Agarwalla et al (2001) ⁴³	2.5: 1
Bindu V et al (2002) ⁴⁷	2.06:1
Welsh O et al(2006) ⁵¹	3.5: 1
Present Study	2.7:1

In present study which involved 100 subjects male to female ratio was 2.7: 1 males are commonly affected by dermatophytosis than females. Most of the studies show similar results with maximum incidence in males. In study conducted by Agarwalla et al (2001)⁴³ male to female ratio was 2.5:1 This male dominance may be due to social factors which makes less number of females to seek medical attention also it may be explained by increase in outdoor physical activity leading to higher exposure.

Table 15: Commonest Clinical Type of Dermatophytosis

Study	Commonest Dermatophytosis	Percentage
Bindu V et al (2002) ⁴⁷	Ti. corporis	54.6%
Sumana MN et al (2002) ⁴⁸	Ti. corporis	48.7%
Agarwalla et al (2001) ⁴³	Ti. corporis	43%
Sanchita Karmakar et al (1995) ⁴⁵	Ti. cruris	34.8%
Aghamirian MR et al(2004) ⁵²	Ti. corporis	20.7%
Aruna Aggarwal et al(2002) ⁵³	Ti. corporis	36.2%
	Ti. cruris	32%
Present Study	Ti. cruris	32%
	Ti. corporis	31%

In present study most common presentation of dermatophytosis studied was Ti. cruris with incidence of 32 (32%). Study conducted by Sanchita Karmakar et al (1995)⁴⁵ also showed similar results.

Most the other studies like study conducted by Bindu V et al (2002)⁴⁷, Sumana MN et al (2002)⁴⁸, Agarwalla et al (2001)⁴³, Aghamirian MR et al(2004)⁵¹ and Aruna Aggarwal et al(2013)^{ref} showed Ti. corporis being most common dermatophytosis with incidence of 54.6%, 48.7%, 43% 20.7% and 36.2% respectively.

Table 16: Commonest Dermatophyte Species isolated

Studies	Dermatophyte	Percentage
Abu Elteen et al (1999) ⁴²	<i>T.mentagrophytes</i>	32.7%
Agarwalla et al (2001) ⁴³	<i>T.rubrum</i>	45.74%
Asticcioli et al (2008) ⁴⁶	<i>T.rubrum</i>	42.3%
Bindu V et al (2002) ⁴⁷	<i>T.rubrum</i>	66.2%
Sumana MN et al (2002) ⁴⁸	<i>T.rubrum</i>	52.7%
Seema Bose et al (2013) ⁴⁴	<i>T.rubrum</i>	33.3%
Present Study	<i>T.rubrum</i>	33%
	<i>T.mentagrophytes</i>	20%

T - Trichophyton

In present study *T.rubrum* was the commonest dermatophyte isolated from 33 clinical samples. Second most common dermatophyte isolated was *T. mentagrophyte* with the incidence of 20. In various studies conducted by Agarwalla et al (2001)⁴³, Asticcioli et al (2008)⁴⁶, Bindu V et al (2002)⁴⁷ and Sumana MN et al (2002)⁴⁸ *T.rubrum* was the commonest isolate with incidence ranging from 42.3% to 66.2%. The present study was comparable with Seema Bose et al (2013)⁴⁴.

Some species of Dermatophytes are endemic in certain parts of the world. Various studies done in India have shown *T.rubrum* as the commonest isolate.

Antifungal susceptibility testing:

In recent years several studies of in vitro susceptibility of dermatophytes have been done and the results have shown considerable variations. This variability is probably due to important methodological differences like preparation of inoculum, incubation at different temperatures, no of days of incubation is different among the laboratories. Following the extensive search for the studies presenting the antifungal susceptibility of dermatophytes, the studies following the CLSI guidelines were only taken as references to compare the results of present study.

Antifungal susceptibility was carried out for all the Dermatophytes isolated (63). Microbroth dilution method was used to determine the MIC for Ketoconazole, Terbinafine, Clotrimazole, and Sertaconazole according to NCCLS (CLSI) M38A (2007) document for antifungal susceptibility testing for filamentous fungi with some modifications. The CLSI approved guidelines recommended separation of the fungal structures (hyphae and conidia) through sedimentation for 15 to 20 min and use of the upper part of the suspension for susceptibility testing.⁵⁴ The separation of hyphae and conidia a crucial step for the determination of MICs for Dermatophytes.

Whatman no 40 was used to filter the inoculum as it retains the hyphal fragment and permits the passage of only microconidia of Dermatophytes.⁵⁵

As there are no guidelines proposed by CLSI for antifungal susceptibility testing of dermatophytes. The procedures recommended are standardized against only Itraconazole, Terbinafine, Griesoflavin, Fluconazole. The antifungals chosen in the study are mainly on the basis of clinical usage in the treatment of dermatophytosis. Sertaconazole is a newer azole available in the treatment of dermatophytosis, the effectiveness of which is not widely studied, nor standardised by CLSI⁵⁶. It promises

to be a better drug in the treatment of resistant dermatophytosis in comparison to other azoles and Terbinafine⁵⁶ Ketoconazole is costly when compared to other drugs, thus is not widely used drug for treatment of dermatophytosis, in low socio economic countries like India. This makes clinicians to prescribe cheaper but clinically effective drugs like Terbinafine, Clotrimazole, and Sertaconazole.

TERBINAFINE:

Table: 17 MIC values for Terbinafine from various studies

Studies	Species	MIC range µg/ml
Santos et al (2005) ⁵⁷	<i>Trichophyton sps</i>	<0.031
Ghannoum et al (2004) ⁵⁸	<i>Trichophyton sps</i>	0.001–0.5
Araujo et al (2009) ⁵⁹	<i>T. rubrum</i>	0.03-0.5
	<i>T. mentagrophytes</i>	0.03-1
	<i>M. gypseum</i>	0.15
Norris et al (1999) ⁶⁰	<i>Trichophyton sps</i>	<0.06
Carrillo-Mun˜oz et al (1997) ⁵⁶	All Dermatophytes	0.03
Present study	<i>T. rubrum</i>	0.03-0.5
	<i>T. mentagrophytes</i>	0.03-1
	<i>T. tonsurans</i>	0.03-0.06
	<i>M. gypseum</i>	0.15

In the present study it was found that for all the species isolated, Terbinafine had low MIC range and this is comparable with most of the other studies like Norris et al⁶⁰, Ghannoum et al⁵⁸.

The results of Santos et al and Carrillo-Mun˜oz et al⁵⁶ is similar to that of the present study. As few isolates in present study showed MIC of <0.03µg/ml

MIC of *T.mentagrophytes* ATCC 9533 which was used as control also showed within the range as in the present study

Ketoconazole:

Table18: MIC values for Ketoconazole from various studies

Studies	Species	MIC range µg/ml
Santos et al (2005) ⁵⁷	<i>T. rubrum</i>	0.0625–2.0
Pujol et al (2002) ⁶¹	All Dermatophytes	0.03-2
Araujo et al (2009) ⁵⁹	<i>T. rubrum</i>	0.03-4
	<i>T.mentagrophyte</i>	0.03-1
Cetinkaya et al (2005) ⁶²	<i>T.rubrum</i>	0.03-8
	<i>T.mentagrophytes</i>	0.25-2
	<i>T.tonsurans</i>	0.12-0.25
Siqueira et al (2008) ⁶³	<i>Trichophyton sps</i>	<0.03-0.5
Present study	<i>T.rubrum</i>	0.03-4
	<i>T.mentagrophytes</i>	0.03-1
	<i>T.tonsurans</i>	0.12-1
	<i>M .gypseum</i>	0.12-0.5

T- Trichophyton

M-Microsporum

Ketoconazole has higher MIC in a study done by Cetinkaya et al ⁶² when compared to the present study this could because of different size of the inoculum used or incubated for longer duration.

The present study had similar results as those of Araujo et al⁵⁹, Santos et al⁵⁷, and Pujol et al⁶¹.

Siqueira et al ⁶³ showed lower MIC than the present study. Few isolates of *T. tonsurans* and *T. mentagrophytes* in the present study showed higher MIC value than the other isolates.

T. mentagrophytes ATCC 9533 which was used as control showed MIC 0.06µg/ml

Clotrimazole:

Table 19: MIC values of Clotrimazole from various study

Studies	Species	MIC µg/ml
Santos et al (2006) ⁵⁵	All Dermatophytes	0.03-0.5
B. Favre et al (2003) ⁶⁴	All Dermatophytes	0.083
Present study	All Dermatophytes	0.03-0.5

Favre et al showed lower MIC value when compared to present study whereas the results of Santos et al ⁵⁵ was similar.

MIC of *T. mentagrophytes* ATCC 9533: 0.03µg/ml

Sertaconazole:

Table 20: Various studies showing MIC values

Studies	Species	MIC $\mu\text{g/ml}$
Carrillo-Mun˜oz et al (1997) ⁵⁶	<i>T.rubrum</i>	0.03-0.62
	<i>T.mentagrophytes</i>	0.03-0.12
	<i>T.tonsurans</i>	0.03-0.12
	<i>M.gypseum</i>	0.3-1.2
Palaci˜n et al. (1992) ⁶⁵	All Dermatophytes	0.24
Present study	<i>T.rubrum</i>	0.03-0.62
	<i>T.mentagrophytes</i>	0.03-0.12
	<i>T.tonsurans</i>	0.03-0.12
	<i>M.gypseum</i>	0.03-0.12

*T- Trichophyton**M-Microsporum*

MIC of Sertaconazole from various studies Palacin et al⁶⁵ and Carrillo Munoz et al⁵⁶ had similar results as the present study. MIC of *T.mentagrophytes* ATCC 9533: 0.06 $\mu\text{g/ml}$

In the present study Terbinafine was found to be more potent when compared to other drugs with lower MIC values (<0.03 $\mu\text{g/ml}$) followed by Sertaconazole and Clotrimazole.

Ketoconazole had higher MIC and also some isolates had MIC more than the normal range.

Ketoconazole had higher MIC and also some isolates had MIC more than the normal range. The susceptibility pattern of the isoltes in the study is comparable to the other studies. This study supports the clinical use of Terbinafine ,Clotrimazole in empirical treatment of dermatophytosis.

CONCLUSION

Dermatophytosis is the most common type of cutaneous fungal infection. It is very common in our country with several contributing factors like hot humid climate, poor hygiene, increased outdoor activities, occupational trauma and immunosuppression.

Dermatophytes isolated included predominately *Trichophyton* species, of which *T.rubrum* was the commonest dermatophyte isolated.

T.mentagrophytes, *T.tonsurans*, *M.gypseum* were other species isolated from clinical samples.

T.mentagrophytes, *T.tonsurans*, *M.gypseum* were other species isolated.

MIC of *T.rubrum*, the commonest isolate, against Terbinafine (0.03-0.5 μ g/ml) Clotrimazole (0.03-0.62 μ g/ml), Sertaconazole (0.03-0.62 μ g/ml) Ketoconazole (0.03-4 μ g/ml). The other isolated species had comparable MIC. Susceptibility of *T.mentagrophytes* and *T.tonsurans* against Ketoconazole was in the higher range.

Periodic evaluation of Antifungal Susceptibility Testing is necessary to know the MIC range of prevailing strains against commonly used drugs so as to guide the empirical treatment of dermatophytosis.

SUMMARY

The present study was conducted in the Department of Microbiology, J.N. medical college, Belgaum for a period of one year from January 2012 to December 2012.

The samples were collected from Dermatology and Venereology OPD at KLE Dr.Prabhakar Kore Charitable hospital & medical research Centre, Belgaum.

- The study was conducted over a period of one year from January 2012 to December 2012.
- Patients who were already on treatment for dermatophytosis were not included in the study
- The present study included 100 clinically diagnosed cases of dermatophytosis.
- *Ti. cruris* (32) was the commonest clinical type followed by *Ti. corporis* (31), *Ti. unguium* (20), *Ti. pedis* (12), *Ti. capitis* (3) and *Ti. manuum* (2).
- Commonest age group affected was between 21-30 years.
- Males were more commonly affected than females with male to female ratio 2.7:1.
- Four species of Dermatophytes were isolated amongst them *T.rubrum* (33) was the commonest species followed by *T.mentagrophytes* (20), *T.tonsurans* (8) and *M.gypseum* (2).
- Terbinafine was found to be more potent than Clotrimazole, Sertaconazole, and Ketoconazole, with lower MIC <0.03µg/ml.

- Our study demonstrated that tested antifungal agents are active against dermatophytes, with species variability. This assures clinician to continue with present protocol of treatment for dermatophytosis with high probability of favourable cure rate.

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ANNEXURE – I : CONSENT FOR PARTICIPATION RESEARCH

TITLE: “Isolation, Identification and antifungal susceptibility of Dermatophytes from clinical samples- One year study”

Study Investigator Dr. _____
Post Graduate Student,
Department of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belgaum – 590 010

Guide Dr. _____

The purpose of research is to Isolate and identify Dermatophytes and their antifungal susceptibility pattern.

You are requested to participate in which will help to provide appropriate and effective treatment. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with Jawaharlal Nehru Medical College. If you decide to participate you are free to withdraw at any time.

PROCEDURE INVOLVED:

Skin samples from clinically diagnosed cases of dermatophytosis will be collected to isolate and identify Dermatophytes and the antifungal susceptibility for the same.

RISKS AND BENEFITS:

There are no risks/minimal risks involved and benefits are to be evaluated.

PRIVACY AND CONFIDENTIALITY:

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except In emergency to protect your rights and welfare.

AUTHORIZATION TO PUBLISH RESULTS:

When the results of research are published or discussed, in a conference no information will be displaced that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION:

You will not have to pay/offer any gifts for participating in the research. You will not be reimbursed for expenses.

In case you have any questions related to the study, you can contact Dr. _____ (Mobile no. _____)

In case you have any questions about your rights as a participant, you can contact In case you have any questions about your rights as a study participant you can contact **Dr. A. S. Godhi (0831-2471350)**.

CONSENT STATEMENT

I undersigned _____ have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left hand thumb print of participant or legally authorized representative.

Participants Name _____

Signature _____

Witness Name _____

Signature _____

Experimenters Name _____

Signature _____

Date :

Place:

ANNEXURE – II

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

Name : Sex :
Age : O.P. No:
DOA : LAB. NO:
Occupation:
Address :

Presenting complaints:

History of presenting illness:

Past history:

- History of similar episode in the past.
- Any treatment taken for similar complaint

Family History

- Any similar complaints in family members.

Personal history:

General Physical Examination:

Local examination :

Systemic Examination :

Laboratory Investigations:

Laboratory findings:

1. Specimen :

2. Direct microscopy:

	Fungal elements Present	Fungal elements absent
KOH		

3. Culture : SDA with cycloheximide and chloramphenicol at 27°C

Macroscopic morphology	First week	Second week	Third week	Forth week
Wooly				
Velvety				
Granular				
Waxy				
Surface of colony				
Pigment production				

Slide culture: Microscopy with LPCB mount :

4. Antifungal susceptibility :

Microbroth dilution :

- Ketoconazole
- Clotrimazole
- Terbinafine
- Sertaconazole

Final report :

ANNEXURE – III: PREPARATION OF MEDIA

Sabouraud's Dextrose Agar :

Glucose – 4 % (40gm)

Peptone -1% (10gm)

Agar -2% (20gm)

Distilled water – 1000ml

pH – 5.6

Autoclave the above mentioned ingredients at 121⁰C for 15 mins. Dispense in tubes and allow it to cool.

Sabouraud's Dextrose Agar with Antibiotics:

Composition of Sabouraud's Dextrose Agar (Emmons Modification)

Dextrose :20gm

Peptone : 10g

Agar :20g

Distilled Water : 1000ml

Final pH : 6.9

The ingredients were dissolved by boiling to it 0.05mg/ml of Chlormphenicol was added and 0.5 mg /ml of Cycloheximide after autoclaving at 121° for 15 minutes, Dispense in Tubes and allow to Cool in slanted position.

POTATO DEXTROSE AGAR

For convenience this medium is generally prepared from dehydrated commercial preparation according to the instruction of the manufacturer. Alternatively this medium may be prepared

From raw materials as follows:

Potatoes :250g

Dextrose :20g

Agar :20g

Distilled water :1 lit

pH : 5.6 ± 0.2

Scrub but do not peel the potato & slice, Boil 200g in 100ml of water for 60 minutes. Squeeze as much of the pulp as possible through a fine sieve. Add agar and boil till dissolved. Add dextrose and make up to 1 liter. Dispense in required amounts taking care to keep solids in suspension. Autoclave at 115° C for 30 minutes. Cool to 50° C & pour in to tubes and allowed to cool in slanted position.

MODIFIED CHRISTENSEN'S UREASE MEDIUM

Peptone : 1.0g

NaCl : 5.0g

Disodium phosphate : 1.2g

Monopotassiumphosphat : 0.8g

Dextrose : 1.5g

Phenol red : 0.012g

Agar : 15.0g

Distilled water : 1000ml

Ph: 6.8±0.2

After dissolving the above ingredients by heat and autoclaved at 121°C for 15 minutes. On cooling 100ml of urea (20% aqueous solution sterilized by solution) was added and medium was poured into slopes

POTASSIUM HYDROXIDE MOUNTS

It is prepared from the following ingredients

Potassium hydroxide -10gms

Glycerol -10ml

Distilled water- 80ml

To a solution of 10% KOH, 10% Glycerol is added to prevent drying. Mix ingredients and store at room temperature.

LACTOPHENOL COTTON BLUE STAIN

The lacto phenol cotton blue (LPCB) is used to study the morphological features of the fungal isolates. It is of two types:

- Plain LPCB

It contains the following ingredients:

Melted phenol -20ml

Lactic acid -20ml

Glycerol-40ml

Cotton blue -0.05 gm

Distilled water- 20ml

Mix all the reagents properly and dissolve 0.05 g of cotton blue stain in the distilled water before mixing with the remaining reagents. The phenol acts as disinfectant, Lactic Acid preserves the morphology of the fungi and glycerol is hygroscopic agent which prevents drying. The cotton blue stains the outer wall of the fungus. Tease out of a fragment of the culture on a glass slide in a drop of LPCB using two teasing needles. Put of a cover slip and examine under the microscope. If the plane LPCB is used the edges of the cover slip can be sealed with nail polish to keep it for longer period of time.

ANNEXURE – IV: MASTER CHART

s.no.	LAB No.	Age	Sex	Sample	Clinical diagnosis	KOH	Culture		Anti fungal susceptibility			Clotrimazole MIC µg/ml
							SDA with Antibiotic	SDA without Antibiotic	Ketoconazole MIC µg/ml	Terbinifine MIC µg/ml	Sertaconazole MIC µg/ml	
1	560	32	F	Nail clipping	Ti. unguium	+		Aspergillus niger				
2	275	80	M	Nail clipping	Ti. unguium	+		Acremonium sps				
3	300	49	F	skin scraping	Ti. mannum	+		No growth				
4	338	19	F	skin scraping	Ti. pedis	+	T.rubrum		0.06	0.03	0.06	0.25
5	339	45	F	skin scraping	Ti. pedis	+	T.rubrum		0.12	0.03	0.03	0.12
6	374	34	M	skin scraping	Ti. cruris	-	T.rubrum		0.12	0.03	0.03	0.025
7	376	55	F	skin scraping	Ti. cruris	+		No growth				
8	375	35	M	skin scraping	Ti. corporis	-		No growth				
9	462	22	M	skin scraping	Ti. pedis	-		No growth				
10	473	62	M	skin scraping	Ti. pedis	+		Curvularia sps				
11	491	23	M	skin scraping	Ti. cruris	+	T.rubrum		0.06	0.12	0.12	0.12
12	555	33	M	skin scraping	Ti. corporis	+	T.rubrum		0.06	0.06	0.12	0.06
13	493	37	M	skin scraping	Ti. corporis	+	T.tonsurans		0.12	0.06	0.12	0.03
14	494	17	M	skin scraping	Ti. corporis	+	T.mentagrophytes		0.06	0.03	0.03	0.03
15	558	42	M	Hair clipping	Ti. capitis	+	T.tonsurans		0.25	0.03	0.03	0.12
16	496	25	M	skin scraping	Ti. cruris	+	T.rubrum		0.5	0.03	0.03	0.06
17	505	40	M	Nail clipping	Ti. unguium	+		Acremonium sps				
18	506	48	F	skin scraping	Ti. corporis	-		No growth				
19	507	60	M	skin scraping	Ti. cruris	+	T.rubrum		0.25	0.12	0.06	0.03
20	508	50	M	skin scraping	Ti. cruris	+	T.rubrum		0.12	0.06	0.06	0.12

21	509	49	F	skin scraping	Ti. corporis	+	T.mentagrophytes	Penicillium sps	0.5	0.5	0.5	0.5
22	510	25	M	skin scraping	Ti. corporis	+	T.rubrum		0.12	<0.03	0.06	0.12
23	530	81	M	Nail clipping	Ti. unguium	+		Acremonium sps				
24	511	23	F	skin scraping	Ti. cruris	-		No growth				
25	516	43	F	skin scraping	Ti. corporis	-	T.mentagrophytes		0.25	0.06	0.12	0.06
26	572	25	M	skin scraping	Ti. corporis	+	T.rubrum		0.25	0.06	0.12	0.12
27	517	20	M	skin scraping	Ti. corporis	-		Penicillium sps				
28	526	62	M	skin scraping	Ti. cruris	+		No growth				
29	525	33	F	skin scraping	Ti. corporis	+	T.rubrum		0.06	0.12	0.5	0.03
30	523	31	M	skin scraping	Ti. corporis	-	T.rubrum		2	0.12	0.5	0.03
31	539	17	M	skin scraping	Ti. corporis	+	T.mentagrophytes		0.06	<0.03	0.5	0.25
32	536	66	M	skin scraping	Ti. cruris	+	T.mentagrophytes		3	0.12	0.5	0.25
33	540	12	M	Hair clipping	Ti. capitis	+		No growth				
34	541	61	M	skin scraping	Ti. corporis	+	T.tonsurans		0.12	<0.03	0.12	0.06
35	550	58	M	Nail clipping	Ti. unguium	-		Aspergillus niger				
36	542	30	M	skin scraping	Ti. corporis	-	T.rubrum		1	0.5	0.06	0.5
37	568	24	M	skin scraping	Ti. corporis	-	T.tonsurans		0.5	0.12	1	0.06
38	574	56	M	skin scraping	Ti. cruris	+	T.tonsurans		0.5	0.06	0.03	0.06
39	545	16	M	skin scraping	Ti. cruris	+	T.rubrum		0.12	0.06	0.12	0.06
40	570	33	F	skin scraping	Ti. cruris	+	T.rubrum		0.12	<0.03	0.12	0.12
41	547	49	M	skin scraping	Ti. corporis	+	T.tonsurans		1	0.06	0.5	0.12
42	566	15	M	skin scraping	Ti. corporis	-	T.rubrum		0.5	0.03	0.5	0.5
43	562	14	M	skin scraping	Ti. cruris	-	T.rubrum		0.5	0.03	0.5	0.5
44	584	28	M	skin scraping	Ti. cruris	+	T.tonsurans		0.25	<0.03	0.03	0.03
45	588	57	F	skin scraping	Ti. corporis	-	T.mentagrophytes		1	0.5	0.03	0.06

46	593	26	F	skin scraping	Ti. corporis	+		Aspergillus niger					
47	596	36	M	skin scraping	Ti. cruris	-		No growth					
48	577	24	M	skin scraping	Ti. cruris	+	T.mentagrophytes		3	0.5	0.12	0.12	
49	579	22	F	skin scraping	Ti. corporis	-		No growth					
50	585	34	M	skin scraping	Ti. cruris	+		Aspergillus niger					
51	607	65	M	Nail clipping	Ti. unguium	+	T.rubrum		2	0.06	0.12	0.06	
52	610	29	M	skin scraping	Ti. pedis	+	T.mentagrophytes		3	0.12	0.06	0.06	
53	613	24	M	skin scraping	Ti. cruris	-		No growth					
54	616	23	M	skin scraping	Ti. cruris	-		No growth					
55	619	25	M	skin scraping	Ti. corporis	-		No growth					
56	620	46	M	skin scraping	Ti. corporis	+	T.rubrum	Penicillium sps	0.5	0.12	0.03	0.5	
57	598	20	M	skin scraping	Ti. pedis	-	T.mentagrophytes		2	0.5	0.03	0.5	
58	623	50	F	Nail clipping	Ti. unguium	-		No growth					
59	626	20	M	Nail clipping	Ti. unguium	+		No growth					
60	629	60	F	Nail clipping	Ti. unguium	-		Curvularia sps					
61	630	52	M	Nail clipping	Ti. unguium	-		No growth					
62	640	37	M	skin scraping	Ti. pedis	-	T.rubrum		0.5	0.5	0.12	0.12	
63	733	40	M	Nail clipping	Ti. unguium	+		Curvularia sps					
64	734	40	M	skin scraping	Ti. pedis	+	M.gypseum		0.5	0.12	0.06	0.06	
65	716	50	M	skin scraping	Ti. mannum	-	T.rubrum		1	0.12	1	0.06	
66	717	72	M	Nail clipping	Ti. unguium	+		Aspergillus niger					
67	723	32	M	skin scraping	Ti. cruris	+		Aspergillus niger					
68	724	30	M	skin scraping	Ti. cruris	+	T.mentagrophytes		2	0.5	0.25	0.25	
69	725	28	M	skin scraping	Ti. cruris	-		No growth					
70	727	30	M	skin scraping	Ti. cruris	+	T.mentagrophytes		0.5	0.03	0.12	0.25	

71	728	23	F	skin scraping	Ti. cruris	-	T.rubrum		2	0.03	0.12	0.25
72	800	23	M	Nail clipping	Ti. unguium	+	T.mentagrophytes		1	0.12	1	0.25
73	808	10	M	skin scraping	Ti. corporis	-	M.gypseum		0.5	0.12	0.25	0.25
74	809	30	M	skin scraping	Ti. corporis	+	T.mentagrophytes		1	<0.03	0.25	0.25
75	859	31	F	skin scraping	Ti. corporis	+	T.rubrum		0.12	0.06	1	0.25
76	865	23	F	Nail clipping	Ti. unguium	-		No growth				
77	870	20	M	Nail clipping	Ti. unguium	-	T.rubrum		0.25	0.5	0.25	0.5
78	871	10	F	Hair clipping	Ti. capitis	-		No growth				
79	872	19	M	skin scraping	Ti. pedis	-	T.rubrum		0.25	0.06	0.12	0.06
80	874	41	M	skin scraping	Ti. pedis	+	T.mentagrophytes		1	0.03	0.12	0.12
81	125	35	M	skin scraping	Ti. corporis	-	T.rubrum		0.25	<0.03	0.12	0.03
82	126	32	M	Nail clipping	Ti. unguium	-	T.mentagrophytes		0.25	0.03	0.25	0.03
83	129	32	M	Nail clipping	Ti. unguium	-	T.tonsurans		2	0.12	0.25	0.03
84	101	52	M	skin scraping	Ti. cruris	+	T.mentagrophytes		0.5	0.25	0.25	0.12
85	102	50	M	skin scraping	Ti. pedis	+	T.mentagrophytes		0.5	0.12	0.06	0.06
86	82	29	M	skin scraping	Ti. pedis	+	T.rubrum		0.5	0.06	0.03	0.06
87	99	12	M	skin scraping	Ti. corporis	+	T.rubrum		0.5	0.06	0.06	0.06
88	130	26	F	Nail clipping	Ti. unguium	+		Curvularia sps				
89	103	60	M	skin scraping	Ti. cruris	-	T.rubrum		1	0.12	0.06	0.12
90	181	56	F	Nail clipping	Ti. unguium	-		No growth				
91	179	20	M	skin scraping	Ti. corporis	+	T.rubrum		0.12	0.12	0.06	
92	178	29	F	skin scraping	Ti. cruris	-		No growth				
93	146	38	F	skin scraping	Ti. cruris	-		No growth				
94	149	45	M	skin scraping	Ti. cruris	+	T.rubrum		2	0.06	0.12	1
95	144	43	F	skin scraping	Ti. cruris	+	T.rubrum		1	0.03	0.12	1

Annexure – IV: Master Chart

96	141	61	M	skin scraping	Ti. cruris	+	T.rubrum		0.03	0.03	0.12	1
97	102	50	M	skin scraping	Ti. corporis	+	T.mentagrophytes		0.03	<0.03	0.25	0.03
98	177	60	F	skin scraping	Ti. cruris	+	T.mentagrophytes		1	0.25	0.25	0.03
99	67	35	F	skin scraping	Ti. corporis	+	T.mentagrophytes		2	0.25	0.06	0.03
100	50	45	M	Nail clipping	Ti. unguium	+		Aspergillus niger				

KEY TO MASTER CHART

M	-	Male
F	-	Female
KOH	-	Potassium hydroxide
+	-	Positive
-	-	Negative
<i>T.rubrum</i>	-	<i>Trichophyton Rubrum</i>
<i>M.gypseum</i>	-	<i>Microsporum gypseum</i>
MIC	-	Minimum Inhibitory Concentration
SDA	-	Sabourads Dextrose Agar



Introduction



Objectives



Review of
Literature



Material & Methods



Results



Discussion



Conclusion



Summary



Bibliography

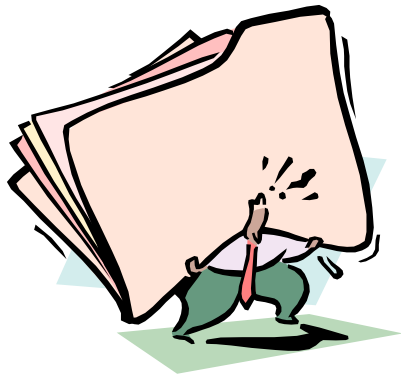


Annexure I: *Consent Form*



Annexure II:

Proforma



Annexure III: *Preparation of Media*



Annexure IV:
Master Chart
