ISOLATION AND IDENTIFICATION OF DERMATOPHYTES AMONG SIDDI COMMUNITY RESIDING IN NORTH KARNATAKA REGION AND MOLECULAR CHARACTERIZATION OF EXOTIC/RARE FUNGI AMONG THEM.

Thesis submitted to
THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI
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For the award of the degree of Doctor of Philosophy in the Faculty of Medicine

By

Mr. Aruna C

(Registration No: KLEU/Ph.D./2014-15/DO1214001)

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NOVEMBER-2021

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LIST OF ABBREVIATIONS

ATCC	:	American type culture collection
BLAST	:	Basic Local Alignment Search Too
Bp	:	Base pairs
CLSI	:	Clinical and laboratory standards institute
CO2	:	Carbon dioxide
CMA	:	Cornmeal agar
DMSO	:	Dimethylsulphoxide
DNA	:	Deoxyribose nucleic acid
dNTPs	:	Deoxynucleotide triphosphates
DPP	:	Dipeptidyl peptidase
DTM	:	Dermatophyte test medium
DW	:	Distilled Water
e.g.	:	For example
EDTA	:	Ethylenediaminetetraacetic acid
Fig/Figs	:	Figure/Figures
gm	:	Gram
mg	:	Milligram
µg	:	Microgram

HCl	:	Hydrochloric acid
HIV	:	Human Immunodeficiency Virus
ITS	:	Internal transcribed spacer
i.e.	:	That is
JNMC	:	Jawaharlal Nehru Medical College
KAHER	:	KLE Academy of Higher Education and Research
Kb	:	Kilo base
kDa	:	Kilo Daltons
KOH	:	Potassium hydroxide
LPCB	:	Lactophenol cotton blue
Ltr	:	Liter
MALDI TOF	:	Matrix-Assisted Laser Desorption/Ionization-Time of Flight
NaOH	:	Sodium hydroxide
NCBI	:	National Center for Biotechnology Information
NDM	:	Non-dermatophytic Mould
Nm	:	Nanometer
PCR	:	Polymerase chain reaction
PDA	:	Potato dextrose agar
RAPD	:	Random amplification of polymorphic

RFLP	:	Restriction Fragment Length Polymorphism
rpm	:	Revolutions per minute
RPMI-1640	:	Roswell park memorial institute
RNA	:	Ribonucleic acid
SDA	:	Sabouraud’s dextrose agar
SE	:	SqualeneEpoxidase
SNP	:	Single nucleotide polymorphisms
Sp	:	Species
Spp	:	species (plural)
SPSS	:	Statistical package for social sciences
TE	:	Tris EDTA
Tris	:	(Hydroxymethyl) aminomethane
UV radiation	:	Ultraviolet radiation
WHO	:	World Health Organization

ABSTRACT

Background: Fungal infection is very common in developing countries due to the life style and illness like diabetes, HIV infection and use of immunosuppressive drugs. Dermatophytosis is a superficial fungal infection caused by keratinophilic fungi belonging to three genera, *Trichophyton*, *Microsporum* and *Epidermophyton* which affects hair, skin and nail. Distribution of the species of dermatophytes varies depending upon the geographic location, ecology and human activities. We have several dermatophyte speciation studies from Indian subcontinent, those were carried out among Indian ethnic group. Siddis/Siddhis are Southeast African descendants that were brought to India as slaves during 16^{th} and 19^{th} centuries by the Portuguese. This study was conducted to determine the clinical profile, Antifungal Susceptibility, species distribution, genotype of predominant and rare dermatophyte isolates in this conserved Siddi community residing in southern part of India.

Materials and Methods: The study was carried out from 2015 to 2017. A total of 1004 clinical samples were collected from 937 Siddi tribal patients with suspected superficial fungal infection, all clinical samples were subjected to direct microscopy using 10% potassium hydroxide (KOH) and inoculated on to Sabouraud dextrose agar (SDA) with antibiotic and incubated at 28°C. Isolates were identified based on its macroscopic and microscopic features and urease hydrolysis. Genomic DNA was extracted by manual phenol-chloroform isoamyl alcohol method and eluted with 50 µl Tris EDTA buffer. Amplification was done by Polymerase chain reaction (PCR) using Internal transcribe spacer (ITS); ITS 1 and ITS 4. Antifungal susceptibility testing was carried out against 13 antifungal drugs, further all terbinafine resistance isolates were analyzed for mutation in Squalene Epoxidase (SE) gene.

Result: A total of 102 samples (10.15%) yielded dermatophytes on culture and a total of 193 samples have shown positive by direct microscopy. Tinea unguium (32.35%) and tinea corporis (27.45%) were the most prevalent clinical condition followed by tinea capitis (25.49%), tinea cruris (13.72%) and tinea pedis (0.98%). *T. mentagrophytes* complex (67.64%) was the most commonly isolated dermatophyte followed by *T. rubrum* (25.49%). Phylogenetic analysis based on the ITS region revealed *T. mentagrophytes interdigitale* complex belongs to "ITS genotype VIII", suspected rare fungi was clustered with *T. rubrum* and *Cladosporium halotolerance*. Voriconazole have shown the lowest MIC (MIC_{50} 0.00781 mg/ltr) and fluconazole have higher MIC (MIC_{50} 2 mg/ltr). Among 25 isolates with higher MIC for terbinafine 4 isolates have shown F397L mutation.

Conclusion: *T. mentagrophytes* type VIII was the prevalent organism causing dermatophytosis among Siddis and patients of 15 to 45 year old age group were the most often affected. Tinea corporis was the most common clinical manifestation. The most prevalent etiological agent was *T. mentagrophytes,* which corresponds to the ITS genotype VIII. Despite their origins in African nations, the distribution of dermatophytes and their etiological agent in the Siddi tribal population is comparable to that of native Indians. *Cladosporium halotolerans*, an indoor fungus, can cause superficial infections like tinea capitis. Voriconazole was the effective antifungal agent, whereas luliconazole and griseofulvin have shown higher MIC. Mutation in the 1191 position which leads to substitution of phenylallamine to lucine at 397thposition in terbinafine resistant *T. mentagrophytes* isolates was observed in the present study.

Key words: Dermatophytes, *Trichophyton*, *Epidermatophyton*, *Microsposporum*, Siddi and Molecular

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1.0 INTRODUCTION

Fungal infection is very common in developing countries due to the life style and illness like diabetes, HIV infection and use of immunosuppressive drugs.[1] Fungi are a unique category of higher organisms that are not related to plants or mammals. Fungal cell wall is made up of chitin whereas animal cell does not have the cell wall and plant cell is encased in cellulose, fungi get food or nourishment by secreting enzymes for external digestion, which they then absorb. These are simple in structure compared to the plant and animal cell. Fungal cell will not divide into tissue or organ, the basic structure of fungus is hyphae which are tubular or filament likes structure or independent single cell. During tissue invasion, several pathogenic fungi modify their development patterns. In tissue, these dimorphic fungi convert from hyphal to single celled form. The vegetative stage of most multicellular fungus involves mass branching known as mycelium. The septate hyphae in the more advanced group have more or less frequent cross walls. Fungi which do not contain hyphae but contains loosely arranged budding cells are known yeast cells. These yeast cells extend from the parent cell before budding, resulting in a chain of elongated cells known as pseudohyphae. Fungi reproduce asexually using microscopic propagules known as spores. Many fungi have the ability to reproduce sexually. Unless two distinct mating strains come into touch, most fungi are heterothallic and do not develop sexual structures. Some fungi are homothallic, meaning they may create sexual structures inside their colonies. The process then progresses to meiosis, which results in the formation of sexual spores. In modern mycological terminology, fungus sexual stage is referred to as telomorph, while its asexual stage is referred to as anamorph.

1.1 Classification of fungi:

The classification and naming of fungus is based on their reproductive bodies and the method in which they are generated. Lately fungus is divided into four groups depending on differences in their reproductive structure.

1. Chytridomycota: It consist of a single class with approximately 1000 species, none of them are pathogenic to human. The fusing of suitable nuclei happens during sexual reproduction, and meiosis occurs to produce motile spores known as zoospores.
2. Zygomycota: There are roughly 175 genera includes 1000 species in this division. The fungus's vegetative state is aseptate. Asexual reproduction happens by forming the spores or sporangiospores, these are non motile and produced in the sac like structure known as sporangium. Sexual reproduction occurs when suitable colonies' nuclei fuse, resulting in the development of enormous single zygospores with thickened walls, on germination, meiosis occurs, and haploid mycelium grows. The Entomophthorales and Mucarales are two significant medical orders in this category.
3. Deuteromycota: There are roughly 3200 genera and 32000 species in this category. The fungus's vegetative body is divided into septa. Asexual reproduction takes place by the formation of spores known as conidia from conidiogenous cell. These conidiogenous cell is not different from mycelium in some species. Condiogenous cells are produced in a specific hyphal structure known as a conidiophore in other species. The fusing of nuclei from suitable colonies is used for sexual reproduction Meiosis haploid spores called as ascospores, and ascospores are produced in sac like formation and these sac

like formations are recognized as ascus. The Ascomycota have primordial transition from a type of primitive to single asci toward an ascocarp with many asci. The telomorph genus of the dimorphic systemic fungal pathogen Ajellomyces is included in this division. Blastiomyces, emmoncia, and Histoplama are anamorphic genera.

4. Basidiomycota: There are around 22,000 species in this category. Thallus is divided into septa. Variables in asexual reproduction conidia are produced by certain species, where as some species do not produce conidia. Few species reproduce sexually by fusing the nuclei after viable colonies, followed by meiosis, which results in the formation of basidiospores on the basidium. Basidia often produces macroscopic structure known as basidiocarp, the spores are often forcefully discharged.

Fungal infection can be divided into three type superficial mycosis, subcutaneous mycosis and systemic mycosis.[2] Among the superficial mycosis dermatophytosis is the most common and contagious infection.[3] Dermatophtes are the keratenophilic fungi infect skin, hair and nail. Because the fungus cannot penetrate into the tissue or organ of an immuno-competent individual, infection is cutaneous and limited to the nonliving cornfield layer.

1.2 Dermatophytosis:

Dermatophytes are the keratonophlic fungi infects skin, hair and nail, dermatophytes are restricted nonliving cornified layers of immune-competent host due to its inability to invade the tissue or organ.[4] Ring worm infection, or tinea infection, is another name for it. Tinea is a preface to the Latin description affected area. The dermatophytic infection ranges from mild to severe depending upon the host

response to the fungal metabolic products, virulence of the infecting species or strain, site of infection and environment of the local geographical location. David Gruby described the causative agent of favus, he explained the clinical features and microscopy of its crust and discussed about its contagious nature of the disease in 1986-1989. Later in 1890 Raimond Sabouraud began his scientific study on dermatophytes.[5] His contribution to dermatophytosis included taxonomy, morphology, methods of cultivation and treatment. He classified the dermatophytes based on clinical features, culture characters and microscopic appearance in four types of genera *Achorion, Epidermophyton, Microsporum,* and *Trichophyton.* Based on spore shape and accessory organs, Chester Emmons reclassified the dermatophtes into three genera: *Epidermophyton, Microsporum,* and *Trichophyton,* and removed Achorion in 1930. Using Vanbreuseghem's hair bait technique, Dawson and Gentles discovered the telomorph of *Trichophyton* (Keratinomyces) *ajelloi* in 1959, which initiated the discovery of teleomorphs of numerous dermatophytes as well as other keratenophilic fungi, further to see the pleomorphism and to study the taxonomy genomic study was carried out.

1.3 Etiological agent:

1.3.1 Anamorphs: Dermatophyes are split into three anamorphic genera which are *Trichophyton, Epidermophyton* and *Microsporum* which comes under Hyphomycetes (also known as Fungi Imperfecti) of anamorphic class under Deuteromycota.

1.3.1.1 ***Epidermatophyton****:* The pathogenic species *Epidermatophyton fluccosum* has ovate with the narrower end at the base to widely club-shaped multicellular (1 to 9 septa) macroconidia (20 to 60 m by 4 to 13 m) having smoother walls and no

microconidia. It depicts a colony with a gritty, crumpled, suede-like texture that grows slowly and is olive to yellow or golden-brown in hue.

1.3.1.2 ***Mirosporum****: Microsporum* has produces the macroconidia with rough surface known as echinulate. It produces large number of macroconidia and lesser number of microconidia except one microsporum which hardly sporulates is *Microsporum audouinii*, after formation of macroconidia, it is usually seen singly on a conidiophore.

Microsporum audoinii**:** *M. audoinii*can be difficult to identify since it sporulates infrequently or does not sporulate at all. It generates grey – white, cream to tan growth on saboraud's dextrose agar with pigmentation on the opposite surface of the growth with reddish brown. The production of terminal chlamydospores distinguishes microscopic finding.

Microsporum canis: This species shows granular buff colored colony and yellow-orange to orange- brown reverse pigmentation. Microscopic features include production of fusiform, echinulated, thick walled and multiseptate macroconidia and few microconidia.

Microsporum gypseum**:** This species shows granular buff colored colony. Microscopic features include production of fusiform, echinulated, thick walled and multiseptate macroconidia and few microconidia.

1.3.1.3 ***Trichophyton***: This species has multi-celled, smooth-walled macroconidia with a variety of shapes (clavate, cylindrical, and cylindrofusiform) that develop alone or in clusters on the conidiophore. Depending on the species, strain, or culture state, macroconidia may be numerous, few or absent.

***Trichophyton rubrum*:** This species produces slow growing flat or heaped up velvety or powdery colony which is highly folded, surface shows white to cream to deep rose, reverse shows wine red or some time yellowish orange in color. Microscopic feature shows the club shaped (tear shaped) microconidia arranged along the hyphae, smooth walled macroconidia.

Trichophyton mentagrophytes: There are zoophilic (*var.interdigitale*) and anthropophilic (*var.mentagrophytes*) variants. Zoophilic strain produces granulated off-white colored colony whereas anthropophillic strain produces white and fluffy colony. Reverse pigment may be present and may resemble the reverse pigment of *T. rubrum*. This species generates spiral hyphae, smooth walled macroconidia, and spherical microconidia clustered along the hyphae.

Trichophyton tonsurans: Shows powdery or granular brown colony, pigmentation may disappear after subculture. This strain may show yellow-brown to red reverse pigmentation. It produces a large number of differently shaped microconidia (elongated or clubbed or balloon shaped). The arthroconidia are also visible in some of the stains.

Trichophyton violaceum: This strain grows slowly and creates a colony that is piled and twisted with a violet or lavender coloured surface. If reverse pigmentation is present, it is purple in colour. Microconidia and macroconidia are absent.

***Trichophyton schoenleinii*:** It generates "favic chandeliers," these will be on tip of the hyphae bearing antler-like forms and produces white to tan heaped up and twisted colonies with no colour on the reverse. It lacks microconidia and macrocondia.

***Trichophyton concentricum*:** shows slow growing colonies in slightly red or beige to brown and these are intricate and later becomes glabrous. It lacks sporulation.

***Trichophyton soudanenase*:** It has a yellow to orange suede colony that is flat to fold and has radiating fringe. Purplish red variation on the surface. The reverse is the same colour as the surface. Arthroconidia often cause septate hyphae to split apart. Reflective branching is characterized by the formation of branches at both a forward and backward angle to the parent hyphae. Microconidia in the shape of teardrops may develop along the hyphae, but macroconidia are not present.

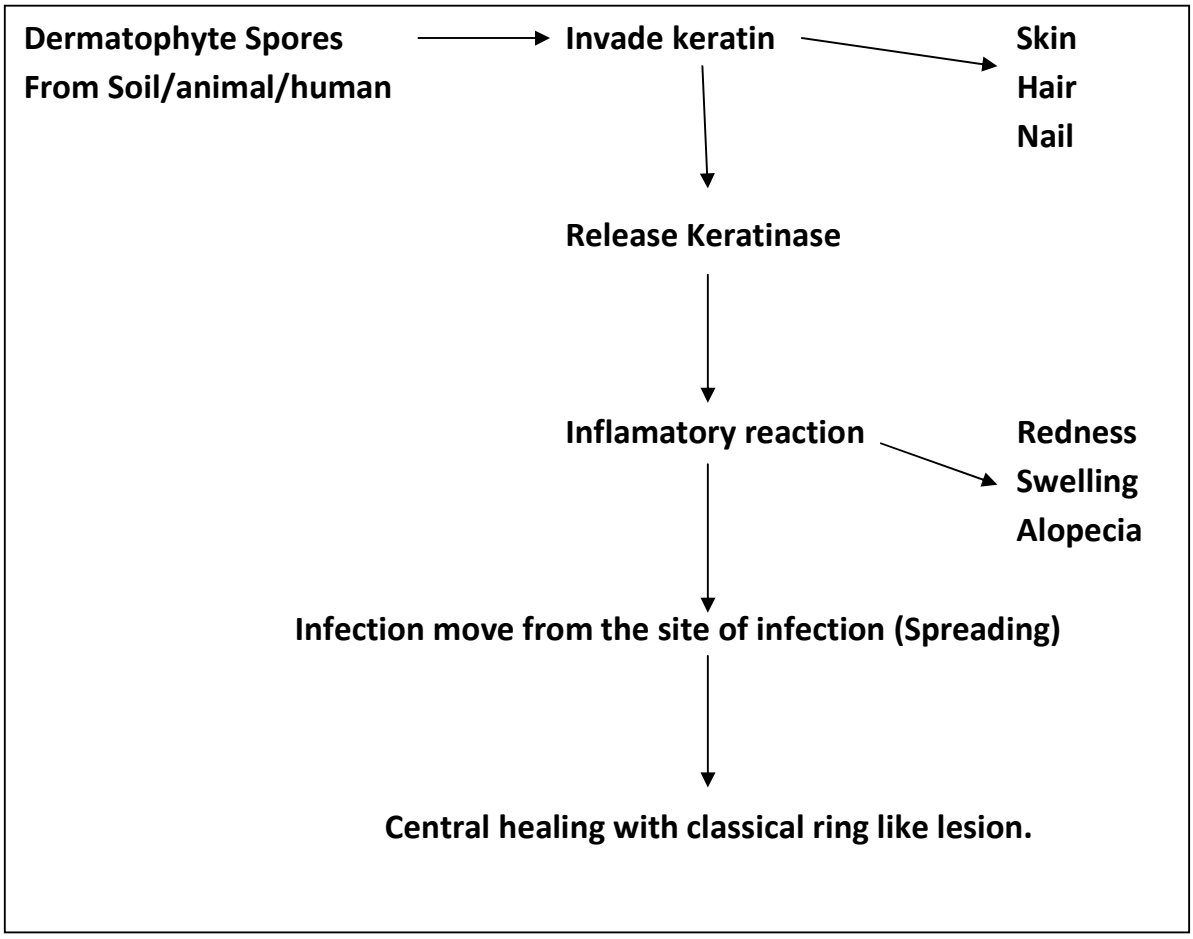
1.3.2 Telomorphs: Few dermatophytes mostly zoophilic and anthropophilic dermatophytes like *Microsporum* and *Trichophyton* may also reproduce sexually by forming ascomycota with asci and ascospores. These belong to the Arthroderma genus, Arthrodermataceae family, Onygenales, phylum Ascomycota.

1.4 Pathogenesis:

Dermatophytes are primary cutaneous pathogens and causes skin, nail and hair infection it invades skin, hair and nail by producing an enzyme keratinase which digest keratin. The formation of mannans is another element that boosts pathogenicity. These are components of the cell wall that have immunosuppressive properties. Mannans may help to escape elimination by the host by suppressing the cell mediated immunity. In few dematophytes pathogenicity human genetics also plays an important role. In few families, tinea unguium is inherited as an autosomal dominant characteristic. *T. concentricum* causes tinea imbricate which is similar to a tinea corporis. It is an autosomal recessive pattern which is inherited.[6]

Many host mediated factor limits the invasion of dermatophytes. Progesterone shows the inhibitory effect on dermatophytes invite which explains the increased incidence in male compared to female. Unsaturated fatty acid present in the sebum inhibits the dermatophytes invitro, which explains the less frequency of dermatophytic infection on adult scalp in contrast to this palm and sole lacks the sebum could explain the persistence of infection in this region.[6]

Fig 1.1 Invasion of dermatophytic fungi into the keratin layer of skin



1.5 Clinical manifestation:

Traditionally the dermtophytes are named after anatomical site involved. The clinical manifestations are as follows.

1.5.1 Tinea corporis: It's a glabrous skin illness that affects the face (but not the beard), trunk, and limbs (includes dorsal side of hands and feet). It is commonly seen word wide and prevalent in person leaving in warm condition. It is seen in all age group. Immuno-compromised patients with disease like diabetes mellitus, Cushing syndrome and HIV infection are predisposing factors for dermatophytoses. Any dermatophyte species is able to cause tinea corporis infection. Anthropophilic dermatophyte *T. rubrum* is the most prevalent cause of tinea corporis all over the world. In children with tinea capitus, *T. tonsurans* is the main cause tinea corporis, person who comes in contact with animals zoophilic organism like *M. canis* and *T. mentagrophytes* var. *mentagrophytes* can cause the infection. Geophilic dermatophytes like *M. gypseum* is more likely to cause the tinea corporis infection.[7] Clinical presentation may vary from non-inflammatory, scaly plaques to inflammatory pustules, with progressive scale and central clearance, an anthropophilic strain of dermatophyte may form annular plaque. Inflammatory lesion which may range from vesicle to pustule can be seen in infection with zoophilic and anthropophlic dermatophyte all lesion of tinea coporis may be pruritic. The dermatophyte may invade the hair follicle and cause perifollicular pustule which resembles bacterial folliculitis and may cause invasion of follicle wall and lead to granuloma formation. Majocchi's granuloma is more frequent in women who shave their legs and have onychomycosis or tinea pedis.

1.5.2Tinea unguim: Tinea unguim is the infection of the fingernail and toenail caused by dermatophytes,[5] whereas onychomycosis is a broad term used to indicate the fingernail and toenail infection caused by dermatophytes, non dermatophytic mould, yeast and rarely bacteria.

Prevalence of Tinea unguim ranges from 3-13% in general population and may increases up to 28% in the age of more than 60 years reduced immune response, poor peripheral circulation, extended infection exposure, and poor cleanliness might all be factors.[8] Majority of onychomycosis, more than 90% is due to dermatophytes. *T. rubrum* and *T. mentagrophtes* is the major causative agent of onychomycosis and *M. gypseum* less commonly seen organism in tinea unguim infection and *T. tonsurans* can be seen in children who has tinea capitis. Non-dermatophytes can appear as secondary infection in onychomycosis after the nail damage. Commonly seen non-dermatophytes in onychomycosis are *Scytalidium* spp. *Scapulariopsis* spp. *Aspergillus* spp. *Candid* spp. and *Fusarium* spp.

Onychomycosis is classified into four types based on the clinical presentation and route of infection.

1. Distal Lateral Subungual Onychomychosis (DLSO): It is the more commonly seen form of onychomychosis. The pathogen migrates proximally through lateral nail groove by penetrating the nail, causing moderate inflammation that can progress to prokeratosis and subungual hyperkeratosis, which can lead to onycholysis and subungual thickening. The infecting agent enters the nail plate through the lateral nail groove and migrates proximally, causing mild inflammation that can progress to prokeratosis and subungual hyperkeratosis,

which can lead to onycholysis and subungual thickening, as well as nail plate separation from nail bed.

2. Whitish Subungual Onychomycosis (WSO): It is less frequently seen than DLSO. Invade the nail plate's surface. Well delineated opaque are seen on nail known as “White Island”. The infection may spread from the plate to the cornified layer and then to the hyponychium and to the nail bed, this causes nails to be mushy, rough and disintegrate. *T. mentagrophytes*is the common etiological agent. Non-dermatophytes like *Aspergillus terreus*, *Acremonium potronii* and *Fusarium oxisporum* can also cause infection.
3. Proximal Subungual Onychomycosis (PSO): It is least commonly seen presentation of onychomycosis in immunocompetent population, however the infection can be seen in immune-compromised patients, especially in AIDS patients. Pathogens spreads away from the lunula region by entering the nail plate through the proximal nail fold, causing subungual hyperkeratosis, proximal leukonychia, and proximal nail plate destruction.[(9)] The prevalent causal agent is *T. rubrum*.
4. Total Dystrophic Onychomycosis: The whole nail plate and bed are affected in complete dystrophic onychomycosis, and the nail thickens and becomes dystrophic later. Distal lateral subungual onychomycosis, proximal subungual onychomycosis, and white subungual onychomycosis are the causes of this condition. Rarely dense cream white subungual area is seen. In this condition densely packed somewhat abnormal hyphae can be seen, which are relatively resistance to systemic antifungal drug.

1.5.3 Tinea capitis: Tinea capitis is a disease caused by the dermatophyte which infects the follicle and scalp hair. It is common worldwide commonly seen in prepubescent children. *M. canis* is the common etiological agent worldwide. Some species are endemic to certain areas. *T. violacium* is endemic in Africa, Asia, and Europe, although non-endemic instances have been identified recently.[10] *T. yaunndei, T. gourvilli* and *T. soudanense* are endemic to Africa. Sporadic outbreaks can be caused by *T. mentagrophytes, T. rubrum, T. verricosum, Trichophyton megninii,* and *M. gypseum*[9].

The causative agent of tinea capitis can be classified according to pattern of infection. Sheath around the hair shaft formed by arthroconidia can be seen in ectothrix infection, whereas in endothrix infection arthroconidia is formed within the hair shaft. In favic infection fungal hyphae fragment are seen in linear chain along the longitudinal axis of hair. The may be dry scaly patches alopecia or inflammatory pustules and kirions. There are four types of tinea capitis.

Gray patch Tinea capitis: Hair growth is decreased or short stabbed at the site of infection in a circular or oval strongly defined area of partial alopecia.[9] Hair may have tendency to break just above the infected skin. From the infected region, the lesion may extend peripherally to produce a patch with moderate inflammatory scaling. Some time resembles the dandruff.

Black dot tinea capitis: Endothrix of hair can be seen in this type of infection leads to break arthroconidia laden stub visible.[9,11] When hair color is black the dot appears in black color. In case of other hair color dot appears in the color of hair. *T. tonsurans* is commonly isolated infective agent followed by *T. violaceum and T. rubrum. T. yaunndei, T. gourvilli* and *T. soudanense* are endemic in the parts of Africa.

Inflammatory Tinea capitis: All dermatophyte capable of producing tinea capitis can cause inflammatory tinea capitis the lesion may range from pustular folliculitis to kerions.

Favus: Favus is another type inflammatory tinea capitis caused by *T. schoenleinii* and *Scutula* is a cup-shaped yellow crust that forms within hair follicles and is made up of hyphae, neutrophils, and epithelial cells. It produces bluish white florescence under Wood's lamp.[1]

1.5.4 Tinea barbae: It is the infection of the facial beard area if men caused by dermatophytes. Infection may transferred by contaminated razor used in barbershop.[12] *T. mentagrophytes* and *T. verrucosum* are the two main causative agent followed by *T. rubrum T. megninii*, *T. violaceum*, *T. schoenleinii* and *M. canis*. *T. mentagrophytes* and *T. verricosum* are zoophilic organisms that cause an inflammatory lesion with kerion with swampy development, raised nodule draining pustule and sinus tract, and hair that is loose, damaged, or missing on the affected region.[9] The chin and neck are also affected, with the problem spreading throughout the full beard. Secondary clinical presentation is non inflamatory or sycosiform and resembles the bacterial folliculitis. In case of *T. violaceum* or *T. rubrum* hair in the affected area may be loose, broken and resembles tinea corporis infection.[9]

1.5.5 Tinea cruris: The dermatophytes causes the tinea cruris infection of the, inguinal, upper thigh, pubic and, perianal areas, with the buttocks occasionally affected. Crowded living condition sweating, tight clothes, and communal bathing facilities of the military forces, athletic team, or prisoner can be a predisposing factor for infection.[13] *T. rubrum* is the common eitiological agent followed by *E. fluccosum* and *T. mentagrophytes*. *T. rubrum* and *E. fluccosum* produces non-inflammatory

slightly erythema with distinct active margin. *T. mentagrophytes* produces inflammatory lesion with sever itching sensation.

1.5.6 Tinea pedis and Tinea manum: Tinea pedis is a disease caused by dermatophytes, which causes the infection to the plantar surface of the toe web and foot, and *T. mannum*, is an infection caused by the dermatophytes to the palmer surface of the hand interdigital area. *T. rubrum* and *T. mentagrophytes* are common causative agent followed by *E. fluccosum.*[13]

Clinical infection by tinea pedis generally fall into the following 4 types Moccasin, interdigitale, inflammatory and ulcerative.[8] Moccasin lesion is dry entire plantar surface is involved and extends to lateral foot. Hyperkeratic scales may be produced with fissure occasionally. Maceration, scaly, and fissures of the toe web characterize the interdigitale lesion. Between the fourth and fifth toe web skin is frequently damaged where *T. rubrum* is commonly isolated. Secondary bacterial infection is seen and which may results in dermatophytosis complex. Vesicular bulla, pustules, and vesicular hypersensitivity response are inflammatory lesions that appear on the instep or mid anterior plantar surface. Tiena mannum produces scale on palmar skin which is quite hyper

Table 1.1 Causative agent of dermatophytosis in different clinical condition.

Sl. No.	Clinical condition	Commonly affected area	Commonly isolated etiological agent
1	Tinea corporis	body, shoulders and legs,	*E. floccosum* and *Trichophyton* spp
2	Tinea capitis	Scalp and hair	*Microsporum* spp., *E. floccosum* and *Trichophyton* spp.
3	Tinea ungium (Onychomycosis)	Nail	*T. rubrum* and *T. mentagrophytes*
4	Tinea cruris	Pelvic, perianal and perineal region	*T. rubrum* and *E. floccosum*
5	Tinea manuum	Palms and interdigital areas of hands	*T. rubrum*
6	Tinea pedis (Athlete's foot)	Soles of feet and toes	*E. floccosum* and *Trychophyton* spp
7	Tinea faceae	Face	*Microsporum sp.*

1.6 Immunology:

Dermatophytes are usually found in the stratum corneum's dead keratinized tissue and may lead to mild to severe inflammatory response[(14)]. The fungal infection is not recognized by a particular immune response to the surface cornified layer, although humoral immunity, cell mediated immunity, and specific or non-specific host defenses respond, the dermatophytes are finally eliminated and access into viable tissue is avoided. In the dermatophytosis infection the cells like Neutrophils,

lymphocytes, macrophages, and mast cells fights against dermatophytes as they have unsaturated transferrin, 2-macroglobulin keratinase inhibitor, epidermal desquamation, and macrophages, lymphocytes, macrophages, mast cells and neutrophils. Dermatophytes have two main antigens: peptidogycans and keratinase. Protein portion of the peptidogylcans are responsible for the cell mediated immunity whereas protein portion can stimulate humoral immunity.[14,15]

1.7 Siddi tribals:

Siddis are unique tribe who has African ancestry who are said to be direct ancestors of Africans transported to India by the Arabs, Portuguese, and Dutch. They originate from Ethiopia, Mozambique and Eastern African countries.[16–18] They have been living in India for many generations and never had been to their original land. Some previous studies have shown the genetic relatedness of the Siddi community with Africans.[19–22] However study conducted using autosomal and Y-chromosomal genetic marker has shown Siddis have likely ancestry of sub Saharan Bantus.[21] Many of the African farmers were settled in east African countries during Bantu expansion and Portuguese were predominantly ruling this region during 15^{th} to 17^{th} century and they might have brought them to India and sold to nawabs and sulthans.[23,24] Existence of Siddis were documented in 1100 AD, later in 13 century they brought to India in large group as their slaves.[24] Later in 17^{th} – 19^{th} century Portuguese brought them to India later many have become officials in Muslims and Hindus armies and rose to power in more than one place.[17,25] These Afro-Indians are known as Sidi/Siddi/Sidhi or Habshi/Habsi. Majority of them reside in the western coastal areas of Karnataka and few in Goa, Maharashtra and Gujarat states with nearly total population of 0.25 to 0.30 million.[26,17]

Fig 1.2 Siddi tribals

In Karnataka, Siddis are spread over the forest areas of Uttara Kannada district, Siddis are escaped from Goa and later settled in thick green forest of Uttar Kannada district.[17,25,26] In Uttar Kannada district they are found in Yellapura, Ankola, Mundgod, Sirsi, and Joida taluka places. Few of them are also settled in Khanapura and Khalaghatagi of Belagavi and Dharwad district respectively as both talukas are bordered with Uttar Kannada district.[27,26]

Uttar Kannada district is coastal area, where western ghats passes through. Most of Uttar Kannada district is has thick forest with hill station lies between 13^0 and 15^0 north latitude and 74^0 and 76^0 east longitude. Most of the Siddis are settled in the slop of western ghats with thick forest and valleys having spice and areca nut plantation.[25] Many Siddis are also scattered along the Hubli and Karwar road from east to west and Belagavi and Sirsi road from north to south. Most the Siddis live in small huts having hay roofing and outside wall of the house is built by mud and plastered with cow dung. Some of the Siddis also live in concrete houses.[19] Siddis lives in separate houses close by one another with minimum of 5 houses and maximum of 40 houses can be seen. In 1996 survey total population of Siddi community were 12,153 with 2405 families.[25]

Siddis are adapted to the local regional culture and language. They belong to Christian, Muslim and Hindu religions, lead a simple life and live in small hamlets. Siddis can speak local regional language Kannada. In the region like Yellapur and Ankola they speak Kannada and Konkani, in Haliyal region Muslim Siddis can speak Kannada and Urdu whereas Christian Siddis can speak both Kannada and Konkani.

Siddis are well built and short or tall with strong or medium physique. Siddis have dark complexion of skin. They have peculiar characteristic form of hair. Hairs are woolly, frizzy and curly known as helical hair. They have rough textured hair and very few may have wavy hair. Siddis have reduced beard and mustache.

Most of the Siddis have nuclear family they divide and establish separate house after marriage. Joint families can also be seen in the community. Elder male heads the family and takes important decision in the family. These Siddi tribal community respect elders and have mutual obligation among living family members.

Few recognized people in the community acts as leader or Guru and give guidance to the community. Most of the Siddis follow monogamy, boys get married at the age of 23 and girls get married by 19 years of age.[26] Early marriages are reduced in the community and child marriages are not been practiced in the community. The incidence of inter caste marriage is seen in the community.

Most of the Siddi depends on agriculture for the source of income some of them are involved in forest labor, honey collection, and daily wage labor. Along with the agriculture they also volunteer to work in areca nut gardening, cut trees for forest contractors

Humid and warm atmosphere of the Indian subcontinent is good growth environment for fungi, especially to dermatophytes.[16,18] The most common fungi causing dermatophytosis in this geographic area are *T. rubrum* and *T. mentagrophytes*. Although Siddis have adopted the local social and religious life styles, they form a different physical and genetic group as compared to local native Indians and hence, their health and disease status especially the susceptibility/resistance to certain infections vary. There are very few reports on health and diseases associated with Siddis especially the fungal infections. However, certain geographically restricted fungi have been associated with Siddi community, which are found to be closely related to the isolates reported from Eastern African Countries.[16,18]

Even though this community never had been to their original land and never exposed to their ancestors but some rare infections are still prevalent in this population as compare to local Indians. Among them, some of the rare and exotic fungal infections specially ring worm/tinea infection/dermatophytosis are commonly

seen among this community. Even though such tinea infections are seen in native Indian of the region, but the species causing such infections are totally different and are rare. These species known to cause such infections among negroid people like Africans. Very few cases have been reported but such geographically restricted fungi in the past.[28] This peculiar observation has stimulated us to think can this fungi infection relate their ancestral relation to African origin

1.8 Objectives

1. Isolation and identification of dermatophytes associated with skin infections in Siddi community.
2. The prevalence of dermatophytosis in the Siddi tribal community.
3. Phylogenetic analysis of the exotic and rare fungal isolates
4. A comparison of the genetic relatedness of the research isolates with African isolates.

2.0 REVIEW OF LITERATURE

Fungi are ubiquitous saprophytes generally involves in the decomposition and recycling of organic matters and have capacity to colonize in any environment. European physicians, discovered the fungal etiology of favus, which began medical mycology.[29]

2.1 Historical review:

In India mycotic infection has been mentioned long back in ancient books in Ayurveda mentioned generally as skin diseases and the term "Dadru" was used to indicate the superficial infection. Based on the morphology of the fungus Planek of Vienna introduced classification of skin disease. Authers replaced the term prurrigo with tinea to put down leave tinea favosa (favus), tinea tonsurans and tinea dseclavans.[30] First human pathogen isolated and identified was the fungus by Remak in 1834. First clinical condition considered to be dermatophytosis was favus. Remak observed the presence of filament when examined tinea favosa. These filaments described as fungi by Schonlein (1839) and Weitzman and Summerbell (1995). While fungal infections are common in both temperate and tropical regions, the distribution of dermatophytosis, its etiological agents, and infection rates differ by geographical region and are influenced by a variety of environmental and cultural factors.

During 1841-1844 Gruby explained the clinical features and microscopic characteristics of favus. He was completely unaware of Remak and Schönlein's studies on favus, he explained fungus can invade hair of beard and scalp to cause enctothrix and causative agent was named as *M. audouinii.* He also described the

endothix hair invasion and named the causative agent as *Herpes* (*Trichophyton*) *tonsurans.*[29,31]

Dermatophytes were further classified by Sabouraud, a French dermatologist into four genera i.e., *Achorion, Microsporum, Trichophyton* and *Epidermophyton* based on clinical type and culture characteristics, In 1910, he published his work, in which he explained about conidial characters, colony morphology, taxonomical characters and treatment. Robert W discovered the woods lamp which was used to detect the hair infection by fungus in 1925.[32]

Chester Emmons (1934) categorized dermatophytes based on conidial morphology and accessory structure, emphasizing the anamorphic genera *Epidermophyton, Microsporum,* and *Trichophyton* while excluding *Achorion*.[29]

2.2 Epidemiology:

Dermatophytes, yeasts, and NDM are the most common fungal pathogens relevant in medical practice, according to Ajello (1962) and Weitzman and Summerbell (1995). Fungi are found in about 100,000 different species all over the planet. The capacity to digest keratin distinguishes the approximately 40 distinct species of dermatophytes that have been identified as human diseases. *Trichophyton, Microsporum,* and *Epidermophyton* are the three genera of dermatophytes that cause the majority of cutaneous fungal infections.[33,34] There is a substantial change in the epidemiology of dermatophtes over last few years,[35] epidemiology of dermatophytes are influenced by several factors like migration, climatic factors, growth in tourism, changes in socioeconomic conditions, overcrowding, healthcare, environmental hygiene and culture.[34,36–39] The dermatophyte infection is cosmopolitan etiological

agent vary in the different part of the world. Its incidence of dermatophytes may increased by the risk factors like high humid weather, over population and unhygienic conditions.[34,36,38–43] Its frequency may increase in tropical and subtropical countries.[44] The etiological agent and the anatomical site involved in the infection of the dermatophtes depend upon the geographical area and due to travel. The incidence of the dermatophytic infection may also increase in immuno-compromised patients like trauma associated with diabetes mellitus, patients on chemotherapy, and in HIV patients.[45] Other factors like air tight clothing and shoes during sports activity and communal swimming pool may also increases the incidence of dermatophytes. According to Erbagci et al., the dispersion of dermatophytes isolated from skin and nail diseases has improved significantly during the previous century (2005). *E. floccosum* and *M. audouinii* dominated human pathogenic dermatophytosis in the 1920s, while *T. rubrum* was almost unheard of and although dermatophytic cases of tinea ungium[46,47] accounted for just 2.8 % of 106 mycoses diagnosed in 1938, their prevalence increased to 10.8 % in 1949 and Buchvald and Simaljakova (1995) all detailed these improvements (1995).

Dermatophytosis is the common superficial infection. As per the CDC prevalence of dermatophytosis is 20-25% worldwide. 30 to 70% of adult host are asymptomatic for dermatophytic infection because of which incidence of the infection increase with age.[34,36,38,42,43] *T. mentagrophytes* is the most prevalent cause of dermatophytosis, which may be found in tinea corporis, tinea unguim, tinea faciae, tinea capitis, and tinea cruries. Some investigators have found that *T. rubrum* is the common cause of dermatophytosis.[48] *T. rubrum* was found to have risen from 41.7 % of all dermatophytic isolates in 1950 to 82.7 % in 1993, according to Marakiand Tselentis (1998). According to another study, 37 % of *T. rubrum* isolates were

retrieved from nail samples and 36.4 % from feet, accounting for 73.4 % of all isolates,[49] but recent studies showed the shift of trend from *T. rubrum* to *T. mentagrophytes.*[50–52] Tenia corporis is the common clinical manifestation in dermatophtosis.[50,51]

Men are more likely than women to develop dermtophytosis, according to several studies. According to one of the latest studies, it is more widespread in men (84 %) than in women.[53] Khadri et al. recently published a study on the Clinico-Mycological Profile of dermatophytes in Vijayapur, Karnataka, which found that dermatophytosis is most widespread in the age group of 21-30 years (37.5%), followed by 31-40 years (37.5%), 31-40 years (37.5%), 31-40 years (37.5%), 31-40 years (37.5%), 31-40 years (37.5%), 31-40 years (37.5%), 31-40 years (3 (17.5%). The sex-wise incidence indicated that males (55%) are significantly more likely than females to have it (45%). Tinea corporis was the most common form of dermatophytosis (57.5%). Tinea cruris (15%), tinea facei (10%), and tinea corporis (10%) are the most common clinical types (12.5%).[54] According to Philpot (1997), males may be more susceptible to infection because they are exposed to more fungal pathogens.[55] Increased occurrence may be due to the style of shoes and clothing worn, especially in the army, schools, and athletic events.[56,57] There were also differences in the prevalence of different clinical forms, such as tinea infection of the groin being more prevalent in males, while tinea infection of the foot and nail infection being more common in females.[58]

Interaction with animals, increased use of public sports facilities (especially swimming pools), wearing occlusive training shoes, the occurrence of diabetes mellitus and vascular disorders such as arteriosclerosis, and an ageing population are all factors that contribute to the development of dermatomycoses in socially and economically developing countries. This is in addition to well-known risk factors including a friendly demeanour, male gender, foot injuries, and cigarette smoking. Despite geographic features and predispositions for dermatophytic infections, Ceburkovas et al. (2000) discovered that the dermatophyte continuum is not stagnant[59]. Global sporting events, mass tourism, and rising human migration all contribute to the import and spread of less common species to specific geographic.[60–62] While dermatophytic infection is more prevalent in adults, especially those between the ages of 16 and 45, tinea infection of the scalp is widely believed to be a disease of children and young adolescents.[59,63] The continuum of dermatophytes is not static, despite geographic features and predispositions to dermatophyte infections. Less popular or forgotten species are being introduced and disseminated as a result of growing mass tourism, foreign sporting events, and increased migration.[34]

Dermatophytes grow at surface temperatures of 25-28°C, according to Stiller et al. (1992) and Weitzman and Summerbell (1995), and infection of human skin is facilitated by warm and humid circumstances, as well as seasonal change, which aids fungal infection dissemination.[29] Cutaneous fungal infections are increasingly widespread in tropical areas as a result of these factors. Furthermore, dermatomycoses is more common in populations with low socioeconomic conditions, as well as crowded environments with many opportunities for skin-to-skin transmission.[64] The outbreak distribution of dermatophytes skin infection is exacerbated by inadequate sanitation and the lack or poor quality of medical treatment. The propagation of

zoonotic fungal infections in culture is aided by close proximity to domestic animals and livestock.[34] Despite geographic features and predispositions for dermatophytic infections, Hormonal modifications during puberty cause acidic sebaceous gland secretions, which are responsible for the decline in dermatophyte occurrence.[53] Deirdre and Buckley (2000) and Brouta et al. (2000), on the other hand, record only a few cases of adult infection.[65]

2.3 Etiology:

Many type of dermatophytosis has been reported in India, the commonly isolated etiological agents in India are *T. rubrum*, *T. mentagrophytes.* In the late twentieth century, *T. rubrum* was one of the most common causes of dermatophytosis in Asia, including India. In Jaipur, Rajasthan, Western India, *T. rubrum* reported 46 % and *T. mentagrophytes* reported 14 %in 2008,[66] Lakshmanan et al in 2011 were able to provide comparable findings from Tamilnadu, India for *T. mentagrophytes*. *T. rubrum* was discovered in 79 % of the cases, while *T. mentagrophytes* was found in 14.5 % of the cases.[67] *T. rubrum* was the most common dermatophyte in Goa, 38.2 % in Western India, followed by 27.2 % *T. mentagrophytes.*[68] Further many other researchers have also shown the highest prevalence of *T. rubrum* in the dermatophytosis infection.[69,49] There was an increased incidence of *T. mentagrophytes* was reported in south India in 2012 by Hanumanthappa et al. 58.9% *T. rubrum* was demonstrated where as *T. mentagrophytes* was accounted for 24.6%.[70] Agarwal et al. have demonstrated 34 % *T. rubrum*, and 38 % *T. mentagrophytes* in northwest India.[71] However, *T. rubrum* was found in 66 % of the studied samples in North India in 2014, whereas *T. mentagrophytes* was found in 19 %. Ramaraj et al. have shown 45 % of *T. mentagrophytes* and 49 % of *T. rubrum.*[72]

In 2016, in north Karnataka region the commonly isolated causative agent of dermatopytosisis *T. metagrophytes* with prevalence of 48.3%.[73] Bhatia and Sharmain from Himachal Pradesh, India, conducted epidemiological study of dermatophytoses, and discovered 63.5% increase in *T. mentagrophytes* and *T. rubrum* 35.1 %.[74] Ramaraj et al. in the year 2016 isolated *T. mentagrophytes* and reported 44.75 % from Chennai which is a next most widespread causative agent of dermatophytosis followed by *T. rubrum* with the prevalence of 48.95%.[72] In multicentric study conducted by P. Nenoff et al. showed higher prevalence of *T. mentagrophytes* in 93.21% and only 6.79% *T. rubrum* prevalence was observed.[68] Mahajan et al. performed a study called clinico-mycological study infectious dermatophytes and their antifungal susceptibility to the medications in a tertiary care centre in Uttar Pradesh, India. *T. mentagrophytes* was the commonly discovered species, with a 75.9% isolation rate. *T. interdigitale* was found in 63 (94 %) of 67 dermatophytes.[75]

Similar situation seen after second world was where there was shift in trend of etiological agent, where *T. rubrum* has replaced *T. mentagrophytes. T. mentagrophytes* was the predominant causative agent of dermatophytosis before 1935 and thereafter increase in the prevalence of *T. rubrum* was observed Isolates of *T. mentagrophytes* per 100 dermatophytosis cultures dropped 80% - 20 % from 1935 to 1954, according to researchers in New York, *T. rubrum*, on the other hand, was the opposite. *T. mentagrophytes* incidence decreased from 13 % in 1935 to 2 % in 1954, whereas *T. rubrum* incidence increased from 1.8 % in 1935 to 10 % in 1954.[68,76] Gong et al. were able to establish that highly discriminating microsatellites are linked to population divergence and genetic diversity in *T. rubrum*. They were the first to employ highly discriminating molecular markers to provide information about *T. rubrum* populations, suggesting that *T. rubrum* populations in densely populated cities

may be more adaptive due to greater genetic diversity under selective pressures. The shift was thought to be caused by external elements such as humidity, temperature, and stress, as well as internal factors such as the host-parasite relationship, host susceptibility, and immunological components.[77]

Malhotra et al. found in 1979 that tinea capitis (94%) was the most frequent dermatophytoses among Libyan children. The most prevalent dermatophyte species was *T. schoenleinii* (69.5 %), followed by *M. audouinii* (23.8%). Mikhasik et al. identified *T.rubrum* (71.3%) and *T. interdigitale* (28.7%) as the most common causative agents in foot mycoses in Russia in 1990.[78]*T. rubrum* was the most well-known dermatophyte in north and central Europe, primarily associated with tinea corporis and tinea cruris infections. Twenty isolates of *T. viollaceum* have been identified as being linked to tinea capitis and tinea corporis in Melbourne, Australia, over a 32-year period. Monod et al. (2002) identified *T. rubrum* as the most prevalent etiological agent in Switzerland, followed by *T. mentagrophytes* and *M. canis.*[79] In 2002, Lacroix et al. reported that 45 % of runners in France were infected with *T. interdigitale* sores on their feet, whereas 31 % were asymptomatic. In tinea pedis, the primary etiological agents were *T. interdigitale* and *T. rubrum.*[80]

T. rubrum was the predominant causative agent in Cleveland, Ohio, USA, Foster et al. reported *T. rubrum* caused skin mycotic infection with recurrence which was accounted for 64.4% in 1999 to 79.3% in 2002.[81] In 2004, Brilhante et al. found that 46.4 % of Brazilians had dermatophytoses. The most prevalent isolates were *T. tonsurans* (54.41 %), *M. canis* (38.97 %), *T. rubrum* (4.41 %), *T. mentagrophytes* (1.47 %), and *M. gypseum* (0.74 %)[82]. Ioannidou et al. (2006) studied the epidemiology of onychomycosis in Crete, Greece, and found that 83.3% of patients

tested positive between 1992 and 2001. Onychomycosis was found in 24.3% of patients, with infected nails found in 10.7% of cases, with 36.7 % men and 63.3% females.[83]

The presence of the uncommon geographically limited dermatophytes *T. yaoundei* and *T. soudananse* in the migrating Siddi ethnic population has been shown in several investigations.[16,18] In 1993, Hemashettar B.M. has isolated *T. yaoundei* in Siddi community. *T. yaoundei* was isolated from a 6-year-old Siddi kid who had numerous greyish white scaly lesions of varying sizes scattered all over his head and had characteristic negroid features. These lesions had started 6 to 8 months prior, according to the child's father. The patient had never left the North Karnataka area of South India, nor had he made contact with anyone who had lately visited Africa.[18] Hemashettar & Nadig identified *T. soudanense*, another geographically confined African dermatophyte, from a local patient in South India in 1980. The patient had never been abroad of the country, and the illness had no clear cause. The discovery of this unique fungus on India's west coast suggests the possibility of an African dermatophyte hotspot nearby.[16] *T. soudanense* was isolated outside of Africa as a result of its isolation, it's been suggested that its existence outside of Africa might be a relic of the historic slave trade that took place between the 15th and 19th centuries.

Studies conducted in the other part of the world have also reported the presence of rare dermatophytes in the migrated African population. Jacquelyn R. Coloe et al. have shown the presence of *T. violaceum* species (4.2%) and *T. soudanense* (1.6%) in children with tinea capitis. Reported 86.2% of cases were seen in the African originated patients. The spread of *T. violaceum* outside of Africa has been linked to emigration from Africa.[84]

In Stockholm, Sweden, a tinea capitis pandemic caused by *T. violaceum* was reported (68 % of 92 isolates). 83% of these infants were born to African immigrants, with Somalia accounting for 41 %. *T. violaceum* was found to be present in 87 % of Somali individuals with tinea capitis.[85] *T. violaceum* discoveries among African immigrants have been documented in Florence Italy (24), Bordeaux France (25), Hamilton New Zealand (26), and Melbourne Australia (27). Between 1983 and 1994, 209% rise in tinea capitis was seen in African Americans in California, according to Lobato et al.[86] Athanassios Kolivras et. al. reported the isolation of *T. soudanense* in 35 cases (28.69%), *T. violaceum* in 22 cases (18.03%) of 122 children from Brussels Belgium. The infections linked with the bulk of TC cases in Brussels and other large European cities are anthropophilic dermatophytes, which represent primarily North and Black African population migrations.[87]

Only a few times have *T. violaceum* and *T. soudanense*, which are common causes of tinea capitis in Africa and West Asia, been identified from patients in the United States. Among 14,696 dermatophytes isolated from patients at 54 locations across the United States from 1985 to 1987, only 12 were recognized as *T. violaceum* and only 2 as *T. soudanense.*[88-91]

Shelley S. Magill et al. observed 25 *T. violaceum* and *T. soudanense* isolates from 24 patients. To isolate these organism in Baltimore, Maryland in 2005 at the Mycology laboratory of John Hopkins hospital, 16 (67%) patients of 24 have given the country of origin where 14 (88%) patients of 16 patients were migrated from eastern African countries like Kenya, Uganda, Somalia, Tanzania, and Ethiopia and West African countries like Liberia and the Congo. 2 patients of 16 patients have given the history of US origin. 11 (69%) patients of 16 patients were living in US

since 2 years or less and 5 (31%) patients were living in US since more than 2 years.[92]

2.4 Molecular characterization of Dermatophytes.

Physiology and feeding are less important in traditional dermatophyte classification than gross and microscopic appearance. However, because of their overlapping characteristics, unpredictability, and pleomorphism, identifying isolates has proven difficult. To go beyond traditional techniques of identification, many chemotaxonomic procedures have been developed and establish interspecies relationships. Disc electrophoresis of culture filtrate proteins,[93] pyrolysis–gas–liquid chromatography for fatty acid analysis,[29,94] polyacrylamide gradient gel electrophoresis of total cell protein extracts for zymogram patterns,[95] and isoelectric focusing of somatic extracts in thin-layer polyacrylamide gels are among them.[96,97] The investigation carried out by Davidson et al. and shown the base content of chromosomal DNA from 55 isolates of dermatophytes representing 34 species using CsCl density gradient centrifugation.[98] All dermatophytes species (*Microsporum, Trichophyton*, and *Epidermophyton*) examined had G+C content that fell within a short range of 48.7 to 50.0 mol%. And suggested that dermatophytes have great overall phenetic and genetic similarities, its classifications based on present phenotypical features cannot be entirely justified. Following that, taxonomy of the three dermatophyte taxa was determined using DNA homology by Davidson and Mackenzie,[99] purified DNA from ten isolates reannealed by hydroxyapatite chromatography. *T. terrestre* is the only exception, showed less similarity with *A. benhamiae* (*T. mentagrophytes*) and *T. rubrum*, DNA homology values of 65 to 80 % corresponded to species within a genus in 25% and 24 %, respectively, and Their

findings were in accordance with existing categorization system, notably in speciating different genera, whereas *A. incurvatum* (*M. gypseum*), which shown lowered similarity with *M. canis* of 28 %. The results of DNA hybridization between 2 strains of *T. rubrum* and *A. benhamiae* showed a close relationship of 73% and 76 % respectively. Mitochondrial DNA was used for the identify taxonomy as genetic marker during 1980.[100,101]

Twenty two *T. interdigitale* isolated in Japan was investigated for its relation with other *T. mentagrophytes* complex by analyzing restriction enzyme like HaeIII, MspI, and HindIII.[100] They found *T. interdigitale* and *T. mentagrophytes* were showing close relation to the *A. vanbreuseghem*ii when compared when restriction profiles of their *T. mentagrophytes* var. *interdigitale* isolates with *A. simii*, *A. benhamiae*, and *A. vanbreuseghemii*. *A. simii* shown the different restriction profiles from *A. vanbreuseghemii's* MspI and HindIII and *A. benhamiae* was different from the other species and to identify the taxonomic connections of *Trichophyton* species and create a sequence diverse based phylogenetic tree, researchers utilized 5 endonucleases which includes HaeIII, MspI, HindIII, XbaI, and BglII.[101] Seven groupings of *Trichophyton* species were identified. *T. rubrum* was divided into 2 groups group I and group II, group I was found to be more closer to *A. benhamiae* genetically when compared to group II, further Davidson and Mackenzie reported *A. benhamiae* and *T. rubrum* were having 76% similarity in the DNA homology. *T. mentagrophytes* var. *erinacei* was closely linked to *A. benhamiae*. Similler restriction pattern was shown by *T. rubrum* type II, *T. tonsurans*, and *A. vanbreuseghemii*, suggesting that they are related or identical. According to Ajello et al., *T. quinckeanum* (*T. mentagrophytes* var. *quinckeanum*) and *T. schoenleinii* showed restriction profiles comparable to *A. vanbreuseghemii*. Pleomorphic strains were

identified via mtDNA analysis. The authors came to the conclusion that traditional taxonomy based on morphology does not always match results from mtDNA restriction profile research.

Walberg et al. (2006) examined clinical specimens from 346 patients with suspected onychomycosis using 18S PCR, sequencing, and database searches in tandem with standard agar culture.[102] Using the ribosomal DNA (rDNA) gene complex in fungus to signify gene order and the position of the internal transcribed spacer, Yang et al. in 2008 developed a nested PCR approach that can be utilized directly on clinical samples and enhanced sensitivity detection (ITS).[103] Through the amplified products of the ITS1-5.8 SrDNA-ITS2 areas, Mirhendi et al. 2008 investigated the use of PCR followed by enzymatic digestion for differentiating of *T. rubrum* and *T. mentagrophytes*.[104] Mirzahoseini et al. 2009 developed a PCR-RFLP analysis of the PCR-amplified ITS region of rDNA for fast identification of dermatophytes in clinical specimens, as well as demonstrating that this approach was a quick and accurate method for identifying major pathogenic dermatophytes. Using a common reverse primer and two distinct forward primers, Malinovschi et al. in 2009 designed the PCR reactions to be efficient under the same PCR processes, allowing the detection of two fungi from one reaction volume.[105]

Rezaei M et al. in 2012 used the universal fungal primers ITS1 and ITS4 to amplify the ITS1-5.8S ITS2 region of rDNA from various dermatophyte species and digested the PCR followed by MvaI-RFLP was shown to be a helpful and reliable method for identifying and distinguishing many pathogenic species, and it could be utilized for fast screening of even closely related dermatophyte species.[106]

2.5 Antifungal susceptibility Testing:

In 1958 Genetles have demonstrated the griseofulvin oral therapy in guinea pigs by experimental dermatophytosis, revolutionized dermatophytosis treatment and was the first significant change in tinea capitis treatment since Sabouraud's work.[107] Dermatophytoses usually respond well to topical antifungal treatment, however it might be ineffective for severe infections/infections involving the nails or scalp, which necessitate the use of systemic antifungal agents. The vast majority of antifungals are fungistatic, with skin concentrations achieved when applied topically; dermatophytes grow more slowly and are eliminated with skin renewal, resulting in recovery.[108] However in the dermatophytosis the infected nail in case of tinea unguium will not respond to the antifungal agent.[109]

Since past few years the recurrence of dermatophytosis has observed by many researchers, which have developed resistance to routinely used antifungal medications.

The amount, location, and clinical type of infection must all be considered, as well as the etiologic agent, the antifungal's spectrum of activity and pharmacokinetics, any potential pharmacodynamic drug-drug interactions, adverse effects, cost, and a risk/benefit analysis. Azole antifungal medications reduce the synthesis of ergosterol, a major component of fungal plasma membranes, by inhibiting the cytochrome P-450-dependent enzyme lanosterol demethylase azole antifungal medicines with two or three nitrogens in the azole ring are classified as imidazoles (e.g., ketoconazole and miconazole, clotrimazole) or triazoles (e.g., ketoconazole and miconazole, clotrimazole) in clinical use (e.g., itraconazole and fluconazole). Triazoles have a wide range of applications in the treatment of both superficial and systemic fungal

infections, whereas imidazoles are only used for superficial mycoses. Triazoles also have a greater affinity for fungal cytochrome P-450 enzymes than human cytochrome P-450 enzymes, which contributes to their improved safety profile.[110,111]

Triazoles (itraconazole and fluconazole), imidazoles (ketoconazole), allylamines (terbinafine), and griseofulvin have all been proven to be effective against dermatophytes.[106,112] Recurrent dermatophytosis might be seen in case of treatment failure and the in vitro antifungal susceptibility test can assist us in overcoming the problem, selecting an appropriate antifungal drug, and optimizing tinea infection therapy. Many of scientific report evident the good result of MIC even though there is no standard susceptibility protocol is available for many of the antifungal agents.[113,114] Other reasons like poor adherence to treatment, use of steroid, medicine taken by oneself, and resistance to antifungal agent are the factors contributing factors for the current danger situation. In recent years, India has seen a number of instances with unique, atypical, and chronic/relapse/recalcitrant manifestations[115].

The mechanism of antifungal resistance, in vivo correlation and invitro tests for susceptibility of antimycotic agents for dermatophytes, have not been well investigated. There is currently no breakpoint that can be used to lead us to antifungal treatment. Relapse is prevalent during tinea unguium treatment,[116] and it is linked to terbinafine resistance to some extent.[117,118] For *T. rubrum* isolates, terbinafine drug resistance and higher MIC of drug was reported by Mukherjee et al. to observe significant relationship.[117] In those isolates, they discovered leu393Phe and Phe397Leu substitution due to squalene epoxidase (SE) gene mutation.[118,119]

Onychomycosis instances that did not react to SE inhibitors (allylamines) have been recorded, even with low MICs.[116,120] Dermatophytosis is often treated with the triazole and imidazole groups of medicines. In dermatophytes, azole resistance has recently been discovered developed fluconazole resistance in *T. rubrum* isolates in 19% of cases.[121,122]

3.0 METHODOLOGY

Present study was conducted in Siddi residing area of North Karnataka Region in Uttar Kannada, Belagavi and Dharwad Districts of Karnataka, India during the year 2015 to 2017. Samples were collected by conducting Skin camps, visiting directly Siddi residing area and in coordination with District health authorities. (Annexure No 6) Patients belong to Siddi tribal community with clinically suspected superficial infection was enrolled in the study.

Sample size 1000 samples was estimated using the formula $N=Z^2\alpha Xpq/d^2$, since there is no data on community based on fungal infection prevalence in this part of Karnataka, it was assumed to be 50% (p), confidence interval of 99% and absolute error of 4% (d) were considered. Patient belongs to Siddi tribal community with superficial infection and all age group of both sex was enrolled in the study. Pregnant women and patients with conditions like leprosy, scabies and psoriasis was excluded.

A total of 1004 samples from 937 patients were obtained with superficial infection Patients were examined for superficial infection and Samples like Skin scraping, Nail and Hair samples were collected depending on site of infection as per standard protocol. Further all samples were shipped to Microbiology department, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India.

Questionnaire was prepared (Annexure No 2) using question about age, sex, previous fungal infection, animal contact, soil contact, infected human contact, seasonal variation, type of house, type of family. The clinical examination was done by observing site of the lesion, type of lesion, multiple infections, presence of spreading erythematus lesion, type of onychomycosis like proximal subungual

onychomycosis, distal lateral subungual onycomycosis, whitish subungual onychomycosis, endothrix, total dystrophy of nail and hyperkeratosis in infected nail was observed.

The KAHER's institutional Human Ethics Committee gave their ethical clearance (Annexure No 1) (Institutional Ethical Clearance Reference Number KLE/Ethic/2015-16/D-51). Singed informed consent (Annexure No 2) was taken from patient or patient guardian after interviewing the patient.

3.1 Specimen collection:

Skin, nail and hair samples were collected aseptically from patient with superficial infection. All equipments were disinfected with 70% ethanol and dried before use. Samples were collected in sterile black card sheet paper with required patient details and kept in zip lock bag and transported to the laboratory for the further process.

3.1.1 Skin sample:

Patient skin was disinfected with 70% alcohol before collecting the sample. Using the blunt ended scalpel the active side of the lesion was scraped, and a significant volume of material was obtained. In case of insufficient sample sticky tape was pressed on the lesion and transferred to glass slide for transportation to the laboratory.[(29)]

3.1.2 Nail sample:

The infected area was cleansed with 70% alcohol, and the clippings were gathered with a sterile nail cutter. Discolored dystrophic or brittle nail was collected

from the infected area. Nail sample was collected by reaching as far as possible from the free edge and full thickness of the nail was included. In case of superficial whitish onychomycosis scraping from the white spot was collected after discarding the upper most layers.[123,29]

3.1.3 Hair sample:

Infected hair sample was selected and collected by removing those completely using epilation forceps. Scraping was collected with blunt scalpel blade. Specimen should contain hair stub, plugged follicle and skin scales.[124]

Fig 3.1: Sample collection

3.2 Sample transportation:

Sample was transported to the laboratory in sterile black card sheet kept in leak proof zip lock bag and transported at normal room temperature and stored in the laboratory at room temperature at 15-30^0 C.

3.3 Direct Microscopy:

All specimens were subjected to direct microscopy to see the fungal element in the specimen using potassium hydroxide (KOH) mount. KOH is a powerful alkali that is used to clean specimens so that fungus may be observed. Two to three drops of KOH were combined with the material on a clean grease-free glass slide, which was then covered with a sterile cover slip and examined under a microscope with a low power (10x) and a high power (40x) objective lens to check if fungal hyphae were present.[29]

3.3.1 Nail Samples:

Nail fragments are treated with 10-30% KOH and incubated at room 15-30^0 C for 30 minutes. When the nails are digested 2 to 3 drops of mixture was transferred on grease free clean glass slide and cover with clean glass cover slip without forming any air bubble. Excess KOH was removed by blotting and observed for fungal filament under microscope using 10x and 40x objective lens and 10x eye piece.

Observation: Arthrospores are arising from fragmented hyphae forming the rectangular spores which are the characteristics feature of dermatophytic infection. Arthrospore formation cannot be seen in other mould infection of nail[29].

3.3.2 Hair sample:

Hair samples collected and cut into 5mm above the root then treated with 20% KOH. Incubated at 15-30^{0}C for 20 min. 2 to 3 drops of the mixture is placed on clean grease free glass slide and covered with clean cover slip without forming air bubble, excess KOH was by blotting and observed for fungal filament under microscope using 10x and 40x objective lens and 10x eye piece.[29]

Observation: Arthrospores are seen and their arrangements as large arthrospores (Endothrix), small arthrospores (Exothrix) and favus type of infection.[29]

3.3.3 Skin sample:

Skin scraping is treated with 10% KOH for 20 min at 15-30^{0}C and observed under microscope using 10x and 40x objective lens.[29]

Observation: branched mycelium with or without arthrospores was seen.

3.4 Isolation of Dermatophytes:

Samples were inoculated on two sets of tubes one containing Sabouraud's Dextrose Agar (SDA) with antibiotics (Containing mycological peptone 10 G/ml Dextrose 40G/ml and Agar 15G/ml with Chloramphenicol and Cycloheximide*)*. Sabouraud's Dextrose Agar with antibiotic is used for selective isolation of pathogenic fungi from highly contaminated area like skin, hair and nail samples. Chloramphenicol inhibits the bacterial contamination whereas Cycloheximide inhibits many saprophytic contamination. Part of the sample was also inoculated on another tube containing plain Sabouraud's Dextrose agar (Containing mycological peptone 10 G/ml Dextrose 40G/ml and Agar 15G/ml).[125][29]

3.4.1 Macroscopy:

All isolates were studied macroscopic characteristic of the colony e.g. Rate of growth, surface texture, consistency, pigment production on both front (forward) and reverse of the tube and whether produced color diffuses in the media or not was also observed.

3.4.2 Microscopy:

3.4.2.1 Lacto phenol cotton blue tease mount:

All isolates were observed microscopically for the identification. Areal mycelium was taken with the help of sterile inoculation loop and needle on a clean grease free glass slide with lacto phenol cotton blue. With the help of two sterile teasing needles mycelium was teased smoothly, clean coverslip placed on lacto phenol cotton blue stain (LPCB) excess stain was bloated and observed under microscope using 40x objective lens. Identification was made based on type of microconidia and macroconidia and hyphal arrangement.[29]

3.4.2.2 Tape technique:

Take 2 cm long cello tape touch sterile forceps/stick on one of the tape and touch lightly the colony with the sticky end of the tape. Place the tape with the surface containing on lacto phenol cotton blue on clean glass slide, detach the tape from the stick. Observe under microscope using 40x objective lens.

Slide culture: slide culture was done when ever there was difficulty in identification of non sporulating moulds by tease mount. Cornmeal agar, potato dextrose, and other nutritionally deficient media were employed to boost sporulation.

A filter paper, V or U shaped glass rod, microscopic slide, and cover slip were placed in a 100 mm glass petridish, and the entire setup was covered in craft paper and autoclaved at 121^0 C for minutes. Aseptically cut 1 cm square agar blocks from CMA or PDA were transferred to the glass slide in the setup, a small amount of colony was inoculated on four sides of the agar block, a cover slip was placed on the inoculated agar block with the help of sterile forceps, and 1 to 1.5 ml of sterile water was added to the pertridish without disturbing the agar block. Water keeps the agar block moist and prevents it from drying out. We also can add 5-20% glycerin to sterile water which prevents the condensation of moisture on the glass slide. Incubate the setup at 15-30^0C in dark place. When mature conidia or spores are observed slide culture will be ready to take down and observe under microscope. A little quantity of LPCB (mounting medium) is put on a clean glass slide, the cover slip is gently removed from the agar block using sterile forceps, and the slide is swiftly passed through the blue section of the flame to heat fix the fungus and spores (over heating can damage or collapse the hyphae). Place the coverslip carefully on mounting medium without forming any air bubble, wipe the excess stain (mounting medium). Use nail polish to seal the edge. After removing the agar block from the apparatus, a second slide may be made from the tiny slide. Apply a drop of LPCB on the cover slip and seal it with nail paint. Observed using a 40x objective lens under a microscope.[29,124–126]

Urease test: Urease test is done to differentiate the *T. mentagrophytes* from *T. rubrum* as *T. metagrophytes* produces urease enzyme and has the ability to hydrolyse urea and shows urease test positive where as *T. rubrum* shows negative result. Christensen's urea agar slant is used for the urease test, isolate was inoculated in urease media and incubated at 15-30^0C color change from yellow to pink indicates the positive result.[29]

3.5 Antifungal susceptibility testing:

3.5.1 Microdilution technique:

This study was performed on 102 dermatophtes isolates including *T. mentagrophtes, T. rubrum, T. tonsurance, T. terestreae* and *M. gypseum*. All isolates were inoculated in distilled water and maintained at room temperature before testing and subcultured on PDA and incubated at 28^0C for two weeks *Candida parapsilosis* ATCC 22019 was used as controlled strain. Broth micro dilution for all study isolates was performed.

3.5.2 Antifungal agent:

13 different antifungal drugs obtained from manufacturer *viz.* sertaconazole, terbinafine, naftifine, miconazole, luliconazole, ciclopiroxolamine, ketoconazole, clotimazole, 0.00781to 4 µg/ml for voriconazole, itraconazole, amorolfine, fluconazole and griseofulvin were tested against study isolates, all antifungal agents were dissolved in distilled water except ketoconazole which was dissolved in dimethyl sulfoxide. Twofold serial dilution was done from stock solution to get a final concentration ranging from 0.0156 to 8 µg/ml for sertaconazole, 0.0156 to 8 µg/ml for terbinafine, 0.03125 to 16µg/ml for naftifine, , 0.03125 to 16µg/ml for miconazole, 0.03125 to 16µg/ml for luliconazole, 0.03125 to 16µg/ml for ciclopiroxolamine, 0.03125 to 16µg/ml for ketoconazole, 0.03125 to 16µg/ml for clotimazole, 0.00781to 4 µg/ml for voriconazole, 0.00781to 4 µg/ml for itraconazole, 0.00781to 4 µg/ml for amorolfine, 0.125 to 64µg/ml for fluconazole and 0.25 to 128 µg/ml for griseofulvin.[127,128]

3.5.3 Preparation of Inoculum:

Inoculum was prepared from 7 days old growth on PDA, fungal growth was treated with 2 to 3 ml of normal saline, and surface of the colony is scraped with sterile 1ml micropipette tip to get the mixture of fungal hyphae and conidia, the mixture was transferred to sterile tube and incubated at 28^0C for 20 minutes. Optical density of the mixture containing fungal hyphae and conidia was read at 530 nm and transmittance was adjusted to 65-70% (2-4×10cells mL^{-1}).[127,128]

Inoculum is mixed with Roswell Park Memorial Institute (RPMI) 1640 medium to get the final concentration of approximately 4-5×104 cells mL^{-1}. 100µl of Mixture is inoculated in micro titer plate containing 100 µl serially diluted antifungal drug of specific concentration and incubated at 28^0C.[128][129]

3.5.4 Results Reading:

MIC: The lowest dosage of an antifungal drug that prevents the organism from growing which can be visually detected. The amount of the growth can be compared with the amount of the growth in the negative control.

For flucytosine and azoles like fluconazole and ketoconazole slight turbid end point is allowed above the MIC which improved the interlaboratory agreement. When compared to the growth of the control (drug free) media, turbity permitted corresponds to develop at a rate of 50 % or more slower.

The lowest medication concentration that stops obvious observable growth by 100 % is interpreted as the MIC for itraconazole and novel triazoles, posaconazole, ravuconazole, and voriconazole.[128][129]

3.6 Molecular Characterization

3.6.1 Purification of fungal DNA:

Purification of fungal genome was done by phenol chloroform method. Isolates were grown on SDA at 28^0C for 7 days. Mycelial mat of fungal growth was transferred to the sterile mortar aseptically, and liquid nitrogen was added to the mortar containing mycelia mat and grained quickly to make powder. 600 µl of lysis buffer (100 mM TrisHCl [pH8.0], 50mM EDTA, 3% SDS) added to the mixture and transfer to a micro-centrifuge tube. Add 1.4µl proteinase k (20mg/ml) and incubated at 56^0C for 30 minutes in water bath. Mixture was transferred to sterile microfuge tube and added 650 µl of phenol chloroform (1:1) and centrifuged at 12000 rpm for 15 minutes, top aqueous phase was transferred to another microfuge tube without disturbing the interphase, an equal amount of chloroform isoamyl alcohol (24:1) was added, and the mixture is centrifuged for 5 minutes at 12000 rpm. Upper aqueous phase is transferred into a fresh sterile tube. $1/10^{th}$ volume of sodium acetate and two volumes of chilled ethanol was added to allow the DNA to precipitate, gently invert the tube by mixing the content incubate the tubes at 2- 8^0 C overnight. Centrifuge the mixture at 12000 rpm for 1-5 minutes, discard the supernatant and the deposited pellet are washed with 70% ethanol. Allow the pellets to dry at 37^0 C till ethanol evaporates completely. Resuspend the pallets in 50µl of tris-EDTA buffer (TE buffer). The DNA is allowed to dissolve at least for 6 hours. 1µl of RNase is added and incubated at 37^0 C for 30 minutes.[126][129]

3.6.2 Quantification of DNA:

Purified DNA was checked for its purity and concentration by NanoDrop 2000 spectrophotometer. Blank was adjusted using 1µl elution (TE) buffer before measuring to ensure accurate result, once the blank is adjusted 1µl sample was placed on the NanoDrop 2000 sensor. Concentration was measured and displayed on the computer screen. Purity of the extracted DNA was measured by 260/280 ratio. For DNA 260/280 ratio should be in the range of 1.8 – 2.0 for RNA 260/280 ratio must be above 2.0 Contamination by residual phenol, guanidine, or the other reagent employed in the purification process is indicated by an abnormal 260/280 ratio. The 260/280 ratio may change if the nucleic acid content is very low.[129]

3.6.3 Polymerase chain reaction:

The universal primers ITS 1 and ITS 4 were used in a uniplex polymerase chain reaction (PCR). Each dNTP is at a concentration of 200 µM, each primer is at a concentration of 25 pmol, Taq polymerase is at a concentration of 1 U µ1, Taq buffer is at a concentration of 5 µl, and template DNA is at a concentration of 10 µl. To make the final volume of 50 l, nuclease-free water was added, and the PCR was performed using an Eppendorf thermal cycler. PCR amplification was carried out with initial denaturation at 94^0C followed by 35 cycles of annealing at 56^0C for 30 seconds and extension at 72^0C for 1 minute. Final extension was done at 72^0C for 10 minutes.[129]

3.6.4 Gel electrophoresis

Gel electrophoresis was done to see the presence of PCR products and to verify the length of the DNA molecule, DNA molecule the gel was separated depending upon the size of the molecule. The amplified products were seen on

agarose gel of 1.5% stained with ethedium bromide. Agarose gel was prepared by mixing in 1x Tris-Acetate EDTA buffer (TAE) buffer (40 mMTris base, 40 mM acetic acid, 1mM EDTA) and stained with ethedium bromide (final concentration is 0.5 μg/μL). The PCR products and the molecular size marker (6-7 μL) was stained with methylene blue (loading dye) and loaded into the agarose gel ran at 100 volts for 45-60 minutes until the line covers approximately 70 – 80 % of the way down the gel. Gel is visualized by Alpha-lmager® HP (Multi image light cabinet), Alpha innotech and the picture was taken with the help of Alpha-Imager software.[129]

3.6.5 DNA Sequencing:

ITS 1 and ITS 4 primers were used to perform sequencing PCR for both strands. BigDye Terminator Cycle sequencer kit version 3.1 and ABI 3130 genomic analyzer used to perform purification and analysis (Applied Biosystems).

Sample was resuspended in 10μl HiDi (Formamide provided by AB). The pallets were mixed properly and subjected to shot spin and incubated at 37^0 C for 15 minutes. Again pellets were mixed proper by tapping with short spin. Reaction mixture was treated at 95^0 C for 15 minutes and rapidly chilled in ice for 10 minutes. Load the sample into 96 wells plate as per the well description in the worksheet.[129]

ITS sequence were analyzed using NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov), Westerdijk Fungal Biodiversity Institute CBS database (http://www.westerdijkinstitute.nl/ Collections/ BioloMICSSequences.aspx) and the International Society for Human and Animal Mycology (ISHAM) ITS database (http://its.mycologylab.org/BioloMICSSequences.aspx).

3.6.6 Phylogenetic analysis:

The Clulstal X2 programme was used to align the sequences utilizing the multiple sequence alignment method. Version 7 of the Molecular Evolutionary Genetics Analysis programme (MEGA-7) was used to export the sequence alignments (MEGA-7). The Kimura 2 parameter model with neighbor joining was used to create a phylogenetic tree with 1000 bootstrapping iterations.[129][130] Factorial analysis was carried out using distance matrix generated by DARwin software 6.0.12.[131]

4.0 RESULTS

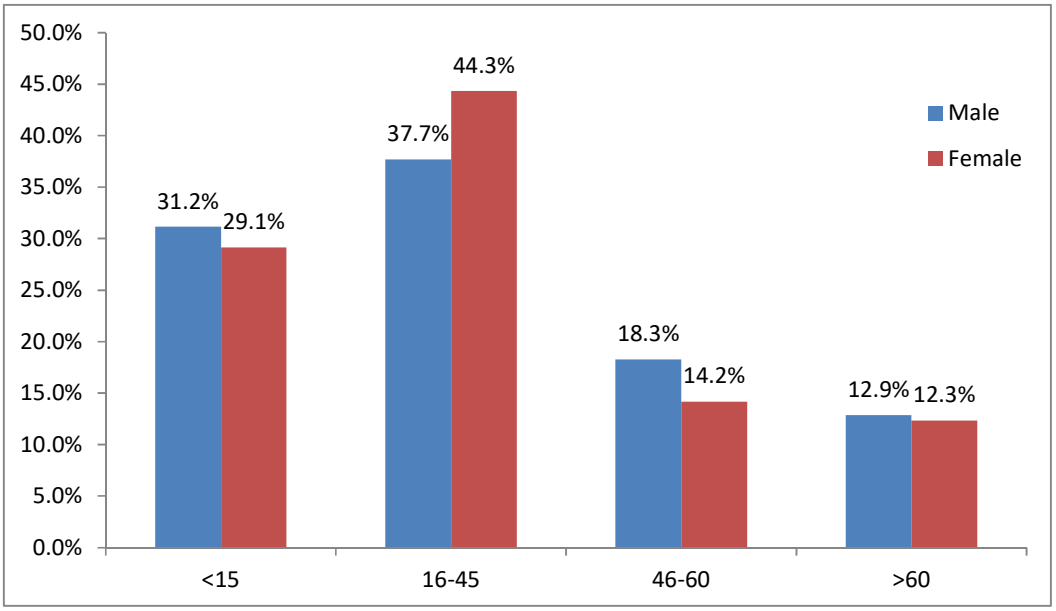
4.1 Basic Clinical data:

We have tested 937 Siddi patients for superficial fungal infection, generating 1004 samples in total. During the years 2015 to 2017, sample collection was carried out from the Siddi population by holding health camps, physically visiting the Siddi dwelling region, and working with the Uttar Kannada district health officials. Skin scrapping (n=382), hair (n=244) and nail (n=378) samples were taken from suspected patients belongs to Siddi ethnic group. 478 were male and 526 were female, majority of the patients were female (52.4%) compared to male (47.6%) with median age of 31 years with the interquartile range of 13 to 50 years.

Table 4.1: Age and sex wise distribution

	Age Group				Total
	<15 Years	16-45 Years	46-60 Years	>60 Years	
Male	138	167	81	57	443
Female	144	219	70	61	494
Total	282	386	151	118	937

Fig 4.1: Age and sex wise distribution



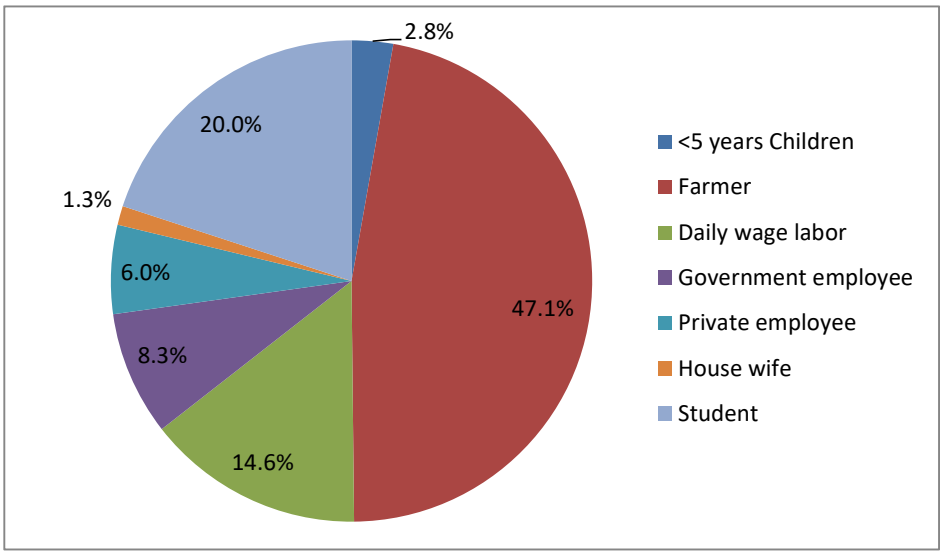
4.2 Occupation wise distribution:

Among 937 Siddi patients suspected for superficial fungal infection most of the patients were uneducated (73.7%) the infection commonly seen in farmers (47.1%) followed by students (20%), and daily wage labors (14.6%).

Table 4.2: showing Occupation wise distribution.

Sl. No	Occupation	Frequency	Percentage (%)
1	<5 years	26	2.8
2	Farmer	441	47.1
3	Daily wage labor	137	14.6
4	Government Employee	78	8.3
5	Private Employee	56	6.0
6	House Wife	12	1.3
7	Student	187	20.0
Total		937	100.0

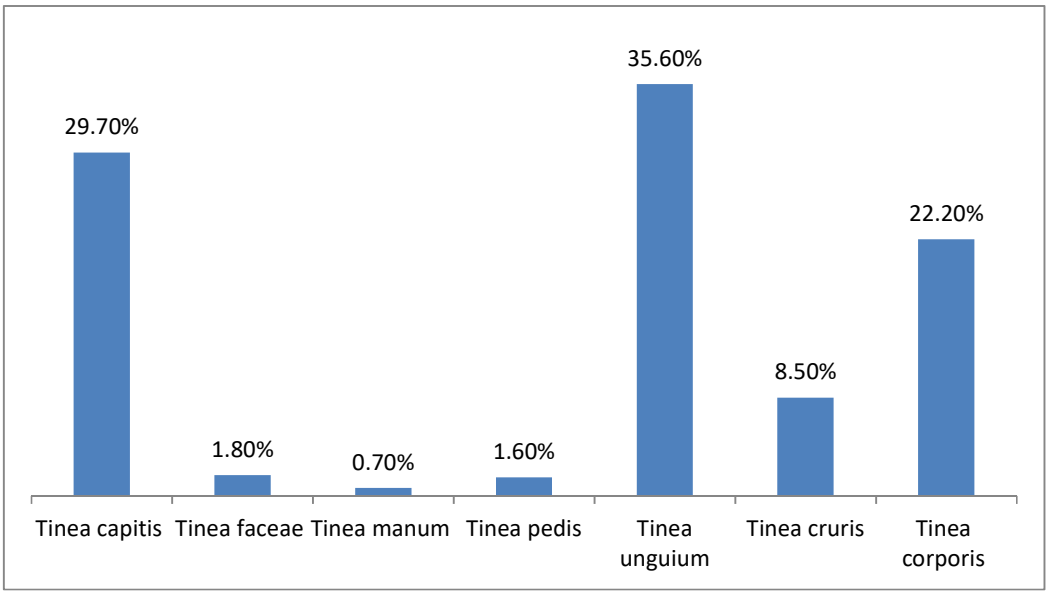
Fig 4.2: showing Occupation wise distribution.



4.3 Distribution of Clinical Manifestation:

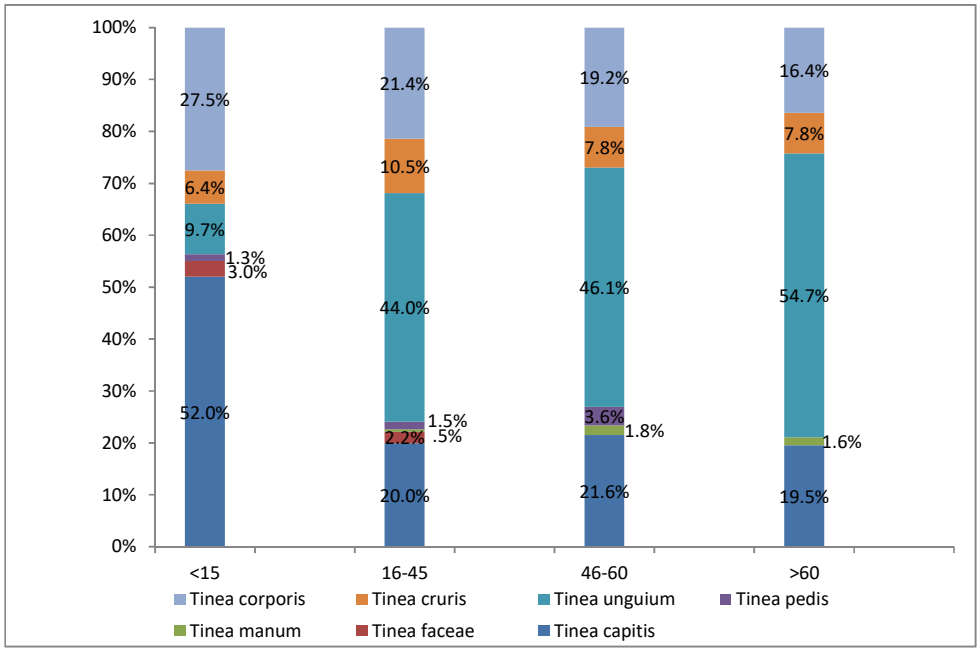
Tenia unguium (35.6%) was the commonly encountered clinical type followed by tiniea capitis seen in 29.7%, tinea corporis in 22.2%, tinea cruris in 8.5%, tinea faceae in 1.8%, tinea pedis in 1.6% and tinea manum in 0.7%.

Fig 4.3: Distribution of clinical condition



Tinea unguam was commonly seen in more than 15 years of age followed by tenia corporis whereas in below 15 years of age tinea capitus commonly seen infection followed by tenia unguium and tenia corpories.

Fig 4.4: Age and clinical condition wise distribution of dermatophytes.



4.4 Basic Laboratory Identification:

Out of 1004 sample collected from 937 patients 102 (10.15%) sample have shown culture positive and 193 samples were positive by direct microscopy, The majority of the patients (n=48, 53.33 %) were female, 28.5 years was the median age [IQR 15-40 years]. Agriculturists accounted for the majority (n=39, 43.33 %), with students (n=21, 23.33 %), field workers (n=17, 18.88 %), office employees (n=11, 12.22 %), and others (n=2, 2.22 %) following closely behind.

The majority of the patients (n=75, or 83.33 %) had a single site lesion. Patients with multiple site involvement accounted for 15 (16.66%) of the total. Tinea unguium (n=24, 32%) was the most frequent isolated lesion, followed by tinea corporis (n=23, 30.66%), tinea capitis (n=23, 30.66%), tinea cruris (n=4, 5.3%), and tinea pedis (n=1, 1.33%).

The tinea corporis and tinea cruris combination (n=6, 40%) was the most prevalent among patients with multiple site involvement, followed by tinea unguium and tinea corporis (n=5 33%).

Fig 4.5: Distribution of clinical features in culture positive isolates.

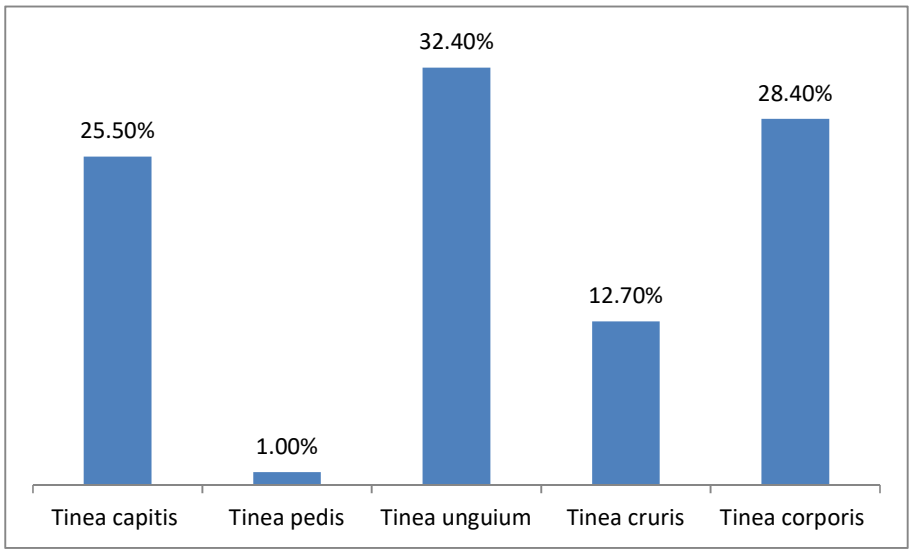
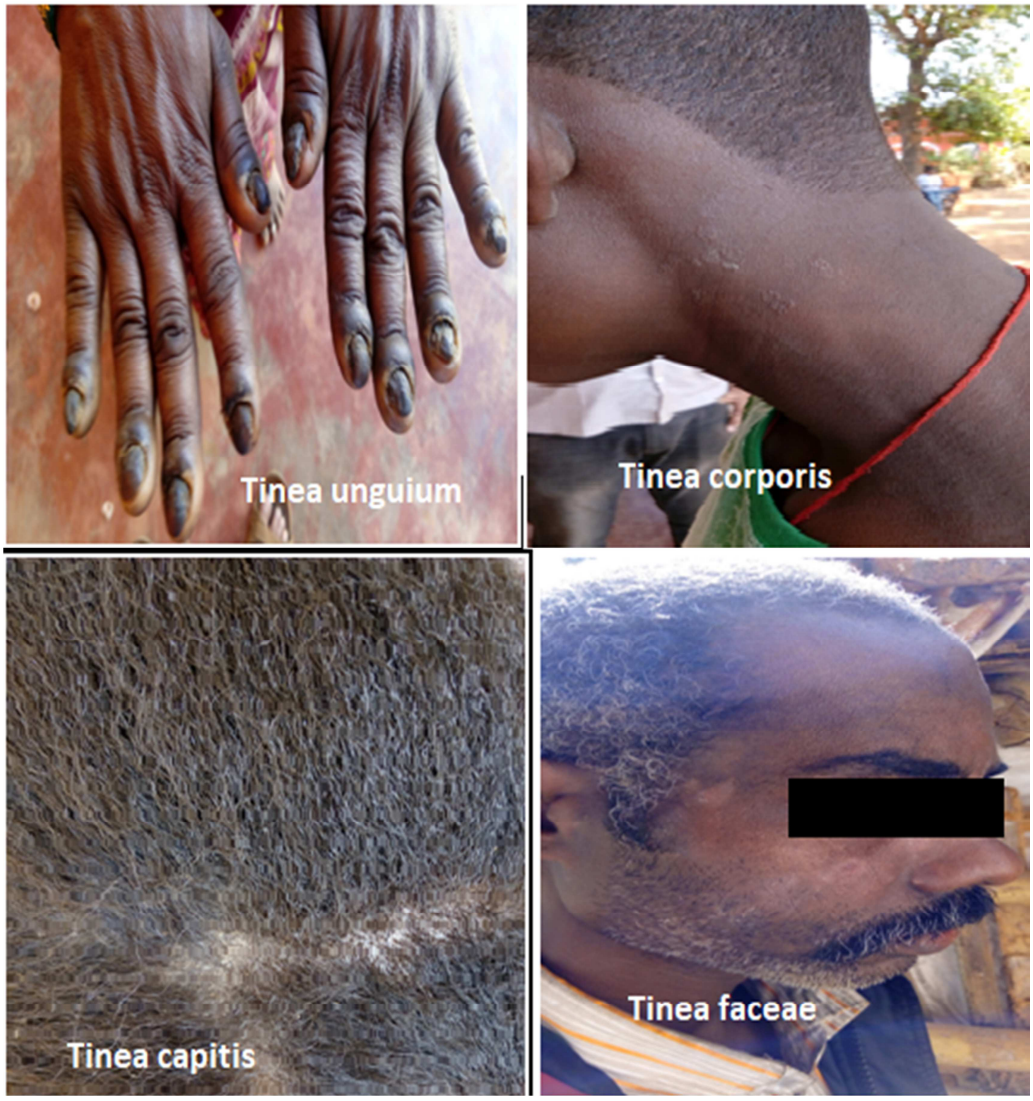
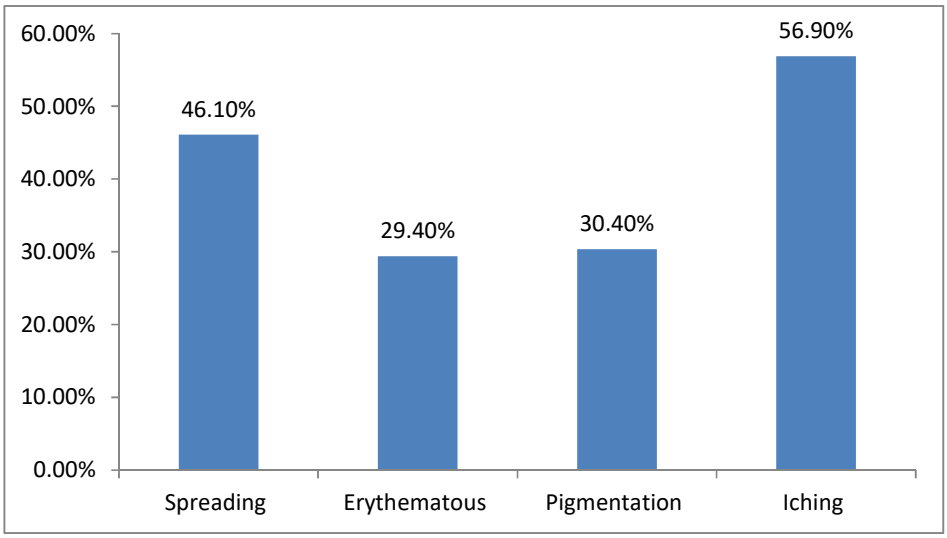


Fig 4.6: Clinical case of dermatophytosis



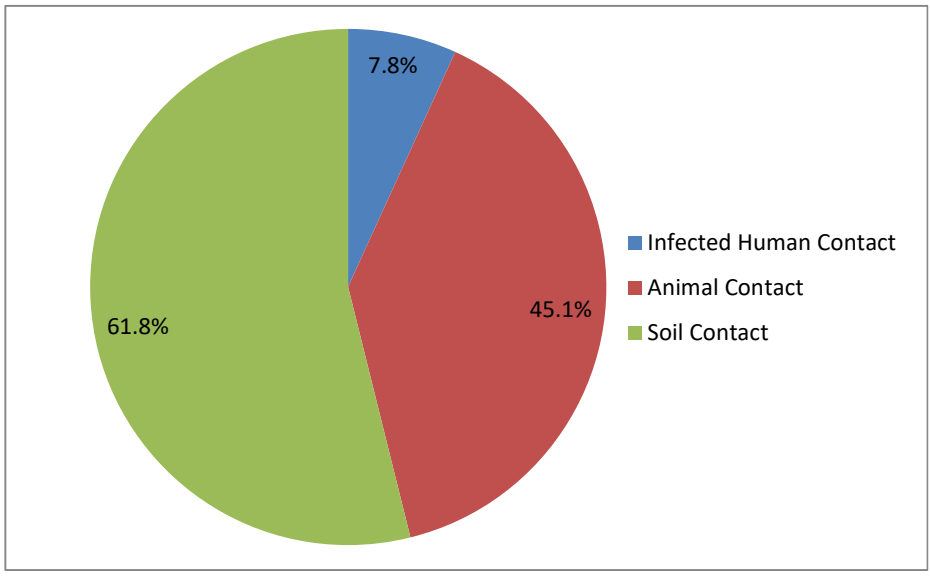
Spreading and erythematous lesions were observed in 45 (50.0%) and 27 (30.0%) of the patients, respectively. Only 28 (31.11 %) of the patients had pigmented lesions.

Fig 4.7: Distribution of clinical features in tinea corporis cases.



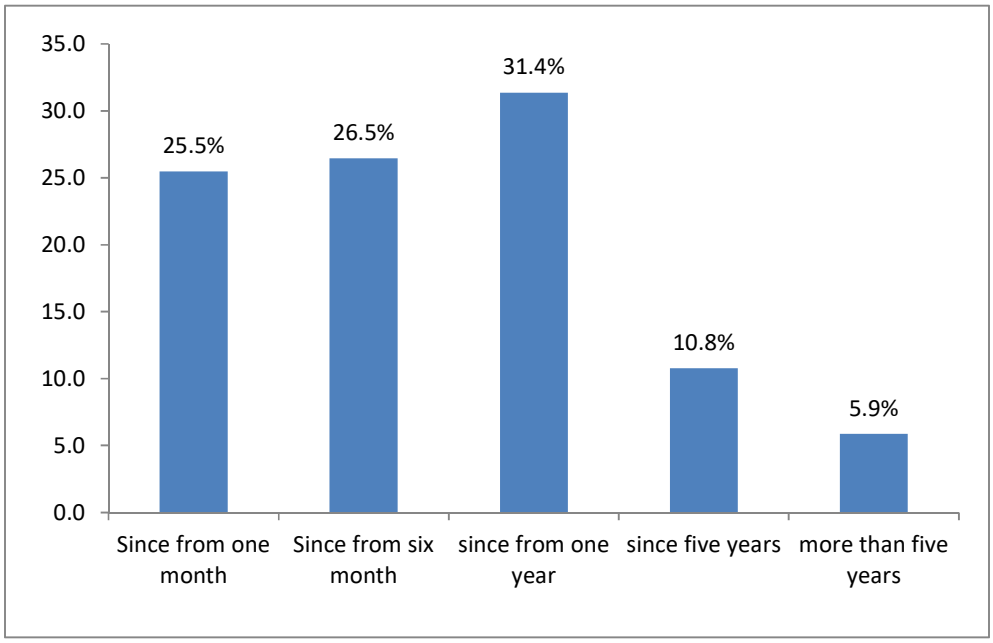
In 15 (16.66%) of the patients, the infection recurred or relapsed. Only four patients (4%) were able to provide precise corticosteroids dosages while using a combination of antibacterial and antifungal medications (Candid B, Itch-guard and Betnovate). Previous contact with an infected human, animal, or soil was reported in 6 (6.66%), 43 (47.7%), and 56 (62.2%) of the cases, respectively.

Fig 4.8: History of infection source contact.



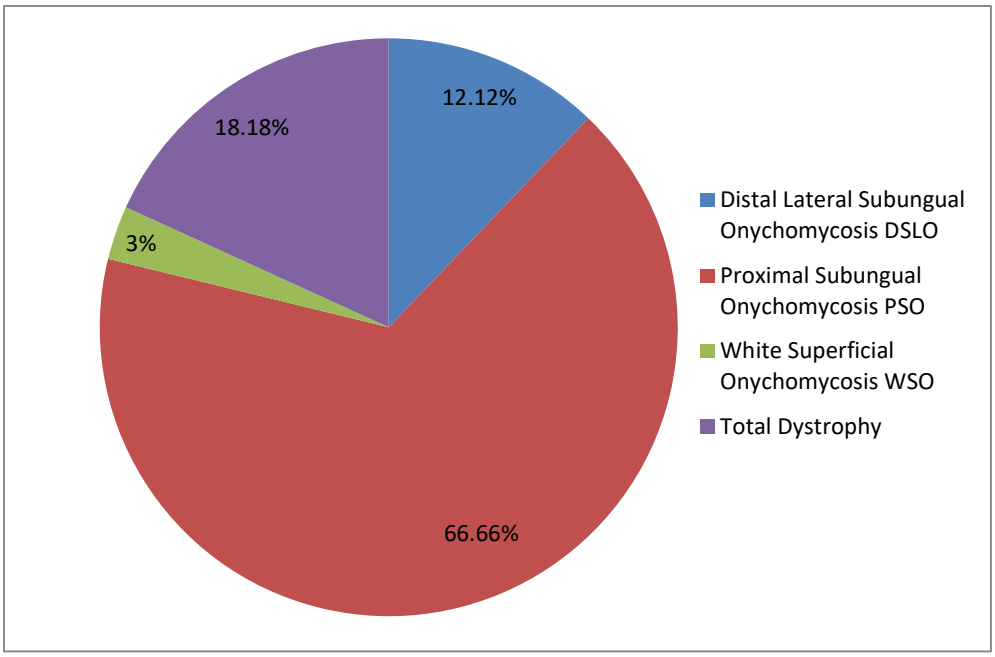
Lesions lasting 1-2 months were found in 25 (27.77 %) of patients, whereas infection lasted from 2 to 6 months in 23 (25.55 %) of patients, and infection lasted from 6 to 12 months in 25 (27.77 %) of patients. However, 17 individuals (18.88%) admit to having had an infection in the previous 5 years. Only 5 (5.55 %) of the patients had diabetes mellitus.

Fig 4.9: History of duration of infection



In Onychochomycosis Proximal subungaual onychomycosis was commonly seen clinical condition in 66.66% cases followed by Distal lateral subungual onychomycosis was seen in 12.12%, Total dystrophy of nail in 18.18% and Whitish superficial onychomycosis in 3%.

Fig 4.10: Clinical features distribution in tinea unguium cases.



T. mentagrophytes complex (67.64 %) was the common causative agent, followed by *T. rubrum* (25.49 %), *T. tonsurans* (2.94 %), *M. gypseum* (2.94 %) and *T. terrestre* (1, 0.98 %), were the most common agents. *T. mentagrophytes* was commonly seen in tinea corporis and tinea captis (30.4%), followed by tinea unguium (26.1%) and tinea cruris, *T. rubrum* was commonly seen tinea unguium (46.2%) followed by tinea corporis and tinea cruris (19.2%) and tinea capitus (15.4%). *M. gypseum* was seen in tinea corporis, tinea capitus and tinea unguium, *T. tonsurans* was seen in tinea corporis and tinea unguium, *T. terrestre* was seen in tinea unguium.

Table 4.3: Distribution of etiological agent in the study.

Fungus Isolated	Frequency	%
T. mentagrophytes	69	67.6
T. rubrum	26	25.5
M. gypseum	3	2.9
T. tonsurance	3	2.9
T. terrestre	1	1.0
Total	102	100.0

Fig 4.11: Distribution of etiological agent in the study.

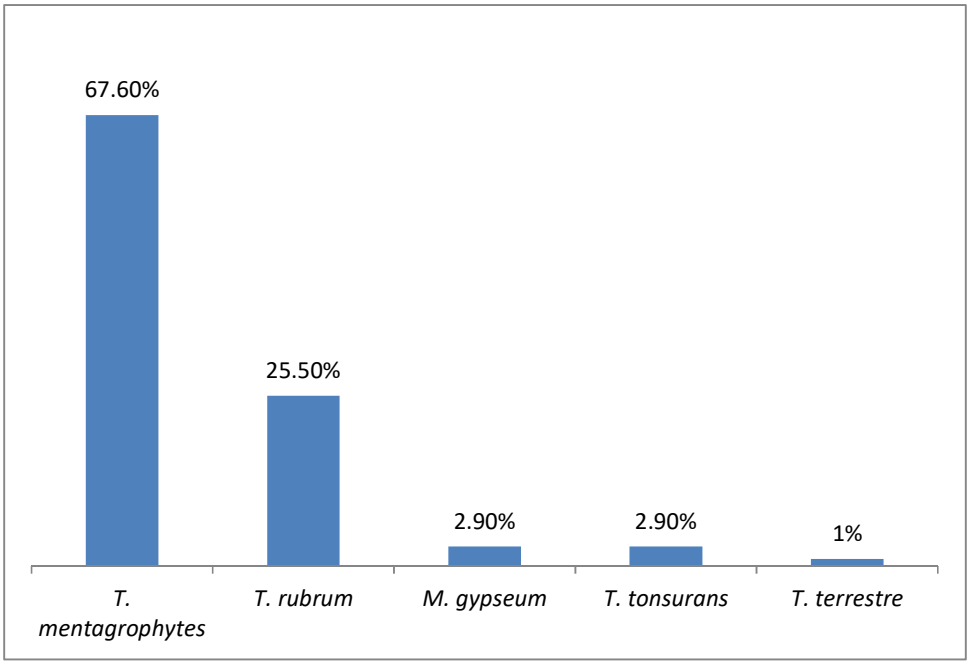


Table 4.4: Distribution of dermatophytes in different clinical condition

	Tinea capitus	**Tinea pedis**	**Tinea unguium**	**Tinea cruris**	**Tinea corporis**
T. mentagrophytes	21	1	18	8	21
T. rubrum	4	0	12	5	5
M. gypseum	1	0	1	0	1
T. tonsurans	0	0	1	0	2
T. terrestre	0	0	1	0	0
Total	26	1	33	13	29

Fig 4.12: Growth of *T. rubrum* Sabouraud Dextrose Agar

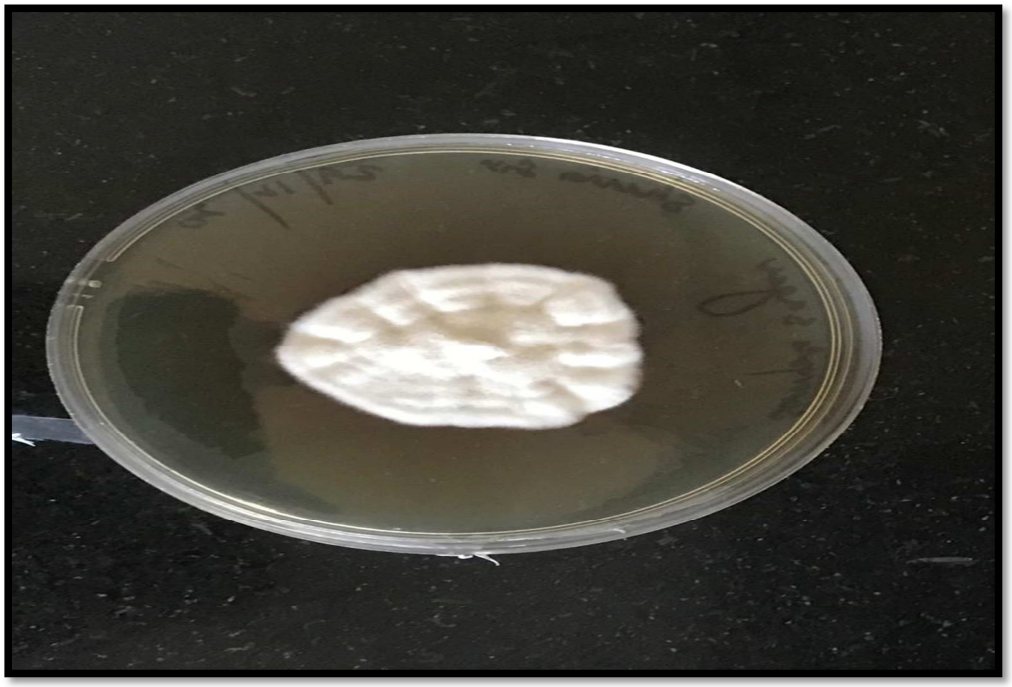


Fig 4.13: Growth of *T. mentagrophytes* on Sabouraud Dextrose Agar

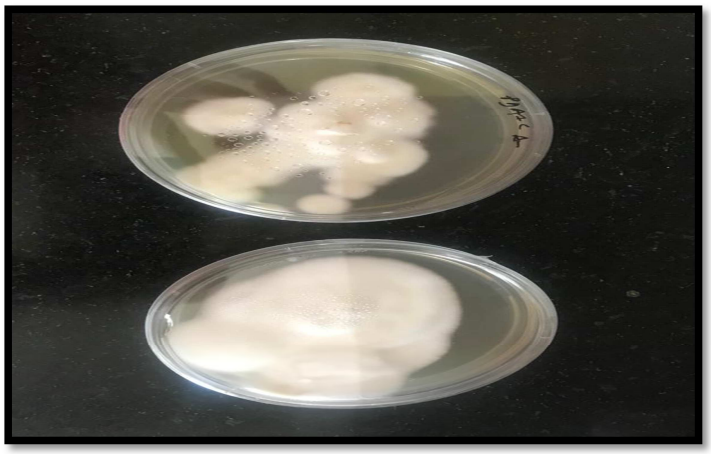


Fig 4.14: Hyphae bearing Microconidia of *T. rubrum*

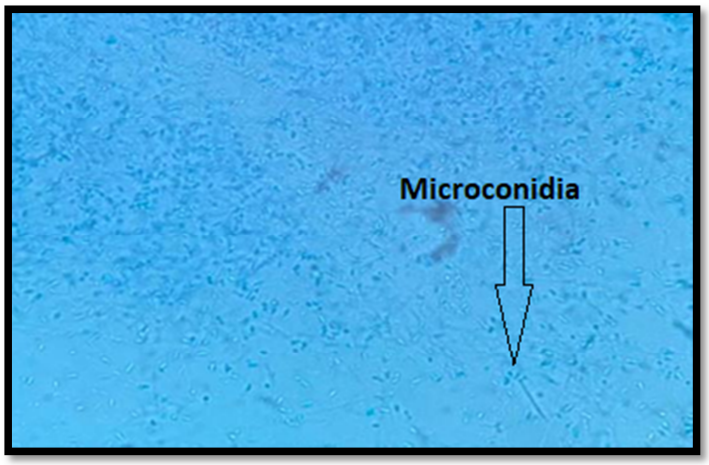
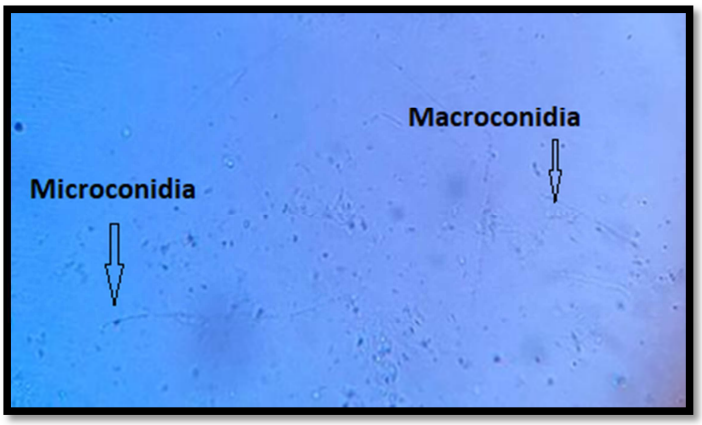
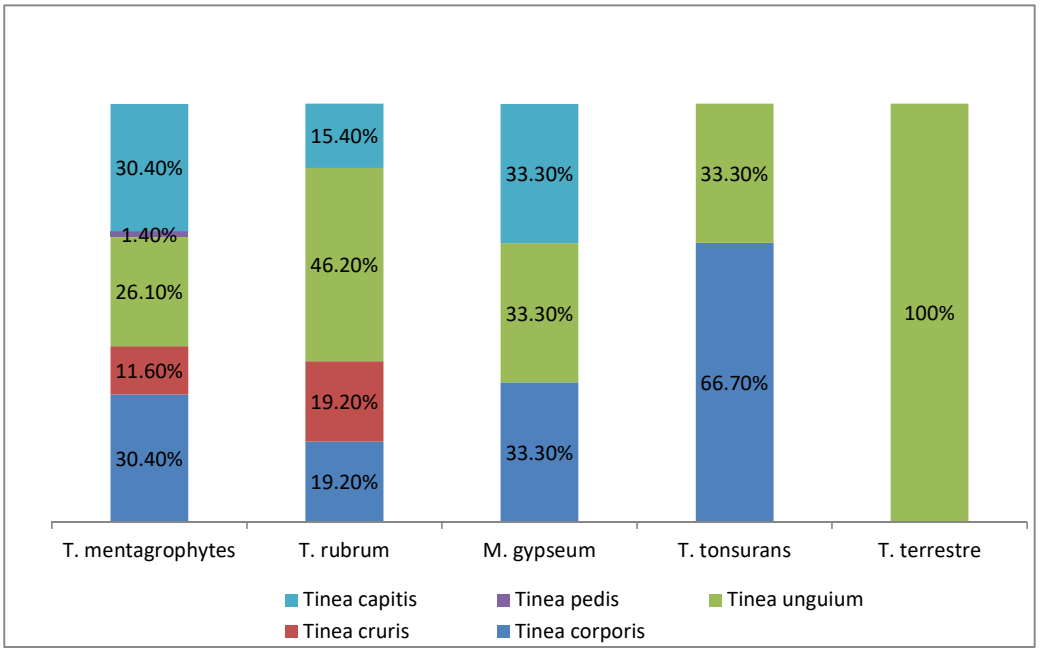


Fig 4.15: Microconidia of *T. mentagrophytes*



The tinea corporis and tinea cruris combination (n=6, 40%) was the most prevalent among patients with multiple site involvement, followed by tinea unguium and tinea corporis (n=5 33%). Spreading and erythematous lesions were observed in 45 (50.0%) and 27 (30.0%) of the patients, respectively. Only 28 (31.11 %) of the patients had pigmented lesions. In 15 (16.66%) of the patients, the infection recurred or relapsed. Only four patients (4%) were able to provide precise corticosteroids dosages while using a combination of antibacterial and antifungal medications (Candid B, Itch-guard and Betnovate). Previous contact with an infected human, animal, or soil was reported in 6 (6.66%), 43 (47.7%), and 56 (62.2%) of the cases, respectively. Lesions lasting 1-2 months were found in 25 (27.77 %) of patients, whereas infection lasted from 2 to 6 months in 23 (25.55 %) of patients, and infection lasted from 6 to 12 months in 25 (27.77 %) of patients. However, 17 individuals (18.88%) admit to having had an infection in the previous 5 years. Only 5 (5.55 %) of the patients had diabetes mellitus.

Fig 4.16: Distribution of dermatophytes in different clinical condition



Candida species was the commonly seen non dermatophytic fungi in 57.1 % of cases followed by *Aspergillus species* 21.4%, *Fusarium species* 17.9% and *Cladosporium halotolarence* 3.6%. *Candida species* (87.7%), *Aspergillus species* (100%) and *Fusarium species* (83.3%) was commonly seen in Tinea unguium, whearas *Cladosporium halotolerence* which is considered as indoor fungus was seen in tinea capitus in twins on repeated sample collection.

Table 4.5: Distribution of Non Dermatophytic fungi in the study population

	Tinea capitus	Tinea unguium	Tinea corporis
Candida species	1	28	3
Fusarium species	0	10	0
Aspergillus species	0	10	2
Cladosporium halotolarance	2	0	0
Total	3	48	5

Fig 4.17: Distribution of Non Dermatophytic fungi in the Study population

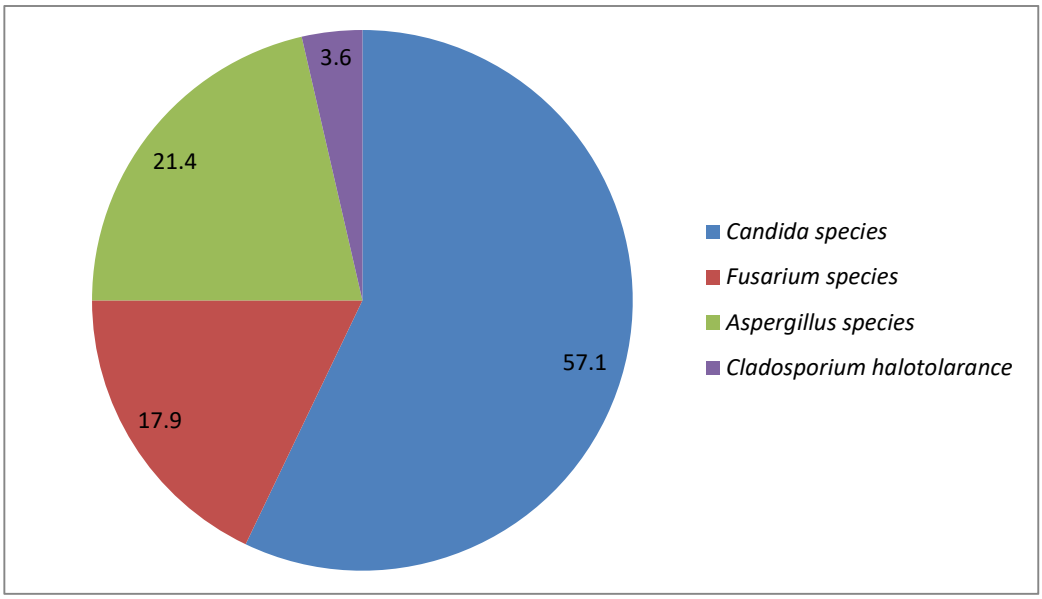
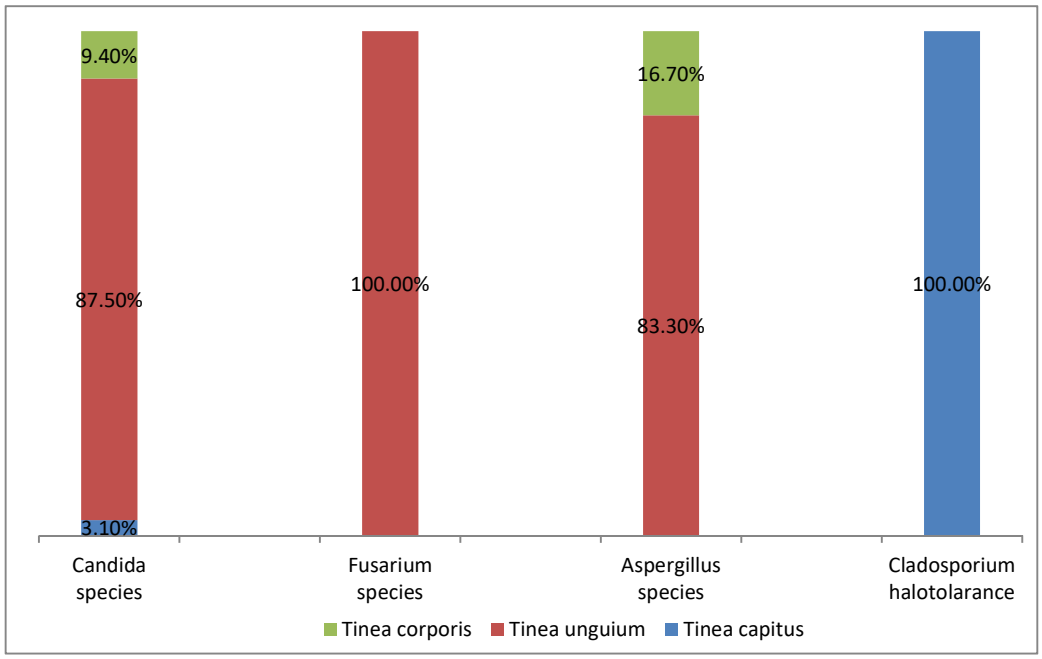


Fig 4.18: Table Distribution of Non Dermatophytic fungi in the Study population



4.5 Molecular characterization of suspected rare fungi:

4.5.1 *Trichophyton rubrum*:

Two dermaphytes isolates from the have shown microscopic and macroscopic appearance of African origin fungi. One isolate was obtained from a 9 years old child with tenia capitis since from one month and with no erythema. He also had a history of dirt and animal exposure. The fungus produced a slow-growing, flat, white colony with a suede-like texture, which eventually became yellow-orange in colour. Conidia were not visible under the microscope.

Another isolate was slowly growing, with colonies waxy glabrous white without conidia after 9 days which was isolated from a 14 year old girl since one month suffering from onychomycosis who had a history of contact with animal and soil.

Both the isolates were amplified using the pan fungal primer ITS 1 and ITS 4 amplification was seen in length 690bp (Fig:4.20) for both suspected rare dermatophytes. Both the isolates were sequenced at ITS region and identified as *T. rubrum* using molecular tool like NCBI BLAST. The sequenced data was submitted in the NCBI Genebank with accession number MT280227 and MT280226. Phylogentic analysis of study isolates was carried out with standard isolates retrieved acquired from the NCBI database [T. CBS 374.92 (NR 144901), *T. violaceum T. soudanense* IHEM 19751 (NR 155948) *T. soudanense* IHEM 19751 (NR 155948) *T. soudanense* IHE *T. rubrum* CBS 392.58 (MH857821) and *T. rubrum* CBS 392.58 (MH857821) *T. mentagrophytes* was taken as out group (KY761968). *T. rubrum* standard strain was found to be grouped with both study isolates (Fig: 4.21). Factorial analysis both the suspected rare isolates was done using the DARwin software 6.0.12 which clearly showed grouping of isolates with *T. rubrum* (Fig: 4.22).

Fig 4.19: Growth of suspected rare dermatophytes on Sabouraud Dextrose Agar

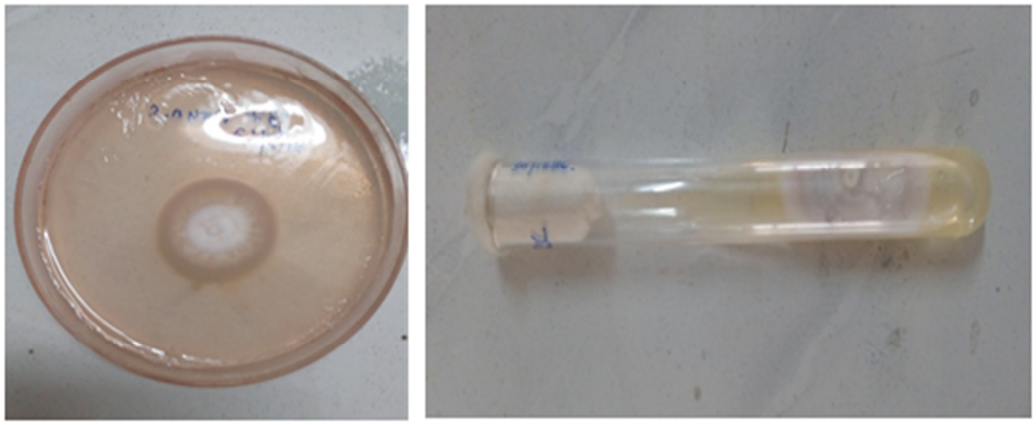


Fig 4.20: Agarose gel electrophoresis of suspected rare dermatophytes Lane 1 & 2: Suspected rare dermatophytes and 3: *T. rubrum*

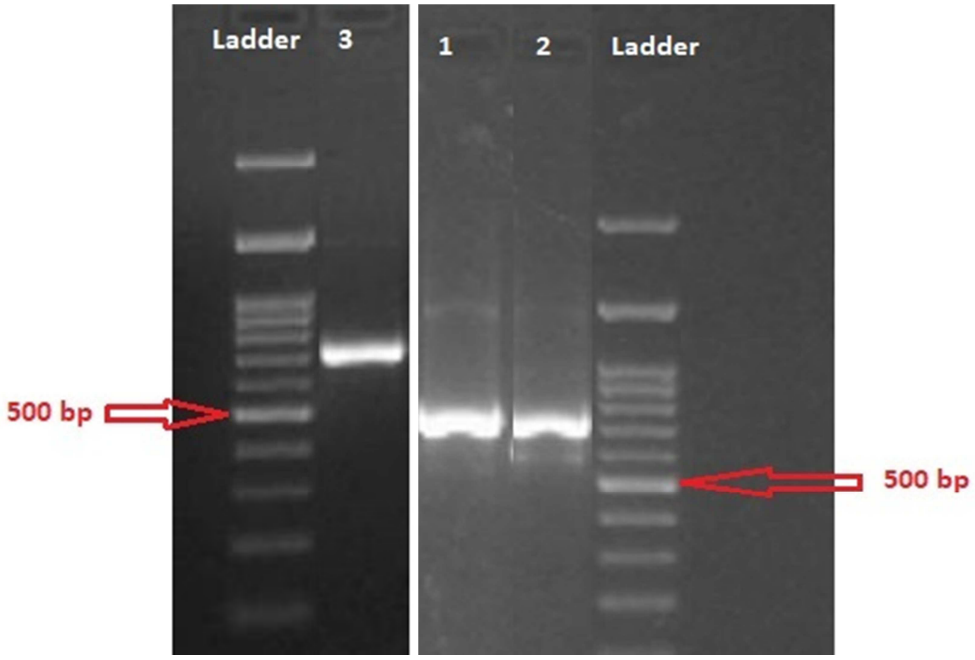


Fig 4.21: Phylogenetic analysis of suspected rare dermatophytes.

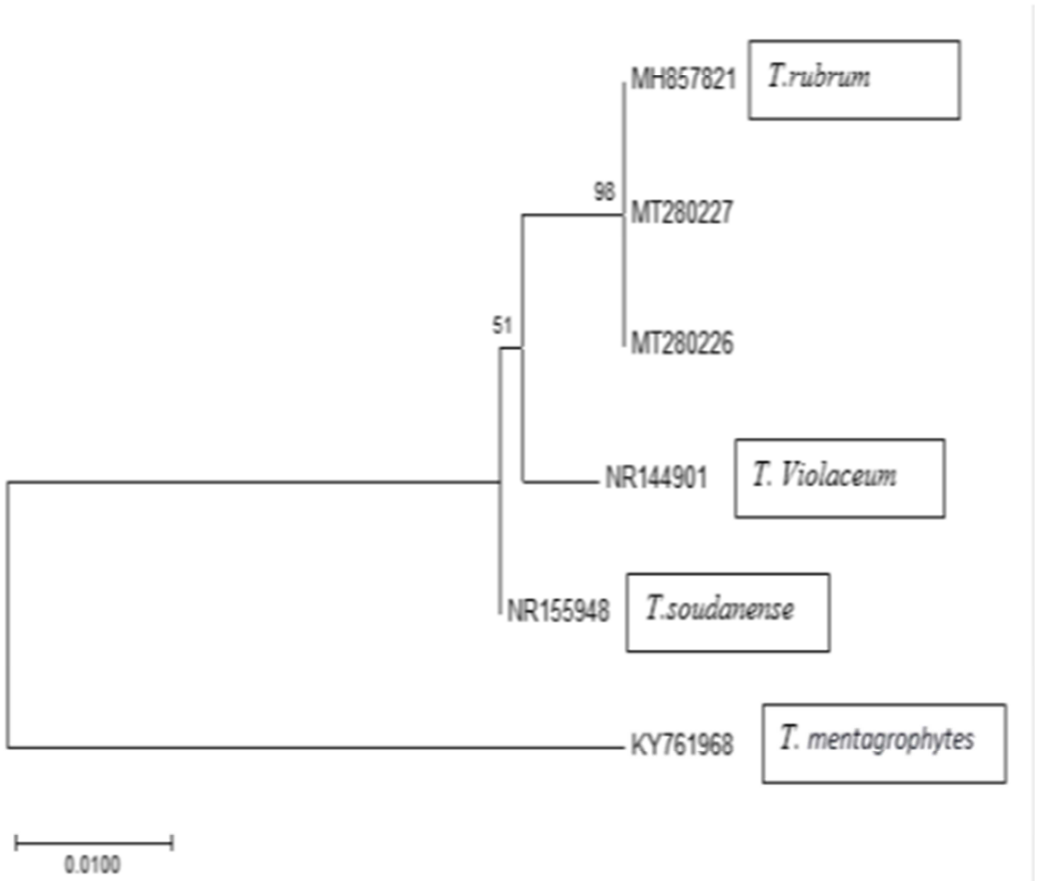
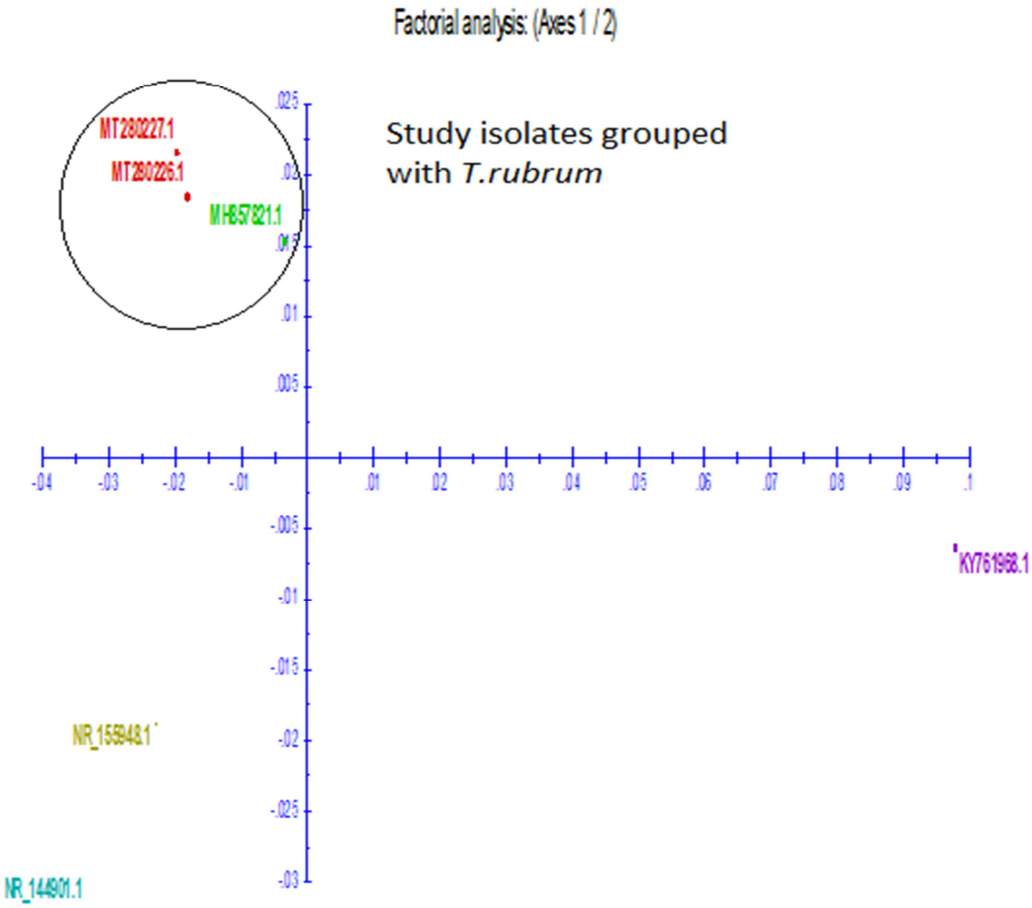


Fig 4.22: Factorial analysis of ITS sequence of suspected rare dermatophytes



4.5.2 *Cladosporium* species

Non Dermatophytic mould *Cladosporium species* was identified in 7 years old twin patients with tinea capitis infection lesion since one year. Greenish colony was in with velvety surface. Conidia were ovoidal and terminal with unbranched cylindrical conidiophores. PCR and Sequencing was performed using ITS1 and ITS4 pan fungal primers and identified as *Cladosporium halotolerans* and submitted to NCBI Gene bank (accession number MT588811 and MT588810). Phylogentic analysis was carried out using the *Cladosporium* reference stains [*Cladosporium halotolerans* (LN834374, LN834375 and DQ780364), *Cl. angustisporum* (LN834356), *Cl. asperulatum* (LN834357), *Cl. allicinum* (LN834353), *Cl. cladosporioides* (LN834358), *Cl. flabelliforme* (LN834361), *Cl. funiculosum* (LN834364), *Cl. herbarum* (LN834378), *Cl. subinflatum* (LN834391), *Cl. tenuissimum* (LN834398)] study isolates were clustered with the *Cl. halotolerans* Reference strain (Fig: 4.26).

Fig 4.23: 7 years old twins with Tinea capitis infection

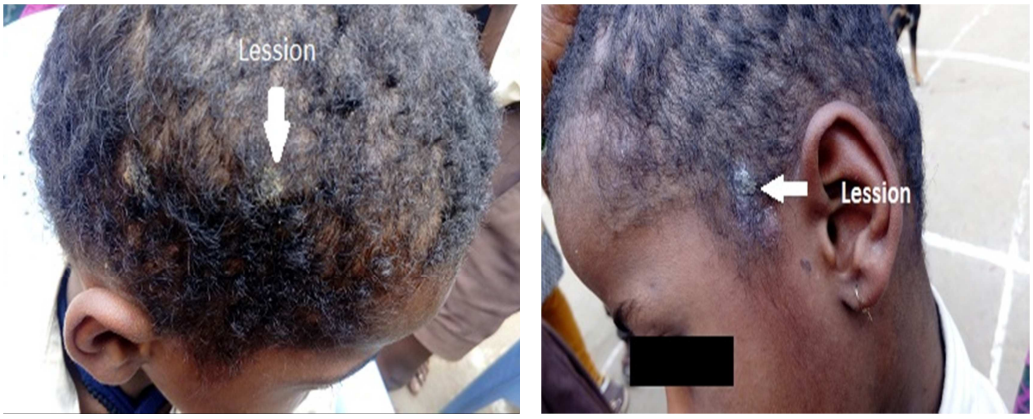


Fig 4.24: Growth of *Cladosporium* species on Sabouraud Dextrose Agar

Fig 4.25: Agarose gel electrophoresis showing the application of *Cladosporium* spp Lane 1&2: *Cladosporium halotolerence*

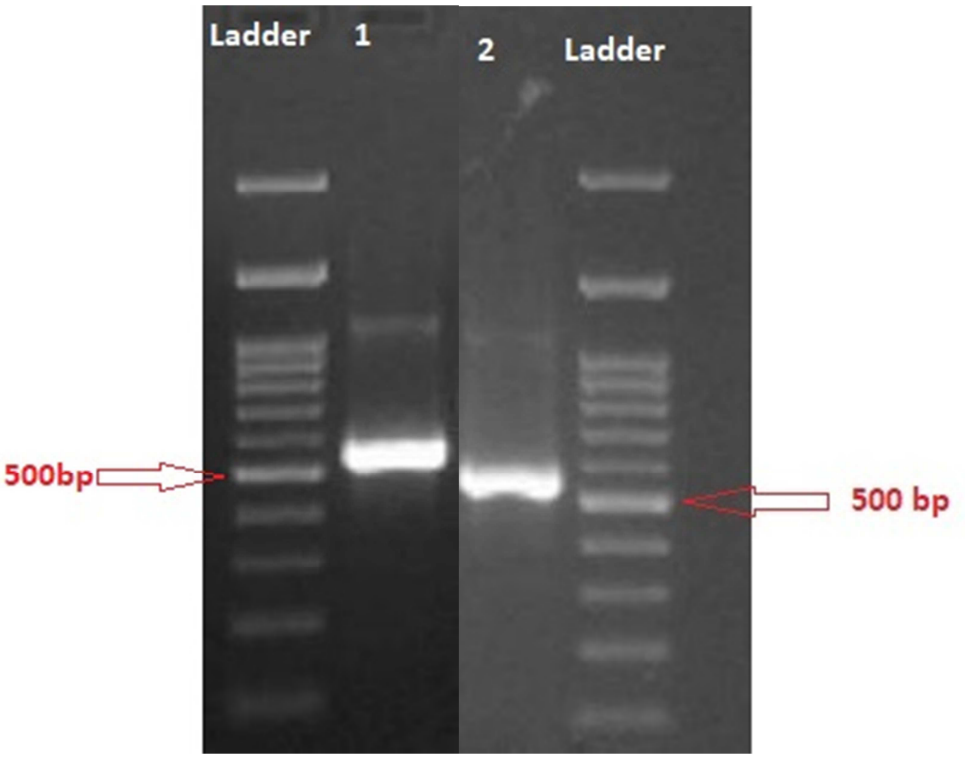
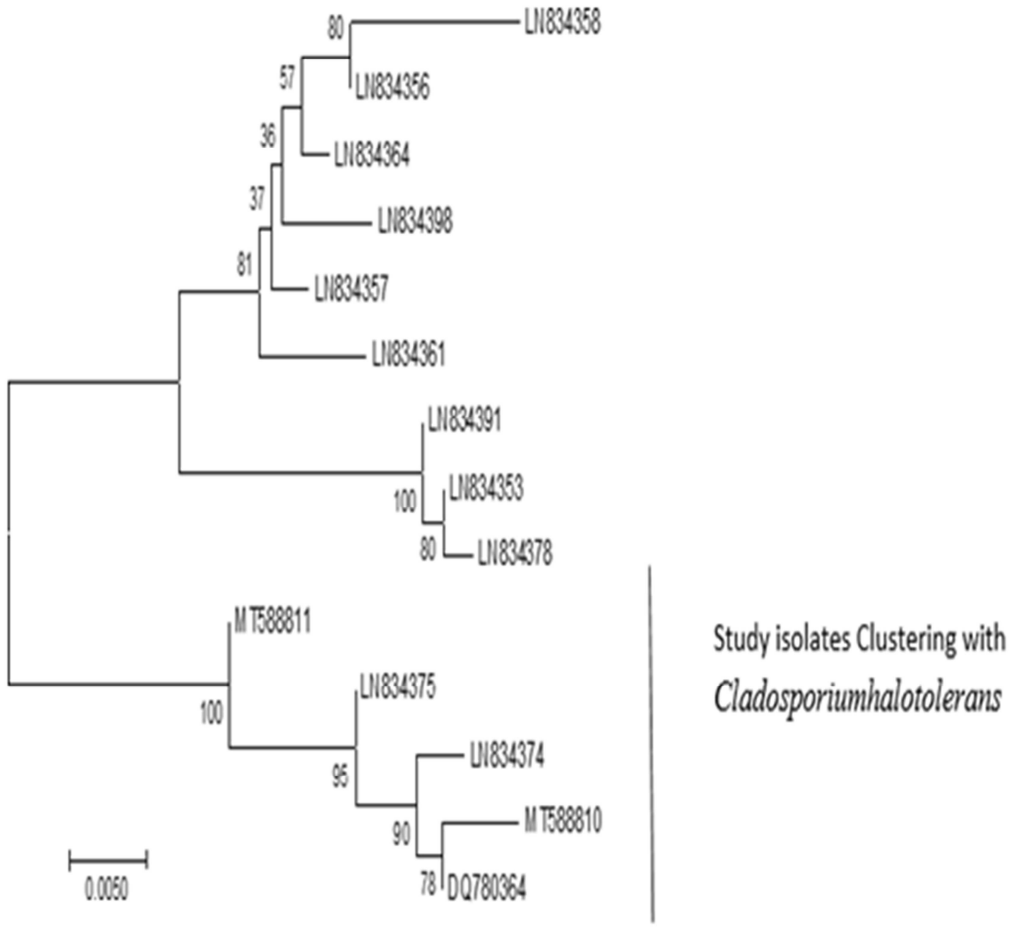


Fig 4.26: Molecular Characterization of *Cladosporium Species*.



4.6 Molecular Typing of *Trichophyton mentagrophytes*

Molecular typing of phenotypically identified 51 representative *T. mentagrophytes* isolates (Ref Table: 4.13) were carried out by sequencing the Internal transcribed spacer (ITS) region. Phylogenetic analysis of study isolates along with different 'ITS Types' of *T. mentagrophytes* [Type I (KC595991), Type II (KP308375 and AJ270790), Type III (KJ606099), Type IV (AF170452), Type V (KJ606098 and KT253558), Type VI (MK722518), Type VII (KT253558), Type VIII (KY761968) and Type IX (MK447613)] was done further phylogenetic analysis have shown all the

study isolates have clustered with the *T. mentagrophytes* type VIII (Fig: 4.28). To confirm the genetic relatedness seen in the genetic dendogram the factorial analysis was carried out, which is a different approach to analyze the species relatedness which clearly grouped the study isolates with *T. mentagrophytes* type VIII and separated all other genotypes (Fig: 4.29).

Fig 4.27: Agarose gel electrophoresis of *T. mentagrophytes* Lane 1-9: "*T. mentagrophytes* type VIII"

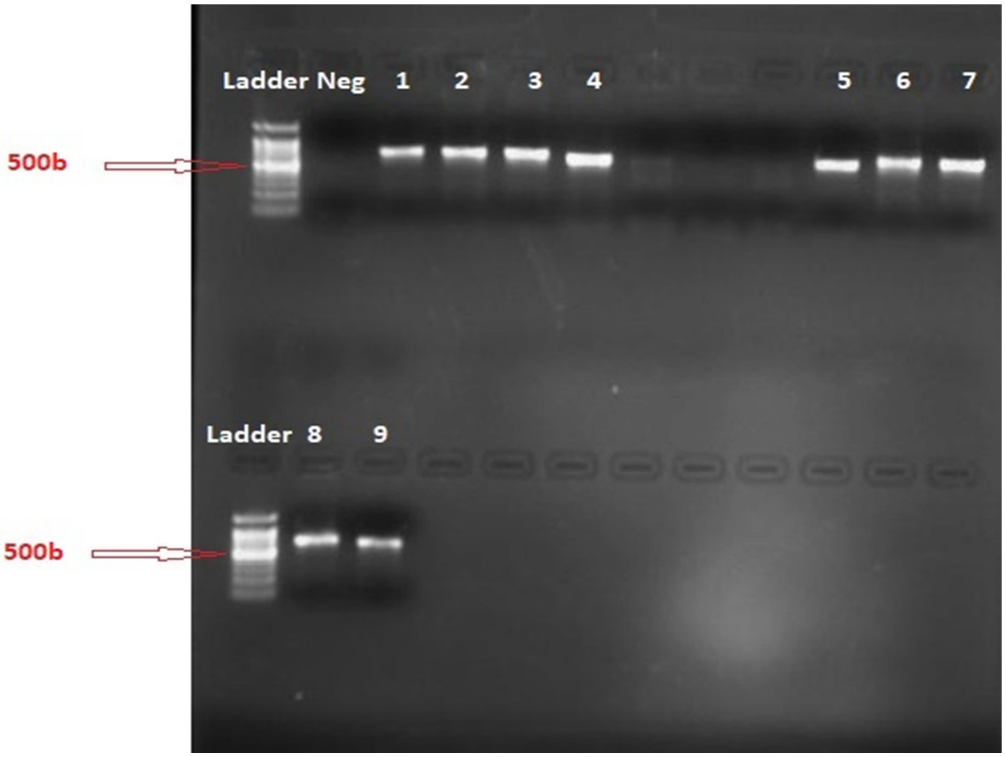


Fig 4.28: Neighbor–joining (NJ) derived dendrogram of internal transcribe spacer (ITS) gene based sequences. All *T. mentagrophytes* isolates have clustered with *T. mentagrophytes* ITS genotype VIII

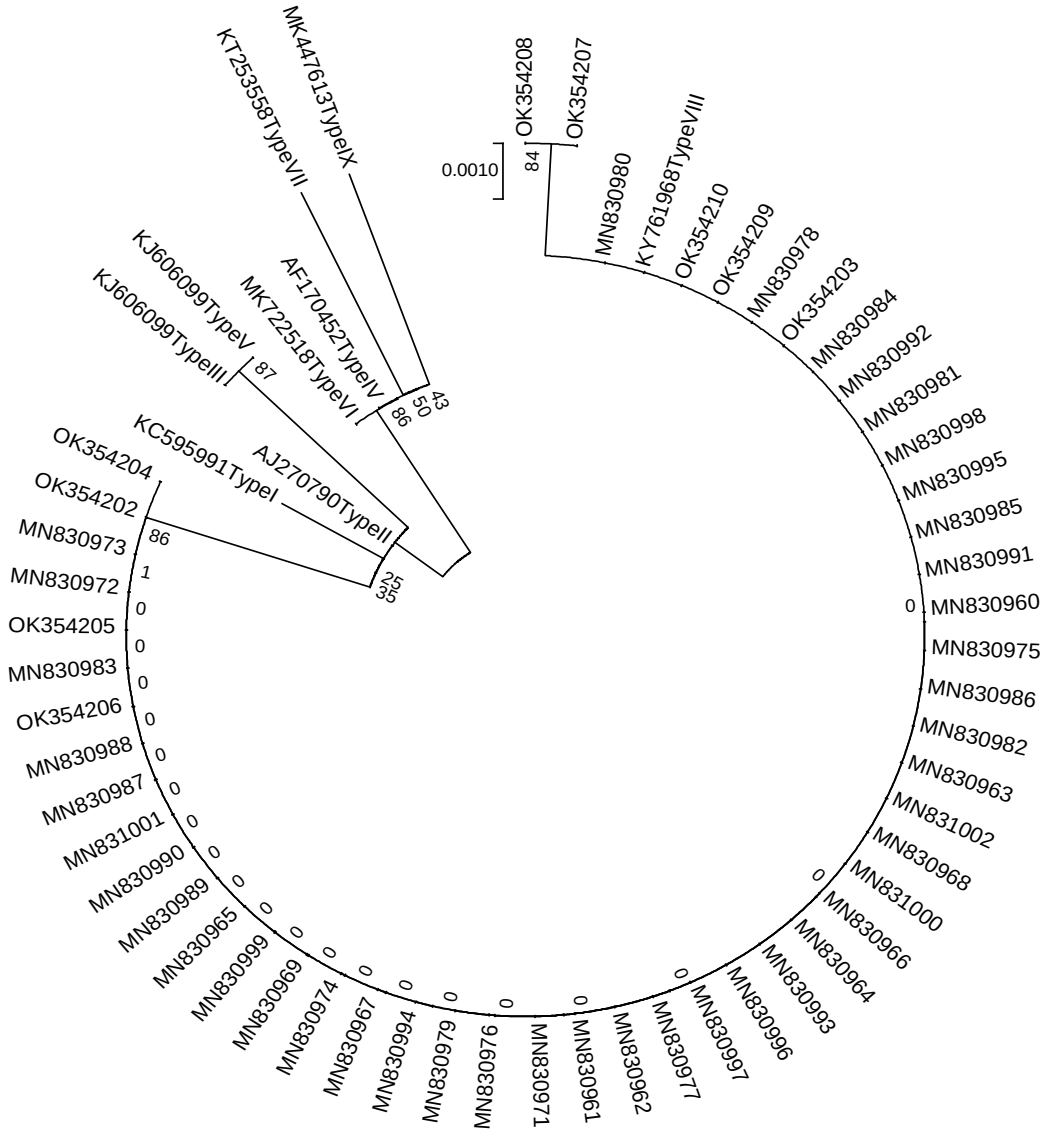
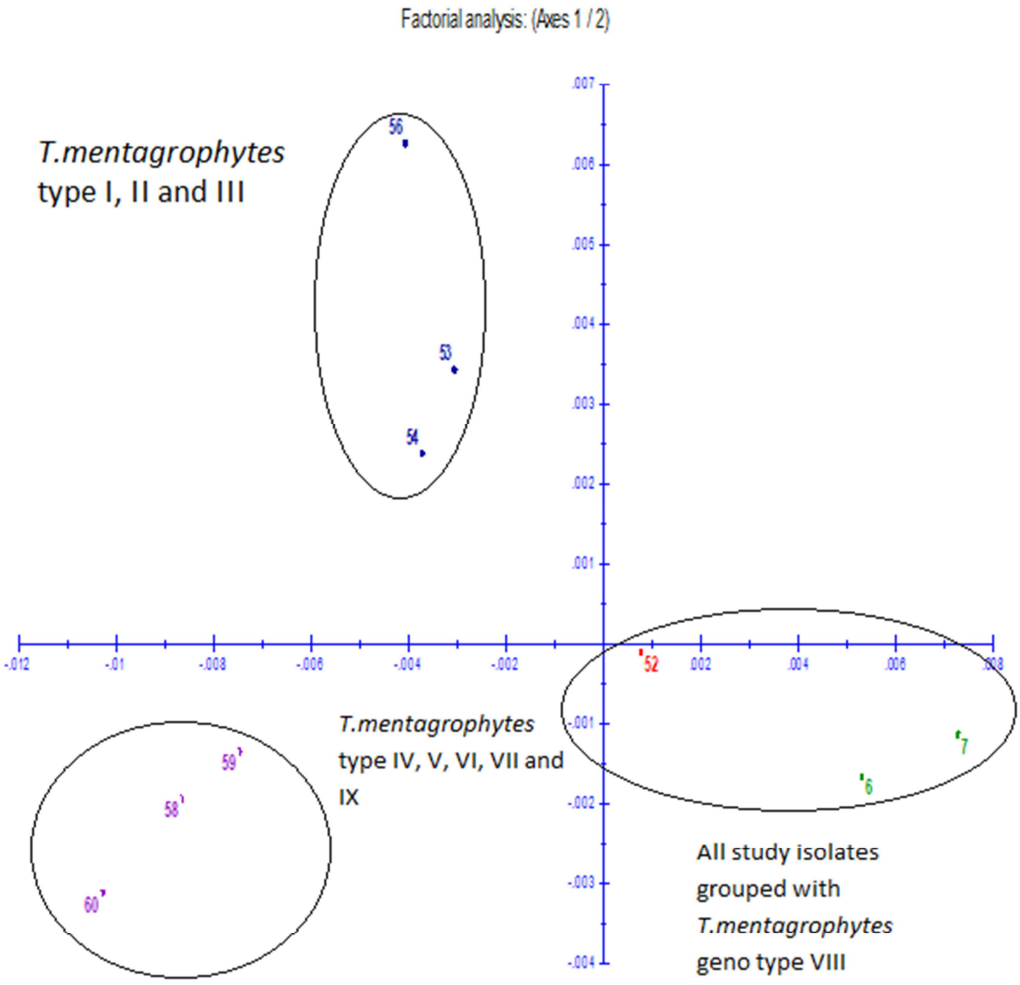


Fig 4.29: Factorial analysis of ITS sequence of 51 *T. mentagrophytes* study isolates with *T. mentagrophytes* genotype I to IX



4.7 Antifungal susceptibility testing (AFST):

AFST of 102 isolates was performed against 13 antifungal agents (terbinafine, sertaconazole, itraconazole, amorolfine, voriconazole, clotrimazole, ketoconazole, naftifine, ciclopiroxolamine, miconazole, griseofulvin, fluconazole and luliconazole). Azole like Voriconazole and itrconazole have shown the potent activity against dermatophytes whereas in allylamine group terbinafine was more potent than naftifine. Floconazole, terbinafine and nafifine have shown the higher MIC_{90} value,

whereas amorolfine, voriconazole, itraconazole, luliconazole, ketoconazole and clotrimazole have shown the low MIC_{90} values. The MIC_{90} of griseofulvine and ciclopiroxolamine were 2 mg/liter while MIC_{90} of miconazole and sertaconazole were 0.5mg/liter.

Table 4.6: MIC of 13 antifungal drugs against Dermatophyte isolates

Antifungal agent	Number of isolates	Range	MIC_{50}	MIC_{90}	GM
Sertaconazol	102	0.0156-8	0.03125	0.5	0.046948
Naftifin	102	0.03125-16	0.0625	8	0.260404
Terbinafine	102	0.0156-8	0.0156	8	0.066281
Miconazole	102	0.03125-16	0.0625	0.5	0.090209
Luliconazole	102	0.03125-16	0.0625	0.125	0.056386
Ciclopiroxolamine	102	0.03125-16	1	2	0.553655
Voriconazole	102	0.00781-4	0.00781	0.03125	0.01223
Itraconazole	102	0.00781-4	0.03125	0.0625	0.026537
Amorolfine	102	0.00781-4	0.00781	0.03125	0.012737
Ketoconazole	102	0.00781-4	0.03125	0.25	0.048932
Clotrimazole	102	0.03125-16	0.0625	0.25	0.091443
Fluconazole	102	0.125-64	0.5	16	1.092362
Griseofulvin	102	0.25-128	0.25	2	0.358391

Table 4.7: MIC of 13 antifungal drugs against Dermatophyte isolates.

Antifungal agent	No. of isolates	No. of isolates with MIC (mg/liter) of													
		0.0078	0.015	0.03	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64
Sertaconazol	**102**	0	46	17	11	10	2	11	5	0	0	0	0	0	0
Naftifin	**102**	0	0	44	11	3	5	3	3	6	9	8	10	0	0
Terbinafine	**102**	0	52	8	3	3	2	9	3	7	3	3	9	0	0
Miconazole	**102**	0	0	47	5	15	21	13	0	1	0	0	0	0	0
Luliconazole	**102**	0	0	32	58	5	6	0	0	0	0	0	0	1	0
Ciclopiroxolamine	**102**	0	0	19	1	0	0	25	20	37	0	0	0	0	0
Viriconazole	**102**	68	8	23	2	0	0	1	0	0	0	0	0	0	0
Itraconazole	**102**	28	20	19	25	4	5	1	0	0	0	0	0	0	0
Amorolfine	**102**	56	20	26	0	0	0	0	0	0	0	0	0	0	0
Ketoconazole	**102**	29	0	25	7	10	29	1	0	1	0	0	0	0	0
Clotrimazole	**102**	0	0	41	11	13	29	7	0	1	0	0	0	0	0
Fluconazole	**102**	0	0	0	0	30	8	17	5	2	3	22	14	0	1
Griseofulvin	**102**	0	0	0	0	0	81	0	10	11	0	0	0	0	0

Azoles like voriconazole have shown high potency against all the isolates [*T. mentagrophytes* (GM. 0.03mg/liter), *T. rubrum* (GM 0.010), *M. gypseum* (GM 0.007mg/liter)], *T. tonsurans* (GM 0.007mg/liter) Single isolate *T. terrestre* has shown the lower MIC against voriconazole and amorolfine (0.0156mg/liter). Allylamine group showed high MIC_{90} against *T. mentagrophytes* [terbinafine (0.11mg/liter and naftifine (0.49mg/liter)], and showed low MIC_{90} against *T.rubrum* [terbinafine (0.021mg/liter and naftifine (0.069mg/liter)], *M. gypseum* [terbinafine (0.0156mg/liter and naftifine (0.039 mg/liter)] and *T. tonsurans* [terbinafine (0.031mg/liter and naftifine (0.049mg/liter)]. Fluconazole shown the higher MIC_{90} in *T. mentagrophytes* (16mg/liter), *T. rubrum*(8mg/liter) and *M. gypseum* (8mg/liter) where as ciclopiroxolamine shown higher MIC_{90} in *T. tonsurans* (1mg/liter).

Table 4.8: MIC of *T. mentagrophytes* isolates (n=69) against 13 antifungal drugs

Antifungal gent	MIC $_{50}$	MIC_{90}	GM
Sertaconazol	0.03125	0.5	0.063096
Naftifin	0.25	16	0.490055
Terbinafine	0.125	>8	0.117899
Miconazole	0.125	0.5	0.116512
Luliconazole	0.0625	0.125	0.057021
Ciclopiroxolamine	1	2	0.669098
Voriconazole	0.00781	0.03125	0.013301
Itraconazole	0.03125	0.125	0.031554
Amorolfine	0.0156	0.03125	0.014411
Ketoconazole	0.0625	0.25	0.055397
Clotrimazole	0.125	0.25	0.101226
Fluconazole	2	16	1.619616
Griseofulvin	0.25	2	0.369903

Table 4.9: MIC of *T. rubrum* isolates against 13 (n=26) antifungal drugs

Antifungal gent	MIC_{50}	MIC_{90}	GM
Sertaconazol	0.0156	0.125	0.024555
Naftifin	0.03125	0.5	0.069533
Terbinafine	0.0156	0.03125	0.021488
Miconazole	0.03125	0.25	0.0547
Luliconazole	0.0625	0.0625	0.05186
Ciclopiroxolamine	0.5	2	0.42609
Voriconazole	0.00781	0.03125	0.010754
Itraconazole	0.0156	0.0625	0.017375
Amorolfine	0.00781	0.0156	0.009927
Ketoconazole	0.03125	0.25	0.031247
Clotrimazole	0.03125	0.25	0.064189
Fluconazole	0.25	8	0.437602
Griseofulvin	0.25	1	0.335196

Table 4.10: MIC of *M. gypseum* isolates (n=3) against 13 antifungal drugs

Antifungal gent	MIC $_{50}$	MIC_{90}	GM
Sertaconazol	0.03125	0.03125	0.02479
Naftifin	0.03125	0.03125	0.039373
Terbinafine	0.0156	0.0156	0.0156
Miconazole	0.03125	0.03125	0.03125
Luliconazole	0.03125	0.0625	0.049606
Ciclopiroxolamine	0.5	1	0.25
Voriconazole	0.00781	0.00781	0.00781
Itraconazole	0.03125	0.03125	0.02479
Amorolfine	0.00781	0.0156	0.009836
Ketoconazole	0.03125	0.25	0.039368
Clotrimazole	0.0625	0.125	0.0625
Fluconazole	0.5	8	1
Griseofulvin	0.25	0.25	0.25

Table 4.11: MIC of *T. tonsurans* isolates (n=3) against 13 antifungal drugs

Antifungal gent	MIC $_{50}$	MIC_{90}	GM
Sertaconazol	0.0156	0.03125	0.019665
Naftifin	0.0625	0.0625	0.049606
Terbinafine	0.03125	0.0625	0.031233
Miconazole	0.03125	0.125	0.049606
Luliconazole	0.0625	0.0625	0.0625
Ciclopiroxolamine	0.03125	1	0.099213
Voriconazole	0.00781	0.00781	0.00781
Itraconazole	0.0156	0.03125	0.015615
Amorolfine	0.00781	0.00781	0.00781
Ketoconazole	0.25	0.25	0.125
Clotrimazole	0.25	0.25	0.25
Fluconazole	0.5	0.5	0.39685
Griseofulvin	0.25	0.25	0.25

Table 4.12: Antifungal Susceptibility of *T. terrestre* isolate (n=1) against 13 antifungal drugs.

Antifunala Agent	MIC
Sertaconazol	0.125
Naftifin	1
Terbinafine	0.25
Miconazole	0.125
Luliconazole	0.25
Ciclopiroxolamine	2
Voriconazole	0.0156
Itraconazole	0.0625
Amorolfine	0.0156
Ketoconazole	0.125
Clotrimazole	0.125
Fluconazole	1
Griseofulvin	2

Out of 102 isolates 25 isolates have shown the higher MIC for terbinafine drug (>1mg/ltr). Three isolates have shown the MIC of 1mg/ltr, 7 isolates have shown MIC of 2mg/ltr, 3 isolates have shown MIC of 4mg/ltr, 3 isolates have shown the MIC of 8mg/ltr and 9 isolates have shown the MIC of >8mg/ltr. All 25 isolates shown resistance to terbinafine were *T. mentagrophytes* complex. Most of the isolates which shown higher MIC for terbinafine have also shown higher MIC for naftifine. Out of 25 isolates with higher tebinafine MICs 24 isolates have shown higher MIC for naftifine.

The squalene epoxidase gene DNA sequences of dermatophyte with higher MIC was compared with the reference *T. mentagrophytes* isolates sequence retrieved from NCBI genebank with terbinafine resistance and known mutation in the squalene epoxidase gene (SE) with C1191A (MH618771 and MH618768) and T1189C (KX906451 and KX906452) transvesion. *T.mentagrophytes* (KX906458 and KX906459) susceptible to terbinafine without any mutations in the SE gene used as control. Among 25 isolates with higher MIC for terbinafine 4 isolates have shown F397L mutation.

Fig 4.30: Mutation in SE gene of terbinafine resistant *T. mentagrophytes*

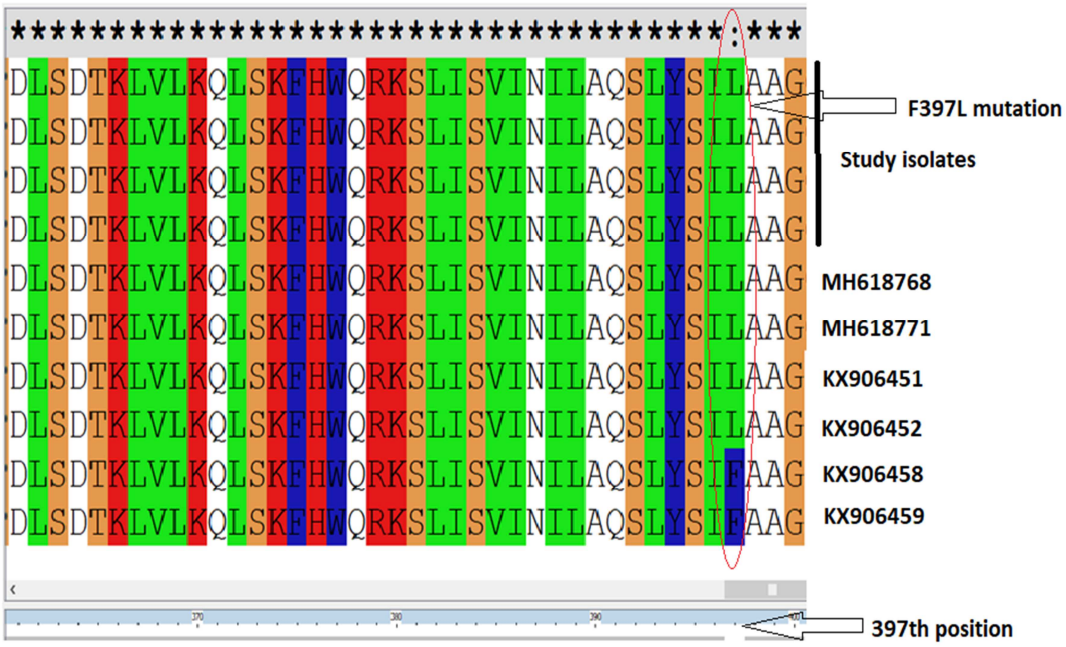


Table 4.13: NCBI Genebank accession number of study isolates

SI No	Organism Name	NCBI Gen Bank Accession No
1.	*T. mentagrophytes*	OK354208
2.	*T. mentagrophytes*	OK354207
3.	*T. mentagrophytes*	MN83098
4.	*T. mentagrophytes*	OK354210
5.	*T. mentagrophytes*	OK354209
6.	*T. mentagrophytes*	MN 30978
7.	*T. mentagrophytes*	OK354203
8.	*T. mentagrophytes*	MN830984
9.	*T. mentagrophytes*	MN830992
10.	*T. mentagrophytes*	OK354204
11.	*T. mentagrophytes*	MN830981
12.	*T. mentagrophytes*	OK354202
13.	*T.mentagrophytes*	MN830998
14.	*T. mentagrophytes*	MN830995
15.	*T. mentagrophytes*	MN830973
16.	*T. mentagrophytes*	MN830 985
17.	*T. mentagrophytes*	MN830972
18.	*T.mentagrophytes*	MN830991
19.	*T. mentagrophytes*	OK354205
20.	*T. mentagrophytes*	MN830960
21.	*T. mentagrophytes*	MN830983

22.	*T. mentagrophytes*	MN830975
23.	*T. mentagrophytes*	OK354206
24.	*T. mentagrophytes*	MN830986
25.	*T. mentagrophytes*	MN830982
26.	*T. mentagrophytes*	MN830988
27.	*T. mentagrophytes*	MN830963
28.	*T. mentagrophytes*	MN830987
29.	*T. mentagrophytes*	MN831002
30.	*T. mentagrophytes*	MN831 001
31.	*T. mentagrophytes*	MN630966
32.	*T.mentagrophytes*	MN830990
33.	*T.mentagrophytes*	MN831000
34.	*T. mentagrophytes*	MN830989
35.	*T.mentagrophytes*	MNB30999
36.	*T.mentagrophytes*	MN830965
37.	*T. mentagrophytes*	MN 830969
38.	*T.mentagrophytes*	MN830966
39.	*T.mentagrophytes*	MN830974
40.	*T. mentagrophytes*	MN830967
41.	*T. mentagrophytes*	MN830994
42.	*T. mentagrophytes*	MN830964
43.	*T. mentagrophytes*	MN830979
44.	*T. mentagrophytes*	MN8 30993
45.	*T. mentagrophytes*	MN830976

46.	*T. mentagrophytes*	MN830971
47.	*T. mentagrophytes*	MN830961
48.	*T. mentagrophytes*	MN830962
49.	*T. mentagrophytes*	MN830996
50.	*T. mentagrophytes*	MN830997
51.	*T. mentagrophytes*	MN830977
52.	*Cladosporium halotolerans*	MT588811
53.	*Cladosporium halotolerans*	MT588810
54.	*T. rubrum*	MT280227
55.	*T. rubrum*	MT280226
56.	*T. mentagrophytes* squalene epoxidase gene	Submission ID 2506064
57.	*T. mentagrophytes* squalene epoxidase gene	Submission ID 2506064
58.	*T. mentagrophytes* squalene epoxidase gene	Submission ID 2506064
59.	*T. mentagrophytes* squalene epoxidase gene	Submission ID 2506064

5.0 DISCUSSION

Dermatophytes are fungi that cause slow-moving infectious diseases in people. This fungus is only found in a certain geographical region.[81,38] Dermatophytosis is more common in persons with a low socioeconomic position and poor personal cleanliness, according to several epidemiological researchers.[132] Dermatophyte distribution varies by area, and is impacted by variables such as climate fluctuation and population socioeconomic level.[133] It occurs more frequently in tropical and subtropical areas, where dermatophytes thrive due to favorable environmental circumstances. The dermatophytosis causative agent spectrum has shifted dramatically during the last 100 years throughout the world.[35]

Dermatophytic infection rates are growing by the day, according to a new study, as it has become one of the most prevalent infectious illnesses with a global distribution. Because there are numerous illnesses with similar clinical signs, it is hard to diagnose ring worm infection only on the basis of clinical manifestations. Dermatophytosis mimics the clinical characteristics of atopic dermatitis, seborrhoeic dermatitis, contact dermatitis, psoriasis, candida intertrigo, erythema and eczema,[134] it is diagnosis of tenia infection would be difficult in immune compromised patientss. Because of the dermatophyte's infectious nature, it's important to get a diagnosis as soon as possible.[135]

This study was conducted to evaluate the clinicomycological pattern if dermatophytosis in Siddi tribal community who are residing in Uttar Kannada, Dharwad and Belagavi district of north Karnataka region. Total of 1004 cases of

superficial fungal infection were included to assess the clinical pattern of dermatophytosis and see the presence of rare or exotic fungi in the tribal community.

5.1 Sex wise distribution

Females (52.4%) were commonly affected compared to male (47.6%) more prevalence of dermatohytosis in female may be due to reduction in personal inhibitions, a reversal of societal limitations, and a greater awareness of their own well-being. In contradictory to the studies done in the other parts of native Indians where male have the highest prevalence compared to female patients. A study from south India shown female were more affected with male and female ratio of and 2.7:1 and 1.6:1 and in north India study conducted have shown the male and female ratio of and 4:1.[136,74] Whereas study conducted by Kaaman and Torssander (1983) have shown 38% of dermatophytosis in male patients.[137] Similar identification was found by other investigators also, In Baroda, Singh and Beena (2003) found 39.4 % of male patients with dermatophytosis.[138] Sandy (2008) reported similar results, with 210 positive diagnoses by direct inspection and 180 positive diagnoses by culture out of 590 samples examined. Increased incidence of dermatophytosis in female might due to most of the females are involved in agricultural activity, daily wage work in the village, poor hygiene and frequent sharing of cloths towel and other households.[139]

5.2 Age wise distribution:

Patients between the ages of 15 and 45 were the most often afflicted. Because this group of patients is socially engaged, the chances of them coming into touch with infected persons are significant. They may also get the disease while working in jobs like farming or physical labor.[11] These young people frequently migrate in and out of

their communities in search of jobs in other parts of the state. As a result, the risk of dermatophyte transmission throughout the population is increased. According to Vyas et al., 2013, 75 % (45/60) of dermatophytic infections occurred in children aged 5 to 10, with 50 % (30/60) of the dermatophytes identified from tinea capitis clinical type.[140] In North India, Malik et al., 2014 and Bhagra et al., 2014 found that dermatophytic infections were prevalent in the 35.1% in 11 to 20 years and28 % in 21 to 30 years age groups, respectively.[141,142] According to Mahale et al., 2014 from South India, Infection with dermatophytes was identified in 23.1% of children aged 1 to 10 years and 23.1% of children aged 41 to 50 years.[143] According to Gupta et. al. 2014, dermatophytes were often infected in those above the age of 60 (32 %).[144]

5.3 Occupation wise distribution and socio-economic category:

The Siddi community is a low-income population that mostly relies on agricultural and daily wage employment. They participate in income-generating activities such as fuel wood collecting or daily wage employment for local landlords. Around 72% of the patients were illiterate, whereas 24 % were students or had finished their elementary school. According to a recent poll, 40% of the Siddi population eats only two meals per day and is at risk of malnutrition.[145] As people live in overcrowded tiny hamlets with inadequate sanitation and regularly share clothing, towels, and other household goods, the risk of dermatophyte transmission can't be ruled out. Among Siddi tribals, farmers (47.1%) had contracted the greatest number of infections followed by students (20%), daily wage labors(14.6%), children less than 5 years of age(2.8%), house wife(1.3%) and others (14.3%). Increased incidence of dermatophytosis was observed in farmers in this tribal community as most of the population in the community dependent on the agriculture and also

inhabitants with domestic animal like buffalo, cow, dog and goat. This might result in the transfer of geophilic and zoophilic dermatophyte species to humans as in this study overall majority organism isolated was *T. mentagrophytes* which is a zoophilic organism. Agriculture was the most common occupation among the patients, according to Hay and Moore (2004).[146]

Increased incidence of dermatophytic infection in students might be due to Certain factors, such as low parental education, are likely to reduce or eliminate the high prevalence of dermatophytes in the student population because it was their responsibility to teach their children the principles of hygienic and healthy living and most of the siddi tribals live in the interior forest region of the western Ghats and most of the students reach school by walking bare foot. According to Romani and Chepchirchir et al. (2009) majority of the school children affected by dermatophytosis under the age of 12 years indicating that ringworm infection is primarily a prepubertal illness, this might be due to poor hygiene and lack of saturated fatty acids, which act as a natural anti-dermatophytic mechanism.[147,148] Aside from this innate propensity, the level of exposure to etiological substances is also crucial.

5.4 Clinical Pattern of Dermatophytosis:

Tinea unguium was the common clinical presentation which is similar to the study conducted by Gosh et.al where they discovered that most prevalent clinical type was tinea unguim in 74.58 % of cases but the most common clinical type was tinea corporis in other regions of India, contrary to the current study. According to Jain et al., 2014, the most frequent clinical form seen was tinea corporis 35.2 %, followed by in 22.4 % of tinea cruris, 11.2 % of tinea capitis, 7.1 % of tinea mannum,6.6 % of tinea pedis, and % of tinea unguium.[149] Whereas tinea corpories is the commonly

Seen clinical condition in India in native Indians.Whereas in America and Africa, the prevalence was 0.07 % and 0.6 %.

We found tinea capitis is the common clinical type after the tinea unguium seen in 29.7%, followed by tinea corporis in 22.2%, tinea cruris in 8.5%, tinea facea in 1.8%, tinea pedis in 1.6% and tinea mannum in 0.7%. The present study show tinea unguium, tinea capitis and tinea corporis is the frequent clinical type of clinical presentation observed in the Siddi tribal community. Increased incidence of tinea capitis was observed in the tribal community whereas tinea capitus was not commonly seen in other part of India however Vyas et al., 2013 found that tinea capitus as the predominant clinical type in his study conducted Jaipur India.[(140)] Tinea capitis is the commonest clinical presentation in Africa countries.[(150)] The present study has shown the tinea capitis infection is commonly seen in the children below the age of 15 year. Simonnet et al. discovered that tinea capitis infection was more frequent in children under the age of puberty than in adults in a 2011 research. In a research done by Hanumanthappa et al in 2012, tinea capitis was shown to be prevalent in children aged 10 to 15 years.[(70)] In Africa, tinea capitis was the most frequent clinical pattern, accounting for 26.9% of all cases.[(151)] The scalp lesions ranged from typical alopecia to postulation in chronic infections, inflammatory to non-inflammatory type, and few patients reported with numerous small plaques of alopecia such as "trichophytic" type (64.2%) and large plaques of alopecia such as "microscopic" type (34.6%), and a few reported with nonspecific lesions (6.2%). (Kechia et al., 2014).[(152)] Sharing hair accessories, carrying things on the head, and overcrowding are the primary predisposing risk factors for scalp infections, according to Ayanlowo et al., 2014.[(153)]

5.5 Direct Microscopy and Culture:

Environmental factors such as pH, temperature, light, incubation time, and culture media all influence dermatophyte development and antigen production. Temperature is a key environmental element that influences microbial growth and metabolite synthesis. Every fungus has a set of cardinal temperatures at which it grows at its fastest. Most fungi thrive at temperatures between 25 and 35 degrees Celsius. Similarly, pH is an essential element that impacts the development of dermatophytes. Every fungus, like temperature, has a pH range in which it thrives. Fungi thrive best when the pH is between 4.2 and 9.3.[154,155,156]

In the Present Study out of 1004 samples 129 (12.8%) samples were positive for Dematophytosis from 937 patients by direct microscopy and Culture of which 27 (20.9%) were positive by only direct microcopy, 5 (0.49%) were positive by only culture and 97 were positive by both culture and direct microscopy. Many epidemiological studies in India have shown high positivity rate of direct microscopy that the culture. Direct microscopy positive rates varied from 38 % to 96 %, with the lowest being 38.2 % in Meghalaya, East India and the highest being 96 % in Mysore, South India.[157,158] As per the epidemiological investigation conducted by Sofia Maraki et al. in 2006 have shown mycological positivity rate of 10.1% for dermaophytosis from 2910 patients.[83] The culture positivity rate in epidemiological studies with high direct microscopy positivity rates varied from 29.3% to 87.43%, with a low of 29.3% and a high of 87.43%.[157,159] It's possible that the low positive rate in culture is attributable to earlier antifungal therapy.[143] As a result, when sampling, patients should be selected based on certain criteria and a sufficient quantity of material should be obtained.[142]

Study conducted by Madhavi et al. in 2011 shown higher rate of culture positivity (58%) compared to direct microscopy (43%).[160] The low positive rate in direct microscopy might be owing to a strong inflammatory reaction that causes the fungal components to become blind or use of antifungal before sample collection.[142] Finally, the value of culture technique plays an important part in infection laboratory diagnosis.

5.6 Etiological Agent:

Out of 1004 sample 102 (10.15%) samples have shown the dermatophytic growth in which *T. mentagrophytes* (67.6%) was the prevalent dermatophyte isolated in the present study followed by *T. rubrum* (25.5%), *T. tonsurans* (2.9%), *M. gypseum* (2.9%) and *T. terrestre* (2.9%). Majority of the patients were infected by zoophilic dermatophytes *T. mentagraphytes* which is accordance with the study conducted by Bhatia and Sharma in 2014 in Himachal Pradesh, *T. mentagrophytes* was the most frequent species (63.5%), while *T. rubrum* (35.1%) was the second most common.[74] On the contrary to our study Mistry et al in 2014 have shown *T. rubrum* (53.9%) was the commonest dermatohyte isolates followed by *T. mentagrophytes* (27.9%).[161] *T. mentagrophytes* was commonly isolated in tinea corporis (30.4%) tinea capitus (30.4%), and tinea ungium (26.1%) whereas *T. rubrum* was commonly seen in tinea unguium (42.2%), tinea corporis (19.2%), tinea cruris (19.2%) and tinea capitis (15.4%) a study conducted by Sajjan and Mangalgi in 2012 have shown that most prevalent species identified from scalp infections were *T.rubrum* (50%) and *T. mentagrophytes*(47.36%).[162] These two dermatophyte species were often isolated from all tinea infections in India, according to several researchers. *T. tonsurans* (22.3 %) was isolated from 206 patients, according to Jha and Murthy (2013), and this

dermatophyte was the second most prevalent species in Mysore, South India.[163] *T. tonsurans* (91.8 %) was commonly isolated species from scalp infections in Northern California, according to Mirmirani and Tucker (2013).[164] Tinea capitis and tinea corporis are common sources of *T. tonsurans*, an anthropophilic dermatophyte. *T. tonsurans* is found in around 1-2% of tinea faciei, mannum, pedis, and unguium, according to several researchers. In the present study *T. tonsurans* is isolated in tinea unguium (33.3%) and tinea corporis (66.7%).

M. gypseum is isolated tinea capitis, tinea unguium and in tinea corporis which is commonly seen in tinea capitis infection.[70,133] Furthermore, *Microsporum* species were isolated less often in India. Species like *M. nanum* and *M. cookie* were among the *Microsporum* species isolated.[140,161] In India, *M. ferrugineum* is a non-endemic anthropophilic pathogen that was identified from 13 cases (or 9.75 %) in Lucknow.[165]

T. soudanense was less frequently isolated from scalp and nail infections in Kolkata, East India.[166,167,152] But it was the most commonly isolated dermatophyte species from scalp infections Africa.[159,152] *T. yaoundei* and *T. soudananse* were isolated from patients with tinea capitis in previous investigations done on the Siddi population.[18,16] We examined the population of this community for superficial infections in the hopes of identifying uncommon dermatophytes among this distinct group that derives from Africa. Single isolate have shown the colony morphology and microscopic feature similar to *T. soudanense* (1, 0.98 %) in our investigation, further it was identified as *T. rubrum* after molecular characterization despite the fact that it is a geographically limited dermatophyte often isolated in African nations. Exotic fungi have also been found in migrating African populations in India and other areas of the

world.[18,16,168] From 2001 to 2006, *T. soudanense* accounted for 1.6 % of cases in Columbus, Ohio, according to a retrospective analysis[84] *T. soudanense* was also isolated from children with tinea capitis who had relocated from African nations by researchers from Belgium, Finland, and Sweden.[169,170,171] *T. soudanense* was isolated in 0.38% of all patients from eastern and western Africa in Baltimore, Maryland.[92] *T. verrucosum* was the most common dermatophyte species isolated from onychomycosis and scalp infections with only a few workers reporting that it was isolated from skin diseases less often.[159,143]

T. concentricum (tinea corporis and cruris), *T. megninii* (tinea corporis and cruris), *M. fulvum* (tinea corporis, capitis, and barbae), *T.terrestre* (tinea corporis, mannum, and capitis), *T. equinum, T. simii* (tinea corporis and pedis), and *M. pracex* and *M. langeronii* were seldom isolated from skin and scalp infections, respectively.[159,149] according to Simonnet et al., 2011, who also reported that the latter was isolated from a patient who returned from Sub-Saharan Africa.[172]

The tinea corporis and tinea cruris combination (n=6, 40%) was the most prevalent among patients with multiple site involvement, followed by tinea unguium and tinea corporis (n=5 33%). Spreading and erythematous lesions were observed in 45 (50.0%) and 27 (30.0%) of the patients, respectively. Only 28 (31.11 %) of the patients had pigmented lesions. In 15 (16.66%) of the patients, the infection recurred or relapsed. Only four patients (4%) were able to provide precise corticosteroids dosages while using a combination of antibacterial and antifungal medications (Candid B, Itch-guard and Betnovate). However, rather than a typical skin lesion, the majority of the patients developed a wide spreading lesion with little erythema, comparable to lesions treated with corticosteroids.[68] Despite the fact that only 16.6 %

of patients had a history of relapse or recurrence, and only 4% had a complete history of corticosteroid usage, 31.1% of patients had corticosteroid modified tinea. As many as 4% of patients said they bought topical creams from a local medical store without consulting an authorized clinical practitioner. As previously reported, these topical steroid users had adverse reactions including as hypopigmentation and striae.[68,74,173] Previous contact with an infected human, animal, or soil was reported in 6 (6.66%), 43 (47.7%), and 56 (62.2%) of the cases, respectively. Lesions lasting 1-2 months were found in 25 (27.77 %) of patients, whereas infection lasted from 2 to 6 months in 23 (25.55 %) of patients, and infection lasted from 6 to 12 months in 25 (27.77 %) of patients. However, 17 individuals (18.88%) admit to having had an infection in the previous 5 years. Only 5 (5.55 %) of the patients had diabetes mellitus.

The spread of dermatophytosis and its epidemiology has been comparable to other parts of India due to changing environment and migration of the community people in and out of the allocated area for Siddi community.

The prevalence of 5.97 % was observed for non-dermatophytes, such as yeast and moulds in this research. It appears that tinea unguium was the most frequent clinical form, seen in 85.71 % of all cases. The risk factor for increased tinea unguium prevalence in superficial mycosis by non-dermatophytes could be due to frequent contact with soil during daily life, age, a history of similar infection, or frequent sharing of footwear. Tinea corporis and tinea capitis were found in 8.92% and 5.35% of the patients, respectively. In 2015, Kaur et al. discovered that nail infection is the most prevalent location for superficial infection, while Lakshmanan et al. reported in 2015 that the most prevalent location of superficial infection is the skin, followed by the nail and hair. Outdoor laborers, such as farmers (57.14 %) and daily wage

employees (16.07 %), had the greatest infection rate, which was consistent with research conducted in other regions of India.[11,174,175,68] *Candida* species (20.25%) was the most common pathogen, followed by *Aspergillus* species (12.75%), *Fusarium* species (10.62%), and *Cladosporium halotolerans* (2, 1.26 %). In 2020, Hazarika et al. found that NDM were more common than yeast in causing superficial infection.[176] In 2015, Kaur et al. discovered that NDM was the most prevalent cause of superficial mycosis, followed by dermatophytes and yeasts.[1] According to Weinberg et al. in 2003 non-dermatophytes made up roughly 10% of the causative agents of onychomycosis,[177] however Batawi et al. 2007 found that 68.75 % of the causative agents of onychomycosis were non-dermatophytes and 0.1 % was dermatophytes.[178] Candida and *Aspergillus* species were commonly isolated from infections of the trunk, groin, scalp, and nails. Several non-dermatophytes have been isolated from onychomycosis, including *Fusarium* species, *Penicillium* species, *Scopulariopsis* species, *Helminthosporium* species (11 cases), and *Trichosporon* species (1 case), according to a few researchers *Curvularia* species (2.5%) and Paecilomyces species (0.6 %) were isolated from trunk, groin, and nail infections, respectively infections of the scalp.[140-143,149]

5.7 Molecular Characterization of Dermatophytes:

5.7.1 Molecular typing of *Trichophyton mentagrophytes/interdigitale* complex:

There is an increase in the incidence of superficial dermatophytosis in India since from last 4 to 5 years of time. Studies from different region of India have shown that an increasing trend in the prevalence of dermatophytosis.[179] There is a notable striking change in clinical features, response to the treatment and recurrent rate of the dermatophytosis many studies have also shown the prevalence of *T. mentagrophytes* as the common etiological agent for the dermatophytosis whereas *T. interdigitale* has been recognized as the infection causing fungi of the chronic, relapsing dermatophytosis epidemic in India. Many studies have confirmed the organism by the convention method and have not used the molecular method like polymerase chain reaction (PCR) to provide evidence.[68,180] To substantiate the problem Pietro Nenoff et. al. have characterize the causative agent of superficial dermatophytosis by sequencing ITS region and (TEF)-1α gene, *T. metagrophytes* (92.62%) was the predominant causative agent to cause dermatophytosis followed by *T. rubrum* (7.38%) and shown the remarkable shift from *T. rubrum* to *T. mentagrophytes.* further sequencing data have shown that all 138 isolates of *T. mentagrophytes* belongs to "ITS genotype VIII" they proposed to call as "Indian genotype".

In the present study *T. mentagrophytes* was the predominant dermatophyte to identify in the tribal community.[181] 51 isolates out of 68 isolates were sequenced in the ITS region. The phylogenetic tree was drawn. All isolates were clustered with ITS genotype VIII as per the classification of Heidemann et al.[171] Due to recent taxonomic instability among dermatophytes, it is difficult to differentiate *T. mentagrophytes* from *T. interdigitale* using phenotypic methods.[182,183]

However, only a few studies have proven that the ITS region of ribosomal DNA can distinguish between genotypes of the *T. mentagrophyte/T. interdigitale* species complex.[181,125] These various genotypes were also linked to clinical symptoms, geographic location, and antifungal resistance[182,183]. Because the population was preserved, we thought the *T. mentagrophytes* identified from the Siddi community may be unique. Representative isolates phenotypically classified as *T. metagrophytes* were submitted to phylogenetic analysis based on the ITS region in this study. As previously stated, all of the sample isolates utilized in this investigation belonged to the ITS genotype VIII, which is the most prevalent infectious agent of dermatophytosis in India.[68,125] The prevalence of *T. mentagrophytes* ITS genotype VIII in the Siddi community might be related to local social and religious adaptations, as well as the fact that they are in close touch with other civilized parts of the state.[184]

5.7.2 Molecular characterization of *Trichophyton rubrum*:

The Siddi are an Indian tribal group having African origins who have resided in India for over five generations.[18,17] The existence of rare African dermatophytes among the Siddi tribal people in India was identified by researchers. In 1980, *T. soudanense*, and in 1993, *T. yaoundei* were identified in the community.[18,16] Only a little amount of research from across the world has indicated the prevalence of this illness in native and migrated African population.[185,186,168] To check for the existence of such uncommon African dermatophytes, we tested over 2000 members of the Siddi community in India's north Karnataka area. 102 Dermatophytes were grown on culture media out of the 1004 samples, with 9.6% prevalence. In the Siddi tribal community tinea unguium was the major clinical condition seen in 32%, tinea

corporis was seen in 27.45%, tinea capitis in 25.49%, tinea cruris in 13.72%, tinea pedis in 13.72% and tinea faceae in 0.98 %. In 67.64 % of cases, Major etiological agent causing dermatophytosis in the tribal community was *T. mentagrophytes*, followed by *T. rubrum* in 25.49 %, Although two dermatophytes with phenotypic features of African dermatophytes such as *T. violaceum* and *T. soudanense* were discovered in 2 separate individuals suffering with tinea capitis and tinea unguium respectively, further by sequencing ITS region both isolates have been identified as *T. rubrum*. Both isolates grew slowly and did not produce macro or micro conidia. *T. violaceum* has been found in populations of native local provenance in India and other areas of the world, according to studies. Despite the fact that it is classified as an African dermatophyte, many researchers have shown the presence of *T. soudanense* in many region of the world in migrated African, according to multiple studies, and native population is rarely affected[185,186,168]. S. S. Magill et al. in Baltimore Maryland reported *T. violaceum* and *T. soudanense* infection in 88% of migrated population of eastern Africa and West Africa who is staying America for 3 months to 5 years, and US native population have shown the 12 % of infection. *T. violaceum*, and *T. soudanense* was observed in 82.7 % of Africans immigrated to the United States, 4.93 % in African Americans and 3.7 % in native white population(92), whereas Elizabeth Gaviria Morales et. al observed prevalence of 77.27 % of *T. violaceum* infection in migrated patients from Africa and 15.90 % of infection in the native population.[185,186] In the year 2000 Graser et al. studied the anthropophilic dermatophytes taxa and noticed short molecular distance, and the majority of them were renamed *T. rubrum* or *T. violaceum*. *T. violaceum* was first discovered by Sabouraud in 1902 which is major infection causing fungi of tinea capitis which is slow growing and rarely sporulating organism. Later *T. rubrum* was discovered which

usually causes tinea corporis and tinea pedis. Later on majority of the species were added to *T. rubrum* complex to explain the phenotypic variation, further many experimental methodologies were used to reevaluate the characters but the difference between the clinical site and the associated morphological difference of *T. rubrum* and *T. violaceum* remain unresolved.[187]

We discovered no such uncommon dermatophyte of African species in the current investigation. It's possible that the lack of uncommon exotic African dermatophytes in the Siddi community, a migratory African group, is related to their residence in India for more than five generations,[26] Other researchers, on the other hand, have observed infection with African dermatophytes in African-origin people who have lived in the area for one or two generations.[185,186,168] According to a observation published in 2018, there has been a six-times increase in geographically restricted dermatophytes commonly seen in African countries among African ancestry children living in Canada for one or two generations.[168] A thorough investigation of population of African ancestry in other regions of India might show the occurrence of regionally limited dermatophytes.

De Hoog et al. have done molecular phylogeny and shown *T. violaceum* is almost impossible to differentiate from *T. rubrum* based on multilocus sequencing results, which includes sequencing of regions like ITS, rDNA, LSU tubulin, and 60S L10 rDNA. Study conducted by Huilin Su et al. showed MALDI TOF can differentiate the *T. violaceum* from *T. rubrum* and *T. soudanense* but it has insufficient discriminatory power to differentiate *T. rubrum* and *T. soudanense*. Further other studies have also shown that adding the *T. soudanense* in the MALDI TOF database can mislead the identification of *T. rubrum*. Even though *T. violaceum*,

T. rubrum and *T. soudanense* shows the logical differences in clinical symptoms, physiology, colony appearance and molecular investigations, none of them are strictly used parameters for diagnosis. Further genetic entities of *T. violaceum*, *T. rubrum* and *T. soudanense* are similar this might be due to short time evolution and in sympatric evolution, these are distinct entities with imperfect lineage sorting. This type of changes has been observed in recently evolving dermatophytes. In dermatophytes, the ITS region of rDNA is utilized to identify taxa as well as to study their evolutionary relationships.[188] Our findings confirm a suspected phenotypically similar dermatophyte of African species as *T. rubrum* by sequencing at ITS region. Many additional studies have discovered these African anthropophilic dermatophyte species in migratory African populations all over the world.[185,168,189] Because the Siddi tribal group has resided in India for five generations and has adapted to local religious customs, our investigation revealed no such unique exotic dermatophyte.

5.7.3 Molecular characterization of *Cladosporium halotolerence*:

Superficial mycosis due to non dermatophytes was seen with the prevalence of 5.97% in the present research. Tinea unguim was the common clinical condition seen in 85.71% of cases, frequent contact with soil and sharing footware regularly could be the possible reason. Other researchers have also shown the similar observation,[1] whereas in contrast to our study Lakshmanan et al. found skin to be the most common site of superficial infection in 2015, followed by nail and hair.[190] Outdoor laborers, such as farmers (57.14 %) and daily wage employees (16.07 %), had the greatest infection rate, which was consistent with research conducted in other regions of India.[191,192,193] Candida species (20.25%) was the most common pathogen, followed by *Aspergillus* species (12.75%), *Fusarium* species (10.62%), and

Cladosporium halotolerans (10.32%). (2, 1.26 %). In 2020, Hazarika et al. found that NDM were more common than yeast in causing superficial infection.[176] In 2015, Kaur et al. discovered that NDM was the most prevalent cause of superficial mycosis, followed by dermatophytes and yeasts.[1] *Cladosporium halotolerans* was isolated from twins with tinea capitis in order to confirm the infection with *Cladosporium halotolerans*. Repeat samples were taken from both patients who showed *Cladosporium halotolerans* growth. *Cladosporium* is commonly thought of as an indoor fungus that is separated from environmental sources and geographical location, yet many *Cladosporium* spp are significant infective agent that infects plants, animals, and humans.[194,195] *Cladosporium* species isolated in most cases lack molecular confirmation; however, *Cladosporium* species isolated in this investigation were sequenced utilizing the ITS region and identified as *Cladosporiumn halotolerans*. The isolation of fungi such as *Cladosporium halotolerants* in the Siddi population may be due to their low socioeconomic condition and reduced cleanliness, as the bulk of the Siddi tribal group lives in poverty.

5.8 Antifungal susceptibility Testing:

Dermatophytoses are a major financial burden on medical establishment all over the world, with a global prevalence of 20-25 %.[87] As a result, the persistent dermatophytosis that has emerged in India has become a worrying medico-economic problem. Aside from dermatophytosis worldwide relevance as the most prevalent superficial infection, in vitro susceptibility testing is critical for determining developing resistance patterns among causative dermatophytes. Furthermore, because many antifungal medications have liver and cardiac adverse effects, it is critical to evaluate novel antifungal drugs. It's great to see new antifungal medicines with

excellent efficacy against dermatophytes being developed. All 102 dermatophytes were subjected to antifungal susceptibility testing against 13 antifungal drugs and resistance mechanism to the terbinafine drug. Geometric mean of voriconazole, itraconazole and amorolfine has shown low geometric mean of 0.013 mg/ltr, 0.031 mg/ltr and 0.014 mg/ltr respectively for *T. mentagrophytes*. Whereas amorolfine have shown the lowest geometric mean of 0.009 mg/ltr for *T. rubrum* followed by voriconazole and itraconazole of 0.010 mg/ltr and 0.017 mg/lit. In case of *M. gypseum* and *T. tonsurans* voriconazole have shown the low geometric mean value (0.00781 mg/ltr). voriconazole have shown the good activity against all dermatopphytes which in agreement the other studies conducted globally, whereas in a stud a study conducted by Salehi et al. have compared the sensitivity pattern of triazole, uliconazole and lanocollnzole with the standard drugs like azoles and allelamines and showed luliconazole and lanoconazole have shown the potent activity against ketoconazole, terbinafine and griseofulvin which are considered as the first line of drug in the present days. In the present study luliconazole and griseofulvin have shown the higher MIC. luliconazole have shown the 0.0625mg/ltr and 0.125mg/ltr of MIC 50 and MIC 90 respectively whereas griseofulvin have shown the 0.25mg/lit and 2mg/lit of MIC50 and MIC 90 respectively. Luliconazole have shown low MIC 90 of 0.0625mg/ltr against *T. rubrum* when compared to *T. mentagrophytes* which is 0.125mg/ltr. terbinafine, itraconazole and griseofulvin have shown lower MIC 90 against *T. rubrum* compared to *T. mentagrophytes*, in contrast to our study lower MIC 90 was observed by Salehi et al. in *T. mentagrophytes* isolates to terbinafine, itraconazole and griseofulvine when compared to *T.rubrum*. Fluconazole have shown the low potency against all the isolates in the present study with the geometric mean of 1.09mg/ltr. Overall MIC 50 and MIC 90 of fluconazole against study isolates were

0.5mg/ltr and 16mg/ltr respectively. 19% Resistance of dermatophytes against azole group of drug has been reported in the previous studies[121]. In agreement to our study many have reported resistance against fluconazole antifungal drug. Hryncewicz-GwodzA, have reported resistance of fluconazole in the *T. rubrum* isolate.[122]

Allelamines like terbinafine and naftifine have shown reduced susceptibility against *T. metagrophytes* which is the commonly isolated dermatophytes in the present study. 25 isolates of *T. mentagrophytes* have shown the higher MIC against terbinafine which is considered as one of the effective antifungal drug against dermatophytes. Even though the Siddi tribal population stays in the rural area of North Karnataka region many the community members visit other cities of India and most of them are adapted to the local and social lifestyle of India could be the possible reason to acquire the resistance for terbinafine drug. Other researchers in India have also reported the increase resistance against terbinafine drug. Shivaprakash M. Rudramurthy et al. reported higher MIC in 20 *Trichphyton* species in North India out of 133 isolates. Ashutosh Singh et al. reported 29.85 % terbinafine resistance in *Trichophyton* species in 2017 in North India. Scanty reports are available in India on the susceptibility of dermatophytes against terbinafine antifungal agent.[117,119] In the last two decades, L393F and F397L substitutions were observed due to point mutation in the SE gene have been linked to terbinafine resistance in *T. rubrum*[117,119]. Sixteen *T. rubrum* isolates and one *T. interdigitale* isolates with point mutations resulting to L393F, L393S, F397I, F397L, F397V, F415V, and H440T changes have been reported by Yamada et al.(196). Present study we identified the mutation which leads to the substitution of phenylalanine to leucine at 397 position (Phe397Leu) in 4 *T. metagrophytes* isolates with higher MIC. Drug resistance due to the effect of amino acid substitution might aid in the understanding of drug and enzyme interactions. In

S.cerevisiae isolates, Atomic three-dimensional (3D) modeling of SE protein was demonstrated by Nowosielski et al.[197] The greatest drug-enzyme interaction was found at F402, F420, F417, C416, V92, and T90 in an amino acid found in the C-terminal region of SE. The SE residue F402 matches to Phe397 in the *T. rubrum* SE gene.[196] The amino acid change at this location has a big influence on drug-enzyme interactions. It's unclear if terbinafine resistance develops spontaneously or as a result of medication use. Out of 20 *Trychophyton* isolates showing higher MIC for terbinafine drug only three have had terbinafine exposure history out of which only one isolate had shown the mutation in T1189C position of SE gene DNA sequence. In the absence of successive isolates, it's difficult to say if our isolates were mainly resistant or developed resistance over treatment. Additional genetic evolutionary investigations of isolates from the same patient in a row might help to clarify the question of primary and secondary resistance.

6.0 SUMMARY

Dermatophytosis was found to be present with 9.6 % prevalence in Siddi tribe population, *T. mentagrophytes* complex was shown to be the most prevalent organism causing dermatophytosis among Siddis in this investigation. The distribution of dermatophytes in this population matched those of previous studies conducted across India. Patients in the 15 to 45 year old age group were the most often afflicted, as this group of patients are more socially engaged, the chances of them coming into touch with infected persons are high. They may also get the disease while working in jobs like farming or physical labor. These young people frequently migrate in and out of their communities in search of jobs in other parts of the state. As a result, the risk of dermatophyte transmission throughout the population is increased. Diabetes mellitus was previously thought to be a significant risk factor for the development of dermatophytes but only 5.5 % of patients in our research were diabetics, which is consistent with earlier findings from India. As previously stated, tinea corporis was the most common clinical manifestation. Tinea cruris was found to be less common in our study than in other Indian studies. The fact that the majority of patients hesitated/refused to show the infection ingroinsregion might explain the decreased rate of tinea cruris in our research. This preserved tribal community's high dermatophyte load might be attributable to their particular lifestyle and socioeconomic circumstances. The Siddi community is a low-income population that mostly relies on agricultural and daily wage employment. They participate in income-generating activities such as fuel wood collecting or daily wage employment for local landlords. Around 72 % of the patients were illiterate, whereas 24 % were students or had finished their elementary school. According to a recent poll, 40% of the Siddi

population eats only two meals per day and is at risk of malnutrition. Because people live in overcrowded tiny hamlets with inadequate sanitation and regularly share clothing, towels, and other household goods, the risk of dermatophyte transmission can't be ruled out.

The most prevalent etiological agent is *T. mentagrophytes*, which corresponds to the ITS genotype VIII. Despite their origins in African nations, the distribution of dermatophytes and their etiological agent in the Siddi tribal population is comparable to that of native Indians. The fact that this group has existed for many generations and has adapted to local social and religious traditions, as well as frequent interaction with nearby native Indians, might all be factors. Antifungal susceptibility testing of dermatophytes in the community might help us learn more about antifungal sensitivity patterns against dermatophytes in the community. Rare African species of dermatopytes were not found in the migrated Siddi tribal population, which might be attributed to the community's long history in India and adaptation to local social and religious customs.

Non-dermatophytes such as yeast and non dermatophytic moulds, in addition to dermatophytes, were shown to be responsible for superficial infection in the Siddi tribal group, with a prevalence incidence of 5.97 %. In 85.71 % of patients, tinea ungum was the most prevalent clinical disease that caused superficial infection by non-dermatophytes. In the Siddi tribal community, *Cladosporium halotolerans*, an indoor fungus, can cause superficial infections like tinea capitis. A detailed study of the Siddi tribal community and native Indians to discover such superficial fungal infections may reveal interesting findings, which may aid clinician diagnosis and change treatment approaches.

All 102 dermatophyte isolates were subjected to antifungal susceptibility testing against 13 antifungal drugs. Voriconazole was the effective antifungal agent, whereas luliconazole and griseofulvin have shown higher MIC. Reduced susceptibility was observed against allylamine like terbinafine and naftifine. Present study shown mutation in the 1191^{th} position which leads to substitution of phenylallamine to lucine at 397^{th} position in Squalene Epoxidase (SE) gene.

7.0 CONCLUSION

The prevalence of dermatophytosis in the Siddi tribal population was found to be 9.6 %, with *Trichophyton mentarophytes* type VIII being the most commonly isolated etiological agent. Despite the fact that the Siddi community originated from African countries, the etiological agents, clinical presentation and distribution of dermatophytosis are similar to that of native Indians. Non-dermatophyte mould such as *Cladosporium halotolerance*, an indoor fungus, can cause superficial infections such as tinea capitis especially among Siddi tribal community.

Most effective antifungal agent was voriconazole, but allylamines such as terbinafine showed lower susceptibility with the F397L mutation in Squalene Epoxidase (SE) gene.

Current study has been focused on the African migrant Siddi tribal population in north Karnataka region, while similar hamlets are also found in Andhra Pradesh, Maharashtra, and Goa. Thus, a systematic analysis of dermatophytosis among such other African migrants using more advanced genetic techniques such as next generation sequencing and whole genome sequencing might disclose newer findings.

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ANNEXURE I: ETHICAL CLEARANCE LETTER

KLE UNIVERSITY
(Formerly known as KLE Academy of Higher Education & Research, Belagavi)
[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956 vide Government of India Notification No.F.9-19/2000-U.3(A)]
'Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Director, Academic Affairs
JNMC Campus, Nehru Nagar, Belagavi-590 010, Karnataka State, India
☎: 0831-2444444/2493779 FAX: 0831-2493777 Web: http://www.kleuniversity.edu.in E-mail: info@kleuniversity.edu.in

Ref.No.KLEU/Ethic/2015-16/D- 51 Date: 21-5-2015.

To,
Mr. Aruna C
Department of Microbiology,
J. N. Medical College, Belagavi
Ph.D. Scholar 2014-15

Dear Research Scholar,

Sub:- Regarding Ethical Clearance.

The KLE University **Ethics Committee on Human Subjects** for Ph. D Research Project met on **23rd March 2015** to consider your application for approval of the research project **"Isolation and identification of Dermatophytes among Siddhi community residing in North Karnataka region and molecular characterization of Exotic / Rare fungi among them".**

As there are no ethical issues involved in your proposed research project, the committee has provided approval for this research project.

You are requested to report to Ethical Committee in case of the following:

1. Any deviation from or change of the protocol.
2. All serious adverse events.
3. Any changes in study documents.

(Dr. Anita Dalal)
Member Secretary,
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belagavi.

DIRECTOR ACADEMIC AFFAIRS KLE UNIVERSITY

(Dr. Anil Hogade)
Chairman
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belagavi.

CC to: - The Director Academic Affairs, KLE University, Belagavi.
- The Director Research Foundation, KLE University, Belagavi.
- The Registrar, KLE University, Belagavi

ANNEXURE II: INFORMATION SHEET AND PATIENT CONSENT

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ :
ಉತ್ತರ ಕರ್ನಾಟಕ ಭಾಗದಲ್ಲಿ ನೆಲೆಸಿರುವ ಸಿದ್ಧಿ ಜನಾಂಗದವರಲ್ಲಿನ ಶಿಲಿಂದ್ರಗಳನ್ನು (ಚರ್ಮರೋಗ) ಗುರುತಿಸುವುದು ಮತ್ತು ಅವಗಳಲ್ಲಿ ಬಂದತಂಹ ಅಪರೂಪದ ಶಿಲಿಂದ್ರಗಳನ್ನು ಆಣ್ವಿಕ ಗುಣ ಪರೀಶೀಲನೆ (ಮೊಲೀಕ್ಯೂಲರ್ ಕ್ಯಾರ್ಯಾಕ್ಟ್ರಾಜೆಶನ್) ಅಧ್ಯಾಯನ ಮಾಡುವುದು.

ತನಿಖೆದಾರರು : ಅರುಣಾ ಸಿ.

ಸಂಶೋಧನ ಮಾರ್ಗದರ್ಶಕರು :
ಡಾ. ಮಹಾಂತೇಶ ಬಾ. ನಾಗಮೋತಿ ಬೆಳಗಾವಿ.

ದಿನಾಂಕ :

ಅವತರಣೆ : ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ಕೋರುತ್ತಿದ್ದೇವೆ. ಈ ಪತ್ರವು ತಮಗೆ ನಮ್ಮ ಅಧ್ಯಯನದ ಮಾಹಿತಿಯನ್ನು ಕೊಡುತ್ತದೆ. ನಮ್ಮ ತಂಡದ ಸದಸ್ಯರೊಬ್ಬರು ಈ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ವಿವರವಾಗಿ ತಿಳಿಸಿಕೊಡುತ್ತಾರೆ ಹಾಗೂ ತಮ್ಮ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸುತ್ತಾರೆ. ತಾವು ಇದರಲ್ಲಿ ಭಾಗವಹಿಸುವ ಅಥವಾ ಭಾಗವಹಿಸದೇ ಇರುವ ನಿರ್ಧಾರ ತೆಗೆದುಕೊಳ್ಳುವ ಮುಂಚೆ ಕೆಳಗಿನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಿ ತಿಳಿದುಕೊಳ್ಳಿ.

ಅಧ್ಯಯನದ ಉದ್ದೇಶ :

ನೂರಾರು ವರ್ಷಗಳಿಂದ ವಲಸೆ ಬಂದ ಸಿದ್ಧಿ ಜನಾಂಗದವರು ಭಾರತದಲ್ಲಿ ಸಣ್ಣ ಸಣ್ಣ ಗುಂಪಾಗಿ ಕಾಡಿನ ಹತ್ತಿರ ನೆಲೆಸಿರುತ್ತಾರೆ. ಹಾಗೆಯೇ ಒಂದು ಗುಂಪು ಉತ್ತರ ಕನಾಟಕದ ಸೀಮೆಯಲ್ಲಿ ಉತ್ತರಕನ್ನಡ, ಬೆಳಗಾವಿ ಮತ್ತು ಇತರ ಜಿಲ್ಲೆಗಳಲ್ಲಿ ಇರುತ್ತವೆ. ಅವರು ಇಲ್ಲಿ ಇದ್ದರು ಅವರ ದೇಹದಾರ್ಢತೆ, ಸಂಸ್ಕೃತಿ, ಆಚರಣೆ ಮತ್ತು ನಡತೆಗಳನ್ನು ಇಂದಿಗೂ ಕೂಡಾ ಅವರು ಭಾರತೀಯರಿಗಿಂದ ಭಿನ್ನವಾಗಿ ಆಚರಿಸುತ್ತಿದ್ದಾರೆ. ಹಾಗೆಯೇ ಅವರಲ್ಲಿರುವ ಕೆಲವೊಂದು ವೈದ್ಯಕೀಯ ವಿಷಯಗಳು ಭಾರತೀಯರಿಗಿಂತ ಬೇರೆ ಆಗಿವೆ. ಇದರಲ್ಲಿ ಅವರಲ್ಲಿ ಆಗುವ ಬೂಷ್ಟುರೋಗವುಸಹಿತ ಬೇರೆ ಆಗಿದೆ. ಈ ರೋಗವನ್ನು ಮಾಡುವ ಜೀವಾಣು (ಮಿಣಿಜೀವಿಗಳು) ಭಾರತೀಯರಲ್ಲಿ ಕಾಣುವುದಿಲ್ಲ. ಮೇಲ್ನೋಟಕ್ಕೆ ಈ ರೋಗವು ಎಲ್ಲರಲ್ಲೂ ಒಂದೇ ತರ ಇರುತ್ತದೆ. ಈ ಮಿಣಿಜೀವಿಗಳು ಆಫ್ರಿಕಾದವರಲ್ಲಿಯೂ ಇದೇ ರೋಗವನ್ನು ಉಂಟು ಮಾಡುತ್ತವೆ. ಆದ್ದರಿಂದ ಈ ವಿಷಯವನ್ನು (ಮಿಣಿಜೀವಿ) ನಾವು ಸಿದ್ಧಿಜನಾಂಗದವರು ಮೂಲತಃ ಆಫ್ರಿಕಾದವರೆಂದು ಹೇಳಲು ಸಾಧ್ಯವಾಗುತ್ತದೆ. ಈ ಜೀವಾಣುಗಷ ಅನುವಂಶೀಯತೆಯಿಂದ ನಾವು ಸಿದ್ಧಿಜನಾಂಗದ ಪೂರ್ವಜರ ನೆಲೆಯನ್ನು ಹುಡುಕುವುದಕ್ಕೆ ಸಾಧ್ಯವಾಗುತ್ತದೆ.

ತಮ್ಮ ಭಾಗದಿಂದ (ಸಿದ್ಧಿಜನಾಂಗದ) 1000 ಜನ (ಗಂಡು, ಹೆಣ್ಣು, ಮಕ್ಕಳು) ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ತಮ್ಮನ್ನು ಆಹ್ವಾನಿಸಬಹುದು. ಅದಕ್ಕೆ ನಾವು ತಮ್ಮ ಸಮ್ಮತಿ ಪತ್ರವನ್ನು ತೆಗೆದುಕೊಳ್ಳುತ್ತಿದ್ದೇವೆ. ನಂತರ ತಾವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಾಗುತ್ತೀರಿ.

ಅರ್ಹತೆಗಳು ;

ತಾವು ಅಧ್ಯಯನದ ಭಾಗವಾಗುವ ಬಗ್ಗೆ ಈ ಕೆಳಗಿನ ಅಂಶಗಳನ್ನು ಪ್ರಮಾಣೀಕರಿಸಬೇಕು.

1. ತಾವು ಸಿದ್ಧಿಜನಾಂಗದವರಾಗಿರಬೇಕು.
2. ತಾವು ಬೂಷ್ಟು ರೋಗ (ಚರ್ಮದ) ದಿಂದ ಬಳಲುತ್ತಿರಬೇಕು.
3. ತಾವು ಈ ರೋಗಕ್ಕೆ ಮಲಾಮು ಮತ್ತು ಗುಳಿಗೆಗಳನ್ನು ತೆಗೆದುಕೊಳ್ಳುತ್ತಿರಬಾರದು.

ಕಾರ್ಯವಿಧಾನಗಳು :

ತಾವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಅರ್ಹವಿದ್ದು ಮತ್ತು ಒಪ್ಪಿಗೆ ನೀಡಿದರೆ ತಮ್ಮಿಂದ ನಾವು ಈ ಕೆಳಗಿನ ಅಂಗಾಂಶಗಳನ್ನು ದವಾಖಾನೆಗಳಲ್ಲಿ ಸಂಗ್ರಹಿಸುತ್ತೇವೆ.

1. ಸೋಂಕಿನೀಂದ ಕೂಡಿದ ಒಣಗಿದ ಚರ್ಮ (ದೇಹದ ಯಾವದೇ ಭಾಗದ)
2. ಸೋಂಕಿನಿಂದ ಕೂಡಿದ ತಲೆಕೂದಲು/ಉಗುರು

ನಾವು ಒಂದು ಸಾರಿ ಇವುಗಳನ್ನು ಸಂಗ್ರಹಿಸಿದ ನಂತರ, ನಾವು ಮತ್ತೆ ಇವುಗಳನ್ನು ಮತ್ತೆ ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. (ಬಹುದು) ಆದ್ದರಿಂದ ನಾವು ಪತ್ರ ಅಥವಾ ಮೊಬೈಲ್ (ಜಂಗಮವಾಣಿ) ಕರೆಗಳನ್ನು ಮಾಡುತ್ತೇವೆ. ಆಗ ತಾವು ಮೇಲ್ಕಾಣಿಸಿದ ನಮೂನೆಗಳನ್ನು ಕೊಡಬಹುದು.

ಸಂಭವನೀಯ ತೊಂದರೆ/ಅಪಾಯಗಳು.

ತಾವು ಮೇಲ್ಕಾಣಿಸಿದ ಅಂಗಾಂಶಗಳನ್ನು ಕೊಡುವುದರಿಂದ, ತಮ್ಮ ದೇಹಕ್ಕೆ ಯಾವುದೇ ಗಾಯ/ನೋವು/ಅಲರ್ಜಿ ಆಗುವುದಿಲ್ಲ.

ನಮೂನೆಯ ಪರೀಕ್ಷೆ ಹಾಗೂ ಅದರ ಪ್ರಮಾಣ ಪತ್ರ.

ನಾವು ತಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಅಂಗಾಂಶಗಳನ್ನು ಬೆಳಗಾವಿಯ ಜವಾಹರಲಾಲ ನೆಹರೂ ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಾಲಯದ ಮಿಣಿಜೀವಶಾಸ್ತ್ರ ವಿಭಾಗದಲ್ಲಿ ತಪಾಸಿಸಿ ಮತ್ತು ಬೆಳೆಸುತ್ತೇವೆ. ಇದರ ಪ್ರಮಾಣ ಪತ್ರಗಳನ್ನು ತಮಗೆ ದೂರವಾಣಿ ಹಾಗೂ ಅಂಚೆಯ ಮೂಲಕ ತಲುಪಿಸುತ್ತೇವೆ. ನಂತರ ಅದನ್ನು ಪಿಜಿಐ ಚಂಡಿಗರ್ಹದಲ್ಲಿ ತಪಸಿಸಲಾಗುವುದು.

ಲಾಭಗಳು :

ಇದರಿಂದ ನಾವು ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಪುಷ್ಟಿಕರಿಸಲಾಗುವುದು. ಸಿದ್ಧಿ ಜನಾಂಗದವರು ಆಫ್ರಿಕಾದಿಂದ ವಲಸೆ ಬಂದು ಭಾರತದಲ್ಲಿ ನೆಲೆಸಿದ್ದಾರೆ, ಹೇಗೆಂದರೆ :

1. ಚರ್ಮರೋಗವನ್ನು ಮಾಡುವ ಕ್ರಿಮಿಜೀವಿಗಳು (ಜೀವಾಣು) ಯಾವ ಭಾರತೀಯರಲ್ಲಿ ವರದಿಯಾಗಿರುವುದಿಲ್ಲ.
2. ಈ ಜೀವಾಣುಗಳು ಕೇವಲ ಆಫ್ರಿಕಾದವರಲ್ಲಿ ಮತ್ತು ಸಿದ್ಧಿ ಜನಾಂಗದವರಲ್ಲಿ ರೋಗಗಳನ್ನು ಮಾಡುತ್ತವೆ.
3. ತಮ್ಮಿಂದ ಆಕರಿಸಿದ ನಮೂನೆಯಲ್ಲಿ ಹುಟ್ಟಿದ ಬೂಷ್ಟು ರೋಗಾಣುವಿನ ಅನುವಂಶೀಯ ಗುಣಗಳನ್ನು ಹೋಲಿಸಿದಾಗ, ಎರಡೂ ಒಂದೇ ಮೂಲ ಎಂದು ಸಾಧಿಸಿದರೆ, ಆಗ ನಾವು ಸಿದ್ಧಿಜನಾಂಗದ ಮೂಲವು ಆಫ್ರೀಕಾದಿಂದ ಎಂದು ಹೋಲಿಕೆ ಮಾಡಬಹುದಾಗಿದೆ.

ಈಅಂಶವು ಈಗಾಗಲೇ ಪ್ರತಿಪಾದಿಸಿ ವರದಿ ಆಗಿದೆ. ನಮ್ಮ ಈ ಅಧ್ಯಯನವು ಅದನ್ನು ಮತ್ತಷ್ಟು ಪುಷ್ಟಿಕರಿಸುವುದು ಎಂದು ನಮ್ಮ ನಂಬಿಕೆ. ಆದ್ದರಿಂದ ಈ ಅನ್ವೇಷಣೆಯಲ್ಲಿ ತಮ್ಮ ಹಾಗೂ ತಮ್ಮ ಜನಾಂಗದ ಪಾತ್ರ ಬಹಳ ಪೂರಕವಾಗಿದೆ.

ಈ ಅಧ್ಯಯನವು/ದ

1. ಭಾರತೀಯ ಆರ್ಯುವಿಜ್ಞಾನ ಅನುಸಂಧಾನ ಪರಿಷದ್ ಅವರ ಹಣಕಾಸಿನ ಸಹಾಯದಿಂದ ನಡೆಸಲ್ಪಡುತ್ತದೆ.
2. ಇದರಲ್ಲಿ ಭಾಗವಹಿಸಲ್ಪಡುವ ತಮಗೆ ನಾವು ಹಣಕಾಸಿನ ನೆರವು ನೀಡುವುದಿಲ್ಲ ಹಾಗೂ ತಮ್ಮಿಂದ ನಾವು ಹಣವನ್ನು ವಸೂಲು ಮಾಡಲಾಗುವುದಿಲ್ಲ.
3. ಇಲ್ಲಿ ತಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ಸ್ವಯಂ ಪ್ರೇರಿತವಾಗಿರುತ್ತದೆ. ತಾವು ಬೇಡ ಎಂದರೆ ನಾವು ತಮಗೆ ಒತ್ತಡ ಹಾಕುವುದಿಲ್ಲ.
4. ನಮ್ಮ ತಂಡದ ವೈದ್ಯರು ಬೇಡೆಂದರೆ ತಮ್ಮನ್ನು ನಾವು ಇದರಲ್ಲಿ ಅಂಗೀಕಾರ ಮಾಡುವುದಿಲ್ಲ.
5. ಇದರಲ್ಲಿ ಭಾಗವಹಿಸುವುದರಿಂದ ತಮಗೆ ವಿನಾದರೂ ತ್ರಾಸು, ಹಿಂಸೆ, ಅಲರ್ಜಿ ಎನಿಸಿದರೆ ತಾವು ತಕ್ಷಣ ಡಾ. ಮಹಾಂತೇಶ ಬಾ. ನಾಗಮೋತಿ ಪ್ರಾಧ್ಯಾಪಕರು ಮಿಣಿ ಜೀವಶಾಸ್ತ್ರ ವಿಭಾಗ ಕೆ.ಎಲ್.ಇ. ವಿಶ್ವವಿದ್ಯಾಲಯ

ಬೆಳಗಾವಿ–10 ಇವರಿಗೆ ಸಂಪರ್ಕಿಸಬೇಕು. (ಅವರ ದೂರವಾಣಿ ಸಂಖ್ಯೆ : 094481–41347, ಕಛೇರಿ 0831–2423222 4070)

6. ಈ ಪತ್ರದ ಪ್ರತಿಯನ್ನು ತಮಗೆ ನಾವು ಕೊಡುತ್ತೇವೆ.
7. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ತಮ್ಮ ಬಾಗವಹಿಸುವಿಕೆಯನ್ನು ನಾವು ಸಾಧ್ಯವಾದಷ್ಟು ಮಟ್ಟಿಗೆ ಗೌಪ್ಯವಾಗಿರುತ್ತೇವೆ.

ಇದರ ಬಗ್ಗೆ ಕೆಳಗೆ ಕಾಣಿಸಿದ ಸಂಸ್ಥೆಗಳವರು/ವಿಶೇಷಜ್ಞರು ತಪಾಸಣೆ/ ವಿಚಾರಿಸಬಹುದು.

ಅ. ಮಾನವ ಸಂಬಂಧಿತ ಅಧ್ಯಯನಗಳ ಬಗ್ಗೆ ನಿಗಾವಹಿಸುವ ಸ್ವಾಯತ್ತ ಸಂಸ್ಥೆಗಳು.

ಆ. ಜವಾಹರಲಾಲ ನೆಹರೂ ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಾಲಯ ಬೆಳಗಾವಿ/ಜಿಜಿಐ ಚಂಡಿಗರ್ಹ ಮಾನವ ಹಕ್ಕು ಮತ್ತು ನೀತಿ ಸಂಹಿತೆ ಸಮೀತಿ.

ಇ. ಅಧ್ಯಯನ ತಂಡ ಹಾಗೂ ಪ್ರಾಯೋಜಕರು.

8. ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳನ್ನು ಮುದ್ರಿಸಬಹುದು/ಸಭೆಗಳಲ್ಲಿ ಹಂಚಿಕೊಳಬಹುದು. ಆಗ ತಮ್ಮ ಬಗ್ಗೆ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿಡುತ್ತೇವೆ.
9. ಈ ಸಮ್ಮತಿ ಪತ್ರಕ್ಕೆ ತಾವು ಸಹಿ ಮಾಡುವುದರ ಮೂಲಕ ತಾವು ನ್ಯಾಯಯುತ ಹಕ್ಕುಗಳನ್ನು ಕಳೆದುಕೊಳ್ಳುವುದಿಲ್ಲ.
10. ಇದಕ್ಕೆ ಸಹಿ ಅಥವಾ ಹೆಬ್ಬೆಟ್ಟಿನ ಬೆರಳಿನ ಅಚ್ಚನ್ನು ತಾವು ಮಾಡಿರುವಿರಿ ಎಂದಾದರೆ ತಮ್ಮ ಇಲ್ಲ ಪ್ರಶ್ನೆ/ಸಂದೇಹಗಳನ್ನು ನಾವು ಉತ್ತರಿಸಿದ್ದೇವೆ ಹಾಗೂ ತಾವು ಸ್ವಇಚ್ಛೆಯಿಂದ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುತ್ತಿರುವಿರಿ ಎಂದು ಖಚಿತವಾಗುತ್ತದೆ.

1. ಪಾಲ್ಗೊಳ್ಳುವವರ ಹೆಸರು
ಸಹಿ/ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು

2. ಸಾಕ್ಷಿದಾರರ ಹೆಸರು
ಸಹಿ/ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು

3) ಸಮ್ಮತಿ ಪಡೆಯುವರ ಹೆಸರು/ಸಹಿ

4) ಸಂಶೋಧಕರ ಹೆಸರು/ಸಹಿ

ಈ ಸಂಶೋಧನಾ ಪ್ರಯೋಗದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿಗೆ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ :
ಉತ್ತರ ಕರ್ನಾಟಕ ಭಾಗದಲ್ಲಿ ನೆಲೆಸಿರುವ ಸಿದ್ಧಿ ಜನಾಂಗದವರಲ್ಲಿನ ಶಿಲಿಂಧ್ರಗಳನ್ನು (ಚರ್ಮರೋಗ) ಗುರುತಿಸುವುದು ಮತ್ತು ಅವಗಳಲ್ಲಿ ಬಂದತಂಹ ಅಪರೂಪದ ಶಿಲಿಂಧ್ರಗಳನ್ನು ಆಣ್ವಿಕ ಗುಣ ಪರೀಶೀಲನೆ (ಮೊಲೀಕ್ಯೂಲರ್ ಕ್ಯಾರ್ಯಾಕ್ಟ್ರಜೆಶನ್) ಅಧ್ಯಾಯನ ಮಾಡುವುದು.

ತನಿಖೆದಾರರು : ಅರುಣಾ ಸಿ.

ಸಂಶೋಧನ ಮಾರ್ಗದರ್ಶಕರು :
ಡಾ. ಮಹಾಂತೇಶ ಬಾ. ನಾಗಮೋತಿ ಬೆಳಗಾವಿ.

ಈ ಕೆಳಗೆ ಸಹಿ ಮಾಡಿದ _____ ನಾನು ಒಪ್ಪಿಗೆ ಪತ್ರದಲ್ಲಿ ಕೊಟ್ಟಿರುವ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ಎಲ್ಲ ಮಾಹಿತಿಯನ್ನು ಪೂರ್ಣವಾಗಿ ಓದಿರುತ್ತೇನೆ/ನನಗೆ ಓದಿ ಹೇಳಲಾಗಿದೆ. ನನ್ನ ಹೆಸರನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುವದು ಎಂಬುದು ನನಗೆ ತಿಳಿದಿದೆ. ನನಗೆ ಯಾವ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುತ್ತಾರೆ ಎಂಬುದರ ಬಗ್ಗೆ ತಿಳಿದಿದೆ ಮತ್ತು ನನಗೆ ಇದರಿಂದ ಯಾವುದೇ ರೀತಿಯ ಹಾನಿಯಾಗುವುದಿಲ್ಲ. ನಾನು ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿಕೊಂಡುತ್ತೇನೆ. ಈ ಒಪ್ಪಿಗೆ ಪತ್ರಕ್ಕೆ ಸಹಿ ಮಾಡುವುದರಿಂದ ನಾನು ನನ್ನ ಯಾವುದೇ ಕಾಯಿದೆ ಬದ್ಧ ಹಕ್ಕುಗಳನ್ನು ಬಿಟ್ಟುಕೊಟ್ಟಿರುವುದಿಲ್ಲ. ಸಂಶೋಧಕರು ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳನ್ನು ಭೋಧನೆಗಾಗಿ ಮತ್ತು ವೈದ್ಯಕೀಯ ಪ್ರಕಟಣೆಗಳಲ್ಲಿ ಪ್ರಕಟಿಸಲು ಉಪಯೋಗಿಸುವರು. ನಾನು ಯಾವುದೇ ಸಂದರ್ಭದಲ್ಲಿ ಈ ಅಧ್ಯಯನದಿಂದ ಹೊರಹೋಗಬಹುದು. ಈ ಒಪ್ಪಿಗೆ ಪತ್ರದ ಒಂದು ಪ್ರತಿಯನ್ನು ನನಗೆ ಕೊಡಲಗುವುದು.

ಭಾಗವಹಿಸುವವರ ಸಹಿ : _____

ಭಾಗವಹಿಸುವವರ ಹೆಸರು : _____

ತನಿಖೆದಾರರ ಹೆಸರು : _____

ತನಿಖೆದಾರರ ಸಹಿ : _____

ದಿನಾಂಕ ;

शिर्षक : - उत्तर कर्नाटक भागातील राहणाऱ्या सीध्दी लोकांचे बुरशी (चर्म रोग) वळकण्यासाठी आणि त्याच्यातुन दुर्मिळ बुरशींचा आण्विक गुणपरीक्षन (मॉलीक्युलर कॅरेक्टराजेशन्) अध्यायन करणे.

संशोधक : अरुणा. सी

संशोधक मार्गदर्शक : डॉ. महानतेश नागमुर्ती, बेळगांव.

उत्तर कर्नाटकातील सिध्दी लोकांचे पुर्वज आफ्रिकेत होते हे सिध्द करण्यासाठीच्या आमच्या संशोधन कार्यात सहभागी होण्यासाठी तुम्हाला आमंत्रीत केले आहे. जरी हे सिध्दी लोक फार वर्षापासुन भारतात व्दास्तव्य करुन आहेत . व परत त्यांच्या पुर्वजांच्या देशात गेलेले नाहीत तरी सुध्दा त्यांच्यातील काही रोगामध्ये साधर्म्य आहे. ह्या रोगातील साम्य इतर उत्तर कर्नाटक भागातील भारतीय लोकांच्यात दिसत नाही ह्या निदर्शनावरुन असे सिध्द करता येईल की सिध्दी लोकांचे पुर्वज आफ्रिकेत होते. ह्या संशोधनात भाग घेण्यापुर्वी तुम्हाला ह्या संशोधनाचे कारण, त्यातील फायदे व तोटे माहीत असणे आवश्यक आहे त्यासाठी तुम्हाला दिलेल्या माहिती पत्रकावरुन याची माहिती मिळेल.

ह्या संशोधनाची संपुर्ण माहिती घेतल्यानंतर तुमची ह्या संशोधनात भाग घेण्यास मान्यता असेल तर, समंती पत्रावर सही किंवा आंगठ्याचा ठसा उमटवुन तुम्ही स्वखुशिने ह्या अभ्यासात भाग घेऊ शकता.

* तुमचा ह्या संशोधनातील सहभाग स्वखुशिने असेल .

*तुम्ही ह्या संशोधनातील सहभाग केंव्हाही काढून घेऊ शकता व त्याचा परिणाम तुमच्या नेहमीच्या वैद्यकिय सेवेवर होणार नाही.

संशोधनाचे कारंण : टिनीयॉ (रिंगवर्म / डरमॅटोफायरोसिस) हा रोग जगभर बऱ्याच लोकांच्यात आढळून हा रोग जगातील बऱ्याच भागात आढळतो . काही भागात जास्त आढळतो तर काही भागात आढळत नाही. या बद्दल जास्त संशोधन झालेले नाही, फार थोड्या इतर लोकांच्यात हा रोग आढळतो हे यामागील कारण असु शकेल बाकीच्या देशात हा रोग फार प्रमाणात आढळुन येत नाही. पाश्चिमात्य देशात बुशी (फणस) बद्दल सर्वक्षण केले गेले आहे . हे सर्वेक्षण सर्व जनसणुदाय व मुलांच्यात केले गेले आहे.

शंभर वर्षापासून सिध्दी लोक - भारतात वास्तव्यास आहेत २०-३० वर्षापुर्वी असे आढळुन आले आहे की ह्या भागात राहणाऱ्या सिध्दी लोकांच्यातील टिनीया (बुशी) ह्या

रोगाचे कारण 'टी याऊनडी' व 'टी स्कोनलेनी' हे जंतू आहेत. हे जंतू भारतातील इतर लोकांच्यात व इतर भागातील आफ्रिकन लोकांच्यात दिसून आले नाहीत. नवीन संशोधन ह्या लोकांवर (सिध्दी) केलेले नाही. कारण एकल तर हे लोक मागासलेले आहेत व शहरापासुन लांब रहात आहेत.

हे संशोधन जे. एन मेडीकल कॉलेज व पी. जी. आय चंदीगड इंडिया यांच्या संयुक्त सहभागाने केलेले आहे . ह्या संशोधनासाठी १००० सिधी लोकांची तपासणी करण्यात येणार आहे. ज्यांना टिनीया (बुर्शी) रोगाची लागण झालेली आहे .

ह्या संशेधनात भाग घेण्याची पात्रता : -

ह्या संशोधनात भाग घेऊ इच्छीणाऱ्यांसाठी समंती पत्रावर सही करुन सहभागी करुन घेतले जाईल. (जर ते खालील गोष्टीस पात्र असतील तर)

1. भाग घेणारी व्यक्ती सिध्दी समाजातील असेल.
2. त्या व्यक्तीला बुर्शी रोग किंवा रोग झाल्याचा संशय असेल .
3. लहान, थोर, स्त्री व पुरुष सर्व संशोधनात सहभागी होऊ शकतील.
4. सिध्दी समाजातील लोक ज्यांना बुर्शी रोग झाला आहे व ते त्यावरील उपचार घेत आहेत.

तसेच ह्या अभ्यासाचा भाग म्हणून वाय, एच. आय. व्ही तपासणी, पुर्वीचे रोग निदानाच्या माहीती वरुन भाग न घेणाऱ्या लोकांचे कारण समजु शकेल.

कार्य पध्दत : जर तुम्ही ह्या संशोधनात भाग घेण्यास तयार असाल व पात्र असाल तर तुमचे शिक्षण प्राप्ती, कौटुंबिक माहिती व आजाराबद्दलची प्राथमिक माहिती घेतली जाईल. त्यानंतर तुमच्या त्वचेचा नखांचा व केसाचा नमुना घेऊन जे. एन. मेडिकल कॉलेजला व पी. जी. आय. चंदिगडला तपासणीसाढी पाठवला जाअईल. त्याचे निदान तुम्हाला फोनवरुन किंवा पोस्टाने कळविण्यात येईल. आठवड्याच्या शेवटी एक व महिन्याभरात दुसरा तपशलि (रिपोर्ट) मिळेल. त्याचा उपयोग तुम्हाला दिल्या जाणाऱ्या बुर्शी रोगावरील उपचारासाठी होईल .

जोखीम त्रास व खर्च : वरील तपासणीचा त्रास होत नाही . रोगावरील उपचाराचा खर्च संशोधनात येत नाही. किंवा संशोधकाकडून दिला जाणार नाही.

फायदे : - तुम्ही जरी अभ्यासात भाग घेऊ शकला नाहीत तरी तुम्हाला प्राथमिक तपासणीचा तपशलि (रिपोर्ट) दिला जाईल व त्याचा उपयोग तुमच्या रोगाच्या उपचारासाठी होईल. तसेच सिध्दी लोकांचे पूर्वज आफ्रिकेत होते हे सिध्द करण्यासाठी होईल.

गुप्तता :- ह्या संशोधनातील सर्व माहिती गुप्त ठेवण्यात येईल. ह्या संशोधनातील तुमची माहिती कायद्याने द्यावी लागली तरच उघड केली जाईल. तेंव्हा हे संशोधन प्रकाशीत होईल तेंव्हा तुमचे नांव व वैयक्तिक माहिती उजेडात आणली जाणार नाही किंवा गुप्त ठेवली जाईल. ही माहिती जे. एन . मेडिकल कॉलेजच्या इथिकल कमिटी. पी. जी. आय . चंदिगड व भारतीय वैद्यकिय अनुसंधान परिषद किंवा अभ्यासक बंधु शकतात.

अधिक माहिती :- ह्या संशोधनासंबंधी अधिक माहिती किंवा संबंधीत प्रश्नासाठी खालील व्यक्तिशी संपर्क साधावा.

1) डॉ. महांतेश बि. नागमोती. प्राध्यापक. के. एल. ई विद्यापीठ जे. एन. मेडीकल कॉलेज बेळगांव. फोन 0831-273777 - 4070 मोबाईल - 9448141342.

संशोधनातील सहभाग थांबवायचा असल्यास त्याप्रमाणे आम्हास माहिती देऊन सहभाग थांवविण्याचा तुम्हास अधिकार आहे. ह्या संशोधनातील सहभागासाठी तुम्ही बांधील नाही. तुमचा सहभाग तुम्ही केंव्हाही काढून घेऊ शकता व त्यासाठी तुम्ही अभ्यास केंद्र किंवा अभ्यासकाशी केंव्हाही संपर्क साधु शकता.

संशोधनात भाग घेतल्यांशी संपर्क :- तुमच्या बरोगाचे निदान (रिपोर्ट) फोन करुन किंवा पोस्टोने कळविण्यात येअईल. तसेच निदान प्राथमिक आरोग्य केंद्राच्या वैद्याधिकाऱ्याला कळवण्यात येईल व त्याचा उपयोग उपचारासाठी केला जाईल. तेसच रोग निदानाची लिखित प्रत दिली जाईल.

सूक्ष्म संतूंचा संचंय : तुमच्या त्वचा, नखे व केसावरील नमुन्यातुन मिळालेले सूक्ष्म जंतू जे. एन. मेडिकल कॉलेज किंवा पी. जी आय चंदीगड या ठिकाणी साठवून ठेवण्यात येतील व पुढील संशोधनासाठी वापरले जातील.

सही :- तुमच्या सहीमुळे तुम्ही तुमचा कोणताही कायदेशीर हक्क गमावणार नाही . तसेच ह्या संशोधनासाठी सहभाग तुम्ही केंव्हाही काढून घेऊ शकता. तुमची सही किंवा आंगठ्याचा ठसा, तुम्ही ह्या संशोधनासाठी तयार तयार असल्याचे व तपासणी नमुने घेण्यास राजी असल्याचे , तसेच तुमच्या प्रश्नांचे व व शंकाचे उत्तर दिले आहे असे दर्शविते.

संशोधनात भाग घेणाऱ्याचे संपुर्ण नांव / सही / आंगठ्याचा ठसा

साक्षिदाराचे नांव / सही /आंगठ्याचा ठसा व तारीख

माहिती घेणाऱ्याचे व संमती देणाऱ्याचे नांव / सही व तारीख

संशोधकाचे नांव व सही व तारीख

संशोधन अध्ययनात भाग घेण्यास कबुली

शिर्षक : - उत्तर कर्नाटक भागातील राहणाऱ्या सीध्दी लोकांचे बुरशी (चर्म रोग) वळकण्यासाठी आणि त्याच्यातुन दुर्मिळ बुरशींचा आण्विक गुणपरीक्षन (मॉलीक्युलर कॅरेक्ट्राजेशन्) अध्यायन करणे.

संशोधक : अरुणा. सी

संशोधक मार्गदर्शक : डॉ. महानतेश नागमुर्ती, बेळगांव.

मी _____ हे संमति पत्र वाचले आहे. मला वाचून दाखवून यातील सर्व माहिती सविस्तरपणे स्पष्ट करुन सांगितले आहे हे अध्ययन कशासाठी केले जाते, मला काय विचारले जाईल आणि मला कोणतीही ईजा होणार नाही माझे नाव कुठेही उघड होणार नाही याची मला माहिती आहे. या अध्ययनातून गोळा केलेल्या माझ्या माहितीचा उपयोग शिकविण्यासाठी वैद्यकी प्रकाशनासाठी आणि वैज्ञानिक सभांमध्ये सादरीकरणासाठी केले जाईल हे मला माहित आहे. मी स्वइच्छेने या अध्ययनात भाग घेतला आहे मी कोणत्याही कारणासाठी या अध्ययनातून भाग काढून घेअईन किंवा संशोधक केंव्हांही, कोणत्याही कारणासाठी मला काढून टाकतील मी संमतिपत्रावर सही करुन कोणतेही कायदेशीर हक्क सोडत नाही मला कबूलीची व संपतिपत्राची प्रत दिली जाईल.

सहभागी व्यक्ती चे नाव ----------

सहभागी व्यक्तीची सही ----------

साक्षीदार सही ----------

संशोधकाचे नांव ----------

संशोधकाची सही ----------

दिनांक : ----------

Protocol title: **Isolation and Identification of Dermatophytes among Siddi community residing in North Karnataka region and Molecular characterization of Exotic/Rare fungi** among them.

Research Scholar: Mr. Aruna C
Research Guide: Dr. Mahantesh B Nagamoti

Introduction: You are being invited to take part in this research study which we are conducting to prove the origin of Siddhi community is from Africa. Even though this community settled in India since many years and not visited their original place, but still they have some unique disease among them. Such unique infections are not reported in naval Indians of the same region. Considering this observation we are trying to prove that the Siddhi community is related to Africa.

Before you decide whether or not to take part in this research study, you need to know the purpose, the possible risk and benefits to you. This consent form provides that information. After the study has been fully explained to you, you can decide whether or not you want to participate. Once you understand the study, and if you agree to take part, you will be asked sign this consent form or make thumb impression in front of some one.

- Your participation in this study is entirely voluntary.
- You may decide not to take part or to withdraw from the study at any time without losing benefits of your routine medical care

Purpose of the Study: Tenia (Ring worm/Dermatophytosis) infection is a common health problem in the world. These infections are seen in all parts of the world. Some of them are endemic in some areas and restricted to a population. Rarely these infections are reported from other parts. The study on these geographically restricted fungi is less notified. There are very scanty reports have mentioned about their occurrence among general population. This may be due to their fewer occurrences among other countries. There are few surveys from western groups regarding the indigenous fungal infection have been performed. These are in the form of surveys among general population including children. It was observed that, T. *yaoundei* and T. *schoenleinii* causing Tenia infection among the Siddi community nearly 20-30 years back. These species are not reported from Indian and other than Africans in the world. Since then there are no new findings and observations have been made in this neglected community. There are no systemic studies have been done on this group even though much technical advances have occurred in the last two decades in India. This may be due to this community is socially and educationally backward and stay away from the city population.

This study is being conducted as collaboration between Jawaharlal Nehru Medical College (JNMC), Belagavi and Post Graduate Institute of Medical Education and Research, Chandigarh India. In this study we will screen 1000 Siddhi patients, who are having Tenia (fungal) infection for identification of the causative agent.

Eligibility to Participate: After reading this study form and discussing with the study counselor, if you agree to join this study, you will be asked to sign this consent form. After you have signed this form, we will ask you few questions to see if you meet requirement for joining the study. We will confirm the following:

- Person belongs to Siddhi community
- Siddhi people suspected and diagnosed as case of Tenia infection
- Adult children of both sex will be included
- Siddhis having Tenia infection and on antifungal treatment

The study counselor will then let you know if you are eligible to participate in the study. If you decide not to take part in the study or if you do not meet the eligibility requirement, we may still use the information collected from you to determine your eligibility. As part of this determination, some. basic information like your age, your HIV disease condition, your previous diagnosis may help us determine whether there are patterns or common reasons why people do not join the study. However we will not use your name in any publication related to this study.

Procedure: If you agree to participate and are eligible, we will collect information about you and your overall health including your education, family, income and previous illnesses. Then we will collect skin/nail from affected area and infected hair. These clinical samples will be shifted for testing at JNMC Belagavi and the primary report will be informed by phone and by post. The isolates further tested at PG! Chandigarh. You will get one preliminary report at the end of one week of collection of samples and second report before a month. This will help you for undergoing specific fungal treatment.

Risks/Discomforts and cost of participation: The procedure described above i; generally harmless. The treatment is not a part of the study and is not paid for by the study.

Potential Benefits: Whether you participate or not, you will receive the report of the screening test that may indicate your fungal disease status. If you participate in this study, you will receive the test report which will help to treat your fungal infections. There are no direct benefits from participating in this study. Information learned from the results of tests in this study may provide valuable support regarding prevailing Dermatophytes among Sidd community and their relation to prove that Siddi are direct descendents of Africans.

Confidentiality: Your study records and test results will be kept confidential. We can assure you that we will not reveal your participation in this study. Your personal information may be disclosed if required by law. Any publication of the study will not use your name or identify you personally. Your record may review by the Ethics committee of the Jawaharlal Nehru Medical College (JNMC), PGI Chandigarh and Indian Council of Medical Research (ICMR), as well as the study staff and investigators and the study monitors.

Problem/questions: For Question about this study Please contact Dr. Mahantesh B

Nagamoti, Professor, Department of Microbiology, J N Medical College, KLE University, Belagavi. Tel•. 0831-273777 extn:4070, cell no: 9448141342.

Termination of Participation: You have the right to stop participating in the study at any time by informing us about your intent to do so. You may visit the clinic to indicate your preference or contact the study investigator by phone, as follows: Dr. Mahantesh B Nagamoti, Professor, Department of Microbiology, J N Medical College, KLE University, Belagavi. Tel: 0831273777 extn: 4070, cell no: 9448141342.

Contacting you: We will inform yourtest reports by cell phone or by post. The report will be informed to your PHC Medical Officer for your further treatment. Hard copy of the same will be given to you.

Storage of Specimens: The organism isolated from your sample may be storage locally or in PGI (with usual protectors of identity) for an indefinite length of time and used for other Ethics Committee approved research studies on fungi.

Signatures: You are not waiving any of your legal rights by singing this consent form. You are free to refuse all or any part of these activities, without any problem. When you sign or make your thumb print on the form bellow, that means that your questions and concerns have been answered and you willing agree to undergo the study procedures.

_____

Participants full name with signature/thumb impression and date

_____

Witness name with signature and date

_____

Name of the person obtaining informed consent with signature and date

_____

Name of the investigator or designee with signature and date

INFORMED CONSENT FORM

title: Isolation and Identification of Dermatophytes among Siddi community Protoc residin in North Karnataka region and Molecular characterization of Exotic/Rare fungi am Ong

Rese h Scholar: Mr. Aruna C
Res ch Guide: Dr. Mahantesh B Nagamoti

he details of the research study in which I am expected to participate, for which I have to undergo history taking, clinical examination have been explained to me.

I willingly, under no pressure from the researcher agree to take part in this study, and agree to participate in all investigations. I may withdraw at any time. I am not giving up any of my legal rights by signing this form.

My signature below indicates that I have read this entire consent form or it has been read to me, and had all my questions answered. I will be given a copy of this consent form.

SIGNATURE OF THE PARTICIPANT OR LEGALLY AUTHORIZED REPRESENTATIVE

Participants Name	Participants Signature
Investigators Name	Investigators Signature
Witness's Name	Witness's Signature
Date	
Plac e	

ANNEXURE III: CLINICAL CASE SHEET

Isolation and Identification of Dermatophytes in Siddhi community residing in North Karnataka region and molecular characterization of Exotic/Rare fungi among them

Clinical Case Report

PID No:

Date:

Name:…….. Age:…….. Sex:……

Address: ……

1. Patient Belongs to Siddhi Community? Yes/No
2. Whether patient is suffering from fungal infection? Yes/No
3. Whether patient is on medication with antifungal agent? Yes/No

Patient questionnaire

1. Date of Birth:……
2. Marital Status:……
3. Education Status:……..
4. Occupation:……
5. Type of house:……..
6. Type of family:……
7. No of family members:……..
8. When the fungal infection is noticed:……..
9. Have you ever had such skin disease in the past?
   If yes,
   Since When:……
   Which area of the body:……..
   Whether treatment was taken? Yes/No
10. Number of family members in your family:……..
11. Any member in the family is suffering from such condition?
    If yes,
    How many Members:……..
    Since when:……..
    Any medication taken:……

12. Duration.....
13. Location.....
14. Site: ..... Secondary site: .....
15. Clinical Diagnosis.....
16. Lesion Description:
17. Sample.....

a) Spreading: YES ☐ NO ☐ **If Nail:**

b) Erythema: YES ☐ NO ☐ DLSO: ☐ PSO:☐ WSO:☐ Endonyx: ☐

c) Pigmentation: YES ☐ NO ☐ Total dystrophy: ☐Color: .....

d) Itching: YES ☐ NO ☐ Hyperkeratosis: YES ☐ NO ☐

18. Multiple infection: YES ☐ NO ☐
19. Seasonal variation:
20. Self-treatment: YES ☐ NO ☐
    If YES, medication used: .....
21. Diabetic: YES ☐ NO ☐ Other Co morbidity.....
22. H/o: Animal Contact: YES ☐ NO ☐ Which Animal;
23. Infected Human contact: YES ☐ NO ☐
24. Soil Contact: YES ☐ NO ☐
25. Other suspected source.....
26. On-going therapy: YES ☐ NO ☐
    If YES, then ..... Duration.....Dose.....
    Antifungals ..... Duration..... Dose.....
27. Prescribed treatment:
    Antifungal..... Duration.....Dose.....

Follow up: Relapse: YES ☐ NO ☐

Mycological Investigation:

- Microscopy: KOH mount.....
- Macroscopy: SDA with Cycloheximide:
  At 28^0C .....
  At 37^0C.....
  SDA with Chloramphenicol (28^0C)
  .....
- Isolates: 1.....
  Other isolate (if any) .....

ANNEXURE IV: PUBLICATIONS

Jemds.com Original Research Article

Isolation and Molecular Characterization of *Trichophyton rubrum* from Siddi Tribal Community Residing in North Karnataka Region, India

Aruna Chowdappa[1], Shivakumar Patil[2], Mahantesh B. Nagmoti[3]

[1]Department of Microbiology, Jawaharlal Nehru Medical College, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India. [2]Department of Dermatology, Jawaharlal Nehru Medical College, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India. [3]Department of Microbiology, Jawaharlal Nehru Medical College, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India.

ABSTRACT

BACKGROUND
Siddi community is the tribal community of African origin living in north Karnataka region since many generations. Few studies have shown the presence of rare dermatophytes of African origin in the Siddi tribal community. This study was performed to detect the presence of such dermatophytes of African origin in the Siddi community.

METHODS
A total of 1004 samples were collected from 937 patients with superficial infections out of which 102 samples have shown the dermatophytic growth on culture media. All the dermatophytes were identified by phenotypic and physiological characters.

RESULTS
Tinea unguium was the prevalent clinical condition and *Trichophyton mentagrophytes* was the commonest etiological agent to cause dermatophytosis. Two isolates have shown similar macroscopic and microscopic features of as dermatophyte of African origin species (*Trichophyton soudanense*) and subjected to sequencing of internal transcribed spacer region (ITS) and identified as *Trichophyton rubrum.*

CONCLUSIONS
Therefore, presence of rare African species was not seen in the migrated tribal community may be due to the existence of community since more than 5 generations and have adapted to local social and religious practices.

KEY WORDS
Siddi, Dermatophytes, *Trichophyton, Microsporon, Epidermophyton* and Tribal

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BACKGROUND

Dermatophytosis is the superficial fungal infection which mainly affects hair, nail and skin. Dermatophytes produce similar clinical symptoms which are difficult to be distinguished by clinical examination, and these infections are commonly known as tinea infections which are classified based on the site of involvement.[1] There are three ecological group of dermatophytes are known they are anthropophilic, zoophilic and geophilic. Anthropophilic species spreads from human to human and are confined to host. Animals serve as host for zoophilic species and spreads from animals to human. Geophilic species spreads from soil to human.[2] Distribution of the dermatophytes are depends on the geographic location, migration, climate condition, Immune status of the host, socioeconomic condition and age.[3,4] This superficial fungal infection is caused by three genera namely *Trichophyton, Microsporon* and *Epidermophyton;* of these *Trichophyton* is most common, which has 15 species and many verities found within the species.[1] *Trichophyton* has considerable variation in their morphology as well as in geographical distribution. *T. rubrum* can be seen in many part of the world whereas *Trichophyton violaceum,* and *T. soudanense* found in trans-Sudan belt and central African regions.[1,5,6]

Siddis are the tribal group having African ancestry brought to India during the 16th – 19th century by Portuguese and residing in Indian states like Gujarat, Karnataka, Andhra Pradesh, and Telangana.[7,8] In Karnataka Siddis are distributed in Uttar Kannada District, although Siddi are residing in India since many generations and adapted to local and social lifestyle they form separate physical and genetic makeup compared to native Indians.[9,10] Several previous study suggested that Siddi genomes have closest relation to the Africans.[7,8] Few literature have shown the existence of rare geographically restricted African origin dermatophytes in the community.[9,10] In the present study we screened Siddi tribal population to see the presence of rare geographically restricted dermatophytes.

METHODS

The study was conducted during 2015 to 2017 in north Karnataka region with the approval from institutional ethical committee (KLE/Ethic/2015-16/D-51). A total of 1004 samples were collected from 937 Siddi tribal population with suspected superficial fungal infection with required clinical details. Further all samples were subjected to direct microscopy by using 10% potassium hydroxide (KOH) and inoculated on to Sabouraud dextrose Agarose (SDA) (Hi-media) with cycloheximide (0.5% 0.5 mg/ml), chlortetracycline (0.1%0.1 mg/ml) and Gentamicin (0.1% 0.1 mg/ml). Inoculated plates were incubated for 4 weeks after inoculation before designating the sample as no growth at 28°C. Identification of dermatophytes were done on the basis of Macroscopic and Microscopic features and urease hydrolysis.

DNA Extraction, PCR and Sequencing

Genomic DNA was extracted by phenol-chloroform isoamyl alcohol method as previously described (15) and eluted with 50 µl Tris-EDTA buffer. Amplification was carried out by polymerase chain reaction (PCR) using internal transcribe spacer (ITS); ITS 1 and ITS 4 primer (ITS1, 5' TCCGTAGGTGAACCTTGCGG 3', and ITS 4, 5' TCCTCCGCTTATTGATATGC 3'), amplification was done with final volume of 50 µl containing 0.5 µg of template DNA, 20 µl of Emerald Amp GT PCR Master Mix (2X premix composed of a DNA polymerase, optimized reaction buffer, dNTPs, and a density reagent) 15 pmol of each primer. PCR was performed in a thermo cycler (Eppendorf) with initial denaturation of 94°C for 6 minutes followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute 30 second and final extension at 72°C for 10 minutes. Separation of PCR products were carried out on 1.5% agarose gel stained with ethidium bromide followed by visualized in UV transilluminator and imaged. PCR product were subjected to sequencing both strands using ITS 1 and ITS 4 primer and Big Dye Terminator Cycle sequencing kit version 3.1 (Applied Biosystems), ABI 3130 genetic analyzer (Applied Biosystems) and analysis of all sequencing reaction. Sequences were compared with the GenBank DNA database using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov), the ISHAM ITS database (http://its.mycologylab.org/BioloMICSSequences.aspx), and the CBS database (http://www.westerdijkinstitute.nl/Collections/BioloMICSSequences.aspx).

Phylogenetic Analysis

Phylogenetic analysis of standard sequences retrieved from NCBI and study isolates were carried out by aligning sequences using multiple sequence alignment mode in ClustalX2 software. The aligned sequences were exported to Molecular Evolutionary Genetics Analysis software version 7 (MEGA7).[11] Neighbor joining tree was constructed with 1000 bootstrapping replicates by using Kimura 2 parameter model

RESULTS

A total of 937 patients were screened for superficial fungal infection, a total of 102 samples showed positive by both microscopy and culture from 90 patients for dermatophytes. Dermatophytes isolated are *T. mentagrophytes* [69 (67.64%)], *T. rubrum* [24 (25.49%)], *Trichophyton tonsurans* [3 (2.94%)], *Microsporum gypseum* [3 (2.94%)] and *Trichophyton terrestre* [1 (0.98%)]. In the present study two isolates have shown the macroscopic and macroscopic feature of dermatophyte of African origin, isolated from nine year old child who was suffering from tinea capitis since one month had history of soil and animal contact, lesions were spreading without any erythema and a fourteen year old girl with tinea unguium since one month with history of soil and animal contact. Both patients did not have history of antifungal treatment as well as self-medication.

Mycological Characters

One isolate shown slow growing growth after 9 days the colonies were waxy glabrous white without any macroconidia and microconidia the colony characters and microscopy were overlapping with the *T. rubrum*. Another strain was also slow growing, colony was flat and whitish suede-like texture later colony turned to yellow-orange in colour and microscopically no conidia, reflective branching was seen rarely.

PCR and Its Sequencing

Two phenotypically suspected dermatophytes of African species were sequenced and identified as *T. rubrum* (accession no: MT280227 and MT280226).

Phylogenetic analysis was done with the study isolates and standard CBS stains retrieved from the NCBI database [*T. violaceum* CBS 374.92 (NR_144901), *T. soudanense* IHEM 19751 (NR_155948) and *T. rubrum* CBS 392.58 (MH857821)] T. Mentagrophytes (KY761968) was taken as out-group. Both the isolate clustered with CBS reference strain *T. rubrum*.

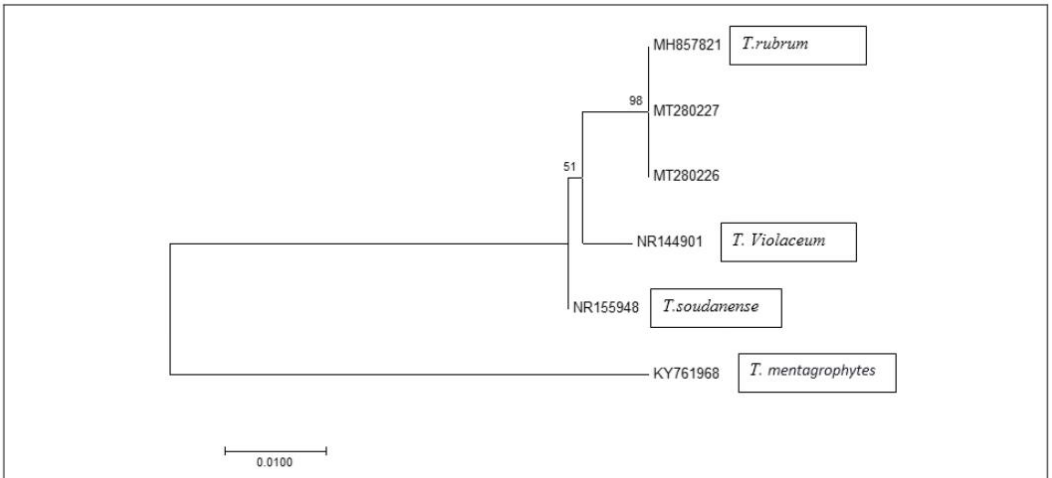

Figure 1. Neighbour–Joining (NJ) derived dendrogram of Internal Transcribe Spacer (ITS) gene-based sequences. Phylogenetic analysis was done with the study isolates and standard CBS stains retrieved from the NCBI database. (Trichophyton violaceum CBS 374.92 (NR_144901), Trichophyton soudanense IHEM 19751 (NR_155948), and Trichophyton rubrum CBS 392.58 (MH857821). Trichophyton mentagrophytes (KY761968) was taken as out-group. All isolates are clustered with T. rubrum

DISCUSSION

Siddi community is the tribal community residing in many parts of India who has African origin and residing in India since more than five generations.[9,12] Investigators in India have shown the presence of rare dermatophytes of African species in the Siddi tribal community. Hemashettar and Nadigir isolated *T. soudanense* in 1980 and in 1993 Hemashetter isolated *Trichophyton yaoundei* in the community.[9,10] Few literature around the world have shown the presence of such infection in the native and African origin population.[5,6,13–16] To see the presence of such rare dermatophytes of African species we have screened more than 2000 Siddi community persons residing in the north Karnataka region of India. Out of 1004 samples 102 samples have grown dermatophytes on culture media with prevalence of 9.6%. Tinea unguium (32%) was the most prevalent infection seen in the community followed by tinea corporis (27.45%), tenia capitis (25.49%), tinea cruris (13.72%) and tinea pedis (0.98%).

T. mentagrophytes was the commonest etiological agent seen in 67.64% followed by *T. rubrum* (25.49%) to cause dermatophytosis, whereas two dermatophytes which have shown phenotypic characters of dermatophytes of African origin like *T. violaceum* and *T. soudanense* from two different patients with tinea capitis and tinea unguium respectively were identified as *T. rubrum*. both isolates were slow growing without any macro or micro conidia. Studies have shown the emergence of *T. violaceum* in the population of native local origin in India as well as in different parts of world.[17–20] even though it is considered as dermatophyte of African Species,[5,6,13,14] whereas many studies have shown *T. soudanense* is isolated in migrated African community in many parts of the world and rarely infecting the people of native origin.[6,13,14] In an attempt to isolate *T. violaceum*, and *T. soudanense* in Baltimore, Maryland, Shelley S. Magill et al have reported 88% of *T. violaceum*, and *T. soudanense* infection in migrated population of eastern Africa and west Africa living in America since three months to five years and 12% of infection U S origin population.[14] In a retrospective study conducted by Konstantin V. Grigoryan et al and Elizabeth Gaviria Morales et al in Rochester, Minnesota, United States and Southern Switzerland respectively to observe the presence of *T. violaceum*, and *T. soudanense* have reported the infection in 82.7% of Africans migrated to America, 4.93% in African Americans and 3.7% in White population in Rochester, Minnesota, United States, whereas in southern Switzerland 77.27% *T. violaceum* infection was seen in African patients and 15.90% infection in originally from Switzerland.[5,6] In the present study we have not found any such rare dermatophyte of African species. The possible reason for the decreased

prevalence or absence of rare exotic dermatophytes of African origin in Siddi community, a migrated African population might be due to their existence in India since more than five-generation,[7] whereas others researchers have reported infection with dermatophyte of African species in the African origin population who are residing since one or two-generation.[5,6,13]

A study conducted by Danielle Marcoux et al in 2018 have showed there was six fold rise in dermatophytes of African species in children of African origin residing in Canada since one or two generation.[13] A detailed study on this African origin population residing in other parts of India may reveal the presence geographically restricted dermatophytes. Antifungal susceptibility testing of isolates may also reveal the sensitivity pattern against antifungal drugs, and helps the clinician to treat dermatophytosis infection in such particular area.

In dermatophytes, ITS region of rDNA is used for species identification as well as for understanding phylogenetic relationship between the species.[21] This study confirms phenotypically identified dermatophyte of African species as *T. rubrum*, based on ITS region sequencing. Presence of these African anthropophilic dermatophyte species was observed in migrated African population in many other previous studies around the world.[5,13,15] Whereas our study revealed absence of such rare exotic dermatophyte and *T. mentagrophytes* complex as prevalent etiological agent to cause dermatophytosis in Siddi tribal community as they stay in India since five generation and adapted to local and religious lifestyle.

CONCLUSIONS

Presence of rare African species was not seen in the migrated Siddi tribal community may be due to the existence of community in India for more than 5 generations and have adapted to local social and religious practices.

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SUSCEPTIBILITY

MIC and Upper Limit of Wild-Type Distribution for 13 Antifungal Agents against a *Trichophyton mentagrophytes-Trichophyton interdigitale* Complex of Indian Origin

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ABSTRACT Dermatophytosis due to the *Trichophyton mentagrophytes-Trichophyton interdigitale* complex is being increasingly reported across India. Reports of therapeutic failure have surfaced recently, but there are no clinical break points (CBP) or epidemiological cutoffs (ECVs) available to guide the treatment of dermatophytosis. In this study, a total of 498 isolates of the *T. mentagrophytes-interdigitale* complex were collected from six medical centers over a period of five years (2014 to 2018). Antifungal susceptibility testing of the isolates was carried out for itraconazole, fluconazole, ketoconazole, voriconazole, luliconazole, sertaconazole, miconazole, clotrimazole, terbinafine, amorolfine, naftifine, ciclopirox olamine, and griseofulvin. The MICs (in mg/liter) comprising >95% of the modeled populations were as follows: 0.06 for miconazole, luliconazole, and amorolfine; 0.25 for voriconazole; 0.5 for itraconazole, ketoconazole, and ciclopirox olamine; 1 for clotrimazole and sertaconazole; 8 for terbinafine; 16 for naftifine; 32 for fluconazole; and 64 for griseofulvin. A high percentage of isolates above the upper limit of the wild-type MIC (UL-WT) were observed for miconazole (29%), luliconazole (13.9%), terbinafine (11.4%), naftifine (5.2%), and voriconazole (4.8%), while they were low for itraconazole (0.2%). Since the MICs of itraconazole were low against the *T. mentagrophytes-interdigitale* complex, this could be considered the choice of first-line treatment. The F397L mutation in the squalene epoxidase (SE) gene was observed in 77.1% of isolates with a terbinafine MIC of ≥1 mg/liter, but no mutation was detected in isolates with a terbinafine MIC of <1 mg/liter. In the absence of CBPs, evaluation of the UL-WT may be beneficial for managing dermatophytosis and monitoring the emergence of isolates with reduced susceptibility.

KEYWORDS MIC, UL-WT, antifungal resistance, dermatophytes, *Trichophyton mentagrophyte*

An indisputable rise in the prevalence of dermatophytosis with an alarming rise in chronic, recalcitrant, relapse, and recurrent cases has been seen in India over the past few years (1–3). The main etiological agents implicated in these infections are members of three different genera, namely *Trichophyton*, *Microsporum,* and *Epidermophyton*, with various species within each genus (4). In contrast to the past, wherein *T.*

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rubrum was the most predominant among the *Trichophyton* species, a notable rise in the incidence of *T. mentagrophytes-interdigitale* complex-associated dermatophytosis has been observed in India in recent times (1, 5). Apart from the changing pathogen epidemiology, several atypical clinical presentations are being reported, perhaps due to the rampant use of corticosteroids and poor compliance to therapy, which results in significant disease-related morbidity in affected patients (4, 6).

In the current clinical scenario, experience-driven consensus guidelines for diagnosing and managing dermatophytosis are needed. Recently, the Expert Consensus on the management of Dermatophytosis (ECTODERM) was published in India to bridge the large void in research related to disease management (3). The current treatment recommendations include the use of terbinafine, itraconazole, sertaconazole, ciclopirox olamine, ketoconazole, naftifine, and newer drugs such as amorolfine and luliconazole, which have shown potent fungicidal activity against *Trichophyton* species (7). However, recent reports of resistance to terbinafine resulting in treatment failure in dermatophytosis caused by species belonging to the *T. mentagrophytes-interdigitale* complex is of great concern to the treating physicians (1, 2, 8). Apart from allylamine resistance, triazole resistance, especially to fluconazole, is high (35.4%) in the Indian isolates, which is also associated with treatment failure (1).

To guide the treatment choice of an appropriate antifungal agent, the Clinical and Laboratory Standards Institute (CLSI) has introduced guidelines for the antifungal susceptibility testing (AFST) for dermatophytes (9, 10). Clinical breakpoints (CBPs), which are based on information derived from clinical studies, help in predicting response to treatment. However, they depend on several factors such as drug MIC, pharmacokinetic (PK)/pharmacodynamic (PD) parameters, treatment outcome in clinical studies, and post-marketing susceptibility data. In view of the lack of CBPs, either the epidemiological cutoff values (ECVs or ECOFFs) or the upper limit of wild-type MIC (UL-WT) may be useful to differentiate between wild type (WT) and non-wild-type (NWT) isolates, as these depend only on the MIC (11). Unfortunately, to date there is no CBP, ECV, or UL-WT established for any of the *Trichophyton* species. Available guidelines for the determination of ECV focus on *Candida*, *Cryptococcus*, and *Aspergillus* species (12). In view of the veritable country-wide epidemic of dermatophytosis predominantly by a single species, the *T. mentagrophytes-interdigitale* complex, and the lack of CBPs, there is an urgent need to define either ECVs or the UL-WT to guide therapy.

In this study, a large collection of *T. mentagrophytes-interdigitale* complex isolates collected from six medical centers in India was tested against 13 antifungal drugs to elucidate the MIC distribution and to define the India-specific UL-WT for each antifungal agent.

RESULTS

The antifungal susceptibility profiles and UL-WT of 13 antifungal drugs against *T. mentagrophytes-interdigitale* complex are depicted in Table 1. The antifungal susceptibility testing (AFST) of all 498 isolates was performed against nine antifungals (fluconazole, itraconazole, voriconazole, clotrimazole, sertaconazole, terbinafine, griseofulvin, amorolfine, and ciclopirox olamine). For AFST to sertaconazole, luliconazole, ketoconazole, and naftifine, a total of 327, 415, 463, and 462 isolates were used, respectively. Among the azoles, itraconazole (geometric mean [GM] 0.08 mg/liter) and miconazole (GM 0.06 mg/liter) exhibited potent activity against the *T. mentagrophytes-interdigitale* complex, whereas among allylamines, terbinafine (GM 0.09 mg/liter) was more potent than naftifine (GM 0.133 mg/liter). Higher MIC_{90} values were observed for fluconazole (16 mg/liter), terbinafine (8 mg/liter), naftifine (8 mg/liter), and griseofulvin (32 mg/liter). The MIC_{90} of ketoconazole, clotrimazole, sertaconazole, and ciclopirox olamine were 0.5 mg/liter. Amorolfine, luliconazole, miconazole, itraconazole, and voriconazole had low MIC_{90} values of 0.06, 0.125, 0.25, 0.25, and 0.25 mg/liter, respectively.

The MIC range, MIC_{50}, MIC_{90}, and GM values for all 13 antifungal drugs against *T. mentagrophytes-interdigitale* complex are summarized in Table 2. Both the 95% and 97.5% MICs were calculated to determine the UL-WT. For the antifungals which showed

TABLE 1 MIC and UL-WT distribution of *T. mentagrophytes-interdigitale* complex isolates against 13 antifungal drugs tested using the CLSI M38-A2 broth microdilution method[a]

Antifungal agent	Total no of isolates	No. of isolates with MIC (mg/liter) of:															UL-WT 95%	UL-WT 97.5%	% NWT 95%	% NWT 97.5%
		0.0078	0.015	0.03	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128				
Miconazole	327			**179**	53	39	33	19	1	2	1						0.06	0.06	29	29
Luliconazole	415			**227**	130	34	13	10	1								0.06	0.125	13.9	5.7
Fluconazole	498		1		2	26	8	24	17	119	**139**	92	37	32	1		32	64	0.2	0
Itraconazole	498	31	47	28	**158**	109	81	42	2								0.5	1	0.2	0
Voriconazole	498	61	17	83	**185**	85	43	18	4	1	1						0.25	0.5	4.8	1.2
Ketoconazole	463	24		34	113	112	**116**	59	3	2							0.5	1	1	0.4
Clotrimazole	498	2	3	34	32	51	**215**	142	16	3							1	1	0.6	0.6
Sertaconazole	498	46		104	58	94	**113**	64	18	1							1	2	0.2	0
Naftifine	462			**247**	55	29	12	18	12	13	24	28	24				16*	16*	5.2	5.2
Terbinafine	498		**209**	88	24	19	17	39	8	18	19	49	6	2			8*	8*	11.4	11.4
Griseofulvin	498						70	9	15	19	29	44	79	**198**	11	24	64*	128*	ND	ND
Amorolfine	498	59	**231**	137	48	8	11	3			1						0.06	0.06	4.6	4.6
Ciclopirox olamine	498			19	2	5	**381**	41	20	30							0.5	0.5	10	10

[a]Most frequently obtained MIC or mode is indicated in boldface type. NWT, non-wild-type; ND, not determined; UL-WT, upper limit of wild-type MIC. The UL-WT for naftifine, terbinafine, and griseofulvin are indicated by * and were calculated based on MIC_{95} and MIC_{97} values.

multimodal, nonsymmetrical, or truncated MIC frequency distribution, both the MIC_{95} and MIC_{97} were documented as UL-WT. The highest 95% UL-WTs were observed for griseofulvin (64 mg/liter) and fluconazole (32 mg/liter), whereas the lowest value of 0.06 mg/liter was found for two azoles (miconazole and luliconazole) and morpholine (amorolfine). High percentages of isolates above the UL-WT were observed for miconazole (29%), luliconazole (13.9%), terbinafine (11.4%), naftifine (5.2%), and voriconazole (4.8%), but low percentages for itraconazole (0.2%), sertaconazole (0.2%), and fluconazole (0.2%).

The phylogenetic analysis by internal transcribed spacer (ITS) dendrogram revealed that all our isolates belong to *T. mentagrophytes* type VIII. Therefore, genotype-based differences in MIC distribution could not be analyzed. Molecular screening for the mechanism of allylamine resistance was performed by sequencing the squalene epoxidase (SE) gene of all isolates with terbinafine at a MIC of ≥1 mg/liter and representative isolates with <1 mg/liter. Of 133 isolates sequenced, the F397L mutation was seen in 74 isolates (55.6%). The MIC range of the isolates with this mutation were between 1 and 32 mg/liter for terbinafine and 0.06 to 16 mg/liter for naftifine. Distribution of the F397L mutation in relation to the terbinafine MICs is provided in Fig. 1. Among 57 isolates with a high terbinafine MIC (≥8 mg/liter), the mutation was observed in 43 isolates (75.4%). Of the 39 isolates with terbinafine MICs between 1 and 4 mg/liter, 31 isolates (79.5%) had the F397L mutation. No mutation was observed in the 37 isolates with MIC values of <1 mg/liter (Fig. 1). While calculating the UL-WT based on the mutation analysis, a lower UL-WT was observed for terbinafine (1 mg/liter versus 8 mg/liter) and naftifine (0.06 mg/liter versus 16 mg/liter) compared to broth microbroth dilution-based results. Thus, the percentage of isolates above the UL-WT was higher both for naftifine (46.5% versus 5.2%) and terbinafine (20.5% versus 11.4%) on mutation analysis versus microbroth dilution method.

DISCUSSION

The testing for susceptibility to any drug is extremely valuable for defining the likely response of infection by a particular organism. CBPs enable us to predict therapeutic outcomes and are based on clinical trial data, global surveys of susceptibility, and PK/PD parameters (13). Either the ECV or the UL-WT offers an alternative for guiding the choice of optimum treatment by virtue of the MIC data. Since neither the CBPs nor the ECVs or UL-WT for *T. mentagrophytes-interdigitale* complex have been defined, we conducted this study to determine the UL-WT to antifungal agents commonly used in the management of dermatophytosis. To the best of our knowledge, this is the first

TABLE 2 Various studies representing the MIC (mg/liter) distribution of *Trichophyton mentagrophytes-interdigitale* complex isolates against antifungal drugs tested using the CLSI M38-A2 broth microdilution method[a]

Reference	Year	Total no. of drugs tested	No. of isolates	Drug	Range	MIC_{50}	MIC_{90}	GM
This study	2020	13	498 (Tm/Ti complex)	Miconazole[b]	0.03–4	0.03	0.25	0.06
				Luliconazole[c]	0.03–1	0.03	0.125	0.05
				Fluconazole	0.0625–64	4	16	3.28
				Itraconazole	0.0078–1	0.06	0.25	0.08
				Voriconazole	0.0078–4	0.06	0.25	0.05
				Ketoconazole[d]	0.0078–2	0.125	0.5	0.120
				Clotrimazole	0.0078–2	0.25	0.5	0.231
				Sertaconazole	0.0078–2	0.125	0.5	0.108
				Naftifine[e]	0.03–16	0.03	8	0.133
				Terbinafine	0.015–32	0.03	8	0.096
				Griseofulvin	0.25–128	16	32	9.27
				Amorolfine	0.0078–4	0.015	0.06	0.024
				Ciclopirox olamine	0.03–2	0.25	0.5	0.289
Rudramurthy et al. (1)	2018	12	88 (Ti)	Fluconazole	2–32	4	16	5.03
				Ketoconazole	0.0625–2	0.125	0.5	0.17
				Sertaconazole	0.03–1	0.125	0.5	0.13
				Clotrimazole	0.125–2	0.25	0.5	0.36
				Voriconazole	0.0312–2	0.125	0.5	0.12
				Itraconazole	0.15–8	0.125	0.5	0.13
				Terbinafine	0.015–32	0.0312	4	0.06
				Naftifine	0.0312–16	0.0312	8	0.1
				Amorolfine	0.007–4	0.0156	0.0625	0.02
				Ciclopirox olamine	0.25–0.5	0.25	0.25	0.25
				Griseofulvin	2–128	32	64	26.31
				Luliconazole	0.0312–0.25	0.0312	0.125	0.05139
Pathania et al. (15)	2018	4	36 (Tm)	Griseofulvin	0.5–128	16	128	
				Fluconazole	0.12–32	4	16	
				Terbinafine	0.015–8	0.125	8	
				Itraconazole	0.015–1	0.063	0.5	
Khurana et al. (8)	2018	11	64 (Ti)	Terbinafine	0.25–≥32	1	32	2.813
				Itraconazole	0.06–≥16	0.25	1	0.287
				Fluconazole	0.5–≥64	16	32	13.205
				Voriconazole	0.06–2	0.25	0.5	0.202
				Ketoconazole	0.25–≥32	0.5	0.5	0.666
				Amphotericin B	0.25–1	0.5	0.5	0.38
				Griseofulvin	0.5–≥8	2	4	2.874
				Miconazole	0.25–≥16	2	4	1.978
				Econazole	0.5–8	2	4	1.836
				Luliconazole	0.0035–0.125	0.007	0.007	0.005
				Sertaconazole	0.25–≥16	1	2	0.842
Rezaei-Matehkolaei et al. (34)	2018	6	66 (Ti)	Efinaconazole	0.002–0.006	0.008	0.016	0.008
				Lanoconazole	0.001–0.008	0.002	0.004	0.002
				Luliconazole	0.0002–0.004	0.0005	0.001	0.0006
				Fluconazole	4–64	8	16	11.07
				Itraconazole	0.03–0.5	0.12	0.25	0.111
				Terbinafine	0.004–0.12	0.015	0.03	0.013
Salehi et al. (19)	2018	8	27 (Ti)	Terbinafine	0.003–0.125	0.01	0.06	0.01
				Griseofulvin	0.03–64	0.12	35.2	0.41
				Itraconazole	0.01–4	0.06	1.3	0.07
				Voriconazole	0.01–16	0.37	8.8	0.41
				Ketoconazole	0.03–4	0.25	2.2	0.32
				Econazole	0.03–0.5	0.06	0.5	0.08
				Lanoconazole	0.03–0.5	0.06	0.5	0.09
				Butenafine	0.03–0.5	0.06	0.5	0.09

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TABLE 2 (Continued)

Reference	Year	Total no. of drugs tested	No. of isolates	Drug	Range	MIC_{50}	MIC_{90}	GM
Singh et al. (2)	2018	11	63 (Ti)	Terbinafine	0.06–32	1	32	2.7
				Itraconazole	0.06–16	0.5	2	0.51
				Voriconazole	0.06–16	0.25	2	0.32
				Fluconazole	0.5–64	32	64	16.7
				Luliconazole	0.003–0.5	0.015	0.06	0.014
				Sertaconazole	0.125–16	2	16	2.3
				Miconazole	0.5–16	2	8	2.44
				Ketoconazole	0.125–64	1	32	1.37
				Clotrimazole	0.008–32	4	4	2.83
				Amphotericin B	0.25–8	0.5	1	0.56
				Griseofulvin	1–32	4	8	4.09
Sadeghi-Nejad et al. (20)	2017	2	3 (Tm)	Ketoconazole	0.78—6.25	1.56	6.25	3.52
				Griseofulvin	12.5—100	25	100	56.25
Baghi et al. (22)	2016	12	52 (Ti)	Luliconazole	0.016–0.032	0.016	0.032	0.02
				Itraconazole	0.031–0.5	0.25	0.5	0.18
				Micafungin	0.5–8	4	8	3.31
				Fluconazole	2–64	32	64	20.8
				Griseofulvin	0.5–4	1	2	0.98
				Terbinafine	0.008–0.125	0.063	0.25	0.071
				Lanoconazole	0.063–0.5	0.25	0.25	0.17
				Econazole	0.031–0.5	0.5	0.5	0.3
				butenafine	0.031–1	0.25	1	0.26
				Caspofungin	0.008–0.032	0.016	0.032	0.016
				Anidulafungin	0.008–0.016	0.008	0.016	0.01
				Tolnaftate	0.008–0.125	0.063	0.25	0.076
Ansari et al. (35)	2015	4	156 (Ti)	Fluconazole	4–128	32	64	27.47
				Itraconazole	0.008–0.25	0.063	0.125	0.062
				Terbinafine	0.004–0.25	0.016	0.125	0.017
				Griseofulvin	0.25–4	1	4	0.93
Badali et al. (36)	2015		13 (Tm)	Amphotericin B	0.125–1	1	2	0.68
				Fluconazole	16–64	32	64	41.77
				Itraconazole	0.5–16	2	16	2.11
				Voriconazole	0.25–2	1	2	1.41
				Posaconazole	0.5–1	1	1	0.85
				Isavuconazole	0.25–2	1	2	0.94
				Caspofungin	0.5–2	1	2	1.05
				Anidulafungin	0.008–0.063	0.031	0.063	0.03
				Terbinafine	0.016–0.31	0.031	0.031	0.03
Adimi et al. (30)	2013	10	136 (Tm)	Terbinafine	0.0156–16	0.0312	16	0.093
				Griseofulvin	0.0312–56	2	256	2.31
				Itraconazole	0.0009–4	0.0625	0.5	0.035
				Ketoconazole	0.0312–32	2	8	0.43
				Fluconazole	0.0625–256	64	256	10.44
				Voriconazole	0.0156–8	0.5	4	0.174
				Clotrimazole	0.0312–32	0.125	2	0.33
				Ciclopirox olamine	0.0312–32	2	16	1.81
				Amorolfine	0.0078–32	0.25	8	0.245
				Naftifine	0.0625–6	0.25	12	0.326
Yenişehirli et al. (37)	2013	6	49 (Tm)	Terbinafine	0.015–0.125	0.06	0.125	0.06
				Amphotericin B	0.03–2	0.125	0.5	0.14
				Miconazole	0.03–2	0.125	2	0.16
				Itraconazole	0.03–0.5	0.25	0.5	0.13
				Ketoconazole	0.03–4	0.25	1	0.2
				Griseofulvin	0.03–16	0.5	2	0.49

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TABLE 2 (Continued)

Reference	Year	Total no. of drugs tested	No. of isolates	Drug	Range	MIC_{50}	MIC_{90}	GM
Carrillo-Muñoz et al. (21)	2013	10	19 (Ti)	Amorolfine		0.015	0.062	
				Bifonazole		0.015	16	
				Clotrimazole		0.015	1	
				Econazole		0.015	1	
				Ketoconazole	0.015	4		
				Miconazole		0.015	4	
				Oxiconazole		0.015	4	
				Sertaconazole	0.015	1		
				Ticonazole		0.015	8	
				Terbinafine		0.015	0.5	
			26 (Tm)	Amorolfine		0.015	0.031	
				Bifonazole		0.061	16	
				Clotrimazole		0.015	1	
				Econazole		0.015	2	
				Ketoconazole	0.015	0.5		
				Miconazole		0.015	8	
				Oxiconazole		0.031	2	
				Sertaconazole	0.031	16		
				Ticonazole		0.015	0.5	
				Terbinafine		0.015	0.015	
Silva et al. (38)	2014	6	24 (Ti)	Ketoconazole	0.031–16	0.25	2	0.46
				Fluconazole	2–64	8	64	5.9
				Griseofulvin	0.125–64	2	32	1.6
				Itraconazole	0.0312–2	0.125	0.5	0.19
				Terbinafine	0.031–2	0.0312	0.0312	0.03
				Voriconazole	0.0312–1	0.062	0.5	0.24
Ataides et al. (39)	2012	4	7 (Tm)	Itraconazole	0.125–16	0.25	16	0.41
				Ketoconazole	0.5–4	0.5	4	1.48
				Griseofulvin	1–8	1	8	0.61
				Terbinafine	0.015–0.062	0.015	0.062	0.01
Zalacain et al. (40)	2011	5	29 (Tm)	Ciclopirox olamine	0.032–0.500	0.25	0.5	0.18
				Fluconazole	32	-	-	ND
				Itraconazole	0.032–1.000	0.5	1	0.35
				Terbinafine	0.016–0.500	0.125	0.5	0.082
				Eberconazole	0.016–1.000	0.25	1	0.18
Bueno et al. (41)	2010	4	18 (Tm)	Itraconazole	0.03–0.5	0.25	0.5	0.2
				Fluconazole	16–64	64	64	50.37
				Terbinafine	0.007–0.06	0.015	0.03	0.014
				Voriconazole	0.03–0.5	0.125	0.5	0.19
Mota et al. (42)	2009	5	14 (Tm)	Fluconazole	4–16	16	16	
				Itraconazole	0.03–0.25	0.125	0.25	
				Ketoconazole	0.03–1	0.125	0.25	
				Terbinafine	0.03–0.5	0.06	0.25	
				Griseofulvin	0.25–1	0.5	0.5	
Valverde et al. (43)	2008	3	30 (Tm)	Itraconazole		0.25	0.5	
				Fluconazole		16	64	
				Voriconazole		0.12	0.25	

[a]GM, geometric mean; Ti, *Trichophyton interdigitale*; Tm, *Trichophyton mentagrophytes*.
[b]327 isolates screened.
[c]415 isolates screened.
[d]463 isolates screened.
[e]462 isolates screened.

multicenter study to propose the UL-WT for the *T. mentagrophytes-interdigitale* complex isolates of Indian origin.

An alarming rise in prevalence and incidence of dermatophytosis has been observed across India (14). Recent reports indicate an epidemic-like situation, with the chief

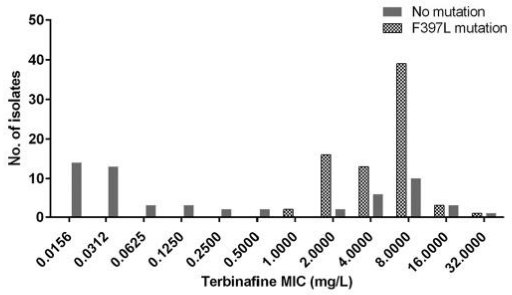

FIG 1 Relationship between mutation in the squalene epoxidase gene and the MIC for terbinafine in 133 *T. mentagrophytes-interdigitale* complex isolates.

etiological agent being *T. mentagrophytes* followed by *T. rubrum* (1, 2, 15). *T. mentagrophytes* is a complex group containing different species. The taxonomy of this species is still not well delineated. Based on the ITS sequences, two major studies from India report that the *T. mentagrophytes* complex causing the epidemic in India belongs to *T. interdigitale* (1, 2). Later, on the basis of multigene sequences like ITS, beta tubulin, translation elongation factor, and calmodulin gene, all the *T. mentragrophytes* complex strains of India were found to be distinct from other clades and were described as *T. mentragrophytes* type VIII. The whole-genome sequence of Indian isolates shares similarity with the recent common ancester, *T. mentagrophytes-interdigitale* complex, and these could not be conclusively differentiated as two species (16). Thus, we characterized all of our isolates as *T. mentagrophytes-interdigitale* complex to preclude any misidentification (1, 16–18). Experts recommend that accurate identification of the implicated fungal pathogen and its AFST may be necessary for the management of the recurrent, relapse, or chronic cases (3). Six medical centers from both North India and South India participated in the present study and a large number of *T. mentagrophytes-interdigitale* complex isolates were evaluated against 13 antifungal agents. The MICs obtained in this study compared with the results of previous studies are provided in Table 2. Previously reported MIC_{50} ranges for *T. mentagrophytes-interdigitale* of various antifungal drugs were similar to those observed in the present study (8, 19–22). The MIC distributions of itraconazole, miconazole, luliconazole, voriconazole, ketoconazole, sertaconazole, naftifine, ciclopirox olamine, and clotrimazole show good susceptibility of *T. mentagrophytes-interdigitale* complex compared to terbinafine, fluconazole, and griseofulvin. Recent reports in Indian literature have revealed the high MIC of terbinafine for *Trichophyton* spp. (1, 2). Clinical evidence of relapse and incomplete mycological cure after standard (250 mg, twice daily for 2 weeks) oral terbinafine therapy have also been reported (23). Since the pharmacodynamic properties of terbinafine remain incompletely described, a cumulative percentage ($\%T_{>MIC}$) of 100% was used as a conservative approach to describe its dosing interval in an animal study (24). According to that study, the use of 250 mg of terbinafine twice daily was appropriate for treatment of dermatophytic infections caused by *T. mentagrophytes* with a MIC of 0.01 mg/liter (24). However, since the tissues infected by dermatophytes are avascular components of the skin and adenexa, the time to attain therapeutic concentrations in them may differ greatly from plasma (24). In the present study, we observed that >40% of the *T. mentagrophytes-interdigitale* complex isolates had a terbinafine MIC of >0.01 mg/liter. A higher dose with a multidosing strategy may be required to treat infections by *T. mentagrophytes-interdigitale* complex isolates with higher MICs. Unfortunately, this may not be clinically practical due to the possibility of drug-related side effects. These findings emphasize that the use of terbinafine as the first line drug against dermatophytic infections is largely unsubstantiated. In view of the contemporary disease epidemiology, it may have lost clinical efficacy against Indian *T.*

mentagrophytes-interdigitale complex and requires further evaluation. However, although serum levels of terbinafine follow a predictive PK/PD, these levels may not parallel the site-specific drug levels. In fact, some studies show that the skin levels of terbinafine exceed trough plasma levels by nearly 10 to 40 times the MIC, indicating little rationale for higher doses (25).

In clinical practice, CBPs are needed to optimize appropriate antifungal treatment. However, CBPs are not defined for any antifungal agent against dermatophytes. In such a scenario, ECVs may help in identifying isolates with antifungal resistance, as has been seen for other molds such as *Aspergillus* spp., Mucorales, and *Sporothrix* spp. (13, 26–28). According to CLSI guidelines, the evaluation of ECVs should follow certain criteria, i.e., (i) the MIC should be determined as per standard guidelines, either by following the protocol of CLSI or the European Committee on Antimicrobial Susceptibility Testing (EUCAST); (ii) large numbers of isolates ($n \geq 100$) should be included; (iii) the MIC of isolates from different laboratories (≥ 3) should be used; and (iv) the AFST should be performed by many investigators (≥ 3) (13). Our study fulfils the above criteria (except for partial fulfillment of the last criteria), and thus we propose the UL-WT instead of an ECV for the *T. mentagrophytes-interdigitale* complex of Indian origin against the antifungal agents in Table 1. For UL-WT determination, isolate identification to the species level is important according to CLSI guidelines (12). In our study, three antifungal agents (terbinafine, naftifine, and griseofulvin) had nonsymmetric, multimodal, or truncated MIC distribution due to which the ECOFF could not be determined using the software. Thus, the UL-WT (in mg/liter) calculated based on the MIC_{95} (27) for terbinafine, naftifine, and griseofulvin was 8, 16, and 64, respectively. Interestingly, the mutation analysis of the SE gene revealed that even isolates with MICs at less than 1 mg/liter for terbinafine and 0.06 mg/liter for naftifine (both allylamines) exhibited the F397L mutation that was absent in all isolates with MICs of <1 mg/liter for terbinafine and 0.03 mg/liter for naftifine. This finding suggests that the UL-WT of these drugs is probably lower than that estimated based on MIC_{95} and MIC_{97}. These findings substantiate our interpretation that the use of terbinafine for first-line management of *T. mentagrophytes-interdigitale* complex infections needs further evaluation. Overall, we noted low MIC and UL-WT values for miconazole, luliconazole, itraconazole, voriconazole, and ketoconazole. Itraconazole has also been found to have a lower MIC than terbinafine against a majority of *T. mentagrophytes* complex isolates and the use of itraconazole as a first line drug in the management of dermatophytosis may be beneficial (29).

The major limitation of this study was that the majority of the isolates were from a single center, the Postgraduate Institute of Medical Education and Research (PGIMER). Also, AFST was performed at a single institute (PGIMER) independently by laboratory persons belonging to all 6 centers. Another concern is that being a tertiary care center, most patients presenting to us might have prior exposure to antifungals, antibiotics, and/or steroids. This could be responsible for the high MIC values to some antifungals (terbinafine, griseofulvin, and fluconazole) and the UL-WT may artificially encompass resistant (non-wild-type) isolates. Additionally, using the Indian *T. mentagrophytes-interdigitale* complex to determine the UL-WT may have limited global applicability, as some antifungals have been shown to have high UL-WT values compared to global data. Thus, future multicentric studies involving isolates with more geographical diversity must be designed to further define the ECVs or UL-WT. The inclusion of isolates belonging to different genotypes from diverse geographic locations will enable the analysis of genotype/clade-related differences in MIC distribution, which could not be performed in the present study. The lack of facilities for AFST of dermatophytes at most Indian institutes limited the testing of isolates at more than one center in the present study. Regular training programs and capacity building at multiple centers could enable the determination of ECVs and CBPs in future studies.

In conclusion, UL-WT values are an important tool for distinguishing WT from NWT isolates and provide a preliminary idea to the treating physician in optimizing antifungal therapy. However, the UL-WT values are based solely on the *in vitro* data of isolates

collected from various centers and are not an interpretative breakpoint. Nonetheless, since breakpoints are not available for dermatophyte infections, these values can assist the clinician to predict whether an isolate is likely to respond to a specific antifungal. Future studies assessing the clinical response to treatment and monitoring the PK and PD of antifungal therapy could be beneficial in establishing CBPs.

MATERIALS AND METHODS

Isolates. A total of 498 clinical isolates of *T. mentagrophytes-interdigitale* complex were collected over a period of 5 years (2014 to 2018) from six tertiary care hospitals in India. All clinical isolates were recovered from skin ($n = 481$), hair ($n = 13$), and nail ($n = 4$) samples at the following medical centers: Postgraduate Institute of Medical Education and Research, Chandigarh ($n = 354$), Government Medical College Haldwani, Nainital ($n = 44$), Jawaharlal Nehru Medical College, Belagavi, Karnataka ($n = 41$), Father Muller Medical College, Mangalore ($n = 32$), Bhasker Medical College & General Hospital, Telangana ($n = 15$), and St. John's Medical College & Hospital, Bangalore ($n = 12$). In cases of multiple isolates from the same patient, only unique clinical isolates were used. The identification of all strains as *T. mentagrophytes-interdigitale* complex was based on culture morphology and microscopic characteristics and confirmed by sequencing of the internal transcribed spacer (ITS) region and 28S region of ribosomal DNA, and beta tubulin (1, 16, 17).

Antifungal susceptibility testing. The AFST of all the isolates was performed at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, and the tests were performed independently by laboratory personnel of respective institutes contributing the isolates. The strains were subcultured on potato dextrose agar (PDA) medium prior to AFST. The broth microdilution technique was performed according to Clinical and Laboratory Standards Institute (CLSI) M38 A2 protocol with minor modifications, as previously described (1, 10, 30). Inoculum suspension with approximately 10^6 CFU/ml conidia was collected from 7 to 14 days culture grown on PDA and quantified microscopically by hemocytometer. The suspension was then diluted to 1:100 according to primary concentration. Double the final concentration (10^3 CFU/ml) of the conidia was adjusted before adding to the drug plates. An initial inoculum corresponding to 65 to 70% transmittance at 530 nm in a spectrophotometer was used. Inoculated plates were incubated at 28°C for 4 days prior to interpretation. In all the isolates, the MICs of azoles, griseofulvin, and amorolfine were documented as the concentration showing prominent inhibition of growth (approximately 80%) compared to that in growth-control wells. For terbinafine, naftifine, luliconazole, ciclopirox olamine, and miconazole, a 100% growth inhibition was documented, as described by Rudramurthy et al. (1). To check the purity of the inoculated plates, 10 μl of the growth from the growth-control well was inoculated onto Sabouraud dextrose agar (10). Panels of 13 topical or systemically applied antifungals commonly used for the treatment of dermatophytosis were tested. The panel included azoles (fluconazole, voriconazole, itraconazole, ketoconazole, sertaconazole, luliconazole, clotrimazole, and miconazole), allylamines (terbinafine and naftifine), a morpholine (amorolfine), an oxaborole/hydroxamic acid (ciclopirox olamine), and an antifungal antibiotic (griseofulvin), all purchased from Sigma-Aldrich, Bengaluru, India. The drugs were dissolved in dimethyl sulfoxide and the final concentrations (in mg/liter) ranged from 0.0625 to 32 for fluconazole; 0.0078 to 4 for voriconazole, itraconazole, and amorolfine; 0.0312 to 16 for ketoconazole, luliconazole, sertaconazole, clotrimazole, ciclopirox olamine, and naftifine; 0.0156 to 64 for terbinafine; 0.0156 to 8 for sertaconazole; and 0.25 to 128 for griseofulvin. *T. mentagrophytes-interdigitale* complex (NCCPF 800035), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), and *Aspergillus flavus* (ATCC 204304) strains were used as quality control measures. The AFST was performed in triplicate for each isolate to ensure quality and reproducibility.

Distribution of MIC and UL-WT determination. The data analysis was performed using an Excel 2018 spreadsheet. The MIC_{50} and MIC_{90} were obtained by a descriptive statistics analysis. In statistical analysis, the modeled population was established based on fitting a normal distribution at the lower end of the MIC and the mean and standard deviation of the normal distribution were calculated to determine the MIC capturing at least 95%, 97.5%, and 99% of the modeled WT population. The Microsoft Excel spreadsheet calculator (ECOFFinder program version XL2000+, http://www.eucast.org/mic_distributions_and_ecoffs/) was used for statistical determination of the UL-WT for the 13 antifungal drugs (26, 31, 32).

The isolate with no mechanisms of acquired resistance or mutational resistance for the antifungal agent tested is defined as the WT. The NWT isolates are those with presumed or known mechanisms of resistance for the antifungal agent being tested. The isolates were classified as WT or NWT depending on whether the MIC was ≤UL-WT or >UL-WT for the above-mentioned antifungals (26, 31, 32).

Phylogenetic analysis. Phylogenetic analysis was performed using the ITS sequences of representative isolates of the present study and sequences of all genotypes described previously (sequences retrieved from NCBI database) (33). *T. quinckeanum* was used as the outgroup due to its high divergence. Sequences were aligned using the multiple sequence alignment mode in ClustalX2 software. The aligned sequences were exported to Molecular Evolutionary Genetics Analysis software version 7 (MEGA 7) and a neighbor joining tree was constructed with 1,000 bootstrapping replicates by using Kimura 2 parameter model.

Mutation analysis for terbinafine. A total of 133 isolates were screened for mutation in the squalene epoxidase gene. The complete gene was sequenced by using the primer pairs SE1aF-5′-CAG AGATAATGCAGCCATCG-3′ and SE1aR-5′-CCGGATTGATGTTCCTAGGT-3′; SE2aF-5′-CCACCAGCGGCGAAT ATAGA-3′ and SE2aR-5′-AGTCCAGTGCCAGACTGATG-3′; and SE3aF-5′-AGTCTGGCACTGGACTCCAA-3′ and SE3aR-5′-ATGATGCAGCGACGGTGACA-3′ (Sigma) as described earlier (1). Bionumerics software

(Applied Maths, Ghent, Belgium) was used for consensus and concatenation of the sequences. The sequences and amino acid sequences depicted by using the ExPASy online tool (https://web.expasy.org/translate/) were aligned.

Data availability. Sequences of the representative isolates have been deposited in GenBank under accession numbers MH517546 to MH517560, MN822738 to MN822771, MN824042 to MN824085, MN830960 to MN831002, MN830945 to MN830959, and MN831003 to MN831103 for the ITS region of ribosomal DNA; MK967531 to MK967551 for the 28S region of ribosomal DNA; MK982906 to MK982926 for BT; and MN836335 to MN836372 for SE.

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Isolation and Molecular Analysis of *Cladosporium Species* from Siddi Tribal Community Residing in North Karnataka Region, India

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Abstract

Dermatophytes are the common etiological agent to cause superficial mycosis, however non-dermatophytes like yeast and non dermatophyte mould (NDM) are emerging as potential agent to cause superficial mycosis. In this study, we evaluated epidemiological, clinical and mycological characters of non-dermatophytes causing superficial infection in the Siddi tribal community. A total of 1004 samples were collected from 937 Siddi community patients with superficial infection. In that 158 samples shown culture positive, *Candida species* (32, 20.25%) was the predominant agent to cause superficial infection other than dermatophytes followed by *Aspergillus species* (12, 7.59%), *Fusarium species* (10, 6.32%) and *cladosporium species* (2, 1.26%). Further, *Cladosporium species* isolated from tineacapitis infection was subjected to PCR, sequencing of ITS region and identified as *Cladosporium halotolerans*. Therefore, this study also revealed non dermatophytic fungi are emerging as important cause of superficial infection.

Key words: *Dermatophytes, Mould, Yeast, Trichophyton, Cladosporium, Onychomycosis and Tinea, Polymerase chain Reaction (PCR)*

Introduction

Superficial mycosis is the fungal infection of skin and its appendages like nail and hair, it is apublic health problem and of worldwide importance in developing countries. Potential reason for proliferation of the infection may be low economic status, poor hygiene, inadequate health facility and exchanging of footwear and cloth[(1)].Even though it is not a life threatening infection but may affect the social life and day to day activities. Dermatophytes are common cause of superficial mycosis worldwide, there is an increase in the infection by non-dermatophytemould (NDM) and yeast has been observed[(1,2)]. Change the incidence of infection by pathogen may affect the clinician capability to diagnose and can change the approach to treat[(3)].

Siddis are tribal community residing in India who brought from eastern African countries before many generations. They leave in different parts of Indian states like Andhrapradesh Maharashtra Gujarat and Karnataka. In Karnataka they reside in North Karnataka region and adapted to local social and religious lifestyle. We under took this study to see the common non dermatophytes causing the superficial fungal infection in Siddi tribal community[(4,5)].

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Materials and Method

Study was conducted during 2015 to 2017in north Karnataka region. A total of 1004 clinical samples like hair nail and skin scrapping were collected from 937 Siddi tribal patients with suspected superficial fungal infection. Clinical details were collected with patient consent. Samples were subjected to direct microscopy

by 10 % pottacium Hydroxide (KOH) and inoculated in pairs in plain SabourdoseDextrose Agar (SDA) and SDA with cycloheximide (0.5% 0.5 mg/ml), chlotetracycline (0.1% 0.1 mg/ml) and Gentamicine (0.1% 0.1 mg/ml) and incubated at room temperature. Cultures were noted for colony character, surface color and color on the reverse. Culture was identified by macroscopic and microscopic examination.

PCR and sequencing

Genomic DNA was extracted by phenol-chloroform isoamyl alcohol method and the extracted genomic material was eluted with 50 µl Tris-EDTA buffer. Polymerase chain reaction was carried out using internal transcribe spacer (ITS); ITS 1 and ITS 4 primer (ITS1, 5' TCCGTAGGTGAACCTTGCGG 3', and ITS 4, 5' TCCTCCGCTTATTGATATGC 3'), with final volume of 50 µl containing 0.5 µg of template DNA, 20 µl of Emerald Amp GT PCR Master Mix (2X premix composed of a DNA polymerase, optimized reaction buffer, dNTPs, and a density reagent) 15 pmol of each primer. PCR was performed in a thermo cycler (Eppendorf) with initial denaturation of 94°C for 6 minutes followed by 35 cycles of 94°C for 30seconds, 58°C for 30 seconds and 72°C for 1 minute 30 second and final extension at 72°C for 10 minutes. PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized in UV transilluminator and imaged. PCR sequencing was done form PCR product using ITS 1 and ITS 4 primer and BigDye Terminator Cycle sequencing kit version 3.1 (Applied Biosystems). ABI 3130 genetic analyzer (Applied Biosystems) was used for purification and analysis of all sequencing reaction. Sequences were compared with the GenBank DNA database using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov), the ISHAM ITS database (http://its.mycologylab.org/BioloMICSSequences.aspx), and the CBS database (http://www.westerdijkinstitute.nl/Collections/BioloMICSSequences.aspx).

Phylogenetic analysis

Phylogenetic analysis of study isolates and standard sequences retrieved from NCBI were done by aligning sequences using multiple sequence alignment mode in ClustalX2 software. The aligned sequences were exported to Molecular Evolutionary Genetics Analysis software version 7 (MEGA7)[6] and neighbor joining tree was constructed using Kimura 2 parameter model with 1000 bootstrapping replicates.

Results

A total of 1004 samples were collected from 937 patients with suspected superficial mycosis. Tineaunguim was the most commonly seen clinical condition followed by tineacorporis and tineacapitis.A total of 158 samples have shown the culture positive. Dermatophytes were the most common etiological agent isolated from 102 (64.55%) cases. Whereas non dermatophytes were isolated from 56 (35.44%) cases. Most of the patients were agriculturist (32, 57.14%) and field workers (9, 16.07%).Commonest clinical condition seen in superficial infection with nondermatophytes was tinea unguium (48, 85.71%) in which 52.08% cases were Proximal subungual onychomycosis (PSO), 22.91% of cases were white subungual onychomycosis, 16.66% of cases were Distal lateral subungual onychomycosis and 6.25% of cases were endonyxonychomycosis whereas, Tineacorporis (5, 8.92%) and Tineacapitis (3, 5.35%) was seen in 5 (8.92%) and 3 (5.35%) cases respectively.

In this study, Candida species (32, 20.25%) was the predominant agent to cause superficial infection other than dermatophytes followed by *Aspergillus species* (12, 7.59%), *Fusarium species* (10, 6.32%) and *cladosporium species* (2, 1.26%). Further repeat sample was collect to confirm the infection with *Cladosporium species*.

Cladosporium species was isolated from twin's patients of age 7years old with tineacapitus infection since from one year. Morphological feature included unbranched cylindrical conidiophores bearing ovoidal to ellipsoidal intercalary and terminal conidia.

Both *Cladosporium specis* were used to sequence ITS region and were identified as *Cladosporium halotolerans*(accession number MT588811 and MT588810) Phylogenetic analysis of both strains were done with standerd *Cladosporium species* stains retrieved from the NCBI database [*Cladosporium halotolerans* (LN834374, LN834375 and DQ780364), *Cladosporium angustisporum* (LN834356), *Cladosporium asperulatum* (LN834357), *Cladosporium allicinum* (LN834353), *Cladosporium cladosporioides* (LN834358), *Cladosporium flabelliforme* (LN834361), *Cladosporium funiculosum* (LN834364), *Cladosporium*

herbarum (LN834378), *Cladosporium subinflatum* (LN834391), *Cladosporium tenuissimum* (LN834398)]. Study isolates have clustered with *Cladosporium halotolerans*.

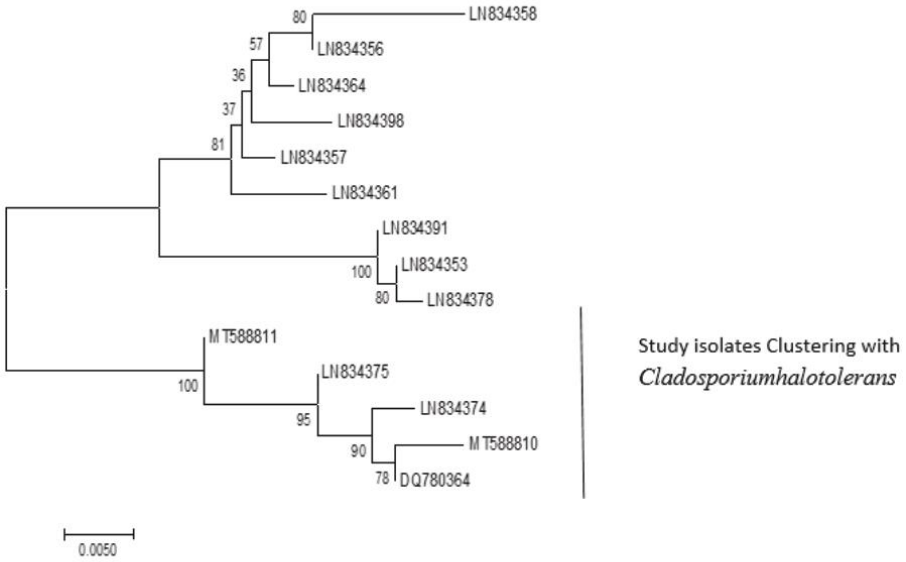

Figure.1 Neighbor–joining (NJ) derived dendrogram of internal transcribe spacer (ITS) gene based sequences. Phylogenetic analysis was done with the study isolates and standerd CBS stains retrived from the NCBI database [*Cladosporiumhalotolerans* **(LN834374, LN834375 and DQ780364),** *Cladosporiumangustisporum* **(LN834356),** *Cladosporiumasperulatum* **(LN834357),** *Cladosporiumallicinum* **(LN834353***), Cladosporiumcladosporioides* **(LN834358),** *Cladosporiumflabelliforme* **(LN834361),** *Cladosporiumfuniculosum* **(LN834364),** *Cladosporiumherbarum* **(LN834378),** *Cladosporiumsubinflatum* **(LN834391),** *Cladosporiumtenuissimum* **(LN834398)] study isolates (MT588811 and MT588810) have clustered with** *Cladosporiumhalotolerans***.**

Discussion

The present study showed 5.97% prevalence of superficial infection with non dermatophytes including yeast and moulds other than dermatophytes. It appears tineaunguium as the common clinical condition accounted for 85.71%, the risk factor for increase prevalence of tineaunguium in superficial mycosis by non dermatophytes might be due to frequent contact with soil during their day today life, Age, history of similar infection or frequent sharing of foot ware. Tineacorporis and tineacapitiswas seen in 8.92% and 5.35% of the cases respectively. Similarly Kaur et.al. in 2015 found nail infection the most common site to cause superficial infection(7),whereas Lakshmanan et al. in 2015 have found skin is the common site of superficial infection followed by nail and hair[8]. Infection rate was highest in the outdoor workers like farmers (57.14%) and daily wage workers (16.07%) which in accordance with studies done in other parts of India[9–11].Candida species (20.25%) was the predominant agent followed by *Aspergillus species*(12, 7.59%), *Fusarium species*(10, 6.32%) and *Cladosporiumhalotolerans*(2, 1.26%). Hazarika et.al in 2020 reported NDM were more prevalent than yeast to cause superficial infection[12]. Kaur et.al. in 2015 have found NDM was the most common agent to cause superficial mycosis followed by dermatophytes and yeasts[7]. *Cladosporium halotolerans*has been isolated from twin's patients with tineacapitis to confirm the infection with *Cladosporium halotolerans* repeat

samples were collected from both the patient shown the growth *Cladosporium halotolerans*.*Cladosporium* usually considered as indoor fungus being isolated in the environmental sources and geographic location, however many species of *Cladosporium species* are important pathogens to cause infection in plants animal and even in humans[13,14]. In most of the cases *Cladosporium species* isolated lack molecular confirmation, however in the present study *Cladosporium species* isolated were sequenced using ITS region and identified as *Cladosporiumhalotolerans*. Possible reason for the isolation of fungus like *Cladosporiumhalotolerans*in Siddi tribal community might be due to their low socioeconomic condition and poor hygiene, as majority of the Siddi tribal community live their life in poverty.

In conclusion, this study showed other than dermatophyte, non-dermatophytes like yeast and non dermatophyticmoulds are also responsible for the superficial infection in the Siddi tribal community with prevalence rate of 5.97%. Tineaungum was the most commonly seen clinical condition to cause superficial infection by non dermatophytes in 85.71% of cases. *Cladosporium halotolerans* which is considered as the indoor fungus can also cause superficial infection like tineacapitis in the Siddi tribal community, a detailed study on Siddi tribal community and native Indians to find out such fungal superficial infection may reveal interesting findings, which may further help clinician diagnosis and may change the approach to treat.

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ANNEXURE V: CERTIFICATES

IAMM-KC's

MICRO-KAR 2019

Certificate of Participation

This is to certify that

Mr. Aruna C.

has participated as a Delegate in the Conference held on 8th & 9th Feb 2019.

He / ~~She~~ has ~~Chaired a Session / Judged an Academic~~

~~Presentation Session~~ / Presented a Paper (Oral / ~~Poster~~).

Dr Praveen C. Shetty
Organising Secretary
MICRO-KAR 2019

Dr Ajantha G. S.
President
IAMM-KC 2018-19

Dr S. K. Joshi
Principal
SDMCMSH, Dharwad

Indian Association of Medical Microbiologists
Karnataka Chapter

XXIII Annual Conference
8th & 9th Feb 2019

SDM College of Medical Sciences & Hospital, Dharwad - 580009

A Constituent of

Sri Dharmasthala Manjunatheshwara University

Organised by
Department of Microbiology

आयुर्विज्ञान सूक्ष्मजीव विज्ञान विभाग
DEPARTMENT OF MEDICAL MICROBIOLOGY
स्नातकोत्तर चिकित्सा शिक्षा एवं अनुसंधान संस्थान, चण्डीगढ़–160 012 (भारत)
POSTGRADUATE INSTITUTE OF MEDICAL EDUCATION & RESEARCH, CHANDIGARH-160 012 (INDIA)

डॉ० शिवाप्रकाश एम. आर.
Dr. Shivaprakash M.R.
एम. डी., एमएनएमएस, एमएनएएसई
M.D., MNAMS, MNASc
प्राचार्य
Professor

संख्या/No./Micro/ 82
दिनांक/Dated 2/11/2018

To Whomsoever concerned

This is to certify that, Mr. Aruna C, Ph.D. Scholar of Microbiology Department, J.N Medical College KAHER Belagavi (Karnataka) has worked in Mycology Division of Department of Medical Microbiology, PGIMER Chandigarh from 30th Sept 2018 to 30th Oct 2018. During his stay in the department he has learnt identification, antifungal susceptibility testing, DNA extraction and Polymerase Chain Reaction and MALDI-TOF for the dermatophytes. I also certify that he had performed all these activities with his hands under my supervision.

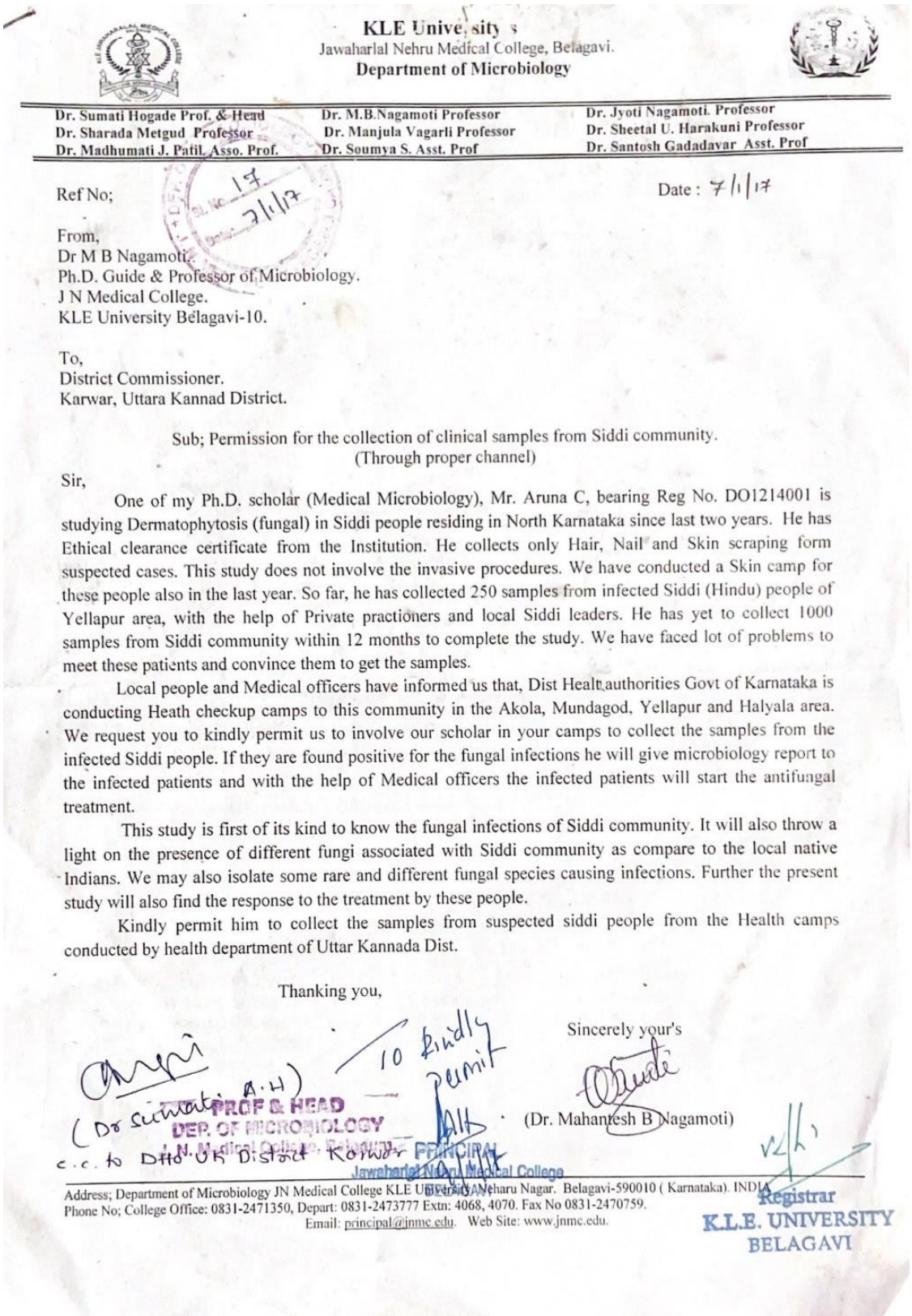
His behavior, character, conduct, work, techniques and timeliness during this stay here were satisfactory.

(M R Shivaprakash)

Dr. M.R. Shivaprakash, MD
Professor,
Department of Medical Microbiology,
Postgraduate Institute of Medical Education and Research,
Chandigarh, India-160012.

दूरभाष/Phone (91)-172-2755156 (off.), (91)-172-2771056 (Res.), 9316175146, Mobile: 7087008162 (PGI Mobile)
फैक्स/Fax. (91)-172-2744401, 2745078, e-mail: mrshivprakash@yahoo.com

ANNEXURE VI: REQUEST LETTER FOR SAMPLE COLLECTION



KLE University's
Jawaharlal Nehru Medical College, Belagavi.
Department of Microbiology

Dr. Sumati Hogade Prof. & Head | Dr. M.B.Nagamoti Professor | Dr. Jyoti Nagamoti. Professor
Dr. Sharada Metgud Professor | Dr. Manjula Vagarli Professor | Dr. Sheetal U. Harakuni Professor
Dr. Madhumati J. Patil. Asso. Prof. | Dr. Soumya S. Asst. Prof | Dr. Santosh Gadadavar Asst. Prof

Ref No;

Date : 7/1/17

From,
Dr M B Nagamoti.
Ph.D. Guide & Professor of Microbiology.
J N Medical College.
KLE University Belagavi-10.

To,
District Commissioner.
Karwar, Uttara Kannad District.

Sub; Permission for the collection of clinical samples from Siddi community.
(Through proper channel)

Sir,

One of my Ph.D. scholar (Medical Microbiology), Mr. Aruna C, bearing Reg No. DO1214001 is studying Dermatophytosis (fungal) in Siddi people residing in North Karnataka since last two years. He has Ethical clearance certificate from the Institution. He collects only Hair, Nail and Skin scraping form suspected cases. This study does not involve the invasive procedures. We have conducted a Skin camp for these people also in the last year. So far, he has collected 250 samples from infected Siddi (Hindu) people of Yellapur area, with the help of Private practioners and local Siddi leaders. He has yet to collect 1000 samples from Siddi community within 12 months to complete the study. We have faced lot of problems to meet these patients and convince them to get the samples.

Local people and Medical officers have informed us that, Dist Healt authorities Govt of Karnataka is conducting Heath checkup camps to this community in the Akola, Mundagod, Yellapur and Halyala area. We request you to kindly permit us to involve our scholar in your camps to collect the samples from the infected Siddi people. If they are found positive for the fungal infections he will give microbiology report to the infected patients and with the help of Medical officers the infected patients will start the antifungal treatment.

This study is first of its kind to know the fungal infections of Siddi community. It will also throw a light on the presence of different fungi associated with Siddi community as compare to the local native Indians. We may also isolate some rare and different fungal species causing infections. Further the present study will also find the response to the treatment by these people.

Kindly permit him to collect the samples from suspected siddi people from the Health camps conducted by health department of Uttar Kannada Dist.

Thanking you,

Sincerely your's

(Dr Sumati A.H)

10 Kindly permit

(Dr. Mahantesh B Nagamoti)

PROF & HEAD
DEP. OF MICROBIOLOGY
J.N. Medical College, Belgavi

PRINCIPAL
Jawaharlal Nehru Medical College

c.c. to DHO UK District Karwar

Registrar
K.L.E. UNIVERSITY
BELAGAVI

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Email: principal@jnmc.edu. Web Site: www.jnmc.edu.