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MOLECULAR METHODS IDENTIFICATION OF DERMATOPHYTES USING (PCR) TECHNIQUES FOR PATIENTS IN AL-ANBAR GOVERNORET- IRAQ

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By Abdulateef Abdulqader HAMMOOD

August 2021

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

MOLECULAR METHODS IDENTIFICATION OF DERMATOPHYTES USING (PCR) TECHNIQUES FOR PATIENTS IN AL-ANBAR GOVERNORET-IRAQ

Abdulateef Abdulqader HAMMOOD

Master of Science in Biology

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August 2021

This study aims to isolate and diagnosis the Dermatophytes by mycological examinations methods in the laboratory, and identify the species that have appeared using the internal transcribed spacer (ITS) region and comparing the current local isolates with those provided by the National Center for Biotechnology Information (NCBI). Dermatophytes are predominantly caused by a group of closely related genera of *Trichophyton*, *Microsporum*, and *Epidermophyton*. These groups of fungi invade the stratum corneum of the skin, hair, and nails. Samples were collected from 100 patients with Dermatophytes in Ramadi Training Hospital, Anbar-Iraq. A portion of each sample was examined microscopically and the remaining portion of each sample was cultured onto plates of Saboraud Dextrose Agar added Chloramphenic and Gentamicin (SDACG). The results showed that out of the 100 cases of dermatophytes, only 60 (60 %) cases were positive by both direct KOH examination and culture, whereas false negative results was recorded in 20 (20 %) of specimens. Culture results of specimens showed nine types of dermatophytes were identified; namely *Trichophyton rubrum* (26.0%), *Microsporum canis* (21.7%), *T.mentagrophytes* and their percentage (19.5), *T. interdigitale* (13.0%), *T. ferrugineum* (8.6%), *T. soudanese* (4.3%), and the rest of the three remaining species were *Epidermophyton floccosum*, *T. tonsurans*, *T. verrcosum* (2.2%). The most common clinical type of infection was tinea corporis (60.9%), and the others were as follows: *Tinea cruris* (19.6%), *T. pedies* (6.5%), *T.faciei* (6.5%), T. capitis (4.3%), and *T. manus* (2.18%). Females (52%) were more affected than men (40%). The countryside (65.2%) was more affected than the city (34.8%). The most affected age group was (31-40) years. Dermatophyte isolates were identified by studying the macroscopic and microscopic characteristics of their colonies. To ensure the correctness of their diagnosis, the primer (region ITS gene rRNA 18S) were used for the diagnosis of dermatophytes. The Polymerase Chain Reaction (PCR) products of Dermatophytes isolates were submitting to Macrogen Company for sequencing. Sequencing analysis showed nine new local strains recording in NCBI with accession numbers MW811374.1, MW811375.1, MW811376.1, MW811377.1, MW811378.1, MW811379.1, MW811380.1, MW811381.1, and MW811382.1. The genetic compatibility between the reference strain and the local strain was 99%.

2021, 89 pages

Keywords: Dermatophytes, Epidemiology, PCR

ÖZET

RAMADİ EĞİTİM HASTANESİNE (IRAK) BAŞVURAN HASTALARDA MOLEKÜLER YÖNTEMLE DERMATOFİTLERİN TANIMLANMASI

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Ağustos 2021

Bu çalışma, Dermatofitleri laboratuvarda mikolojik inceleme yöntemLeri ile izole etmeyi ve teşhis etmeyi ve internal transcribed spacer (ITS) bölgesini kullanarak ortaya çıkan türleri tanımLamayı ve mevcut yerel izolatları Ulusal Biyoteknoloji Bilgi Merkezi (NCBI) tarafından sağlananlarla karşılaştırmayı amaçlamaktadır. Dermatofitlere ağırlıklı olarak yakın ilişkili *Trichophyton*, *Microsporum* ve *Epidermophyton* cinsleri neden olur. Bu mantar grupları deri, saç ve tırnakların stratum korneumunu istila eder. Irak Anbar Ramadi Eğitim Hastanesi'nde Dermatofitli 100 hastadan örnekler toplandı. Her numunenin bir kısmı mikroskobik olarak incelendi ve geri kalan kısmı, Saboraud Dextrose Agar eklenen Kloramfenik ve Gentamisin (SDACG) plakalarına kültürlendi. Çalışmanın sonucunda, 100 dermatofit vakasından sadece 61 (%61) vakanın hem doğrudan KOH muayenesi hem de kültür ile pozitif olduğunu, örneklerin 20'sinde (%20) yanlış negatif sonuçların kaydedildiğini gösterdi. Örneklerin kültür sonuçları, *Trichophyton rubrum* (%23,9), *Microsporum canis* (%21,9), *T. mentagrophytes* (%19,2), *T. interdigitale* (%13,4), *T. Ferrugineum* (%8,7) olmak üzere dokuz tip dermatofitin tanımLandığını göstermiştir ve geri kalan dört tür (%2,2) *Epidermophyton floccosum, T.soudanese, T. tonsurans, T. verrrcosum* olrak belirlendi. En sık görülen klinik enfeksiyon tipi tinea corporis (%61), diğerleri ise *Tinea cruris* (%19,6), *T. pedies* (%6,5), *T. faciei* (%6,5), *T. capitis* (%4,3), ve *T. manus* (%2,18). Kadınlar (%52) erkeklerden (%40) daha fazla etkilenmiştir. Kırsal kesim (%65,2) şehirden (%34,8) daha fazla etkilenmiştir. En çok 31-40 yaş grubu etkilendi. Dermatofit izolatları, kolonilerinin makroskopik ve mikroskobik özellikleri incelenerek tanımLandı. Tanılarının doğruluğunu sağlamak için dermatofitlerin teşhisinde primer (bölge, ITS geni rRNA 18S) kullanıldı. Dermatophytes izolatlarının Polimeraz Zincir Reaksiyonu (PCR) ürünleri sekanslama için Macrogen Company yapıldı. Sekans analizi, NCBI'de erişim numaraları MW811374.1, MW811375.1, MW811376.1, MW811377.1, MW811378.1, MW811379.1, MW811380.1, MW811381.1 ve MW811382.1 olarak kayıtedildi. Referans suş ile yerel suş arasındaki genetik benzerlik %99 bulundu.

2021, 89 sayfa

Anahtar Kelimeler: Dermatofit, Epidemiyoloji, PCR

PREFACE AND ACKNOWLEDGEMENTS

This study entitled "Molecular Methods Al-Anbar Province- Identification of Dermatophytes Using (PCR) Techniques for Patients in Iraq" was written in 2020-2021 and presented to Çankırı Karatekin University Institute of Science as a "Master's Thesis". This epidemiological study aims to identify the types of dermatophytes, the site of infection, the population distribution, the age, and the sex and isolate and identify the Dermatophytes using the ITS region comparing the current local isolates with those provided by the NCBI.

First and foremost, praises and thanks go to the one and only, Allah (SWT), for His showers of blessings throughout my thesis work to complete the thesis successfully. I would like to extend my thanks, respect, and appreciation to my supervisor, Prof. Dr. Özcan ÖZKAN .and the co-supervisor Dr. Jaleel SAMANJE. I also would like to thank the Turkish people and the University of Çankırı Karatekin, both teachers and administrators, for their support.

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Abdulateef Abdulqader HAMMOOD

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CONTENTS

LIST OF ABBREVIATIONS

PCR	polymerase chain reaction
AIDS	Acquired Immune deficiency syndrome
ITS	The internal transcribed spacer
rDNA	Ribosomal Deoxyribonucleic acid
SDA	Saboraud Dextrose Agar
T.	*Trichophyton*
M.	*Microsporum*
E	*Epidermophyton*
KOH	Potassium hydroxide
SDACG	Saboraud Dextrose Agar Chloramphenic Gentamicin
RFLP	Restriction Fragment Length Polymorphism
AFLP	Amplified Fragment Length Polymorphism
RAPD	Random Amplified Polymorphic DNA
rRNA	Ribosomal Ribonucleic Acid
NTS	Non transcribed spacer
IGS	InterGenic spacer
EtBr	Ethidium Bromide
Bp	Base pair
T.	*Tinea*
INSDC	International Nucleotide Sequence Database Collaboration
NCBI	National Center for Biotechnology Information
ENA	European Nucleotide Archive
DDBJ	DNA Data Bank of Japan
ME	Minimum Evolution
MEGA	Molecular Evolutionary Genetic Analysis

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Dermatophytes are pathogenic fungi that can damage the keratin membrane of the skin by producing proteolytic enzymes, which they used to hydrolyze keratin, the major protein element of hair, nails, and skin (Viani *et al.* 2001).

This closely related group of organisms can be categorized into three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton* are grouped according to their habitat as being either anthropophilic (human-associated), zoophilic (animal associated), or geophilic (soil-dwelling) (Whittam and Hay 1997). Furthermore, dermatophytoses are widespread and growing in spread on a global scale, with the rise in their incidence attributed to population aging and the rise in immune-compromised states such as those associated with acquired immune deficiency syndrome (AIDS), diabetes mellitus, organ transplantation, and the use of corticosteroids and antineoplastic agents (Lackner *et al.* 2019). Accurate and quick diagnosis is critical for successful treatment of Dermatophytes, as well as any other dermatophytosis. About half of patients are likely misdiagnosed if the diagnosis is only based on clinical symptoms (Faergemann and Baran 2003). Because most clinical isolates are imperfect fungus, species that do not develop sexual structures in culture, the taxonomy of these fungi is complex. For these fungi to identify the propagation of infections and relapses, strain differentiation within the same species is required. (Abdel-Rahman 2008). The cultivation of dermatophytes on appropriate growth media is depend on an examination of their colonies' gross morphological characteristics (e.g., growth rate, colony topography, surface, and reverse side pigmentation) and microscopic morphology. Further characteristic recognition involves dietary requirements (such as the use of vitamins and amino acids), temperature tolerance, development of urease, in vitro hair perforation, etc. Although culture-based identification is exact and sensitive, it takes time since some species require up to two weeks to establish diagnostic characteristics in culture medium. Furthermore, several dermatophyte strains have unusual characteristics. In recent years, genotypic techniques to identification have shown to be effective in resolving dermatophyte taxonomy issues. Genotypic distinctions, in fact,

are thought to be more permanent and precise than phenotypic features. The polymerase chain reaction (PCR) represents a significant advancement in dermatophyte infection diagnosis (Liu *et al.* 2000). DNA samples obtained from fungal cultures or clinical specimens may be amplified using three primer sets targeting the 18S rDNA and the internal transcribed spacer (ITS) regions (TR1/TR2 and B2F/B4R for 18S rDNA and ITS1/ITS2 for ITS regions), yielding species-specific products *Trichophyton*, *Microsporum*, or *Epidermophyton* (Fari 1998).

1.1 Aims Of The Study

In this study, the types of dermatophytes isolated from dermatophytosis patients who were admitted to Ramadi Teaching Hospital (Iraq) were identified through routine laboratory procedures and molecular method (PCR). To achieve this goal, the following objectives were followed:

1. Collecting a sample of hairs, nails and skin, and microscopic identification of dermatophytes using 10% KOH method.
2. Culturing of clinical samples on Saboraud Dextrose Agar (SDA) plate
3. Comapre between the result of 10% KOH method and Saboraud Dextrose Agar (SDA) plate.
4. Sequencing analysis of the nitrogen bases of the ITS1-5.8S-ITS4 gene segment for some of the isolates under study and determining the similarity ratios between them and the reference samples deposited in the database in the gene bank NCB.

2. LITERATURE REVIEW

2.1 Fungi As Human Pathogens

Only around 500 of the 50000 to 250000 species of fungus that have been identified have been related to human illness, and only about 100 are capable of infecting otherwise healthy people (Ascioglu *et al.* 2003). Human fungal infections, with a few exceptions, are acquired by inhalation, ingestion, or traumatic implantation from an external source in the atmosphere. Fungal infections are classified into many major groups based on the initial location of infection. This clearly demonstrates the diverse groups of fungi's parasitic adaptability and the way in which the infected location is linked to the path by which the fungus enters the host (Haddad and Powderly 2001).

2.2 The Superficial Mycoses

These are skin diseases that affect the outermost layers of the skin, such as the hair, nails, and mucous membranes. Dermatophytoses and superficial forms of candidosis are the principal infections in this community. These disorders affect millions of people throughout the world, yet they are easily identified and usually respond well to treatment. Dermatophytes are only found in keratinized tissues such as the epidermis, hair, and nails. In the face of competition from other keratinophilic species in the environment, the majority of keratinophilic species are unable to live as free-living saprobes and must rely on transit from host to host to survive. These mandatory pathogens appear to have developed from unspecialized saprobic species. Many are no longer capable of sexual reproduction, and some are even capable of asexual reproduction. In general, these species have evolved well to humans, eliciting little or no host inflammatory response (Ericsson *et al.* 2002). Like dermatophytes, the aetiological agents of candidosis are primarily reliant on the living host for their survival but differ from them in the way this is accomplished.

2.3 The Subcutaneous Mycoses

These are infections that affect the dermis, subcutaneous tissues, and nearby bones. These infections are often acquired as a result of the traumatic implantation of organisms that grow as saprobes in the soil and on decaying plants. Among the rural communities of the tropical and subtropical regions of the world, these infections are most commonly found. More widespread transmission of the infection is rare through the blood or lymphatics and typically occurs only if the host is weakened or immunocompromised in any way (Rees *et al*. 1998).

2.4 The Systemic Mycoses

These infections generally start in the lungs, but they can spread to other organs. It is possible to distinguish the species causing systemic fungal infection into twodistinct groups: the real pathogens and the opportunists. The first category includes a small number of species capable of entering and thriving in the tissues of a host with no known predilection, such as *Histoplasma capsulatum* and *Coccidioides immitis*. These species also have distinctive morphological characteristics that tend to contribute to their survival within the host. The second group, the opportunists, consists of species that are less virulent and less well-adapted, such as *Aspergillus fumigatus*, which can only invade an immunocompromised patients tissues (McNeil *et al.* 2001).

2.5 Classification Of Dermatophytes

Dermatophytes are mainly present in asexual states. On the basis of the morphological characteristics of the *Macroconidia*, they are classified into three asexual genera: *Microsporum*, *Trichophyton*, and *Epidermophyton*. *Macroconidia* in the genus *Microsporum* (M) are spindle-shaped. They have 1–12 septa and a strong wall. *Trichophyton* (T) contains oblong and rectangular *Macroconidia* with a thin wall and up to 12 septa in the genus *Trichophyton*. *Macroconidia* in *Epidermophyton* (E) are wider, rounded or oval, thin-walled, and have up to 5 septa. Within each genus, there are several representatives. So far, over 40 distinct dermatophyte species have been identified (AL-Jubori and Hasan 2015).For epidemiological and clinical reasons, it is

useful to classify dermatophytes accordingto their natural habitats into three different groups: geophilic, zoophilic, and anthropophilic dermatophytes. Representatives of all of these groups can cause infections in humans (SemLin *et al.* 2011).

Geophilic dermatophytes are soil-borne pathogens that spread to people via contaminated soil. They may also be found on fruits and vegetables. Infections can happen when children play outside or when professional gardeners work. They can also be passed from animals to people through the soil. Geophilic dermatophytoses are found all over the world and are most common in the spring and summer. *Microsporum gypseum* is the most prevalent geophilic pathogen with a global distribution. *Microsporum fulvum* is a geophilic fungus that can infect humans. (Nenoff *et al.* 2014). Infections caused by zoophilic dermatophytes (Table 2.1) are mostly seen in mammals, although they've also been detected on bird feathers. (Kerwin and Semon 1998).

The disease can spread to human skin after close contact with infected animals. Farmers and veterinarians, as well as those who come into close contact with sick domestic pets, are at danger of contracting the disease. The intimate relationship between humans and their pets aids in the spread of zoophilic dermatophytes. Indirect infection transmitted by infected garments, towels, brushes, or other infected objects should also be taken into account. Infections can also be spread from person to person by skin-to-skin contact. Outbreaks can happen at school or in the home. Both rural and urban populations have been infected with zoophilic dermatophytes (Dolenc-Voljč e*t al.* 2005).

Table 2.1 Anthropophilic, Geophilic and Zoophilic some type dermatophytes, their hosts and geographical distribution (Nenoff *et al.* 2014)

Dermatophyte	Ecology	Hosts	Distribution
Trichophyton rubrum	Anthropophilic	Human	Worldwide
Microsporum canis	Zoophilic	cats, dogs	worldwide, more common in Central and Southern Europe
Epidermophyton floccosum	Anthropophilic	Human	Worldwide
Trichophyton interdigitale	Anthropophilic	Human	Worldwide
Trichophyton violaceum	Anthropophilic	Human	Middle East, India, Western China, North Africa
Microsporum gypseum	Geophilic	soil	Worldwide
Trichophyton soudanense	Anthropophilic	Human	Africa
Trichophyton mentagrophytes var. mentagrophytes	Zoophilic	rodents: hamster, guinea pig	Worldwide

The most frequent zoophilic dermatophytes are *Microsporum canis*, *T. mentagrophytes var. mentagrophytes*, and *T. verrucosum* (Table 2.1). Infections are most commonly spread by cats, dogs, rats, and cattle. *Trichophyton* species of Arthroderma benhamiae, which matches to zoophilic *T. mentagrophytes* isolates, was recently identified as a novel zoophilic pathogen. Small rodents, particularly guinea pigs, are the sources (Nenoff *et al.* 2014).

Zoophilic infections are more often seen on exposed portions of the body in children and teenagers, with a higher affi nity to hairy areas of the skin. Acute inflammatory lesions, pustule development, and deep infiltrates are common clinical manifestations. Infection is typically found in people who are otherwise healthy and immune-competent (Nenoff *et al.* 2014).

Anthropophilic dermatophytes have evolved into highly specialized pathogens that only parasitize people with keratinized tissues. In adult patients, these infections are more frequent on covered areas of the body and have a long-term course. Anthropophilic dermatophytes are most frequently seen on the foot, toenails, groin, and trunk. In industrialized nations, these illnesses are more prevalent. Infected warm and humid floor surfaces in community bathing and sporting facilities are the most common routes of transmission, with direct human contact being rare. It may happen in places like hotels and mosques. Transmission among family members is also frequent, and the major source of infection is the bath at home (Nenoff *et al.* 2014). Anthropogenic dermatophytes may exploit household dust as a reservoir, storing their spores for years (Havlickova *et al.* 2008). *T. rubrum* and *T. mentagrophytes var. interdigitale* are the most prevalent anthropophilic dermatophytes.

Dermatophytes are divided into two categories based on the location of infection in the hair shaft. Ectothrix refers to infections of the hair shaft's outer layer. Endothrix infections are those in which spores are generated within the hair shaft (Weitzman and Summerbell 1995).

Hyphae are the epitome of the Dermatophytes way of life. The main building blocks of dermatophytes are tiny filaments with diameters ranging from 1 to 5 micrometers. *Hyphae* are ideal for growing into skin, hair, and nails because of their filamentous structure, where they excrete harmful enzymes and collect required nutrients. It is made up of creatine (Weitzman and Summerbell 1995). *Hyphae* can effectively fuse if they belong to the same species, however *Hyphae* from other species are rarely compatible, unless in the context of sexual reproduction (This is referred to as vegetative incompatibility). A mycelium is the hyphal network of an individual fungus that has become aggregated (Smijs *et al.* 2008). Dermatophytes are transmitted mostly by spores. Filamentous dermatophytes proliferate asexually by producing tiny, big, and joint (Arthro – micro and macroconidia) spores from unique cells that create conidiogenous cells. In addition, several Chlamydospores, Spiral hyphae, Antler-shaped hyphae or chandeliers, fungal hyphae Bat shaped (*Racquet hyphae*), mycoplastic hyphae (*Pectinate hyphae*), streptococcus organs (Nodular organ filaments), streptococcus

organs (Nodular organ filaments), streptococcus organs (Nodular organ filament) (Mukoma 2000).

Depending on the asexual form (anamorph), dermatophytes can be divided into Two types of the genus *Epidermophyton* and approximately 18 species of the genus *Microsporum*, as well as 25 species of the genus *Trichophyton*, as for the main characteristics of these three genera are.

2.5.1 Epidermophyton

This genus is characterized by the presence of only macroconidia that are thin-walled, multicellular, solitary, with rounded ends and grouped into clusters, and over time, the Conidias transform into chlamydial spores, and the fungus does not form *Microconidia*, this genus contains two types. The pathogen type is *E. floccosum*, which is human-loving (Anthropophiles) which causes a number of skin infections in humans, in addition to causing some pathological conditions in animals (Achterman and Theodore 2012).

The second type is *E. stockdaleae*, which is loving soil (Geophiles) and is not registered as a pathogen for fungal skin diseases (Achterman and Theodore 2012).

2.5.2 Microsporum

Colonies of this genus on a solid medium are powdery or velvety with white or brown color. This genus produces small and large conidia, *Macroconidia* is characterized by multicellular, thick rough walls, characteristic of this genus. They are many in types and few in others. The *Macroconidia* are usually spindle-shaped except for the type *M. nanum*, as there is a variation in their shapes depending on the type, and they are also generated singly. The *Microconidia* are pear-shaped about 2-3 μm, which arise singly along the fungal strand or in clusters, which are usually less of the large conoid. Rarely, there are species that produce either large or *Microconidia*. The typical species for this genus is *M. audouinii* (Sharma *et al.* 2015).

2.5.3 Trichophyton

Colonies of this genus on the solid medium may be powdery, velvety, or waxy. This genus also produces both small and large conidia. *Macroconidia* with thin, smooth walls are Pen-like shape, cylindrical, or spindly shaped, often Sporadic, as for *Microconidia*, they are pear-shaped or irregular in shape in some species. It may arise singly along the fungal strand or as clusters. They are usually found in abundance much more than the large condylomas as some species of this genus rarely produce *Macroconidia,* The typical species for this genus is *T. tonsurans* (Sharma *et al*. 2015).

2.6 The Skin

The biggest organ in the body is the skin. The skin weighs over 5 kg in a 70 kg person and covers a surface area reaching 2 m^2. Human skin consists of a stratified, cellular epidermis and an underlying connective tissue dermis, divided by a dermal-epidermal basement membrane. A layer of subcutaneous fat lies beneath the dermis, separated from the rest of the body by a vestigial layer of striated muscle (Young 2000). The epidermis is firstly made up of keratinocytes and is typically 0.05–0.1 mm thick for the live cell layers. It is created by cell division in the basal layer, which gives birth to the spiny layer. This layer comprises cells that shift outward and develop gradually, creating the granular layer and the stratum corneum. It takes around 30 days for cells to grow from the basal layer to the skin surface. When you have psoriasis, though, it accelerates. Keratinocytes have a cytoskeleton made up of keratin intermediate filaments, which gives them a brick-like appearance. As the epidermis develops, the keratinocytes flatten down. Filaggrin, a filament aggregating protein found in keratohyalin granules, is implicated in this process. Keratin and filaggrin make up 80-90 percent of the epidermis' bulk. The stratum corneum is the epidermis' outermost layer, where the nuclei and cytoplasmic organelles have lost their cells (now called corneocytes). The corneocyte possesses a highly insoluble cornfield envelope generated by cross-linking soluble protein precursors such as involucrin and loricrin inside the plasma membrane, with the latter accounting for 70-85% of the cornfield cell envelope mass. It also comprises lipids produced from lamellar bodies inside the top, living epidermis (fatty acids, sterols, and

ceramides). The stratum corneum may be divided into three biochemical and functional zones: an outside solute absorber, a middle water hydration absorber, and an interior mechanical protection barrier. The dermis is made up of a supporting matrix that is intertwined with a network of polysaccharides and proteins to give skin resilience and exceptional water retention. The thickness of the dermis varies depending on the place of the skin, from less than 0.5 mm to more than 5 mm (Fujiyama *et al.* 1988).

Collagen and elastic tissue are the two primary kinds of protein fiber. Collagen is the major extracellular matrix protein, accounting for 80-85 percent of the dermis' dry weight. The dermis also includes a number of non-collagenous glycoproteins, including as fibronectins, fibulins, and integrins. Cell adhesion and cell motility are aided by these extracellular matrix components. Between the dermal collagen and elastic tissue is the ground material formed by glycosaminoglycan / proteoglycan macromolecules. These make up just 0.1 to 0.3 percent of the dermis' total dry weight, but they serve a critical role in maintaining moisture, thanks to hyaluronic acid's strong water-binding capacity. Water makes up around 60% of the dermis' overall weight. Despite the fact that no vessels pass across the dermal-epidermal junction, the dermis possesses an abundant blood supply. There is a superficial and deep vascular plexus. The exocrine and apocrine glands' cholinergic portions, the smooth muscle and arterioles, and the erector pili muscle, as well as the exocrine sweat glands and adrenergic components, all contribute to the skin's motor innervation. Sensory nerve endings come in a variety of shapes and sizes; some are open, some end in hair follicles, and some have extended ends (Meglinski and Matcher 2002). The skin, like the rest of the body's organs, is susceptible to bacterial, parasite, viral, and fungal diseases. The bacteria that produce these illnesses, as well as the kinds of organisms that are typically resident flora or endemic organisms, are dependent in part on these diseases, and when conditions change, such as when the composition of the skin layers changes, they become pathogenic organisms. Dermatophytes are fungi that come from outside sources and are differentiated by their capacity to examine the keratinized tissues of the skin before moving inside into the dermis layers, where they are stopped from growing (Wagner and Sohnle 1995).

*2.*7 Epidemiology

Till today, it has been recognized that dermatophytoses are the most common fungal infections worldwide, affecting 20–25 % of the world population (Fuller 2009). Dermatophytes have undergone significant modifications over the last 100 years, with significant inter- and intra-continental variations in epidemiological data being reported. The most frequent anthropophilic dermatophytes described in published surveys are *T. rubrum* and *T. mentagrophytes* var interdigitale. Both are sold all over the world (Nenoff *et al.* 2014).

In the year 2013, a study was published in the Middle Euphrates provinces of Iraq (Babylon, Najaf, Dewania and Karbala) indicating that most of the infections of samples taken for dermatophytes were as follows: *Trichophyton tonsurans, T. rubrum, T. violaceum Epidermophyton,* and *T. verrucosum* (Karrema 2014). In Turkey, a study was conducted on the population of Duzce rural area, where the most frequently isolated aetiological agents were *T. rubrum,* and *T. mentagrophytes* (Sahin *et al.* 2004). In the USA it was observed that the *T. tonsurans* and *T. rubrum* the most common fungi (Sinski and Kelley 1987), in southwest Poland among the years 2011–2016 *T. rubrum* accounted for the majority and was followed by *T. tonsurans* (Gawdzik *et al.* 2019), In Greece, A study was conducted in mycology laboratory at the University Hospital of Heraklion, Crete, the most common dermatophyte was *M.canis* followed by *T.rubrum* (Maraki and Mavromanolaki 2016). In a large survey performed by the mycology reference laboratory, Bristol, UK, from 1980 to 2005, *T. rubrum* was the most frequently isolated dermatophyte, followed by *T. interdigitale* (Borman *et al.* 2007). In China, Malaysa, Singapore, Japan and Australia, *T. rubrum* and *T. mentagrophytes var*. interdigitale have been reported as the predominant pathogens (Havlickova *et al.* 2008).

In Africa, very little published material on the status of dermatophyte infection in Africa is available. The most prevalent clinical form is *M. audouinii* and *T. soudanense* (Coulibaly *et al.* 2015).

Different diagnostic procedures and criteria for notifying fungal infections, as well as social and economic reasons, contemporary living, increased travel, and population

movement from the southern to northern hemisphere, have all contributed to changes in the dermatophyte pandemic (Hayette and Sacheli 2015). These alterations point to the urgent need for more research to uncover the next epidemic trend.

2.8 Clinical Types Of Fungi

The clinical types of skin fungi are registered as *Tinea* or ring worm, Thus the word *Tinea* refers to cutworms (Chawing worm) and was used to describe skin fungal infections since the clinical type of the infection is pink scaly patches the color and these spots progressively widen and take a ring-like shape and, in addition to the location of the injury in the body, the term *Tinea* is used to refer to the various clinical types of these injuries and as shown in the following clinical figures (Jha *et al.* 2018).

2.8.1 *Tinea corporis*

Tinea corporis is one of the most prevalent dermatophyte infections. By definition, lesions are limited to the trunk and extremities. All known dermatophytes can cause it, and their incidence reflects the epidemiological condition in each nation. Acute zoophilic dermatophyte infections are more frequent in children and teenagers. Adults are dominated by anthropophilic fungus, the most prevalent of which is *T. rubrum.* (Nenoff *et al.* 2014).

Initially, a little erythematous macule or papule appears. In one to three weeks, typical erythematous annular and ciricinal lesions form, with distinct boundaries and core erythema regression. The borders of the erythema are more pronounced and scaly (Drakensjö and Chryssanthou 2011). With an inflammatory follicular pattern, it may include deeper layers of the skin in the hairy regions of the trunk and limbs. There are several types of tinea corporis that may be distinguished. *T. gladiatorum* infections spread by close personal contact in a wrestling team owing to transmission of dermatophytes infections of this sort (Nenoff *et al.* 1998). Tinea is characterized by erythematous annular lesions with sharp boundaries produced by *Microsporum* types. *Tinea microsporica*, commonly known as microsporia, is a parasitic fungus found in exposed parts of the body. *Microsporia* is also present in limited endemic locations.

This has been discovered in the majority of young children. One-quarter of the infected patients were children under the age of five. There was a normal seasonal fluctuation in the frequency, with a higher incidence between July and October (Dolenc-Voljč *et al.* 2005).

Near contact with infected animals is normally needed for infection. Infections can also be transferred indirectly through human contact. *M. gypseum* infections are common in patients with similar clinical symptoms, although infections with this geophilic dermatophyte are uncommon. *T. rubrum* infections generate a distinct pattern of erythematous lesions, which gradually develop to big erythematous macules or plaques encompassing vast regions of the body (Figure 2.1).

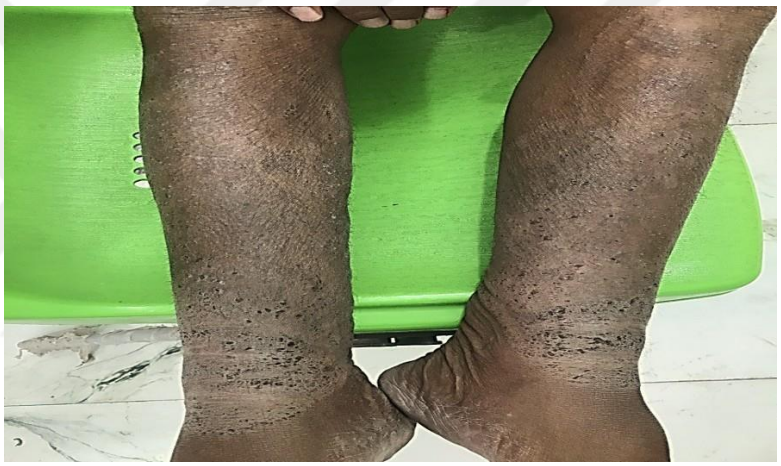


Figure 2.1 Large erythematous macules of *Tinea corporis*

2.8.2 Tinea capitis

In several parts of the world, *Tinea capitis* remains a widespread childhood infection (Elewski 2000). The knowledge of the basic pathogenetic processes as well as the generation of effective immunity improved significantly. Its clinical symptoms range from mild scaling to large inflammatory and pustular plaques, as well as severe alopecia and hair loss. The clinical appearance of scalp ringworm varies depending on the kind of hair invasion, the amount of host resistance, and the degree of inflammatory host reaction. Children from 6 months to 10–12 years make up the majority of those afflicted (Hogewoning *et al.* 2011). *T. capitis* is a fungal infection that can affect adults and is usually caused by anthropophilic fungi. The fundamental lesions of *M. audouinii*

infections are alopecia patches, which are often circular in shape but include numerous damaged hairs. Although there is little inflammation, fine scaling is common. There might be a few or a lot of these patches, placed more or less arbitrarily. The most frequent *Microsporum* species that causes tinea capitis, *M. canis*, has a similar appearance, although lesions are generally more inflamed and irritating (Mapelli *et al.* 2013). With *T. tonsurans* and *T. violaceum* infections, a minimally inflammatory form of patchy hair loss occurs. In this case, the development of black dots (swollen hair shafts) on the surface of the scalp as the affected hair breaks is typical, but they can be difficult to locate due to their sparse distribution. Several areas, sometimes mimicking discoid lupus erythematosus, occasionally seborrhoeic dermatitis, may show limited scaling. Other kinds of alopecia include diffuse and patchy alopecia, which might include solitary, isolated hair shafts and no scales. The following are the most common types of clinics:

- Black Dot (scaling with patchy hair loss) grey patch, and alopecia. But seriously inflammatory and elevated lesions may occur on occasions.

- Kerion: The severest reaction pattern. It is a painful, inflammatory mass in which the remaining hairs are loose. Discharge pus follicles. The affected area is generally small, but much of the scalp can be involved in a broad confluence.

- Scaly lesion of the scalp: In some cases, they are in the form of patches covered with light scales and can resemble alopecia areata, as the hair is easy to pull out in the affected region, loses its strength, becomes grayish in color, and may fall or cut from near distances to the scalp, and sometimes the injury causes slight scratching in the affected area (Wagner and Sohnle 1995).

2.8.3 Tinea facie

Tinea faciei is a dermatophyte infection of the face glabrous tissue, according to the definition. It possesses epidemiological characteristics that are comparable to tinea corporis. All known dermatophytes are capable of causing it. Children are more likely to be infected with zoophilic dermatophytes like *M. canis* and *T. mentagrophytes* var. mentagrophytes, whereas adults are more likely to be infected with anthropophilic

dermatophytes like *M. canis* and *T. mentagrophytes var. mentagrophytes* (Sharma *et al.* 2012). Inoculation of fungus from a pre-existing foot infection can potentially induce Tinea faciei. Skin lesions have an unusual clinical history, with no distinct borders and a lack of size. Exogenous factors such as cosmetics and UV radiation can conceal or aggravate inflammation, resulting in atypical skin lesions. Tinea faciei is commonly misdiagnosed because it resembles other face skin diseases. It is portrayed as a separate clinical entity among dermatophyte infections due to its unusual clinical characteristics (Kasperova *et al.* 2013).

2.8.4 Tinea barbae

Tinea barbae is a typical zoophilic infection localized on the hairy skin of the beard in men. The causative agents that are most common are *T. verrucosum* and *T. mentagrophytes var. mentagrophytes*, most often, farmers and veterinarians get infected. Infection is also transmitted by cattle or rodents (Seeliger and Heymer 1981). *T. barbae* is one of the most serious dermatophyte infections and difficult to treat. There are deep infiltrates of follicular papules and pustules, painful nodules of furunculosis, and inflammatory discharge and crusts. Hairs are quickly removed and fall out.

2.8.5 Tinea cruris

Synonyms: *T. Inguinalis,* ringworm of the groin, jock itch The causative dermatophytes are anthropophilic, most commonly *T. rubrum*, *E. floccosum* and *T. mentagrophytes var. interdigitale*. *T. crurisis* globally distributed, but in warm and humid climates it is more prevalent. Men, in general, are impacted. It usually occurs as a result of a foot autoinfection. Obesity, diabetes, poor personal hygiene, synthetic clothes, and hyperhidrosis can all contribute to this illness. This infection can last a long time, and it is quite uncommon in youngsters (Schönborn and Schuhmann 1973). Sharply marginalized erythematous and itchy patches or plaques can be seen in the groin and inner thighs. Scaling and vesicles may develop around the edges of the lesions (Figure 2.2). It can be delivered unilaterally or bilaterally. The infection can also extend to the scrotum and perianum, the perineum, and the gluteal region (Aditya *et al.* 2003).

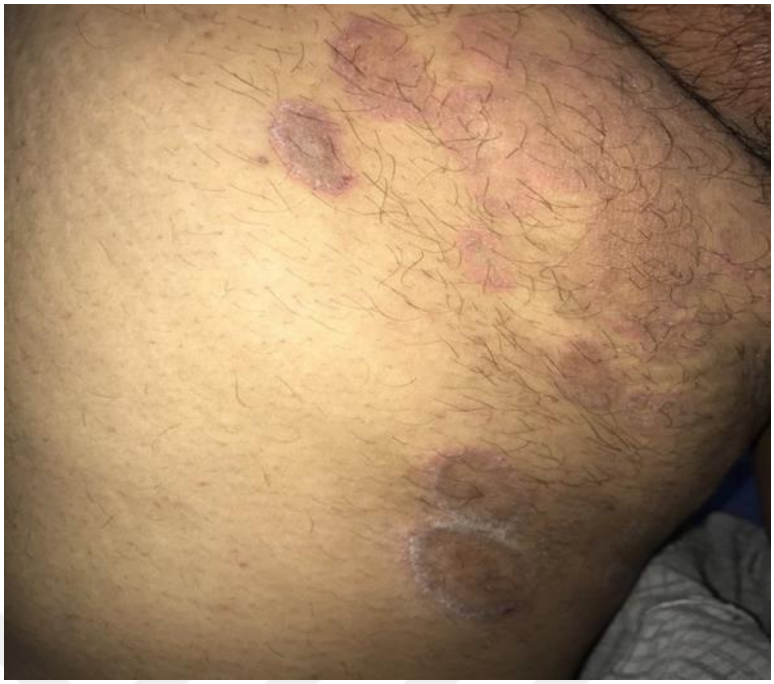


Figure 2.2 *Tinea cruris*

2.8.6 *Tinea pedis*

Synonyms: Athlete's foot, Foot ringworm *T. rubrum* and *T. mentagrophytes var. interdigitale* are nearly often the cause of this illness, which is caused by anthropophilic and dermatophytes. The most common fungus that cause disease. *E. floccosum*, on the other hand, is a less frequent species. (Dolenc-Voljč *et al.* 2005). *T. violaceum* can produce tinea pedis in places where this dermatophyte is common. *T. pedis* at the dorsal location of the foot can be caused by zoophilic dermatophytes on a sporadic basis. Since 1980, the number of *T.rubrum* infections has increased. Infections with *T. rubrum* have been on the rise (Hay and Ashbee 2010). *T. pedis* is one of the most frequent human dermatophyte infections and one of the most common human illnesses in general in underdeveloped countries. The prevalence rate in the general population is around 10% (Dolenc-Voljč *et al.* 2015). Some epidemiological studies havealso shown that adult prevalence can exceed 20% (Perea *et al.* 2000). It can affect up to 70% of individuals in special patient populations (soldiers, athletes, miners). Over 50% of sports-active people have clinical signs of foot disease, most of which are of fungal origin. Up to 70% of people may be affected by it. More than 50% of people who are

involved in sports have clinical symptoms of foot disease, most of which are of fungal origin (Summerbell *et al.* 1989).

Infected people are most likely to spread the disease in sports facilities, swimming pool resorts, hospital wards, and among family members. It is typically spread indirectly by important arthrospores. Direct human connection is a rare mode of transmission. Infections are frequently spread by people who have a foot infection. In a sufficiently warm and humid environment, arthrospores can stay infectious for months or even longer (Sahoo and Mahajan 2016). As a result, preventing this illness is difficult. Infection is more common in middle and older age, and it is more common in men. This infection affects some people more than others. Toe infection can be exacerbated by occlusive footwear and skin maceration. It's odd for people who typically go about barefoot. Obesity, immunosuppression, peripheral vascular disease, trauma, osteoarticular pathology, sports participation, and hyperhidrosis are all factors to consider (Akkus *et al.* 2016). Among risk factors warm climates, it is more common. Children are rarely infected and typically get the infection at swimming pools or from their parents. *T. pedis* may have a chronic course if untreated. It spreads from the skin to the toenails, which are also contaminated at the same time. Tinea pedis may also serve as an accessory for secondary bacterial infection.

2.8.7 Tinea unguium

The term *Tinea unguis* is used to describe a fungal nail infection caused by dermatophytes. Onychomycosis is a broad term that encompasses both yeast-caused nail infections and fungi that aren't dermatophytes. For toenail onychomycosis, the epidemiology is identical to tinea pedis.

The causative dermatophytes that most common are *T. rubrum. var. interdigitale mentagrophytes*, whereas rarely includes *E. floccosum*, *T. tonsurans* and *T. violaceum*. Dermatophytes are responsible for at least 90% of toenail onychomycosis. Toenail onychomycosis, which is similar to *Tinea pedis*, is one of the most common fungal diseases in people. It is frequently linked to tinea pedis. Its incidence in underdeveloped nations has increased in recent decades (Thomas *et al.* 2010). Prevalence rate ranging

from 2.2 to 8.4 % (Roberts 1992) was identified in the first epidemiological studies conducted some decades ago. A larger study conducted later in European countries found prevalence in the adult population of 23 % (Burzykowski *et al.* 2003). The dorsolateral variant is the most common. Discoloration and separation from the nail bed are the first signs of infection in the distal and lateral regions of the nail plate. With time, the free edge of the nail becomes brittle and might shatter (Figure 2.3). It does not result in self-healing. Infection symptoms progress slowly in a proximal manner into the lunula and nail matrix. In a few years, the full length of the nail is usually treated. The first toenail is usually the first to get infected, with the illness spreading to the other nails afterwards. The superficial white onychomycosis is a rare kind of onychomycosis (leukonychia trichophytica). The infection is seen on the dorsal area of the nail plate, with white spots that can progress to the deeper layers of the nail plate.

2.9 Diagnosis Of Dermatophytosis

The morphological characteristics were used to identify the 70,000 described funguses. Fungi, on the other hand, are known for their phenotypic flexibility, a trait that has led to the comment that fungi are "a changeable and treacherous group (Buxton 2000). Different species can be assigned to forms that are in reality closely related. A group of individuals, on the other hand, may have certain physical characteristics yet be genetically separated. As a result, morphological criteria (morphological species recognition, MSR) are insufficient for species recognition (Taylor *et al.* 2000).

With recombinant DNA technology, long stretches of DNA can be cloned, sequenced, and compared for determining whether two nucleic acid molecules are similar. Nucleic acid sequences are scanned and fitted by computer programs into a branching tree pattern based on maximum parsimony. A phylogenetic system that classifies organisms according to their evolutionary sequence, that is, which enables one to determine at a glance the ancestors and derivatives, is therefore being used in recognizing species.

2.10 Microscopic Examination

Direct microscopy and culture have long been the gold standard in dermatologic laboratory diagnosis, and they are still regarded the “gold standard” today. The proper preparation of skin, hair, and nail specimens necessitates the use of skilled professionals. Scrape the scales off the border of the skin lesion, the nailbed and nails, or pluck hairs to acquire specimens. You can use a sterilized scalpel, curette, or tweezers. A drop of 10–20 percent KOH solution is put on a glass slide with the scales (Nenoff *et al.* 2014). This exam is also known as a "native examination." The KOH dissolves keratin, allowing hyphae and spores to be more transparent. The hyphae become more apparent when specific dyes (Parkers blue or lactophenol blue) are applied. Using fluorescent dyes and examining using a fluorescence microscope can improve the sensitivity of microscopic inspection (Dolenc-Voljč *et al.* 2015). Direct microscopy is a simple and accessible approach. It can help a doctor choose the best treatment for a patient. Spores and *Hyphae* are easily visible. It is possible to examine the distribution of spores throughout the hair shaft. The spores of ectothrix species are found in the outer layer of the hair shaft, whereas the spores of endothrix species are found within the hair shaft (Hay and Ashbee 2010).

Microscopic examination, on the other hand, can only identify the existence or absence of fungal components, not their vitality. Dermatophytes only exist as mycelium and arthroconidia within keratinized tissue. *Hyphae* and arthrospores generated by hyphae are morphologically identical in all dermatophyte diseases (AL-Jubori and Hasan 2015). In addition, false-negative microscopic examination results should be taken into account. To avoid false-negative findings, topical antimycotic medications should be ceased for at least one week before to mycological testing (Dolenc-Voljč *et al.* 2015).

2.11 Culture

Culture is an important complement to direct microscopy because it allows dermatophytes to be identified. Sabouraud Dextrose Agar (SDA), the most extensively used in medical mycology, is the most common medium used for dermatophyte isolation (Brun *et al.*2001) amended with chloramphenicol and Gantamicin to inhibit bacterial

and saprophytic fungal contamination. The results of laboratory fungal culture are usually not available for 3–6 weeks (Nenoff *et al.* 2014). Dermatophytes are identified by their gross colony features and microscopic appearance. Color and development rate of dermatophyte cultures vary greatly. Microconidia and macroconidia are species-specific, but macroconidia and microconidia are identical in most species. The shape of hyphae, which might be helical, raquet, or pectinate, can also aid in identification (Hay and Ashbee 2010). The color, physical look, and morphological features of the culture may vary as it grows. It may be difficult to distinguish between various dermatophyte species based on morphological characteristics. Some physiological tests can be additionally used.

2.12 Diagnostic Features Of The Most Important Types Of Ringworm

2.12.1 Epidermophyton floccosum

Epidermophyton floccosum is an anthropophilic dermatophyte with a worldwide distribution which often causes tinea pedis, tinea cruris, tinea corporis and onychomycosis. It is not known to invade hair in vivo and no specific growth requirements have been reported (Žárová 2018).

Colonies are often slow-growing morphologically. White pleomorphic tufts of mycelium may appear in older cultures. In most cases, a deep yellowish-brown reverse pigment is visible. *Microconidia* do not develop. Greenish-brown or khaki-colored, suede-like surface, elevated and folded in the middle, flat perimeter, and submerged fringe of development The *Macroconidia* are smooth, thin-walled, and develop in clusters straight from the *Hyphae*, as evidenced by microscopic morphology. In ancient civilizations, a large number of chlamydospores develop (Žárová 2018).

2.12.2 The *Microsporum* genus

Several *Microsporum* species are involved in human and animal infections, depending on geography and hosts' characteristics. *M. audouinii*, *M. ferrugineum*, *M. nanum*, *M. canis*, and *M. gypseum* are the most often isolated species, and *M. canis*, *M. audouinii*,

M. ferrugineum, and *M. gypseum* are the most often recovered species from human infections (Di Mattia *et al.* 2019).

2.12.2.1. *Microsporum canis*

Microsporum canis is a zoophilic dermatophyte of worldwide distribution and is a frequent cause of ringworm in humans, especially children. Invades hair, skin and rarely nails. Cats and dogs are the main sources of infection. Invaded hairs show an ectothrix infection and fluorensce a bright greenish-yellow under Wood's ultra-violet light (Di Mattia *et al.* 2019).

Morphological description colonies are flat, spreading, and white to cream-colored, with a dense cottony surface which may show some radial grooves. Colonies usually have a bright golden yellow to brownish-yellow reverse pigment, but non-pigmented strains may also occur. *Macroconidia* are typically spindle-shaped with 5-15 cells, verrucose, thick- walled, and often have a terminal knob, 35-110 x 12-25 μm. A few pyriform to clavate microconidia are also present Figure 4.1.

Macroconidia and/or *Microconidia* are often not produced on primary isolation media and it is recommended that subcultures be made onto lactritmel agar and/or boiled polished rice grains to stimulate sporulation (Isa-Isa *et al.* 2010).

Microsporum audouinii is an anthropophilic fungus causing non-inflammatory infections of the scalp and skin, especially in children. Once the cause of epidemics of tinea capitis in Europe and North America, it is now less common. Invaded hairs show an ectothrix infection and usually fluorensce a bright greenish-yellow under Wood's ultraviolet light. Only rarely found in Australasia (Isa-Isa *et al.* 2010). Morphological description of colonies are flat, spreading, and greyish-white to light tanwhite in color, and have a dense suede-like to downy surface, suggestive of mouse fur in texture. Reverse can be yellow-brown to reddish-brown in colour. Some strains may show no reverse pigment. *Macroconidia* and *Microconidia* are rarely produced. Most cultures are sterile or produce only occasional thick-walled terminal or intercalary chlamydospores. When present, macroconidia may resemble those of *M. canis* but are usually longer, smoother, and more irregularly fusiform in shape; microconidia, when

present, are pyriform to clavate in shape and are similar to those seen in other species of Microsporum, Lophophyton, and Nannizzia. Pectinate (comb-like) hyphae and racquet hyphae (a series of hyphal segments swollen at one end) may also be present. *Macroconidia* and *Microconidia* are only rarely producing chlamydospores (West and Kwon-Chung 1980).

2.12.2.2. *Microsporum ferrugineum*

Microsporum ferrugineum is an anthropophilic fungus causing epidemic juvenile tinea capitis in humans. The clinical features are similar to those of infections caused by *M. audouinii*. Invaded hairs show an ectothrix infection and fluorensce a greenish-yellow under Wood's ultra-violet light. Reported from Asia (including China and Japan), Russia, Eastern Europe, and Africa. Morphological description of colonies are slow-growing, forming a waxy, glabrous, convoluted thallus with a cream to buff-colored surface and no reverse pigment. Irregular branching hyphae with prominent cross walls ("bamboo hyphae") and chlamydospores are seen "Bamboo hyphae" are a characteristic of this species (Kobylak *et al.* 2016).

2.12.3 The *Trichophyton* genus

This is the most often isolated genus from clinical samples. It can affect the hairless skin, scalp, and nails. Many species are imputed as being human and animal pathogens, such as: *T. rubrum, T. tonsurans, T.mentagrophytes, T. verrucosum, T. shoenleinii, T. concentricum, T. equinum, T. violaceum*, etc. These dermatophytes are responsible for virtually all cases of infection, with higher or lesser predominance of one species or another in a particular location, due to their extremely variable incidence and dependency on geographic circumstances. Microscopical inspection reveals a great number of pyriform, oval, or spherical *Microconidia* grouped in right angles with the hyphae, bunches, or creating various structures, depending on the species. When *Macroconidia* are present, they take on an extended claviform shape with many septa. In other species, such as *T.schöenleinii*, these fructification structures are occasionally or always missing (Pchelin *et al.* 2019).

2.12.3.1. Trichophyton rubrum

Trichophyton rubrum is an anthropophilic fungus that has become the most widely distributed dermatophyte of humans. It frequently causes chronic infections of skin, nails, and rarely scalps (Su *et al.* 2019). Granulomatous lesions may sometimes occur. Infected hairs do not fluorensce under wood's ultraviolet light and microscopically may show endothrix or ectothrix type of invasion. Morphological description colonies are mostly flat to slightly raised, white to cream, suede-like to downy, with either no reverse pigment or a yellow-brown to wine-red reverse. Most cultures show scanty to moderate numbers of slender clavate to pyriform *Microconidia* Figure 4.3 *Macroconidia* are usually absent, but when present are smooth, thinwalled multiseptate, slender, and cylindrical to cigar-shaped. Older cultures may show numerous chlamydospores with a few clavate to pyriform *Microconidia* (Gräser *et al.* 2000).

2.12.3.2. Trichophyton mentagrophytes

Trichophyton mentagrophytes is a zoophilic fungus with a worldwide distribution and a wide range of animal hosts, including mice, guinea-pigs, kangaroos, cats, horses, sheep, and rabbits produce inflammatory skin or scalp lesions in humans, particularly in rural workers. Kerion of the scalp and beard may occur. Invaded hairs show an ectothrix infection but do not fluorensce under wood's ultra-violet light (Frías-De-León *et al.* 2020). Morphological descriptions for colonies are generally flat white to cream in color, with a powdery to granular surface. Some cultures show central folding or develop raised central tufts or pleomorphic suede-like to downy areas. Reverse pigmentation is usually a yellow-brown to reddish-brown colour. Numerous single-celled *Microconidia* are formed, often in dense clusters. Microconidia are hyaline, smooth-walled, and are predominantly spherical to subspherical in shape.

However occasional clavate to pyriform forms may occur. Varying numbers of spherical chlamydospores, spiral (Nenoff *et al.* 2019). Culture characteristics, microscopic morphology and clinical disease with known animal contacts. *T. mentagrophytes* can be distinguished from *T. interdigitale* by: its granular appearance on the 1% peptone agar; its microscopic morphology of more spherical microconidia and generally greater numbers of macroconidia; and a yellow to brown diffusible pigment is often seen on the

Littman oxgall agar. Both *T. interdigitale* and *T. mentagrophytes* demonstrate profuse growth and alkalinity on BCP milk solids aga hyphae and smooth, thin-walled, clavate-shaped, multicelled *Macroconidia* may also be present (Rønnest *et al.* 2012).

2.12.3.3. Trichophyton interdigitale

Trichophyton interdigitale is an anthropophilic fungus which is a common cause of tinea pedis, particularly the vesicular type, tinea corporis, and sometimes superficial nail plate invasion in humans (Watanabe *et al.* 2019). It is not known to invade hair in vivo but produces hair perforations in vitro. This species may be regarded as a clonal offshoot of the zoophilic *T. mentagrophytes*. Morphological description for colonies are usually flat white to cream in color with a powdery to the suede-like surface and yellowish to pinkish-brown reverse pigment, often becoming a darker red-brown with age. Numerous subspherical to pyriform microconidia, occasional spiral hyphae, and spherical chlamydospores are present, the latter being more abundant in older cultures. Occasional slender, clavate, smooth-walled, multiseptate *Macroconidia* are also present in some cultures (Zhang *et al.* 2019).

2.12.3.4. Trichophyton verrucosum

Trichophyton verrucosum is a zoophilic fungus causing ringworm in cattle. Infections in humans result from direct contact with cattle or infected fomites and are usually highly inflammatory involving the scalp, beard, or exposed areas of the body (Papini *et al.* 2009). Invaded hairs show an ectothrix infection, and fluorescence under Wood's ultra-violet light has been noted in cattle but not in humans (Kim *et al.* 1986).

Morphological description for colonies are slow growing, small, button or disk-shaped, white to cream-coloured, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth. Reverse pigment may vary from no pigmented to yellow—broad, irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are often in chains (Kim *et al.* 1986).The tips of some hyphae are broad and club-shaped and occasionally divided, giving the so-called "antler" effect.

2.12.3.5. *Trichophyton violaceum*

Trichophyton violaceum is an anthropophilic fungus causing inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails, beard and scalp, producing the so-called “black dot” tinea capitis. Distribution is worldwide, particularly in the Near East, Eastern Europe, Russia, and North Africa. Invaded hairs show an endothrix infection and do not fluoresce under Wood’s ultra-violet light (Magill *et al.* 2007).

Morphological descriptions for colonies are very slow growing, glabrous or waxy, heaped and folded and deep violet in colour. Cultures often become pleomorphic, forming white sectors. Occasional non-pigmented strains may occur. Hyphae are relatively broad, tortuous, much branched, and distorted. Young hyphae usually stain well in lactophenol cotton blue, whereas older hyphae stain poorly and show small central fat globules and granules. Typically, no conidia are present, although occasional pyriform *Microconidia* have been observed on enriched media (Mapelli *et al.* 2013). Numerous chlamydospores are usually present, especially in older cultures.

2.13 Serological

In the diagnosis of dermatophyte infections, serological testing is irrelevant. Antibodies to dermatophytes can persist in the blood for years, thus they can't be used to diagnose an acute infection. The trichophytin test is only useful as a marker of cellular immunological response and does not play a substantial role in diagnostic confirmation (Grappel *et al.*1974).

2.14 Molecular

Advanced molecular diagnostics provide successful approaches for rapid diagnosis of pathogenic dermatophytes with high accuracy, sensitivity, and specificity, applicability to a variety of specimen types, ability to provide results unaffected by growth conditions, and high discriminatory power, ensuring identification of fungi that would otherwise be indistinguishable phenoty-wise (Behzadi *et al.* 2013).

By providing species-level identification of fungi through the sequencing of appropriate taxonomic markers, shortening the time required for microbiological confirmation of life-threatening fungal infections, and tracing the molecular epidemiology of fungal diseases, molecular methods have resolved many critical aspects of mycological diagnosis (Gherbawy and Voigt 2010). In molecular diagnostic methods, DNA molecules extracted from clinical samples are amplified by universal primer sets that targeting the regions of 18S rDNA and Internal Transcribed Spacer (ITS) (Alzubaidy and Al-Gburi 2018). The ITS regions are species-specific for the three dermatophyte genera of *Epidermophyton*, *Microsporum*, and *Trichophyton*. This region has four primary advantages over other regions: (1) it is multicopy, so the amount of starting material needed for successful amplification is extremely low; (2) it has well-conserved fungal specific priming sites directly adjacent to multiple highly variable regions; (3) there are many sequences already available for comparison, which greatly facilitates the identification of unknown samples; and (4) it correlates well with morphologically defined species in many groups conventional (Peay *et al.* 2008).

Polymerase chain Reaction (PCR), Nested-PCR, RealTime PCR, Multiplex PCR, (RFLP), (AFLP), (RAPD), and DNA microarray and other nucleic acid-based techniques provide faster, easier, and confident species-level diagnostic approach which may lead to an effective treatment at early stage of dermatophytosis (Gherbawy and Voigt 2010).

Recently, molecular studies have made significant progress in fungal classification and identification, employing methods based on highly performing molecular targets that act as evolutionary clocks for phylogenetic investigations. The set of genes that code for nuclear proteins is one such target. The fact that its sequences encode for multiple-copy loci, whose repeated copies in tandem are synchronized by concerted evolution, and therefore may be considered as a single locus, is the major reason for its effectiveness as an evolutionary marker. Ribosomes are also found in all species and have a similar evolutionary origin (Begum *et al.* 2020). Parts of the molecule are highly conserved and serve as reference points for evolutionary divergence studies. The 5.8S, 18S, and 25–28S rRNA genes (or rDNAs) are transcribed as a 35S to 40S precursors, along with internal and external transcribed spacers (ITS and ETS, respectively), which are spliced

out of the transcript (Abd-Elmegeed *et al.* 2020). The conserved regions alternate with divergent domains (D1 and D2) and highly variable regions (ITS). Between each cluster, there is a non transcribed or intergenic spacer (NTS or IGS) that serves to separate the repeats from one another along the chromosome. A 5S rRNA gene takes a variable position and transcription direction depending on the fungal group. The total length of one DNA repeat is between 7.7 and 24 Kb. For the phylogeny of filamentous fungi, the 18S rDNA (also called the small-subunit, SSU) is most often used either as a full-length sequence or as a subdomain of ca. 600 bp. The divergent domains of the 25–28S (also called the large-subunit, LSU) rDNA are very informative and allow comparisons from high taxonomic levels down to the species level, although only a limited number of variable positions are present (Mochizuki *et al.* 2017).

In the 18S rRNA gene, the variable domains mostly provide insufficient information for diagnostic purposes, and large parts of the molecule must be sequenced to obtain the resolution required for species identification. In contrast, the 5.8S rDNA is too small and has the least variability. The 5S molecule has mainly been used to infer relationships at the order level, where differences could be traced back to the secondary structure of the molecule (Ahmadi *et al.* 2016). The ITS regions are much more variable, and sequences can be aligned with the confidence only between closely related taxa. These regions have been extensively used to assess intraspecies differentiation. In yeasts, the D1 and D2 variable regions of the 25–28S rDNA have been extensively used for species-level identification. A region encompassing the D2 domain has also been exploited to produce a commercial sequencing kit based on the interrogation of libraries of fungal D2 rDNA sequences (MicroSeq D2 LSU rDNA Fungal Identification Kit, Applied Biosystems). The MicroSeq system is composed of PCR and cycle sequencing modules, identification and analysis software, and a D2 sequence library. Also, mitochondrial targets, considered as “multicopy” loci because of the multiplicity of mitochondria per cell, have been exploited as molecular tools for the classification and identification of molds and yeasts. Introns of several protein-encoding genes, such as the b-tubulin, actin, chitin synthase, acetyl coenzyme A synthase, glyceraldehyde-3-phosphate dehydrogenase, lignin peroxidase, or orotidine 50 -monophosphate decarboxylase genes, can also provide information at the species level (Verrier and Monod 2017).

3. MATERIALS AND METHODS

3.1. Material

3.1.1. Instruments and equipment

The instruments and equipment used in this study are listed in Table 3.1.

Table 3.1 The instruments and equipment's used in the study

Equipment	**Manufacturer**	**Origin**
AURA TM PCR Cabinet	BioSan	Germany
Autoclave	Nüve	Turkey
Balance	Kernpfb	Germany
Bio TDB-100, Dry block thermostatbuilt	Bio San	Germany
Centrifuge	Hettich	Germany
Combi-spin	Biosan	Latvia
Distillator	GFL	Germany
Deep-freezer	Hitachi	Japan
Digital camera	Sony	Japan
Document system	Labnet	USA
Electrophoreses	CBS, Scientific	USA
Gel Imaging system	Major science	Taiwan
Haemocytometer	Neubauer	Germany
Hot plate with magnetic stirrer	Gallenkamp	England
Incubator	Memmert	Germany
Laminar air flow cabinet	Techne	UK
Light microscope	Olympus	Japan
Micro spin centrifuge	Myfugene	China
Microwave	Gosonic	China
Mini-Power Supply 300V, 2200V	MAX	China
Multi Gene OptiMax Gradient Thermal Cycler	Labnet	USA
OWL Electrophoresis system	Thermo	USA
Oven	Sailbran	China
Petri Dish	WTW	Germany
pH meter	Memmert	Germany
Pipette	WTW	Germany
Quintus florometer	Promega	USA
Rotary evaporator	Quikfit	England

Table 3.2 The instruments and equipment's used in the study

slides and cover slides	Superestar	India
Standard wire loop (1m)	Himedi	India
Sensitive electronic balance	Gallen Kaamp	England
V-1 plus, Personal Vortex for tubes	Digsystem	Germany

3.1.2. Chemicals and biological materials

The chemicals and biological materials used in this study are listed in Table 3.2.

Table 3.2 Chemical and biological materials used in this study

Chemical	**Manufacturing company**	**Origin**
Agarose	Conda	USA
Absolute ethanol	Hazard	UK
Agar	BDH	UK
Ammonium Chloride	BDH	UK
Calcium Chloride	BDH	UK
Chloramphenicol	LBRAWN	India
Dextrose	Hi-Media	India
Ladder 1000 plus bp	Intron	Korea
Loading dye (6X)	Intron	Korea
Ethanol Absolute	Merck	Germany
Gentamicin	LBRAWN	India
Pre mix pcr	Intron	Korea
Potassium hydroxide	BDH	UK
Red safe staining souluion	Intron	Korea
TBE buffer 10 X	Conda	USA
Yeast extract	BDH	UK
ZR Fungal/Yeast/Bacterial DNA MiniPrep	ZYMO	USA

3.2. Methods

During the period from October 2020 to May 2021, 100 samples were collected from patients diagnosed with dermatophytes in the Consultant Dermatology and Venereology department at Ramadi Teaching Hospital in Anbar Governorate. All individuals included in this study were seen by a medical professional. They were interviewed and answered

questions regarding their location of residence, duration of infection, previous use of antibiotics and location of the infection in the body. Skin, nail and hair samples were placed on clean, strong paper; black were transferred to see tiny particles of scrapings. The paper was folded and taped to form a leakproof packet and transported in an envelope, and the instruments and equipment used in this study are listed in Table 3.1.

3.2.1. Sample collection

3.2.1.1. Collection of skin samples

Samples from dermatophyte infections were collected from the margins instead of the center of lesions. The affected area was sterilized using ethyl alcohol 70% to get rid of bacteria and fungi, and the peripheral edge of lesions were scraped with a scalpel and placed on clean, strong paper black until direct microscopy (Al-Doory 1980).

3.2.1.2. Collection of hair samples

Damaged broken hair samples (10 to 12 hairs) were collected from the affected persons using sterile forceps and the scalp of the head was included if infected with dermatophytes. After being sterilized with 70% ethyl alcohol, the samples were kept between two glass slides until other diagnostic tests were done (Hay 2017).

3.2.1.3. Collection of nail samples

When fungal infections were suspected in nails, they were cleansed with alcohol wipes and scraped deeply enough to obtain recently invaded nail tissue. The most rewarding samples were usually from under the nail, close to the nail bed. If the infection was on the surface of the nail plate, then scrapings were taken from that area. Initial scrapings that were likely to be contaminated, were discarded and only the deeper samples were sent to the laboratory in a packet (Kayarkatte *et al.* 2020).

3.2.2. Methods for direct microscopic examination of specimens

3.2.2.1. Preparation of slide

1. A portion of the specimen was placed a on a labelled slide.
2. One drop of potassium hydroxide (KOH) ink solution was added to the slide.
3. A coverslip was put on the preparation.
4. The preparation was heated gently over the flame, and boiling was avoided.
5. For the required degree of chemical softening, portions of nail samples were heatedagain and sustained heating was kept on, so that the material can be pressed outto afford good visibility; instead, the nail was submerged overnight for complete softening, then clearing in the KOH-ink was applied.
6. In order to detect hyphal segments, spores or conidia, budding yeasts, spherules, orsclerotic bodies, the slides were carefully examined microscopically (40X).
7. During slide preparation, using of cotton swabs was avoided, as the cotton strandscan resemble hyphae.
8. KOH preparations are not permanent types; the fungi can gradually be killed by the reagent, therefore, a small amount of glycerol was added to the preparation to protect the fungus many days.

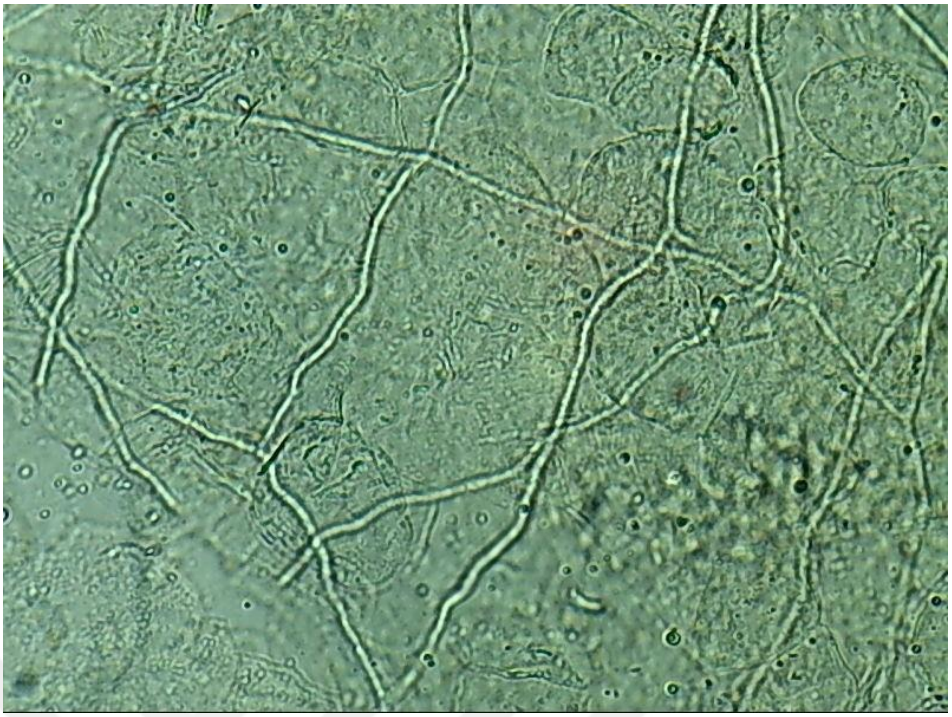


Figure 3.1 The hyphae of dermatophytes with KOH (40X)

3.2.3. Culture

3.2.3.1. Preparation of culture media

The appearance of a fungus colony depends on the medium used, but for comparative purposes, Sabouraud dextrose agar (SDA) medium was conventionally used to obtain colonies which can be compared to others reported in the literature (Ajello *et al.* 1966). Five important colony characteristics were looked for presumptive identification of a dermatophyte culture when it is one to three weeks old: (1) rate of growth (2) general topography (flat, heaped, regularly or irregularly folded) (3) texture (yeast-like, glabrous, powdery, granular, velvety or cottony) (4) surface pigmentation and (5) reverse pigmentation. The (SDA) medium was prepared according to the manufacturer's instructions.

3.2.3.2. Cultivation of specimens

After obtaining the hair, skin and nail samples, they were cut into small pieces, and inoculated with sterile forceps on the media directly, with at least three pieces were

placed in each dish. Each dish was closed using the parafilim so it did not contain any fungal growth and any growth of fungal spores that may be formed and prevent contamination of other dishes. The plates were incubated at 29±2 ºC for 14-21 days, and samples were examined every week to determine the growth of fungi by describing the colony morphology.

3.2.3.3. Examining a fungal colony for microscopic structures

3.2.3.4.Procedure

1. A piece of clear scotch tape was cut and folded back on itself, with the adhesive side turned outward, holding it between the thumb and middle finger.

2. The index finger was used; the adhesive side of the tape was pressed onto the surface of the colony and pulled it away. The aerial hyphae of the colony will stick to the adhesive surface.

3. The tape adhesive side was placed down into a drop of lactophenol-cotton blue or KOH, which was previously placed on the center of a glass slide.

4. The slide was examined microscopically for septate hyaline hyphae, chlamydoconidia, microconidia, and/or macroconidia.

The samples were identified according to describe identification key (Handke 2007, Johns and Kost 2007, Ellis *et al.* 2007).

3.2.4. Molecular diagnosis of dermatophyte isolates

Ten samples were selected from dermatophytes grown on Sabouraud Dextrose Agar (SDA) dishes, which were diagnosed microscopically, and sent for molecular examination.

Table 3.3 Fungal /Bacterial / Yeast DNA ZR MiniPrep™(Catalog No. D6005)

Product Contents ZR Fungal/Bacterial DNA MiniPrep™ (Kit Size)	D 6005 (50 preps.)	Storage Temperature
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)1	50	Room Temp.
Lysis Solution	40 mL	Room Temp.
Fungal/Bacterial DNA Binding Buffer2	100 mL	Room Temp.
DNA Pre-Wash Buffer3	15 mL	Room Temp.
Fungal/Bacterial DNA Wash Buffer	50 mL	Room Temp.

Table 3.3 Fungal /Bacterial / Yeast DNA ZR MiniPrep™(Catalog No. D6005)

DNA Elution Buffer	10 mL	Room Temp.
Zymo-Spin™ IV Spin Filters (Orange Tops)	50	Room Temp.
Zymo-Spin™ IIC Columns	50	Room Temp.
Collection Tubes	150	Room Temp.
Instruction Manual	1	-

3.2.4.1. DNA extraction kit contents

DNA was extracted from dermatophytes according to the protocol of ZYMO/ USA kit asfollows:

1. 50–100 mg (wet weight) fungal or bacterial cells was added (which were resuspended in up to 200 µL of water or isotonic buffer e.g., PBS, or up to 200 mg of tissue to a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm). 750 µL Lysis Solution was added to the tube 2, as in the Table 3.3.

2. Secured in a bead beater fitted with a 2 mL tube holder assembly and processed at maximum speed for ≥ 5 minutes.

3. The ZR BashingBead™ Lysis tube was centrifuged in a microcentrifuge at 10,000x g for 1 minute.

4. Up to 400 µL supernatant was transferred to a Zymo-Spin™ IV Spin Filter (Orange Top) in a Collection Tube and centrifuged at 7,000 x g for 1 minute.
   Note: The base of the Zymo-Spin™ IV Spin Filter (Orange Top) was snapped of prior to use

5. 1,200 µL of Fungal/Bacterial DNA Binding Buffer was added to the filtrate in the collection tube from Step 4.

6. 800 µL of the mixture was transferred from Step 5 to a Zymo-Spin™ IIC Column3 in a collection tube and centrifuged at 10,000 x g for 1 minute.

7. The flow-through was discarded from the collection tube, and step 6 was repeated.

8. 200 µL DNA Pre-wash buffers was added to the Zymo-Spin™ IIC Column in a new collection tube and centrifuged at 10,000 x g for 1 minute.

9. 500 µL Fungal/Bacterial DNA wash buffer was added to the Zymo-Spin™ IIC column and centrifuged at 10,000 x g for 1 minute.

10. The Zymo-Spin™ IIC column was transferred to a clean 1.5 mL microcentrifuge tube and 100 µL (35 µL minimum) DNA Elution Buffer was added directly to the column matrix, and centrifuged at 10,000 x g for 30 seconds to elute the DNA.

11. The optimal condition has been identified for (Initial denaturation and annealing). After performing several experiments to gain this condition, the temperature was changed throughout the work of (Gradient PCR) for all samples to select the optimal condition; the concentration for DNA template was also changed between (1.5-2 µL). These two factors are considered among the important factors in primer annealing with complement.

3.2.4.2. Agarose gel electrophoresis of DNA

Electrophoresis was done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the agarose gel.

3.2.4.3. Preparation of the agarose gel

According to Sambrook *et al.* (1989), the agarose gel was made in 1.5% by melting 1.5 g of agarose in 100 mL of previously made TBE buffer. Agarose was heated to boil then left to cool down at (45-50^{o}C). The gel was poured in the pour plate in which the plate

of agarose support was prepared after fixing the comb to make holes that would hold the samples. The gel was poured gently to avoid making air bubbles and left 30 minutes to cool down. The comb was removed gently from the solid agarose. The plate was fixed to its stand in thee electrophoresis horizontal unit represented by the tank used in the electrophoresis. The tank was filled with TBE buffer where it covered the gel surface.

3.2.4.4. Preparation of sample

Three µL of the processor loading buffer (Intron / Korea) was mixed with 5 µL of the supposed DNA to undergo electrophoresis (loading dye) Figure 3.2. After the mixing process, the process of loading is now to the holes of the gel. An electric current of 7 V \ c2 was exposed for 1-2 h till the tincture reached to the other side of the gel. The gel was tested by a source of the UV with 336 nm after putting the gel in pool containing 3 µL Red safe Nucleic acid staining solution and 500 mL of distilled water.

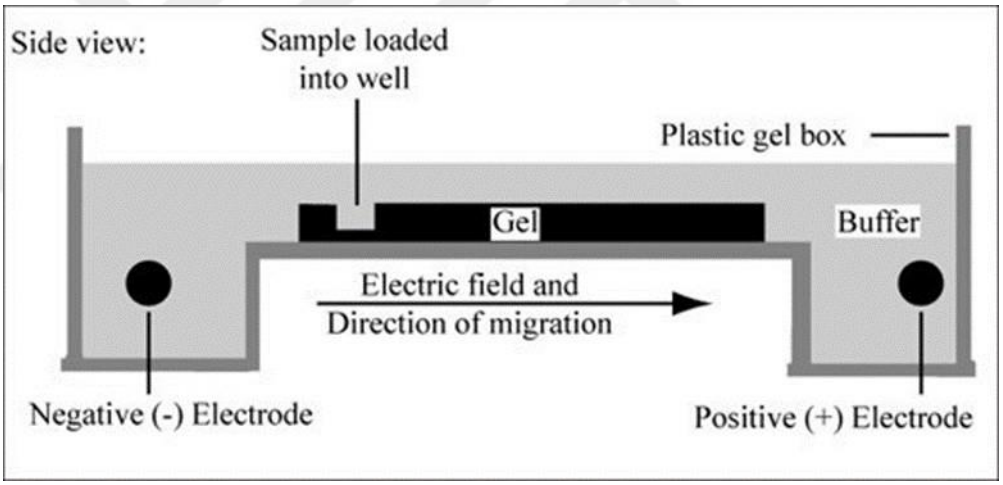


Figure 3.2 The work of the electrophoresis system

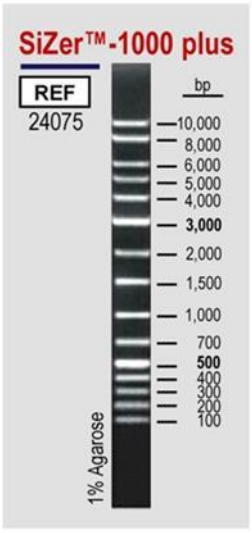


Figure 3.3 Sizer DNA markers intron

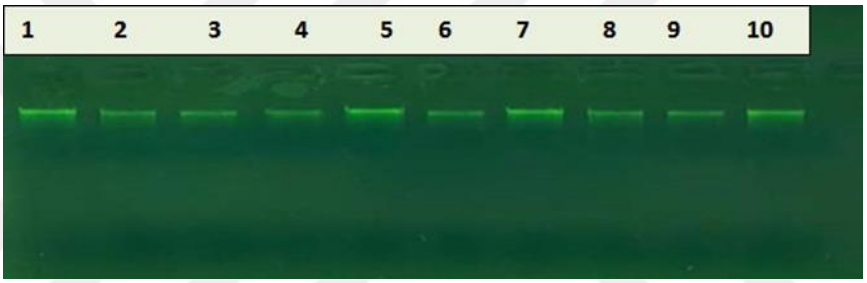


Figure 3.4 Gel electrophoresis of genomic DNA extraction from Fungi, 1.5% agarose gel at 5 vol /cm for 1houre, 1-10 sample of dermatophyes genomes

3.2.5. The primers used in the interaction

The primers were lyophilized. They were dissolved in the free ddH2O to give a final concentration of 100 pmol/µL as stock solution and kept as a stock at -20. To prepare 10 pmol/µL concentrations as work primer, 10 µL of the stock solution was suspended in 90 µL of the free ddH2O water to reach a final volume of 100 µL, and it was investigated by IDT (Integrated DNA Technologies company Canada) (Figure 3.3).

Table 3.4 The specific primer of gene ITS

Primer	Sequence	Tm (^{o}C)	GC (%)	Product size/bp
Forward	5'- TCCGTAGGTGAACCTGCGG -3'	60.3	50 %	550 base pair
Reverse	5' TCCTCCGCTTATTGATATGC-3'	57.8	41 %	

3.2.5. Detection of ITS gene by using PCR

Detection of ITS gene was conducted by using primers for amplification. A fragment of ITS was amplified using a forward primer (ITS1F:5'-TCCGTAGGTGAACCTGCGG-3') and a reverse primer (ITS4R:5' TCCTCCGCTTATTGATATGC-3') (Primers set supplied by IDT (Integrated DNA Technologies company, Canada) (Table 3.4). The PCR amplification was performed in a total volume of 25µL containing 1.5 µL DNA, 5 µL Taq PCR PreMix (Intron, Korea), 1 µL of each primer (10 pmol) then distilled water was added into the tube for a total volume of 25 µL. The thermal cycling conditions (Table 3.5). were done as follows: Denaturation at 94 ^{o}C for 3 min, followed by 35 cycles of 94 ^{o}C for 45s, 52^{o}C for 1 min and 72 ^{o}C for 1 min with final incubation at 72 ^{o}C for 7 min using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm) after staining with the red stain (Intron Korea) (Figure 3.4).

Table 3.5 The optimum condition of detection

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1-	Initial denaturation	95^{o}C	3 min	1
2-	Denaturation - 2	95^{o}C	45sec	35
3-	Annealing	52^{o}C	1 min	
4-	Extension-1	72^{o}C	1 min	
5-	Extension -2	72^{o}C	7 min	1

3.2.5.1. Maxime PCR PreMix kit (i-Taq) 20µL rxn (Cat. No. 25025)

iNtRON's Maxime PCR PreMix Kit (Table 3.6) has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution. Maxime PCR Pre Mix Kit (i-Taq) is the product that is mixed with every component: i-Taq DNA polymerase, dNTP mixture, reaction buffer and so on-in one tube for 1 rxn PCR (Table

3.7). This is the product that can get the best result with the most convenience system. The first reason is that it has every component for PCR, so we can do PCR by just adding a template DNA, primer set and D.W**.** The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. It is suitable for various sample's experience by fast and simple using method.

Table 3.6 The components of the maxime PCR remix kit (i-Taq)

Material	Volume
i-Taq DNA Polymerase	5U/ µL
DNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

Table 3.7 Mixture of the specific interaction for gene diagnosis

Components	Concentration
Taq PCR PreMix	5 µL
Forward primer	10 picomols/µL (1 µL)
Reverse primer	10 picomols/µL (1 µL)
DNA	1.5 µL
Distill water	16.5 µL
Final volume	25 µL

3.2.5.2. Determination of DNA Concentration

3.2.5.3. Procedure

1. 200 µL was added from Tris-EDTA (TE) to 3,800 of D. water to form amixture of 4000 µL. 10 µL was drawn and discarded (ignored), and 10 µL was added from dye (DNA Dye)
2. 200 µL of the mix was drawn for each sample Table 3.8.
3. The series of the following tubes were prepared as follows:
4. Vortex was made for second to mix, and
5. Left on the rack at room temperature for 5 min.
6. The value was extracted from the device immediately.

Table 3.8 Determination of DNA concentration

	Blank	Standard	Sample
Mix	200 µL	200 µL	200 µL
DNA Extraction		2 µL	2 µL

4. RESULTS

4.1.Potassium Hydroxide Microscopy And Culture Examination (SDACG)

Specimens of this study (100 skin scrapping's, hair fragments and nail fragments) It was distributed between males and females (Table 4.1) were examined by direct microscopic examination Figure 4.1 and macroscopically on SDACG media. The result of this study documented that, 92 (92%) cases were infected with dermatophyte fungi. It was shown that (60%) cases showed positive with direct microscopic examination and negative with culture results, and (12%) cases showed positive with direct microscopic and negativewith culture results, while (20%) showed negative with direct and positive with culture results (false-negative), whereas (8%) of them showed negative direct examination and culture results as shown in Table 4.2.

Table 4.1 Distribution of the infected between males and females

No.	Samples	No.	%
1	Skin	63	63
2	Hair	24	24
3	Nail	13	13
Total	-	100	100
P-value			3

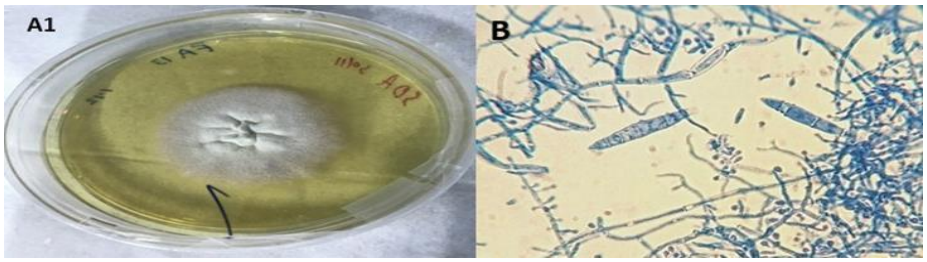


Figure 4.1 *Microspotum canis* grown on (SDA) medium after 14 days of incubation at 30 ^{0}C, A1:Surface view, B- microconidia of M. canis mounted with Lacto phenol cotton blue (40X).

Table 4.2 Relation between direct [m]icroscopic examinations KOH mounts smear and culture method on Saborud dextrose agar (SDA)

Clinical cases examination status	Number	%
Positives in both direct microscopic examination and culture	60	60
Positive direct microscopic examination and negative culture	12	12
Negative direct examination and positive culture	20	20
Negative in both direct examination and culture	8	8
Total	100	100
*P-value	0.001 (H.S)	

*Chi-sequare; H.S; highly significant

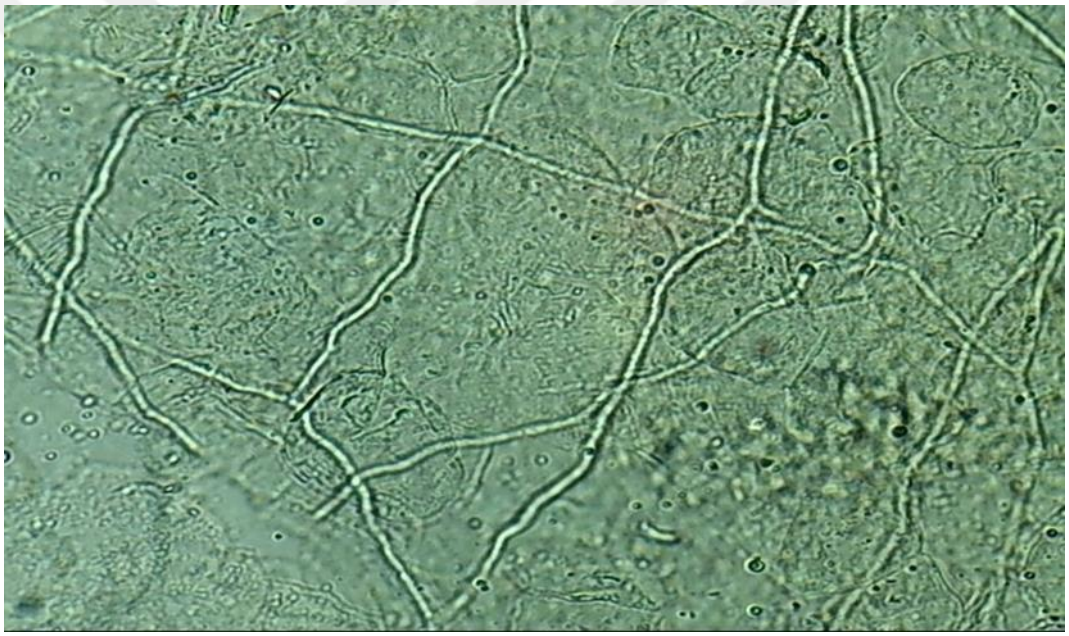


Figure 4.2 Long, thin and branching septate hyphae as a diagnostic of later dermatophyte infections KOH (40X)

4.2. Dermatophyte Infection And Gender

The relationship between dermatophytes infection and gender in this study was demonstrated in Table 4.2. Infection with dermatophytes was the highest among females (52%), while the infection rate among males was (40%), with a highly significant statistical difference at P-value= 0.006.

Table 4.3 Sex distribution of patients with dermatophyte infections

Sex	Number	%
Female	52	56.52
Male	40	43.7
Total	92	100
*P-value	0.006 **(H.S)	

*; Chi-seqaure,**; highly-significant

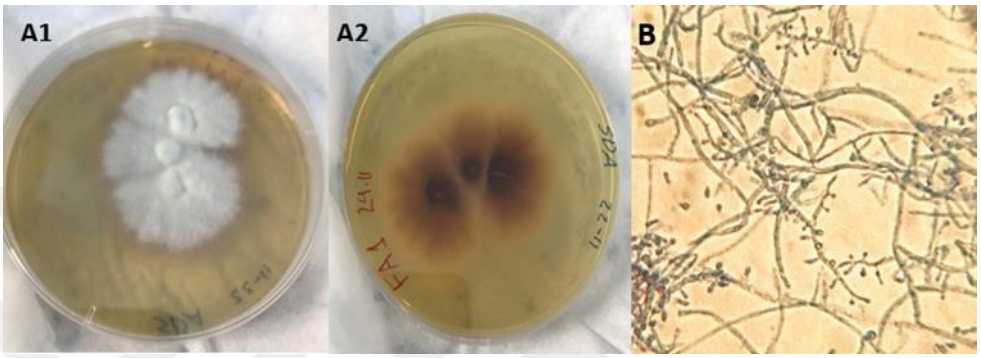


Figure 4.3 *Trichophyton rubrum* grown on SDA at 28 C0 for 7-14 days A1: surface view, A2: revers side B. *Microconidia* mounted with lacto phenol cotton blue (40X)

4.3. Distribution Of Various Dermatophyte Species In Relation To The Site Of Infection

In *Tinea corporis*, out of 56 culture isolates, *T. rubrum* was the commonest isolate 16 (17.4%) cases followed by *M. canis* 20 (21.7%) isolates, followed by *T. interdigitale* 6 (6.6%) followed by *T. mentagrophytes*, *T. ferrugineum* and *T. tonsurans* 4 (4.3%) for each, then *E. floccosum*, *T. soudanese* and *T. verrcosum* 2 (2.2%) for each. In *Tinea cruris*, out of 18 (19.6%) culture isolates, *T. mentagrophytes* canis was the commonest isolate 8 (8.73%) cases followed by *T. rubrum*, *M. canis* and *T. ferrugineum* 2 (2.18%) cases for each, followed by *T. interdigitale* 4 (4.35%).

In *Tinea faciei*, out of 6 (6.5%) culture isolates, *T. mentagrophytes* was the commonest isolate with 4 (4.3%) cases, followed by *T. ferrugineum* 2 (2.22%) cases. In *Tinea pedies*, out of 6 (6.5%) cultures isolates, *T. rubrum*, *T. interdigitale* and *T.*

mentagrophytes recorded 2 (2.17%) for each. In *Tinea capitis*, out of 4 culture isolates, *T. rubrum* 2 (2.33%) and *M. canis* was 2 (2.33%). In *Tinea manus*, out of 2 culture isolates, only *T. rubrum* was 2 (2.2%) as illustrated in Table 4.4.

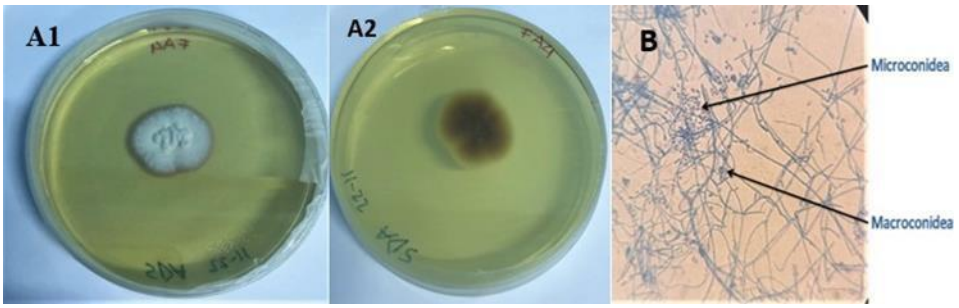


Figure 4.4 *Trichophyton mentagrophytes* grown on SDA at 30 ºC for 14 days A1: Surface view, A2: Reverse side, B. *Microconidia* and *Macroconidea* of *Trichophyton mentagrophytes* mounted with lacto phenol cotton blue (40X)

Table 4.4 Sex distribution of patients with dermatophyte infections

Tinea type	Culture									
	T. rubrum	***M. canis***	***T. mentagrophytes***	***T. interdigitale***	***T. ferrugineum***	***E. floccosum***	***T.soudanese***	***T. tonsurans***	***T. verrcosum***	**Total**
T.corporis	16 (17.4%)	16 (17.4%)	4 (4.3%)	6 (6.6%)	4 (4.3%)	2 (2.2%)	2 (2.2%)	4 (4.3%)	2 (2.2%)	56 (60.9%)
T. cruris	2 (2.18%)	2 (2.18%)	8 (8.73%)	4 (4.35%)	2 (2.18%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	18 (19.6%)
T. faciei	0 (0%)	0 (0%)	4 (4.3%)	0 (0%)	2 (2.22%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (6.5%)
T. pedies	2 (2.17%)	0 (0%)	2 (2.17%)	2 (2.17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (6.5%)
T. capitis	2 (2.33%)	2 (2.33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (4.3%)
T. manus	2 (2.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (2.2%)
Total	24 (26.0%)	20 (21.7%)	18 (19.5)	12 (13.0%)	8 (8.6%)	2 (2.3%)	2 (2.3%)	4 (4.3%)	2 (2.3%)	92 (100%)
***P-value**	**0.01(N.S)									

* Chi-sequare; *; non-significant*

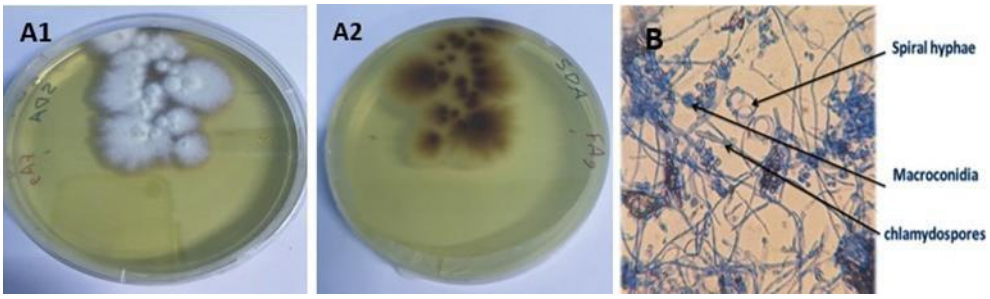


Figure 4.5 *Trichophyton interdigitale* grown on SDA at 30 ºC for 14 days A1:Surface view, A2: Revers side, B. *Microconidia*, spiral hyphae and chlamydospores of *Trichophyton mentagrophytes* mounted with lacto phenol cotton blue (40X)

4.4. Distribution Of Clinical Types Of Isolated Ringworm Infections

The relationship between dermatophyte infections and gender in this study was shown in Table 4.5 Infection with *T. corporis* was the highest among males and females, while *Tinea manuum* infections were the lowest in both gender of patients. Dermatophytes isolated from *T. corporis*, *T. cruris*, *T. capitis*, *T. faciei*, *T. manuum* and *T. pedis* were higher in females than males with a significant difference at (P<0.006).

Table 4.5 Distribution of clinical types of the isolated *Tinea* infections according to gender

Gender	***Tinea* type**						**Total**
	T.manus	***T.capitis***	***T.faciei***	***T.pedies***	***T.cruris***	***T.corporis***	
Male	26 (28.4%)	4 (4.3%)	4 (4.3%)	0 (0%)	4 (4.3%)	0 (0%)	38 (41.3%)
Female	30 (32.6%)	14 (15.3%)	2(2.2%)	6 (6.5%)	0 (0%)	2 (2.18%)	54 (58.7%)
Total	56 (61%)	18 (19.6%)	6 (6.5%)	6 (6.5%)	4 (4.3%)	2 (2.18)	92 (100%)
P-value	0.006 (H.S)						

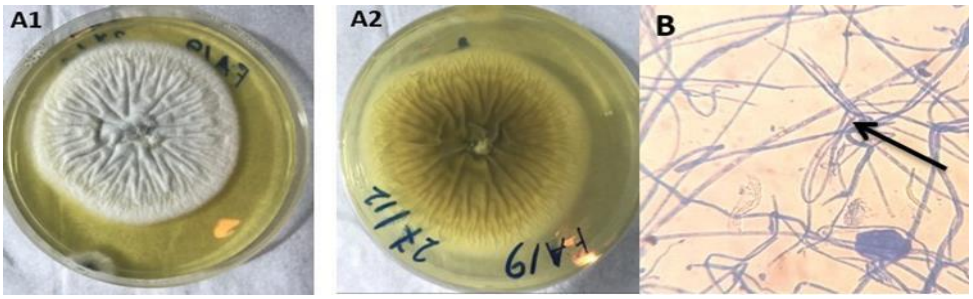


Figure 4.6 *Microsporum ferrugineum* grown on SDA at 30 ºC for 14 days A1:Surface view, A2: Revers side, B. Bamboo hyphae of *Microsporum ferrugineum* mounted with lacto phenol cotton blue (40X)

4.5. Relationship Between Dermatophyte Infection And Residency Of Patients

The results in Table 4.5 revealed that the percentage of infection with drermatophytes was different according to the area of residence countryside and city Table 4.6. Out of sixty, 42 (45.8%) cases of *T.corporis* were picked up from countryside versus 14(15.2%) cases out of 32 (34.8%) cases picked up from city. In this table, it was also observed that there was less cases of *T. cruris 10* (10.9%) out 32 (34.8%) were picked up from city versus 8 (8.7%) out 60 (62.2%) picked up from countryside. The number and percentage of city-resident infected people with *T. faciei* was 4 (4.35%), while in the countryside, the rate of people infected with *T. faciei* was 2 (2.15%). Other cases of tinea infection types showed less cases of infection among both city and countryside residents, with a statistically significant differences at (P= 0.007).

Table 4.6 Relationship between dermatophyte infection and residency of patients

Residence	***Tinea* type**						**Total**
	T.manus	***T.capitis***	***T.pedies***	***T.faciei***	***T.cruris***	***T.corporis***	
City	14 (15.2%)	10 (10.9%)	4 (4.35%)	0 (0%)	2 (2.18%)	2 (2.18%)	32 (34.8%)
Country side	42 (45.8%)	8 (8.7%)	2 (2.15%)	6 (6.5%)	2 (2.12%)	0 (0%)	60 (65.2%)
Total	56 (61%)	18 (19.6%)	6 (6.5%)	6 (6.5%)	4 (4.3%)	2 (2.18%)	92 (100%)
P-value	(0.007) H.S						

4.6. The Relations Between Infection And Age

In Table 4.5 demonstrated the percentage of infection with dermatophyte according to patient's age. The results showed that 14 out of 54 cases of *Tinea corporis* occurred within the age group (21-30) years, and 11 cases of *T. corporis* occurred within the age group (31-40) years. The lowest infection cases (0-7) cases occurred due to other *Tinea* types such as *T. cruris, T. capitis, T. faciei, T. manuum, T. pedis* and *T. unguium* among different age groups., with a statistically significant differences at (P=0.03).

Table 4.7 Distribution of dermatophytosis according to age of patients

Clinical types	Categorial age group (years)							Total n(%)
	(1-10)	**(11-20)**	**(21-30)**	**(31-40)**	**(41-50)**	**(51-60)**	**(61-70)**	
T.corporis	4 (50%)	4 (44.4%)	14 (70%)	11 (44%)	7 (50%)	9 (81.8%)	5 (100%)	54
T. cruris	1 (12.5%)	3 (33.3)	4 (20%)	7 (28%)	3 (21.4%)	0 (0%)	0 (0%)	18
T. capitis	3 (37%)	0 (0%)	1 (5%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)	6
T. faciei	0 (0%)	0 (0%)	0 (0%)	2 (8%)	1 (7.14%)	0 (0%)	0 (0%)	3
T. manuum	0 (0%)	0 (0%)	1 (5%)	1 (4%)	0 (0%)	1 (9%)	0 (0%)	3
T. pedis	0 (0%)	1 (11.1%)	0 (0%)	2 (8%)	1 (7.14%)	0 (0%)	0 (0%)	4
T.unguium	0 (0%)	1 (11.1%)	0 (0%)	0 (0%)	2 (14.2%)	1 (9%)	0 (0%)	4
Total	8	9	20	25	14	11	5	92
n. %	8.69%	9.78%	21.7%	27.1%	15.2%	11.9%	5.43%	100%
***P-value**	(0.03) **S							

4.7.Molecular Confirmatory Diagnosis Of Dermatophytes Isolates Using Conventional PCR Method

4.7.1. DNA quality and purity

After extraction of the DNA of fungus isolates, Nanodrop spectrophotometer at wavelength (260-280) nm was used to measure the quality and purity of fungus genomes. The value ranging from 1,9 to 2 was depended as shown in Table 4.8. Based on the standard values of DNA concentrations for PCR amplification, the values of the

present study were considered efficient values and suitable for the establishment of the DNA extracted with target primers or sequences amplification for 10 selected fungus samples (Applied Biosystems 2008).

Table 4.8 Nanodrop spectrophotometer values of DNA concentrations and purity of selected 10 fungus samples

Sample code	Nucleic acid conc. (ng/mL)	(260/280) purity
1	77	1.8
2	89	1.9
3	112	1.98
4	67	1.8
5	78	1.9
6	222	2
7	67	1.9
8	110	2
9	119	1.9
10	117	1.8

4.8. Amplification Of Extracted DNA With Primers (ITS1)

In the present study, the confirmation process of the ten dermatophyte isolates was also conducted by conventional PCR technique to detect the presence of a specific 5.8S rRNA gene. The extracted genomic DNAs of these isolates were used as a template for amplification with specific primers of Internal Transcribed Spacer1 (ITS1). The results revealed that 10/10 (100%) isolates were classified as dermatophytes with amplicon size equal to 550 bp after band electrophoresis and UV-transilluminated of the product as seen in Figure 4.7. Type of polymorphism of isolate of 18S ribosomal RNA gene as shown in Table 4.8.

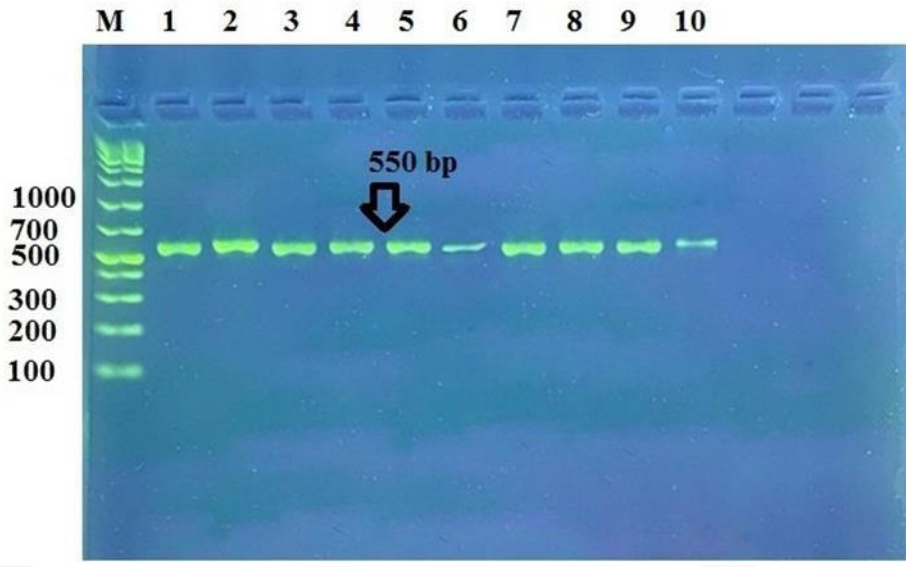


Figure 4.7 Ethidium bromide-stained agarose gel of PCR product amplified with ITS1 primer of dermatophytes (Amplicon size = 550bp), where lane M = marker, 100-1000bp; Lane (1-10) dermatophytes isolates

Table 4.9 Type of polymorphism of isolate of 18S ribosomal RNA gene

Gene: 18S ribosomal RNA gene						
No	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transition	C\T	231	ID: MN894010.1	*M. canis*	99%
	Transvertion	C\G	232			
	Transition	G\A	581			
2	Transvertion	T\G	442	ID: MG889853.1	*T. interdigitale*	99%
	Transvertion	A\C	544			
3	Transition	G\A	316	ID: MF155590.1	*T. rubrum*	99%
	Transvertion	G\T	327			
4	Transition	G\A	192	ID: MG881876.1	*T. mentagrophytes*	99%
	Transition	G\A	677			
5	Transition	G\A	380	ID: AJ252336.1	*M. ferrugineum*	99%
	Transvertion	A\T	382			
6	Transition	C\T	331	ID: MN807401.1	*T.tonsurans*	99%
	Transvertion	G\C	489			
	Transition	G\A	623			
7	Transvertion	G\T	287	ID: MN691058.2	*T. soudanense*	99%
	Transvertion	A\C	291			
	Transvertion	C\G	374			
8	Transition	G\A	305	ID: MT497455.1	*E. floccosum.*	99%
	Transition	C\T	317			
	Transition	C\T	322			
	Transition	T\C	443			
	Transition	T\C	448	ID: MT636892.1	*T.verrucosum*	99%
9	Transvertion	C\G	86			
	Transition	C\T	164			

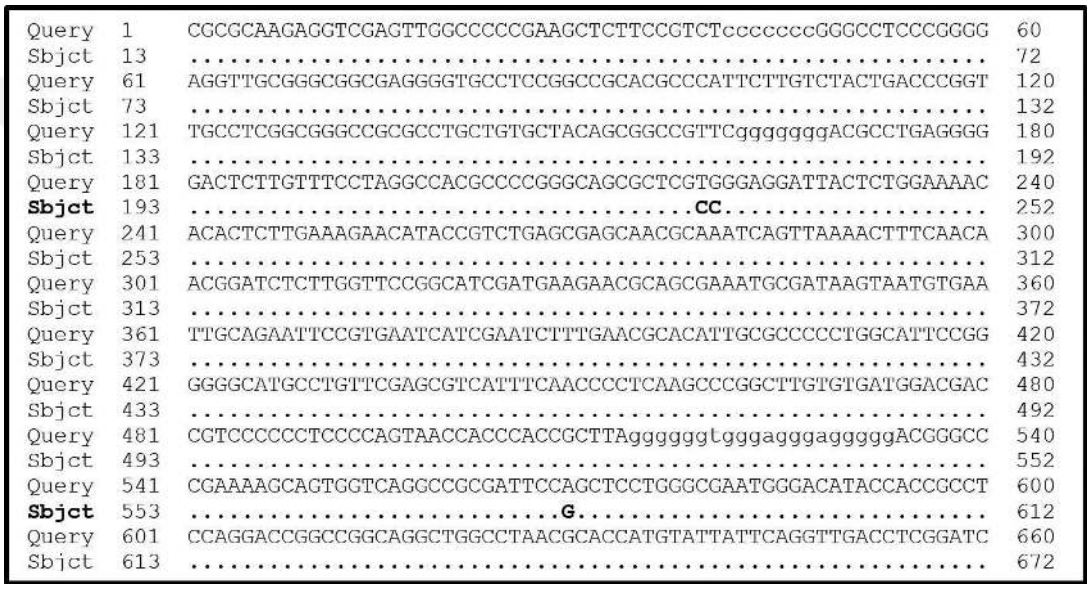
1

Microsporum canis isolate BCH33059 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MN894010.1 Length: 697 Number of Matches: 1

Range 1: 13 to 694 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1217 bits(1349)	0.0	679/682(99%)	0/682(0%)	Plus/Plus



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Query  1    CGCGCAAGAGGTCGAGTTGGCCCCCGAAGCTCTTCCGTCTcccccccGGGCCTCCCGGGG  60
Sbjct  13   ............................................................  72
Query  61   AGGTTGCGGGCGGCGAGGGGTGCCTCCGGCCGCACGCCCATTCTTGTCTACTGACCCGGT  120
Sbjct  73   ............................................................  132
Query  121  TGCCTCGGCGGGCCGCGCCTGCTGTGCTACAGCGGCCGTTCgggggggACGCCTGAGGGG  180
Sbjct  133  ............................................................  192
Query  181  GACTCTTGTTTCCTAGGCCACGCCCCGGGCAGCGCTCGTGGGAGGATTACTCTGGAAAAC  240
Sbjct  193  ..........................................CC..................  252
Query  241  ACACTCTTGAAAGAACATACCGTCTGAGCGAGCAACGCAAATCAGTTAAAACTTTCAACA  300
Sbjct  253  ............................................................  312
Query  301  ACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA  360
Sbjct  313  ............................................................  372
Query  361  TTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGG  420
Sbjct  373  ............................................................  432
Query  421  GGGGCATGCCTGTTCGAGCGTCATTTCAACCCCTCAAGCCCGGCTTGTGTGATGGACGAC  480
Sbjct  433  ............................................................  492
Query  481  CGTCCCCCCTCCCCAGTAACCACCCACCGCTTAgggggggtgggagggagggggACGGGCC  540
Sbjct  493  ............................................................  552
Query  541  CGAAAAGCAGTGGTCAGGCCGCGATTCCAGCTCCTGGGCGAATGGGACATACCACCGCCT  600
Sbjct  553  ............................G...............................  612
Query  601  CCAGGACCGGCCGGCAGGCTGGCCTAACGCACCATGTATTATTCAGGTTGACCTCGGATC  660
Sbjct  613  ............................................................  672
```

2

Trichophyton interdigitale isolate 30816 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MG889853.1 Length: 715 Number of Matches: 1

Range 1: 35 to 667 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1133 bits(1256)	0.0	631/633(99%)	0/633(0%)	Plus/Plus

```
Query  1    CACGATAGGGCCAAACGTCCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCAT  60
Sbjct  35   ............................................................  94
Query  61   TCTTGTCTACCTTACTCGGTTGCCTCGGCGGGCCGCGCTCTTCCAGGAGAGCCGTTCGGC  120
Sbjct  95   ............................................................  154
Query  121  GAGCCTCTCTTTAGTGGCTAAACGCTGGACCGCGCCCGCCGGAGGACAGACGCaaaaaaa  180
Sbjct  155  ............................................................  214
Query  181  TTCTTTCAGAAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAACTTTCAA  240
Sbjct  215  ............................................................  274
Query  241  CAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTG  300
Sbjct  275  ............................................................  334
Query  301  AATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCC  360
Sbjct  335  ............................................................  394
Query  361  GGGGGGCATGCCTGTTCGAGCGTCATTTCAGCCCCTCAAGCCCGGCTGGTGTGATGGACG  420
Sbjct  395  ...............................................T............  454
Query  421  ACCGTCCGGCGCCCCCGTTTTTGGGGGTGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCC  480
Sbjct  455  ............................................................  514
Query  481  GCGATTCCGGCTTCCTAGGCGAATGGGCACCAAACCAGCGCCTCCAGGACCGGCCGCCCT  540
Sbjct  515  ............................A...............................  574
Query  541  GGCCTCAAAATCTGTTTTATACTTATCAGGTTGACCTCGGATCAGGTAGGAATACCCGCT  600
Sbjct  575  ............................................................  634
Query  601  GAACTTAAGCTTAAAAAgggggggggggAAAAA  633
Sbjct  635  .................................  667
```

3

Trichophyton rubrum isolate 613.PTCC small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MF155590.1 Length: 692 Number of Matches: 1

Range 1: 33 to 404 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
663 bits(734)	0.0	370/372(99%)	0/372(0%)	Plus/Plus

```
Query  1
GCGCAGGCCGGAAGCTGGCCCCCCACGATAGGGACCGACGTTCCATCAGGGGTGAGCAGA  60
Sbjct  33
............................................................  92
Query  61
TGTGCGCCGGCCGTACGCCCCCATTCTTGTCTACCTCACCCGGTTGCCTCGGCGGGCCGC  120
Sbjct  93
............................................................  152
Query  121
GCTCCCCCTGCCAGGGAGAGCCGTCCGGCGGGCCCCTTCTGGGAGCCTCGAGCCGGACCG  180
Sbjct  153
............................................................  212
Query  181
CGCCCGCCGGAGGACAGACACCAAGAAAAAATTCTCTGAAGAGCTGTCAGTCTGAGCGTT  240
Sbjct  213
............................................................  272
Query  241
TAGCAAGCACAATCAGTTAAAACTTTCAACAACGGATCTCTTGATTCCGGCATCTATGAA  300
Sbjct  273
.........................................G.........G.....  332
Query  301
GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTT  360
Sbjct  333
............................................................  392
Query  361  TGAACGCACATT  372
Sbjct  393  ............  404
```

4

Trichophyton mentagrophytes isolate 30823 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence and internal transcribed spacer 2, partial sequence

Sequence ID: MG881876.1 Length: 731 Number of Matches: 1

Range 1: 37 to 686 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1164 bits(1290)	0.0	648/650(99%)	0/650(0%)	Plus/Plus

```
Query  1    CCAAACGTCCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATTCTTGTCTAC  60
Sbjct  37   ............................................................  96
Query  61   CTTACTCGGTTGCCTCGGCGGGCCGCGCTTCTTCCAGGAGAGCCGTTCGGCGAGCCTCTC  120
Sbjct  97   ............................................................  156
Query  121  TTTAGTGGCTAAACGCTGGACCGCGCCCGCCGGAGAACAGACGCaaaaaaaTTCTTTCAG  180
Sbjct  157  ................................G...........................  216
Query  181  AAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAATTTTCAACAACGGATC  240
Sbjct  217  ............................................................  276
Query  241  TCTTGCTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA  300
Sbjct  277  ............................................................  336
Query  301  ATTCCGTGAATCATCGAATCTTTGAACGCACATTTGCGCCCCCTGGCATTCCGGGGGGCA  360
Sbjct  337  ............................................................  396
Query  361  TGCCTGTTCGAGCGTCATTTTCAGCCCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTC  420
Sbjct  397  ............................................................  456
Query  421  CGGCGCCCCCGTTTTTTGGGGGTGCGGGACGCCCCCCGAAAAGCAGTGGCCCAGCCCGCG  480
Sbjct  457  ............................................................  516
Query  481  ATTCCGGCTTTCCTAGGCGAATGGGCAACAAAACCAGCGCCCTCCAGAACCGGGCCGCCC  540
Sbjct  517  ............................................................  576
Query  541  TTGGCCCTCAAAAATCTGGTTTAATACTTAATCAGGTTTGACCCTCGGAATCAGGTTAGG  600
Sbjct  577  ............................................................  636
Query  601  AAATACCCCGCTGAACCTTAAGCATATATATACgggggggAGaaaaaaaa  650
Sbjct  637  ....................................G.........  686
```

5

Microsporum ferrugineum mRNA for 5.8S ribosomal RNA, internal transcribed spacer 1 and internal transcribed spacer 2, strain CBS427.63

Sequence ID: AJ252336.1 Length: 768 Number of Matches: 1

Range 1: 131 to 755 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1119 bits(1240)	0.0	623/625(99%)	0/625(0%)	Plus/Plus

```
Query  1    GTGCTCCGGCCGCACGCCCATTCTTGTCTACTGACCCGGTTGCCTCGGCGGGCCGCGCCT  60
Sbjct  131  ............................................................  190
Query  61   GCTGTGCTACAGCGGCCGTTCgggggggACGCCTGAGGGGGACTCTTGTTTCCTAGGCCA  120
Sbjct  191  ............................................................  250
Query  121  CGCCCCGGGCAGCGCTCGCCGGAGGATTACTCTGGAAAACACACTCTTGAAAGAACATAC  180
Sbjct  251  ............................................................  310
Query  181  CGTCTGAGCGAGCAACGCAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGC  240
Sbjct  311  ............................................................  370
Query  241  ATCGATGAAAATCGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCA  300
Sbjct  371  .........G.A.................................................  430
Query  301  TCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCGAGCG  360
Sbjct  431  ............................................................  490
Query  361  TCATTTCAACCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCCCCCTCCCCAACAAC  420
Sbjct  491  ............................................................  550
Query  421  CACCCACCGCTTAgggggggtgggagggaggggggACGCGCCCGAAAAGCAGTGGTCAGGCC  480
Sbjct  551  ............................................................  610
Query  481  GCGATTCCGGCTCCTGGGCGAATGGGACATACCACCGCCTCCAGGACCGGCCGGCAGGCT  540
Sbjct  611  ............................................................  670
Query  541  GGCCTAACGAACCATGTATTATTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAA  600
Sbjct  671  ............................................................  730
Query  601  CTTAAGCATATCAATAAGCGGAGGA  625
Sbjct  731  .........................  755
```

6

Trichophyton tonsurans strain 188 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MN807401.1 Length: 666 Number of Matches: 1

Range 1: 16 to 625 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1087 bits(1205)	0.0	607/610(99%)	0/610(0%)	Plus/Plus

```
Query  1    CGGAGCCGGCCCCCCAGGATAGGGCCAAACGTCCGTCAGGGGTGAGCAGATGTGCGCCGG  60
Sbjct  16   ............................................................  75
Query  61   CCGTACCGCCCCATTCTTGTCTACCTTACTCGGTTGCCTCGGCGGGCCGCGCTCTTCCAG  120
Sbjct  76   ............................................................  135
Query  121  GAGAGCCGTTCGGCGAGCCTCTCTTTATAGCGGCTCAACGCTGGACCGCGCCCGCCGGAG  180
Sbjct  136  ............................................................  195
Query  181  GACAGACGCaaaaaaaaaTTCTTTCAGAAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAA  240
Sbjct  196  ............................................................  255
Query  241  ATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGA  300
Sbjct  256  ............................................................  315
Query  301  AATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACAT  360
Sbjct  316  ............................................................  375
Query  361  TGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAGCCCCTCAAGCC  420
Sbjct  376  ............................................................  435
Query  421  CGGCTTGTGTGATGGACGACCGTCCGGCGCCCCCGTCTCTGGGGGTGCGGGACCCGCCCG  480
Sbjct  436  ............................................................  495
Query  481  AAAAGCAGTGGCCAGGCCGCGATTCCGGCTTTCTAGGCGAATGGGCAACAAACCAGCGCC  540
Sbjct  496  ............................................................  555
Query  541  TCCAGGACCGGCCGCCCTGGCCTCAAAATCTGTTTTATACTTATCAGGTTGACCTCGGAT  600
Sbjct  556  ............................................................  615
Query  601  CAGGTAGAGA  610
Sbjct  616  .......G..  625
```

7

Trichophyton soudanense isolate D15 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MN691058.2 Length: 694 Number of Matches: 1

Range 1: 282 to 630 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
617 bits(683)	9e-179	346/349(99%)	0/349(0%)	Plus/Plus

```
Query  1    AATCATTTACAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCG  60
Sbjct  282  .....G...A.....................................................  341
Query  61   AAATGCGATAAGTAATGTGAATTGCAGAATTCGGTGAATCATCGAATCTTTGAACGCACA  120
Sbjct  342  ..............................C..........................  401
Query  121  TTGCGCCCTCTGGCATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCCTCAAGC  180
Sbjct  402  ............................................................  461
Query  181  CCGGCTTGTGTGATGGACGACCGTCCGGCCCCTCCCTTCGGGGGCGGGACGCGCCCGAAA  240
Sbjct  462  ............................................................  521
Query  241  AGCAGTGGCCAGGCCGCGATTCCGGCTTCCTGGGCGAATGGGCAGCCAAACCAGCGCCCT  300
Sbjct  522  ............................................................  581
Query  301  CAGGACCGGCCGCCCTGGCCCCAATCTTtatatatatatatatatCTTT  349
```

8

Epidermophyton floccosum isolate SS_7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MT497455.1 Length: 764 Number of Matches: 1

Range 1: 231 to 659 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
752 bits (833)	0.0	424/429(99%)	0/429(0%)	Plus/Plus

```
Query  1    CTGGACAGCGCTCGCCGAAGGAGTGATTCTCAGAAATTCTACGAAATCTCCATAGGTGGT  60
Sbjct  231  ............................................................  290
Query  61   TCAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTT  120
Sbjct  291  ..............G...........C....C............................  350
Query  121  CCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTG  180
Sbjct  351  ............................................................  410
Query  181  AATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTTC  240
Sbjct  411  ................................T....T......................  470
Query  241  GAGCGTCATTTCAACCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGACCGCCTTT  300
Sbjct  471  ............................................................  530
Query  301  GCATCCCCCGTTCCACCGGGAGAGGAGAAAGGTGGAGGGGACGCGCCCGAAAAGCAGTGG  360
Sbjct  531  ............................................................  590
Query  361  CCAGGCCGCGATTCCGGGCCCCTGGGCGAATGGGCAACAAAACCAGCGCCTTCAGGACCG  420
Sbjct  591  ............................................................  650
Query  421  GCCGGCTCT  429
Sbjct  651  .........  659
```

9

Trichophyton verrucosum isolate TV68/20 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MT636892.1 Length: 617 Number of Matches: 1

Range 1: 13 to 421 Gen Bank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
729 bits(808)	0.0	407/409 (99%)	0/409 (0%)	Plus/Plus

```
Query  1    ACGATAGGGATCAGCGTTCCATCAGGGGTGTGCAGATGTGCGCCGGCCTTACGCCCCATT  60
Sbjct  13   ............................................................  72
Query  61   CTTGTCTACCTTAGTCGGTTGCCTCGGCGGGCCGCGCTCTCCCCGGAGAGTCGTCCGGCG  120
Sbjct  73   ............C.............................................  132
Query  121  AGCCTCTTCGGGGGCTTTAGCTGGATCGCGCTCGCCGGAGGACAGACATCAAAAAATCTT  180
Sbjct  133  ............................C...........................  192
Query  181  GAAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAATCAGTTAAAACTTTCAACAACGGATC  240
Sbjct  193  ............................................................  252
Query  241  TCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA  300
Sbjct  253  ............................................................  312
Query  301  ATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGGGGGCAT  360
Sbjct  313  ............................................................  372
Query  361  GCCTGTTCGAGCGTCATTTCAACCCCTCAAGCTCGGCTTGTGTGATGGA  409
Sbjct  373  ..............................................  421
Query  1    ACGATAGGGATCAGCGTTCCATCAGGGGTGTGCAGATGTGCGCCGGCCTTACGCCCCATT  60
Sbjct  13   ............................................................  72
```

4.9. Phylogenetic Analysis

Phylogenetic analysis results as shown in Figure 4.9 revealed that dermatophyte local isolates (1-9) were very close together with NCBI-Blast (AbOEzAh). Furthermore, they showed significant differences (P≥0.05) compared to other tested dermatophyte isolates species (Clade 1-9).

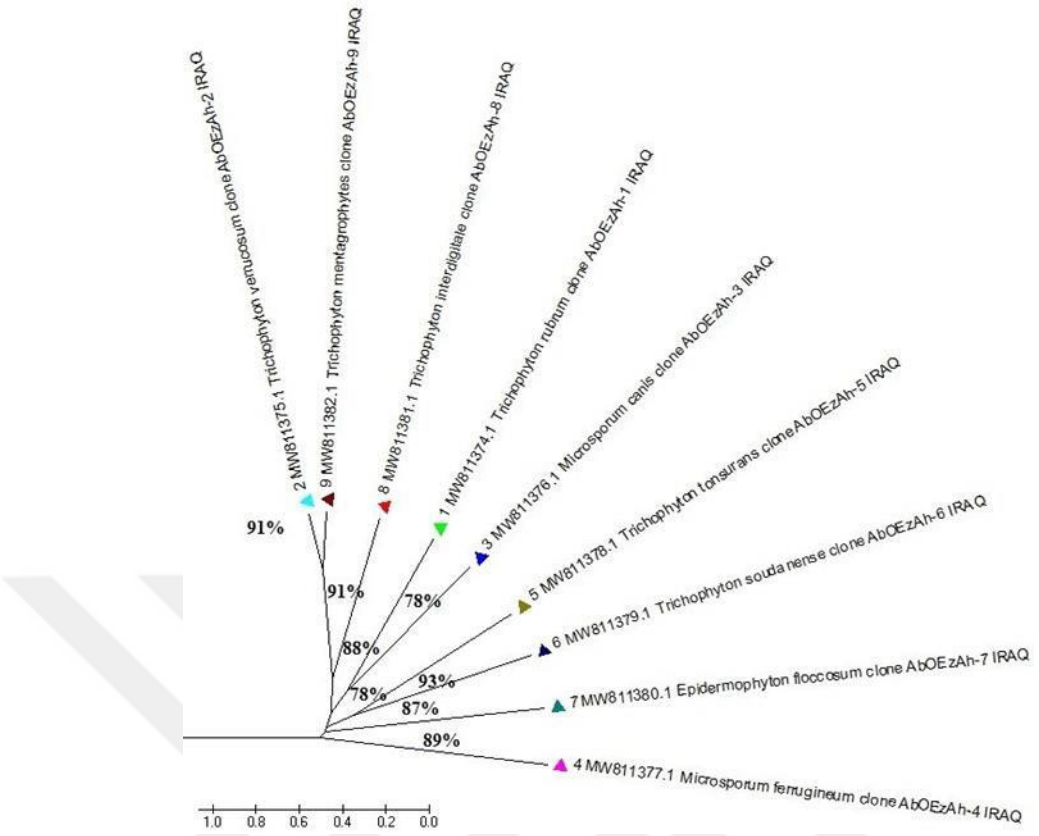

Figure 4.8 Neighbor-joining tree isolate of 18S ribosomal RNA gene

	1	2	3	4	5	6	7	8
1. 1 MW811374.1 Trichophyton rubrum clone AbOEzAh-1 IRAQ								
2. 2 MW811375.1 Trichophyton verrucosum clone AbOEzAh-2 IRAQ	1.7751							
3. 3 MW811376.1 Microsporum canis clone AbOEzAh-3 IRAQ	1.6249	1.9300						
4. 4 MW811377.1 Microsporum ferrugineum clone AbOEzAh-4 IRAQ	2.2220	2.0539	1.9383					
5. 5 MW811378.1 Trichophyton tonsurans clone AbOEzAh-5 IRAQ	2.0053	1.9164	1.8810	2.4711				
6. 6 MW811379.1 Trichophyton soudanense clone AbOEzAh-6 IRAQ	2.2700	2.3457	1.8714	1.8578	1.7394			
7. 7 MW811380.1 Epidermophyton floccosum clone AbOEzAh-7 IRAQ	2.2026	2.1797	1.8592	2.2882	2.0604	2.0631		
8. 8 MW811381.1 Trichophyton interdigitale clone AbOEzAh-8 IRAQ	1.5728	1.0609	2.0234	2.0663	1.6353	2.2913	1.9591	
9. 9 MW811382.1 Trichophyton mentagrophytes clone AbOEzAh-9 IRAQ	2.0135	0.5453	1.8739	2.1159	1.6703	2.2047	2.0171	2.0337

Figure 4.9 Neighbor-joining tree isolate of 18S ribosomal RNA gene

4.10. Recording Nine Iraqi Dermatophyte Isolates In Gene Bank-NCBI

Nine fungi were isolated from clinical specimens of human dermatophytes in Ramadi city, and each sequence had a symbol code No.1 (MW811374.1), No.2 (MW811375.1), No.3 (MW811376.1), No.4 (MW811377.1), No.5 (MW811378.1) No.6 (MW811379.1) No.7 (MW811380.1) No.8 (MW811381.1) and No.9 (MW811382.1) based on 18S rRNA gene sequence submitted to Gene bank under submission code KP979787.1,KP979788.1, KP979789.1, KP979790.1, and KP979791.1. The results of these sequences were analyzed and examined by professional staff in Gene bank in two

working days. All these sequences were accepted in Gene bank and each sequence took accession number (MN894010.1, MG889853.1, MF155590.1, MG881876.1, AJ252336.1, MN807401.1, MN691058.2 and MT497455.1) respectively. These results were recorded and published in the International Nucleotide Sequence Database Collaboration (INSDC). This location contained the database of (NCBI), European Nucleotide Archive (ENA) and DNA Data Bank of Japan (DDBJ).

5. DISCUSSION

Fungal infections are still a major health concern around the world, affecting people of all ages. Therefore, many studies have been conducted concerning different epidemiological economic, control as well as therapeutic features of this infection (Akcaglar *et al.* 2011). Dermatophytosis is the most common human fungal infection, with an estimated (20–25%) of the global population suffering from skin mycoses (Weitzman and Summerbell 1995). Dermatophytes are a group of closely related fungi divided into three genera: *Epidermophyton, Microsporum* and *Trichophyton,* each with several recognized species. These keratinophilic fungi infect humans' and animals' superficial keratinized tissues (skin, hair and nails). Dermatophytes are one of the few fungi that cause communicable diseases, and most dermatophyte strains had a limited geographic range previously (Abdel-Rahman *et al.* 2007).

Dermatophytosis, on the other hand, has recently become one of the most common human infectious diseases worldwide, with a cosmopolitan distribution. Dermatophytosis cannot be easily diagnosed on the basis of clinical manifestations as a number of other conditions mimic the clinical presentation. The differential diagnosis of dermatophytoses includes seborrhoeic dermatitis, atopic dermatitis, contact dermatitis, psoriasis, candidal intertrigo, and erythrasma, eczema (Seebacher *et al.* 2008).

In addition, dermatophytosis is more difficult to detect in immunocompromised patients, such as those with AIDS, asthma, organ transplantation and corticosteroids and antineoplastic agents (Gianni *et al.* 1995). In order to apply appropriate treatment and prevention measures, good laboratory methods for rapid and precise identification of the dermatophytes involved are required. The traditional methods of fungal detection have their own drawbacks, for example, KOH microscopy has low specificity, and fungal culture is associated with low sensitivity and takes a long time. Furthermore, dermatophyte isolates from patients on antifungal treatment generally do not show

characteristic morphology on culture, thus further compromising the results of culture isolation (Liu *et al.* 2000).

The changing profiles of human dermatophytosis among countries have further necessitated the development of improved diagnostic methods for identification of dermatophytes. Thus, newer fungal short-time diagnostic methods are needed for identification of the etiological agent not only for accurate diagnosis but also for post-therapeutic strategies (Shiraki *et al.* 2006). The treatment of dermatophytosis would be most appropriate when the selection of antimicrobial agent is based on the identity of the causative agent.

In this study, direct microscopy was the first method used in the laboratory diagnosis of dermatophytes. The result of this study, documented high rates of positive results appeared in direct examination and culture 60 (60%), while negative results for direct microscopy showed 8 (8%) rate result in table (4.2) was close to the study conducted by each of (Shtayeh and Arda 1985) broadly in Italy and (Najem *et al.* 2016) localy in Al-Nassiriyah city- Iraq. The reason behind the highly positive rates of the microscopy examinations was due to good sampling, sampling from the edges of the infected place (Dolenc-Voljč *et al.* 2015), storage method and culture of samples less than 48 hours from the time of sampling (Rippon 1982).

The direct microscopic examination showed false-negative results in 20 (20%) of specimens. This was relatively in accordance with a study done by Kadhim (2018), who documented that up to (19%) false-negative results. These false-negative results may be related to an insufficient amount of material or a specimen poor in fungal elements, but also to a too short incubation time, a non suitable temperature, or the presence of "contaminants" which can prevent the development of the pathogen.

In addition, low rates were shown in the negative results of the microscopic examinations and cultures, and the reason may be related to a small amount of the sample taken and the use of antibiotics by the patient without consulting a doctor (Slifkin

and Cumbie 1988). The wrong clinical diagnosis may be due to a non-fungal infection such as eczema or allergic diseases (Weitzman and Kane 1991).

In this study, Sabouraud Dextrose Agar medium was chosen, with Chloramphenicol and Gentamicin added to prevent the growth of other bacteria and fungi, and it is considered an ideal medium for the growth of dermatophytes (Al-Janabi and Abdul Husseine 2005). Blue cotton dye was used to identify the macro and microconidia species, and it was a good way to show the diversity between species (Poluri *et al.* 2015).The percentage of infected females in our study appeared high 52 (52%), while it was 48(48%) in infected males as shown in Table 4.3. These results agreed with the findings of Weinstein and Berman 2002 and Gianni *et al.* 1995. The reason may be due to a difference in social or professional behaviour, or a physiological difference, or it may be due to a difference in health habits, which are among the most important factors that contribute to the occurrence of fungal skin infections (Hassan and Mousa 2020).

5.1.The Species Sex And Age-Wise Distribution In Relation To Clinical Types

5.1.1. *Tinea corporis*

Tinea corporis was the main clinical type encountered, and it accounted for 56 (61%) of all infections. This finding was in agreement with our previous data from crete and fromsouth western (Maraki 2012).The results from Greece were identical to a study conductedin Iraq (Al-Khafajii 2014), which did not agree with a study conducted in Turkey by Aktas *et al.* (2009).

The most common type was *T. rubrum* 16 (17.4%), which was the highest among the species. According to several epidemiologic studies worldwide (Maraki 2012) the etiology of tinea corporis is very heterogeneous. In several countries such as central, southern and Eastern Europe, *M. canis* was prevailing, while in western and northern countries of Europe, in the USA and in Latin America such as Mexico, *T. rubrum* is the main aetiological agent. In UK and in the USA, the anthropophilic species *T. tonsurans* is the second most common pathogen of *Tinea corporis* after *T. rubrum* (Seebacher *et*

al. 2008). It is the result of an approach to a study conducted in Iraq by (Najem *et al.* 2014).

Regarding gender, the highest rate of infection was among females 30 (32.6%), while in males, it was 26 (28.4%) cases, which was the highest rate among all clinical types. These results were consistent with an American study performed by (Estrada and Morris 2000), and also in Turkey by Akcaglar *et al.* (2011) who showed that the proportion of females is more influential than males (İnanir *et al.* 2002). The infection rate in the countryside was high 42 (45.8%) patients, due to the proximity to animals and the lack of attention to personal hygiene (Seebacher *et al.* 2008). In the city, there were 14 (15.2%) cases of infection. The age group most affected by *T. corporis* was (21-30) years, with a 14 (70%) percentage which agreed with what was reported in a study in Slovenia, 1995–2002 (Voljc 2005).

5.1.2. *Tinea cruris*

In the present study, *Tinea cruris* was the second commonest clinical type 18 (19.6%). This result was identical to previous results founded by Nasimuddin *et al.* (2014).

M. canis was the most widespread cultural isolation 20 (21.9%), which was consistent with a study in Iraq by Kadhim (2018). However, this result was not identical to a study done in Turkey by Aktas *et al.* (2009). Females were the most influential with a rate of 14 (15.3%) compared to males (4.3%), which was not identical to a study performed in the city of Nasiriyah in Iraq by Najem *et al.* (2016). The infection rate in the city was higher 10 (10.9%) patients, in comparison with countryside 8 (8.7%) patients. The most affected age group by *Tinea cruris* was (31-40) years 7 (28%), as reported by a study in Iran by Ansar *et al.* (2011).

5.1.3. *Tinea faciei*

Ringworm of the face accounted for 6 (6.5%) of all dermatophytosis. Almost all of the cases were diagnosed in females only. The reason may be due to women being more interested in pets than men as shown by others (Atzori *et al.* 2012).

In the present study, the zoophilic species *T. mentagrophytes* accounted for the majority (80%) of the isolates. The aetiological agent of *Tinea faciei* varies according to geographic region. In Northern Greece and Italy, *M. canis* is the predominant agent (Devliotou-Panagiotidou *et al.* 1995), while in Spain and in Switzerland; *T. mentagrophytes* is the most predominant. The infection rate in the city was high 4 (4.35%) patients, while in the countryside, the infection rate was 2 (2.15%). The age group most affected by *T. faciei* was (31-40) years 2(8%).

5.1.4. *Tinea pedies*

Feet and toenails are important reservoirs from which fungal infections could spread to other body sites due to wearing closed shoes and synthetic socks (Rabobe *et al*, 1998). Males were more frequently affected than females. According to Gupta and colleagues, the gender difference may be due to differences in hormone levels that result in a different capacity to inhibit dermatophyte growth (Gupta *et al.*1997).

Three types of fungi caused infection *T. rubrum, T. mentagrophytes* and *T. interdigitale*. These results were identical to a the findings in Turkey (Akcaglar *et al.* 2011). In *Tinea pedies,* the affected persons 6 (6.5%) were all from the countryside only. This could be related to the different types of jobs that rural residents perform compared withcity residents. Moreover, patients living in the countryside have limited access to medical care compared with people living in the cities (Szepietowski *et al.* 2006).The most affected age group by *Tinea pedies* was (31-40) years 2 (8%), and increased steadily with age (Szepietowski *et al.* 2006). This may be attributed to slow-growing nails, reduced peripheral circulation, diabetes mellitus, nail trauma, increased exposure to disease-causing fungi and immunosuppression (Thomas *et al.* 2010).

5.1.5. *Tinea capitis*

Tinea capitis continues to be an important health problem in the world, affecting mainly preschool and school children (1-10) years old. In the current study, Tinea capitis constituted (4.3%) of the total patients, then *M. canis*, followed by *T. rubrum*. The incidence of *Tinea capitis* varies by gender, depending on the infecting organism. When

the causative agent is *M. canis*, the infection rate in boys is usually higher (Elewski 2000). In *Tinea capitis,* the number of people distributed evenly between the countryside and the city, and the number of people was 2 (2.12%). This was identical to the current study, as all of the infected patients were boys.

5.1.6. *Tinea manus*

Tinea manus was the least common clinical presentation, and it was the only type of dermatophyte isolated. This is compatible with (Goyal *et al.* 2015). In *Tinea manus,* the infected people were only in the city, with their number and percentage was 2 (2.18%). Females were more frequently affected than males. The reason may be due to the daily use of household cleaners and not wearing gloves.

Studies have reported that different species of dermatophytes may behave significantly differently as aetiological agents in different regions and years, as well as socioeconomic status (Pontes *et al.* 2002).

5.2.Molecular Detection Of Dermatophytes

After the isolated fungal species were diagnosed by traditional methods based on determining the phenotypic criteria using the taxonomic keys as previously shown, and to ensure the correctness of their diagnosis, the primers (region ITS gene rRNA 18S) were used for the diagnosis of dermatophytes.

The primers in this study were designed from the Genbank NCBI website and using Primer 3 plus software to design the primers and specifics in the PCR assay. Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities of DNA (due to the high copy number of rRNA genes), and it has a high degree of variation even between closely related species. The ITS region is the most widely sequenced DNA region in molecular ecology of fungi (Peay *et al.* 2008), and it has been recommended as the universal fungal barcode sequence (Schoch *et al.* 2012). It has typically been most useful for molecular systematic at the species level and even within species (e.g. to identify geographic races) analysed the nucleotide sequence of ITS1 region of pathogenic dermatophytes and

reported that the nucleotide sequence of ITS1 region were reasonably useful, not only for understanding the phylogenetic relationships among dermatophytes, but also for identification of dermatophytes at the species level (Makimura *et al.* 1999). The amplification of the ITS region of rDNA have been used by various other workers for identification of dermatophytes species. Li *et al.* (2008) could identifiy 17 dermatophyte species using PCR amplification of ITS regions of rRNA.

The results of DNA extraction of fungi isolates were shown using the special kit for this purpose. In addition, electrophoresis in the agarose gel was detected with the use of ethidium bromide dye and examined under ultraviolet light. All isolates contained one single bundle of DNA extracted by taking the same location on the agarose reference plate, as shown in Table 4.7 of the DNA was also extracted using a Nanodrop.

The PCR products of dermatophytes isolate were submitted to Macrogen Company /Korea for sequencing, and the pairwise sequence alignment was conducted using blast. The query of nucleotides depending on subject of nucleotides sequence showed 99% score which means that the isolate was identical to the reference sequence for local isolates.

The molecular evolutionary genetic analysis (MEGA) software is a desktop application designed for comparative analysis of homologous gene sequences either from multigene families or from different species with a special emphasis on inferring evolutionary relationships and pattern of DNA and protein evolution. In addition to the tools for statistical analysis of data, MEGA provides many convenient facilities for the assembly of sequence data sets from files or web-based repositories, and it includes tools for visual presentation of the results obtained in the form of interactive phylogenetic trees and evolutionary distance matrices.

In this study, the nucleotide maximum likelihood method was used for detection of the close relationship of world and local sequences which was a better method of the nucleotide sequences in MEGA 6.0. Also, the Neighbor-Joining (NJ) method was used, which is a simplified version of the minimum evolution (ME) method as shown in Figure 4.9. The NJ method produces an unrooted tree because it does not require the

assumption of a constant rate of evolution. Finding the root requires an out group taxon (Goldman *et al.* 1987).

The PCR amplicons generated were sequenced with the same primers to obtain partial 18S ribosomal RNA gene sequences. The 18S rRNA sequences examined by nucleotide blast and the percentages of similarity were calculated by using method of alignments explorer clustalin Microsoft Mega 6.0 package. The gene sequence of local strain of *Microsporum canis* showed 99% homology with the sequence of local isolate strains MN894010.1 *Trichophyton interdigitale* showed 99% homology with the sequence of local isolate strain MG889853.1. *Trichophyton rubrum* showed 99% homology with the sequence of local isolate strain MF155590.1. *Trichophyton mentagrophytes* showed 99% homology with the sequence of local isolates strain MG881876.1. *Microsporum ferrugineum* showed 99% homology with the sequence of local isolate strain AJ252336.1. *Trichophyton tonsurans* showed 99% homology with the sequence of local isolate strain MN807401.1. *Trichophyton soudanense* showed 99% homology with the sequence of local isolates strain MN691058.2. *Epidermophyton floccosum* showed 99% homology with the sequence of local isolate strain MT497455.1, and *Trichophyton verrucosum* showed 99% homology with the sequence of local isolate strain MT636892.1 as illustrated in Table 4.9.

The result of the sequence of *Microsporum canis* sample that was compared with the isolate MN894010.1 showed two transitions at the site 231-C / T and 581-G / A and one sample Transversion at the site 232-C / G for the sample. *Trichophyton interdigitale* sample that was compared with the isolate MG889853.1 showed two sample transversion at the site 442-T / C, and sample transversion at the site 544 -A / C. *Trichophyton rubrum* sample that was compared with the isolate MF155590.1 showed two sample transitions at the site 316 - G\A, and sample transversion at the site 327 - G\T. *Trichophyton mentagrophytes* sample that was compared with the isolate MG881876.1 showed two sample transitions at two sites 192 -G\A and the site 677 - G\A. *Microsporum ferrugineum* that was compared with isolation AJ252336.1 transition in situ 380 G\A and 581-G / A. Transversion in situ 382 A\T. *Trichophyton tonsurans* was compared with the isolate MN807401.1. Transition in two in situ 331C\T and 623 G\A, transvertion in situ 489 G\C. *Trichophyton soudanense* that was compared

with the isolate MN691058.2, there was transversion in three situ 287 G\T, 291A\C and 374 C\G. *Epidermophyton floccosum* that was compared with isolate MT497455.1, showed transversion in four situ 305G\A, 317C\T, 322C\T, 443T\C and 448T\C. *Trichophyton verrucosum* that was compared with the isolate MT636892.1, showed transversion in situ 86 C\G and transition in situ 164 C\T as observed in Table 4.9.

5.3. The Evolutionary Tree For Dermatophyte Isolates

Evolutionary tree diagram for comparing samples with each other showed that the percentage of convergence was 91% between *Trichophyton verrucosum* and *Trichophyton mentagrophytes* and 91% with *Trichophyton interdigitale*, and the affinity was 78% between *Trichophyton rubrum* and *Microsporum canis*, and the percentage of affinity was 93% between *Trichophyton tonsurans* and *Trichophyton soudanense. Epidermophyton floccosum* showed 89% affinity with *Microsporum ferrugineum* as shown in in Figure 4.3 and Figure 4.4. The problem of diagnosing skin fungi affects most dermatologistsin Iraq. Work must be done by using modern and fast molecular methods in detecting skin fungi types and not to rely only on microscopic examination; in most medical clinics, due to the importance of identifying and diagnosing the types in giving the correct treatment to eliminate skin fungi.

6. CONCLUSION AND RECOMMENDATION

1. The results showed that out of the 100 cases of dermatophytes, only 61 (61 %) caseswere positive dermatophytes by both direct KOH examination and culture, whereasfalse negative results was recorded in 20 (20 %) of total specimens count.

2. Culture results of specimens showed nine types of dermatophytes were identified, namely *T. rubrum* (23.9%), *M. canis* (21.9%), *T.mentagrophytes* and their percentage (19.2), *T. interdigitale* (13.4%), *T. ferrugineum* (8.7%), and the rest of the four remaining species were *E. floccosum*, *T.soudanese*, *T. tonsurans* and *T. verrcosum* (2.2).

3. Females (52%) were more infected than men (40%).
4. The most common clinical type of infection was tinea corporis (61%), and the others were as follows: *Tinea cruris* (19.6%), *T. pedies* (6.5%), *T. faciei* (6.5%), *T. capitis* (4.3%), and *T. manus* (2.18%).

5. The primer (region ITS gene rRNA 18S) were used for the diagnosis of dermatophytes. The PCR products of Dermatophytes isolates were submitting to Macrogen company /Korea for sequencing.

6. The spread of different types of dermatophytes may be due to the migration of thepopulation and their move to live in different areas due to the wars.

7. It is not necessary to rely on microscopic examination only in diagnosing dermatophytes, in which only fungal hyphae are detected and does not specify thespecies.

8. Following molecular methods that take less time to detect the pathogens of dermatophytes.

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APPENDICES

APPENDIX 1. Work permit (Turkish)

APPENDIX 2. Work permit (Arabic)

APPENDIX 1. Work permit (Turkish)

APPENDIX 2. Work permit (Arabic)

CURRICULUM VITAE

Personel Information

Name and Surname : Abdulateef Abdulqader HAMMOOD

Education

High School : Palestine High School 2000 - 2004

Undergraduate : ANBAR University Faculty of Education 2006 – 2010

Work Experience

Year	Position
2011- 2021	Biology teacher,