

**An-Najah National University**  
**Faculty of Graduate Studies**

# **Epidemiology of Dermatophyte Infections in Palestine: an update Study**

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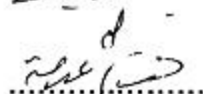
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## ***Dedication***

**To Allah,** *To my dear mother, father, sisters, brothers, hasband  
and my little sweet daughter and son for their patience and  
encouragement, with love and respect*

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## الإقرار

أنا الموقعة أدناه مقدمة الرسالة التي تحمل عنوان:

# Epidemiology of Dermatophyte Infections in Palestine: an update Study

## وبائية الأمراض الجلدية في فلسطين: دراسة للتحديث

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

### Declaration

The work provided in this thesis, unless otherwise referenced is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

**Student's name:**

اسم الطالبة:

**Signature:**

التوقيع:

**Date:**

التاريخ:

## List of Abbreviations

HCL	Hydrochloric acid
DMSO	Dimethyl sulfoxide
dNTP's	Deoxynucleotide triphosphates
EDTA	Ethylenediamine tetraacetic acid
Kac	Potassium acetate
KOH	Potassium hydroxide
□g	Microgram
□l	Microliter
NaCl	Sodium chloride
PCR	Polymerase chain reaction
RAPD	Random amplification of polymorphic DNA
S.D. H <sub>2</sub> O	Sterile distilled water
SDA	Sabouraud dextrose ager
SDS	Sodium dodecyl sulfate
TAE	Tris base, acetic acid and EDTA
Tris	(hydroxymethyl)aminomethane

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**Abstract**

**Background:** Dermatophytes are a group of morphologically and physiologically related molds some of which cause well defined infections: dermatophytoses (tineas or ringworm) .They have two important properties: they are keratinophilic and keratinolytic. This means they have the ability to digest keratin in vitro in their saprophytic state and utilize it as a substrate and some of them can invade tissues in vivo and cause tineas.

**Objectives:** This study was designed to determine the epidemiology including prevalence and occurrence of causative agents of dermatophytosis in patients in Palestine; detecting any changes in the etiological agents during the last 28 years; studying the effective factors such as socioeconomic condition, age, contact with animals and others; studying the correlation between multifocal infections tinea pedis , tinea nail and tinea cruris. The study was also aimed at genotyping identification and how can be used as a molecular tool for rapid diagnosis for dermatophytes also to study the diversity during different dermatophytes species and within the same specie specially *Microsporum canis*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Genetic studies by random amplification of polymorphic DNA (RAPD) have been used to

detect polymorphism of dermatophytes. The genotypical analysis was performed using the RAPD method as rapid molecular tool for diagnosis.

**Results:** In this study, a total number of 220 samples from 188 patients were examined ((137 males (72.9%), and 51 females (27.1%)). Tinea Capitis (27.7%) was the predominant clinical manifestation followed by tinea pedis (23.4%), tinea nail (22.3%), tinea cruris (20.2%) and tinea corporis(6.4%). The dominant causative agent was *Microsporum canis* 104 samples from 138 (75.36%) followed by *Trichophyton rubrum* 31 samples from 138 (22.5%) then *Trichophyton mentagrophytes* 3 samples from 138 (2.1%). The RAPD results showed that all analyzed strains are mainly from three genotypes of *Microsporum canis*, two genotypes of *Trichophyton rubrum* and one genotype of *Trichophyton mentagrophytes*. Thus, based on analysis of the RAPD data, a correlation can be shown between the genotypical patterns of the strains of *Microsporum canis* also between the strains of *Trichophyto rubrum* and *Trichophyton mentagrophytes* from Palestine.

**Conclusions:** The results showed that the predominant causative agent of dermatophytes specis was *Microsporum canis*. This change could be due to several factors as activity related to human and animals' hygiene, interaction between human and human, animal and soil, and changing population over the times.

# **CHAPTER ONE**

## **GENERAL INTRODUCTION**

## CHAPTER ONE

### GENERAL INTRODUCTION

#### 1.1 General properties of fungi

The kingdom Fungi contains many organisms such as dermatophytes, yeasts, *Penicillium* spp., morels and gilled mushrooms. It includes more than 100,000 species. Fungi are considered as a separate kingdom of living organisms at the same level as the animals and the plants (Koichi *et al.*, 1999).

Fungi are eukaryotes, heterotrophic organisms, and spread as colonies of isolated cells (yeasts) or mycelium (a mycelium is a multicellular filament (or hypha) which branches to form a network). Each fungus cell contains one or several haploid or diploid nuclei, fungal cells are delimited by a  $\beta$  1-3 and  $\beta$  1-6 glucan wall and chitin. Fungi reproduce both sexually and asexually often resulting in the production of spores which are wind-disseminated depending on the species and conditions. Spores are generated on hyphae or in micro or macroscopic sporangia which show a limited tissue differentiation. The asexual and sexual forms in the same species are morphologically very different. The asexual forms of fungi are called anamorphs and the sexual forms are called teleomorphs. The term of “holomorph” is used to designate the total organism and a fungal colony where an anamorph and the teleomorph coexist. A holomorph can give rise to an exclusive anamorph species by loss of the teleomorph during evolution. The term of “synanamorph” is used to designate two different anamorphs of the same species (Ajello *et al.*, 1966).

## 1.2 Dermatophytes

Dermatophytes are a group of morphologically and physiologically related molds some of which cause well defined infections: dermatophytoses (tineas or ringworm) (Liu *et al.*, 2011; Azab *et al.*, 2012). They have the ability to digest keratin in vitro in their saprophytic state and utilize it as a substrate and some of them can invade tissues in vivo and cause tineas. However, their morphology in the parasitic growth phase is different from the morphology exhibited in culture or in vitro (McPherson *et al.*, 2008).

Dermatophytes as saprophytes reproduce asexually by simple sporulation of arthroconidia, microconidia and/ or macroconidia which are produced from specialized conidiogenous cells. They also exhibit a range of vegetative structures with typical arrangement on hyphae, chlamydospores, spirals, antler-shaped hyphae (chandeliers), nodular organs, pectinate organs and racquet hyphae (Emmons, 1934; Ajello *et al.*, 1966).

In presumptive identification of a dermatophyte culture, five important colony characteristics should be taken into consideration, when it is one to three weeks old: rate of growth , general topography (flat, heaped, regularly or irregularly folded) , texture (yeast-like, glabrous, powdery, granular, velvety or cottony) , surface pigmentation and reverse pigmentation (Ajello *et al.*, 1966 ).

Based on the above criteria dermatophyte species can be classified, particularly on differences in conidial morphology, into three genera within the Fungi Imperfecti (or Deuteromycotina) namely: *Epidermophyton*, *Microsporum*, and *Trichophyton* (Emmons, 1934; Caputo *et al.*, 2001; Ghannoum *et al.*, 2003).

### **1.3 Ecology**

Dermatophytes have been divided into three ecological groups: geophiles, zoophiles and anthropophiles (Georg, 1959; Achterman *et al.*, 2011). Some of these fungal pathogens probably evolving from their natural habitat in the soil have developed host specificity, resulting in these three groups. Individual dermatophytes differ considerably in their host range and importance as agents of disease in man and animals. The differences in host specificity have been attributed to the differences in keratin of the hosts (Rippon, 1982).

Geophiles are primarily soil-inhabiting and only rarely encountered as agents of ringworm, with the exception of *Microsporum gypseum*. Zoophiles are essentially animal pathogens, although they may cause infection in humans. Anthropophiles are restricted to man, very rarely infecting animals (Georg, 1959).

### **1.4 Geographical distribution of the dermatophytes**

Variations in the distribution pattern of dermatophytes infection among different countries was reported (Ayadi *et al.*, 1993; Staats &



Korstanje, 1995; Weitzman & Summerbell, 1998; Ellabib *et al.*, 2002). This distribution pattern of dermatophytes infection in different part of the world has been attributed to climatic factors, life-style, and prevalence of immunodeficiency diseases in the community and also the reluctance of patients to seek treatment because of embarrassment or minor nature of disease unless the condition becomes sufficiently serious to affect the quality of life (Al sheikh, 2009).

Dermatophytosis, especially tinea capitis, tinea corporis and tinea cruris, occurs worldwide but is very common in tropical countries because dermatophytes grow best in warm and humid environments (Frieden & Howard, 1994). The geographic distribution varies with the organism. *Microsporum canis*, *Microsporum nanum*, *Trichophyton mentagrophytes* and *Trichophyton verrucosum* occur worldwide, while *Trichophyton mentagrophytes* var. *erinacei* is limited to France, Great Britain, Italy and New Zealand (Ginter-Hanselmayer *et al.*, 2007) and *Trichophyton simii* (found in monkeys) occurs only in Asia (Macura, 1993).

### **1.5 Dermatophytosis Transmission**

Infection occurs by contact with arthrospores (asexual spores formed in the hyphae of the parasitic stage) or conidia (sexual spores formed in the “free living” environmental stage). Dermatophytes usually begins in a growing hair or the stratum corneum of the skin and they don't generally invade resting hairs, since the essential nutrients they need for growth were absent or limited. Hyphae spread in the hairs and keratinized skin,

eventually developing infectious arthrospores. Transmission between hosts usually occurs by direct or indirect ways. The direct way is by contact with a symptomatic or asymptomatic host. Infective spores in hair and dermal scales can remain viable for several months to years in the environment and fomites such as brushes and clippers which can be important in indirect transmission. Geophilic dermatophytes, such as *Microsporum gypseum*, are usually acquired directly from the soil (Ajello *et al.*, 1966). In animals arthrospores are transmitted through direct contact with sick or subclinically infected animals, mainly cats, but also with dogs and other species. In sick animals, the infected hair shafts are fragile and hair fragments containing arthrospores are efficient in spreading the infection. In addition, uninfected cats can passively transport arthrospores on their hair, thereby acting as a source of infection. Risk factors include introduction of new animals into a cattery, cat shows, catteries, shelters, mating etc. Indirect contact is important, too; transmission may occur via contaminated collars, brushes, toys etc. Arthrospores are easily spread on dust particles. In households with infected cats, the furniture, wallhangings, clothes and even rooms without access for cats become contaminated. Outdoor cats, especially in rural areas, can be sometimes infected with agents other than *Microsporum canis* dermatophytes. *Microsporum gypseum* is a geophilic fungus living in soil to which cats are exposed by digging. *Trichophyton mentagrophytes* and *Trichophyton quinckeanum* are prevalent in small rodents and their nests, and *Trichophyton verrucosum* is isolated from cattle (Brumm, 1985).

## **1.6 Symptoms and Clinical signs of Dermatophytosis Disease in Human**

Dermatophytes generally grow only in keratinized tissues such as hair, nails and the outer layer of skin. The incubation period is one to two weeks. The fungus usually stops spreading where it contacts living cells or inflammation areas. Mucus membranes are not affected. The clinical signs may vary, depending on the region affected. In humans, pruritus is the most common symptom. The skin lesions are usually characterized by inflammation that is most severe at the edges, with erythema, scaling and occasionally blister formation. Central clearing is sometimes seen, particularly in tinea corporis; this results in the formation of a classic “ringworm” lesion. On the scalp and facial hair, there may be hair loss. Dermatophytes acquired from animals or the soil generally produce more inflammatory lesions in humans than anthropophilic dermatophytes. Dermatophytoses in humans are referred to as “tinea” infections, and are named with reference to the area of the body which is involved (Enemuor & Amedu, 2009).

## **1.7 Clinical manifestations**

A wide range of clinical features have been presented by dermatophytosis, which are influenced by many factors mainly depends on the site of infection, the species, size of inoculum and the host’s immune status. Instead of a single organism causing one sign of the disease, several

species can, in fact, result in a single disease manifestation (Hiruma & Yamaguchi, 2003). The various clinical features are as follows:

- a) Tinea capitis: Ringworm of the scalp, where spores are formed within the hair shaft – endothrix infection or outside it – ectothrix infection and caused by *Microsporum* specially *Microsporum canis* and some of *Trichophyton* species (Fuller *et al.*, 2003).
- b) Tinea Barbae: Infection of the bearded area caused mainly by *Trichophyton mentagrophytes* and *Trichophyton verrucosum*.
- c) Tinea Manuum: Palms infection, mainly caused by *Trichophyton rubrum*.
- d) Tinea Favosa: Chronic, severe infection of the scalp in humans with crust formation around hair shafts. It is caused by *Trichophyton schoenleinii* and is common in Eurasia and Africa.
- e) Tinea Unguium (onychomycosis): Nail plate invasion, mainly in toenails. It is commonly caused by *Trichophyton mentagrophytes* and *Trichophyton rubrum*.
- f) Tinea Imbricata: Chronic infection on the body characterised by concentric rings and caused by *Trichophyton concentricum*. It is geographically restricted to Southeast Asia, Mexico and Central and South America (Weitzman & Summerbell, 1995; Hay, 2003).
- g) Tinea Cruris (Jock itch): Ringworm of the groin with occasional infection of upper thighs and usually seen in men. Frequent etiologic

agents are *Trichophyton rubrum* and *Epidermophyton floccosum* (Havlickova *et al.*, 2008).

- h) Tinea Corporis: Ringworm of the body usually involving the trunk shoulders or limbs and can be caused by any dermatophyte.
- i) Tinea Pedis (Athlete's foot): Ringworm of the feet especially the soles and presents as scaling or macerations between toes. It is caused by some of etiologic agents mainly as *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Sadri *et al.*, 2000).

### **1.8 Multifocal infections tinea pedis, tinea nail and tinea cruris Tinea pedis**

Tinea pedis presents as pruritic, erythematous, inflamed regions on the feet that may be located on the sole (vesicular type) or lateral aspects (moccasin type) of the foot and sometimes between the toes (interdigital type). Three main genera of dermatophyte may cause tinea pedis, *Trichophyton* (*T. rubrum*, *T. mentagrophytes*, and *T. tonsurans*), *Epidermophyton* (*E. floccosum*), and *Microsporum* (*M. canis*) (Hainer, 2003; Vander Straten *et. al.*, 2003).

#### **Tinea unguum (onychomycosis)**

Tinea unguum is a fungal infection of the nail, usually with a dermatophyte, on the matrix, plate, or nail bed commonly associated with tinea pedis. Like tinea pedis infections, *T. rubrum* is the major cause of subungual onychomycosis. Onychomycosis accounts for about one third of

fungal skin infections but only about half of onychomycosis infections are caused by dermatophytes (Roujeau *et al.*, 2004).

### **Tinea cruris**

Tinea cruris, or “jock itch,” forms an erythematous, pruritic patch in the groin area that usually spares the scrotum and penis. The leading edge may include follicular papules and pustules, and the etiology agents are *T. rubrum*, *T. mentagrophytes*, *E. floccosum*, and *M. canis*. The etiology agents are the same as in tinea pedis.

In a closer look for the three multifocal infections, Charif & Elewski in 1997 found that the most cutaneous infections are dermatophytes, and the dermatophyte *T. rubrum* is the major cause of tinea pedis, Onychomycosis and tinea cruris. Also approximately one half of patients with tinea cruris have coexisting tinea pedis. It was suggested that the plantar surface is frequently an overlooked area and it has been suggested that this part of the foot, in particular, acts as a fungal reservoir from which the infection can spread. Consequently, it is important to assess other areas of skin for a coexisting fungal infection. Concomitant dermatophyte infections are particularly common on the hands (tinea manuum), groin (tinea cruris) and fingernails (Szepietowski *et al.*, 2006).

The presence of tinea pedis is also highly correlated with the development of onychomycosis (Sigurgeirsson & Steingrímsson, 2004; Walling *et al.*, 2007) and that have been proven in one large

epidemiological study, that the presence of either interdigital or moccasin forms of tinea pedis increased the risk of onychomycosis approximately four folds (Sigurgeirsson & Steingrímsson, 2004). In turn, onychomycosis can serve as a reservoir for dermatophytes, which can re-infect the skin. The presence of tinea pedis and/or onychomycosis is also a significant risk factor for acute bacterial cellulitis of the leg as well as diabetes-related foot and leg complications in at-risk individuals (Gupta & Humke, 2000; Roujeau *et al.*, 2004), so there is a tight correlation between the multifocal infections tinea pedis, tinea nail and tinea cruris.

## 1.9 Epidemiology

The epidemiology of dermatophyte infection is likely to alter with changing patterns of migration, growth in tourism, and changes in socioeconomic conditions. Dermatophytes endemic to Asia (Macura, 1993) and Africa (Morar *et al.*, 2006), such as *T. soudanense*, *T. violaceum*, and *M. audouinii*, have increased in frequency in Europe and North America as a result of migration (Ginter-Hanselmayer *et al.*, 2007). Changes to the epidemiology of causative agents are also a reflection of changing patterns of dermatophytosis.

Knowledge of the predominant causative species provides a clearer understanding of risk factors for superficial fungal infections and future epidemiologic trends. Improvements in living conditions have generally been associated with an increase in zoophilic dermatophyte and in anthropophilic dermatophyte infections.

*Microsporum canis* is a typical zoophilic dermatophyte. As this pathogen is isolated from healthy domestic cats, they are considered the major natural host of *M. canis*. It was generally thought that subclinical infections are common, especially in longhaired cats over 2 years of age. However, the prevalence of *M. canis* isolation from healthy animals varies greatly between subpopulations, and in many groups the prevalence is low (Mignon & Losson, 1997). Therefore, *M. canis* should not be considered as part of the normal fungal flora of cats, and isolation from a healthy cat indicates either subclinical infection or fomite carriage (DeBoer & Moriello, 2006).

### **1.9.1 Dermatophytes Epidemiology in Palestine**

In 1989, Ali-Shtayeh and Arda published their study on the epidemiology of dermatophytosis in Palestine. The study has shown that the dermatophytic mycobiota of the West Bank comprised about 14 dermatophytes with *T. violaceum* (anthropophilic dermatophyte) being the most predominant aetiological agent accounting for 34-50 % of total isolates; *M. canis* was the next most common aetiological agent accounting for 23% of total isolates (Ali-Shtayeh and Arda, 1989).

An epidemiological study of tinea capitis was also carried out among 7,525 primary school children in the Nablus district in Palestine. Seventyfive cases of tinea capitis (1%) were mycologically proven. The incidence was higher in young children (1.4%) aged from 6 to 10 years than in older children (0.5%) aged from 10 to 14 years. *Trichophyton*



*violaceum* was the most common causative agent (82.7%) followed by *M. canis* (16%) and *T. schoenleinii* (1.3%). Also, other study of tinea capitis were carried out in 2000 among 8,531 primary school children in Nablus district in Palestine also the *Trichophyton violaceum* was the most common causative agent (65.5%) followed by *M. canis* (22.1%) (Ali-Shtayeh *et al.*, 1998, 2002).

In Gaza the most common dermatophytic infection was tinea capitis (50.5%), followed by tinea corporis and tinea unguium (12.6% for each), and tinea versicolor (4.5%). Dermatophytes were significantly the most common isolated pathogens (28/34, 82.4%), followed by *Candida* spp. (5/34, 14.7%) and *Malassezia furfur* (1/34, 2.9%). Among dermatophytes, *Trichophyton* spp. was the most frequent isolate (14/34, 41.2%), followed by *Microsporum* spp. (13/34, 38.2%). The least isolated dermatophyte was *Epidermophyton floccosum* (1/34, 2.9%) (Al Laham *et al.*, 2011).

### 1.9.2 Etiological agents

Dermatophytes can be classified according to their usual habitat into anthropophilic, zoophilic and geophilic organisms. There are approximately 100, 000 species of fungi distributed worldwide. The majority of fungal infections seen in both temperate and tropical countries are superficial infections of the skin.

There are approximately 40 different species of dermatophytes, characterised by their capability to digest keratin and divided among three

genera: *Trichophyton*, *Microsporum* and *Epidermophyton*. A majority of superficial fungal infections of the skin are caused by five or six species of dermatophyte, of which *Trichophyton rubrum* is the most common (Aly, 1994).

The predominant species of dermatophytes vary according to their clinical localization. The management of tinea of the glabrous skin, caused by dermatophyte infections, is a common therapeutic problem for dermatologists (Szepietowski *et al.*, 2006).

Over 90% of tinea capitis dermatophytosis cases worldwide are caused by *Microsporum canis* (Sparkers *et al.*, 2000; DeBoer & Moriello, 2006). Others are caused by infection with *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton quinckeanum*, *Trichophyton rubrum* or other agents. With the exception of *Microsporum gypseum*, all produce proteolytic and keratolytic enzymes that enable them to utilize keratin as the sole source of nutrition after colonization of the dead, keratinized portion of epidermal tissue (mostly stratum corneum and hairs, sometimes nails).

By segmentation and fragmentation of the hyphae, dermatophytes produce arthrospores, which are highly resistant and adhere strongly to keratin also surviving in a dry environment for 12 months or longer (Sparkers *et al.*, 2000). In a humid environment, however, arthrospores are short-lived. High temperatures (100°C) destroy them quickly.

## 1.10 Diagnosis

Other annular rashes are often confused with tinea infections. Eczema and psoriasis are commonly confused with tinea. *Pityriasis versicolor* occurs all over the trunk while *Candida* occurs as a flexural rash at extremes of age or in the immunocompromised, those with diabetes or patients on antibiotics. Treatment with topical steroids often causes confusion, making tinea less scaly and more erythematous (Rashid *et al.*, 2011) Steroid use also makes the 'active' edge and the inactive centre less distinct. Clinically the diagnosis can be difficult but, if it is a possibility, take scrapings for mycology. Other fungal infections look nothing like tinea. Other conditions to consider include: Contact Dermatitis, Seborrhoeic, and Erythrasma (Ellis *et al.*, 2000; Rashid *et al.*, 2011). Microscopy of skin and nail specimens may reveal hyphae and spores. Fungal culture can identify the species but is not always reliable and it can take six weeks to get results, so the use of molecular tool as PCR for identification is needed for fast and accurate diagnosis.

Ultraviolet light (Wood's light) is useful for tinea capitis especially. Fluorescence is produced by the fungus. Fluorescence is not seen with tinea corporis or tinea cruris. Rarely, a biopsy may be needed.

## 1.11 Identification of Dermatophytes

### 1.11.1 Conventional approaches

The diagnosis is not clinically obvious in many cases so the mycological analysis is required. This includes both direct microscopic

examination and cultures. First of all, clinical specimens have to be sampled according to localization and characteristics of the lesions. Direct microscopic examination is usually performed using clearing reagents (KOH or Amman's chloral-lactophenol) (Koneman & Roberts, 1985). Histological analysis is an efficient method, but it is constraining for the patients and, as direct examination, it does not allow precise identification of the pathogen. Cultures are therefore needed, and specific culture media may be used to overcome the growth of rapidly growing contaminating moulds which may hamper the recovery of dermatophytes. Most dermatophytes colonies could be a presumptive identifying by using developed forms and pigmentation which appeared from a fungus colony depends on the used medium. In many reported literature Sabouraud dextrose agar (SDA) medium is conventionally used for comparative purposes. It is used to obtain colonies which can be compared to others (Ajello, 1974; Ameh & Okolo, 2004).

Identification at the species level which may be useful to initiate an appropriate treatment or for setting prophylactic measures relies on macroscopic and microscopic morphology. Subcultures on culture media which stimulate conditions and, for some species, the production of pigments, are often necessary. Additionally, in case of typical isolates, some biochemical or physiological tests may be performed such as the search for urease activity or the in vitro hair perforation test. However, their contribution to species identification is rather limited, and progress is still needed for the development of biochemical or immunological tests and

for the availability of molecular biology-based kits allowing an accurate identification at the species level (Mochizuki *et al.*, 1997; Elewski, 1998; Moriello, 2001).

### **1.11.2 Molecular Biological Methods**

Using molecular methods for the genotyping characteristic of the species of dermatophytes are more specific, precise, rapid and less likely to be affected by external influences such as temperature variations and chemotherapy and can be useful when the identification of a strain is not possible with conventional methods. PCR–RFLP (Blanz *et al.*, 2000; Machouart-Dubach *et al.*, 2001) and sequencing of ITS regions (Harmsen *et al.*, 1999) have been developed. However, most of these molecular approaches are used only in research laboratories. DNA of dermatophytes can also be detected directly in clinical samples (Machouart-Dubach *et al.*, 2001). Indeed, a PCR–ELISA-based kit using dermatophyte-specific probes has been recently commercialized. It has been evaluated for onychomycosis, and its sensitivity was found to be close to that of mycological conventional techniques (Seebacher *et al.*, 2008). This test allows the diagnosis of onychomycosis within only 24–48 h after sampling. However, as no species identification is provided, cultures remain essential with such a kit. This could be definitively solved by the use of oligonucleotide hybridization methods which allow both direct detection of fungal DNA in clinical samples and identification at the species level and an oligonucleotide array has been developed recently which allows correct identification of 17 dermatophyte species (Li *et al.*, 2007).

Random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD is a modification of the PCR in which a single, short and arbitrary oligonucleotide primer, able to anneal and prime at multiple locations throughout the genome, can produce a spectrum of amplification products that are characteristics of the template DNA. RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics, and plant and animal breeding. This is mainly due to the speed, cost and efficiency of the technique to generate large numbers of markers in a short period compared with previous methods. Therefore, RAPD technique can be performed in a moderate laboratory for most of its applications. It also has the advantage that no prior knowledge of the genome under research is necessary (Meyer *et al.*, 1997; Macura *et al.*, 2008). Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers and genotyping for different species, and among the dermatophytes, *Microsporum canis* constitutes the main species involved in infections of cats and dogs (Cabañes 2000; Brilhante *et al.*, 2005).

The importance of using molecular epidemiology tools was once the question regarding the epidemiology of dermatophytes is the issue of reactivation or reinfection. Is an infection the result of a reactivation of a past infection or a new infection from the environment? This question is most easily answered by using molecular tools that have sufficient discrimination power to distinguish closely related species and strains. Appropriate species and strain typing in most organisms usually focuses on the DNA/ rRNA gene cluster, using conserved regions of the DNA/rRNA or internal transcribed spacer (ITS). This type of analysis has been performed for many of the dermatophytes (Jackson *et al.*, 1999; Jackson *et al.*, 2000; Gupta *et al.*, 2001; Gaedigk *et al.*, 2003; Yazdanparast *et al.*, 2003; Rad *et al.*, 2005; Sugita *et al.*, 2006). However, the rRNA repeat is present in multiple copies in the genomes of most eukaryotes. Because of those repeats, PCR complications can occur associated with allelic variation between the repeats and mixing of alleles. Recent molecular epidemiology using DNA (non-rRNA) methods has been performed for the most common dermatophytosis tinea capitis (Abdel-Rahman *et al.*, 2006; Abdel-Rahman *et al.*, 2007). The genetic relationships of fungi, was done by using DNA depending method. The random amplified polymorphic DNA (RAPD) method, have been described as molecular diagnostic tool by some authors to detect dermatopytosis and polymorphism in some fungal strains (Bradley *et al.* 1999; Mukherjee *et al.* 2003).

### **1.12 Aims and Objectives**

The aim of this study was to:

- Determine the dermatophytes epidemiology including prevalence and occurrence of causative agents of dermatophytosis in patients in Palestine; and to detect any changes in the etiological agents during the last 28 years.
- Study the correlation between multifocal infections tinea pedis, tinea nail and tinea cruris.
- Investigate genetic relationship within and between different etiological agents using RAPD –PCR.
- Genotyping identification for different dermatophytes species.
- Study diversity during different dermatophytes species and within the same species.



# **CHAPTER TWO**

# **MATERIALS AND METHODS**

## **CHAPTER TWO**

### **MATERIALS AND METHODS**

#### **2.1 Epidemiology of Dermatophytes Infections**

##### **2.1.1 Sample Collection**

Samples were collected by Dr. Hisham Arda from his Dermatology Clinic in Nablus, 220 clinical specimens were collected from 188 patients with clinical signs of dermatophytosis. Demographic data was collected from all cases including: age, sex, profession, presence of animal in the patient's environments.

##### **2.1.2 Direct Examination**

Direct microscopic examination of skin and hair specimens was performed after digestion in 10% (w/v) potassium hydroxide (KOH) with dimethyl sulfoxide (DMSO) aqueous solution, while nail specimens were digested using 20% KOH. Briefly, a portion of each sample was placed on a sterile empty petri plate and a few drops of KOH solution were added (Lilly *et al.*, 2006). After 5-10 min for skin and hair samples and after 30 min for nail samples, part of this wet preparation was transferred to clean, dry microscopic slide and one drop of Lactophenol cotton blue was added then examined under low (100x) and high (400x) magnification power for the presence of arthroconidia, mycelium and /or spores (Panasiti *et al.*, 2006).

### **2.1.3 Cultures and Isolation of Dermatophytes**

All specimens (irrespective of the negative or positive microscopic examination result) were cultured on Sabouraud's dextrose agar (SDA) supplemented with antibiotics (chloramphenicol 0.05mg/ml, gentamicine, and cycloheximide 0.5mg/ml (Sigma-Aldrich). The use of a selective medium is essential because skin and hair are susceptible to contain many bacteria or conidia of saprophytic fungi (Brun *et al.*, 2001). Cultures were either made on agar plates or agar slants. Cultures were incubated at 25° C, and examined at least twice a week since some morphological traits appeared (Singh *et al.*, 2003), then identified using standard methods.

## **2.2 Genotypical Pattern of Dermatophytes Strains by using RAPD Analysis Primers**

### **2.2.1 Fungal DNA Isolation**

Eighty dermatophytes isolates were taken to do RAPD analysis, fifty three from *Microsporum canis* and twenty four from *Trichophyton rubrum* and three from *Trichophyton mentagrophytes*. A standardized inoculum was prepared from each isolate. Stock inocula were prepared from 10 days old cultures grown on Sabourod dextrose agar at 28 °C. Sterile normal saline solution (0.9%) was added to the agar slant, and the cultures were gently swabbed. The suspension of conidia and hyphal fragments was cultured in a sterile tube, contained 2 mL of Sabouraud liquid medium with 0.5 mg/l cycloheximide (Sigma) and 0.05 mg/l chloramphenicol (Sigma)

solution, twice for each sample and incubated with shaking for up to 8 days at 27°C. After that the pellet was harvested and suspended in 500µl of lysis buffer (400mM Tris-HCl [pH8.0], 60mM EDTA [pH8.0], 150mM NaCl, 1% sodium dodecyl sulfate) and left at room temperature for 10 min. Next, 150µl of potassium acetate Kac [pH8.0] was added and tubes were vortexed and centrifuged (1min, 12,000xg). The supernatant was transferred to a new tube and 500µl isopropyl alcohol was added. The DNA pellet was washed with 500µl of 70% ethanol then dried for 5 min and 50µl of S.D.H<sub>2</sub>O was added, then the concentration was measured by using multiscan plate biotech and all samples were diluted to 30ng/µl (Brillowska-Dabrowska *et al.*, 2007). DNA integrity was performed by agarose gel electrophoresis. One percent agarose gel with 0.5 mg/ml ethidium bromide was used and gel was running at voltage 120V for 30 min in 100 mL from 1X TAE buffer (Tris–EDTA (0.001 mol/L). DNA samples were loaded as follows: 1µl DNA sample, 2µl from 6X sample loading buffer, and 7µl sterile distilled water. The samples were loaded in the well by micropipette. The first well was the 1Kb DNA ladder 5µl. The DNA band was visualized with a UV transilluminator and photographed.

### **2.2.2 RAPD Assays**

RAPD assays were performed with the following random five primers: OPA 01 (5'-CAGGCCCTTC-3'); OPA 02 (5'-TGCCGAGCTG-3'); OPA 03 (5'-AGTCAGCCAC-3'); OPA 04 (5'-AATCGGGCTG-3'); and OPA 05 (5'-AGGGGTCTTG-3') The reactions were performed in 25

μL volumes containing the following: reaction buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 9.0), 0.1% Triton X-100); 2 mmol/L MgCl<sub>2</sub>; 0.5 mmol/L of each deoxynucleoside triphosphates ; 10 pmol of OPA's primer; 1 U of *Taq* DNA polymerase ; and 30 ng of genomic DNA.

### **2.2.3 RAPD-PCR Amplification Program**

RAPD-PCR was done on 96-Well GeneAmp PCR System thermal cycler, and two trails for each primer were done. The amplification parameters consisted of 1 cycle of 4 min of denaturation at 94 °C, 45 cycles of 20 s at 94 °C of denaturation, primer annealing for 1 min at 35 °C and extension for 1 min at 72 °C; with 1 final extension step at 72 °C for 10 min.

Amplification products were separated by electrophoresis in 1.5 % agarose gels, visualized by staining with 0.5 mg/ml ethidium bromide. Gel was running at voltage 120V for 1 hour in 100 mL from 1X TAE buffer (Tris-EDTA (0.001 mol/L)).

PCR products were loaded as follows: 5μl PCR product and 5μl 6X sample loading buffer. The first well was the 1Kb DNA ladder 5μl, the bands were visualized with a UV transilluminator and photographed. All the tests were repeated twice.

### **2.2.4 RAPD-PCR products scoring**

RAPD markers resulting bands were scored for each RAPD primer based on the molecular size. Scored RAPD bands were done by using a

binary system of 0 to the absence of the band and 1 to the band is present for each primer in the two trails. Then the row data was interred to the Statistical Package for the Social Sciences (SPSS) program version 15 for data analysis.

#### **2.2.5 Data analysis**

All RAPD data results were tabulated encoded and statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 15.

# **CHAPTER THREE**

# **RESULTS AND DISCUSSION**

## CHAPTER THREE

### RESULTS AND DISCUSSION

#### 3.1 Epidemiology of Dermatophytes Infections

Fungal infections still create a main health problem all over the world affecting all ages particularly children. Therefore, many studies have been conducted concerning different epidemiological economical, control as well as therapeutic features of this infection (Seebacher *et al.*, 2008). Dermatophytosis is considered as the most common human fungal infections, and about 20–25% of the world's population estimated to have skin mycoses (Havlickova *et al.*, 2008).

In this study, a total number of 220 samples from 188 patients were examined ((137 males (72.9%), and 51 females (27.1%)). Tinea capitis (27.7%) was the predominant clinical manifestation followed by tinea pedis (23.4%), tinea nail (22.3%), tinea cruris (20.2%) and tinea corporis(6.4%) (Table 3.1).

There were many studies in many countries in different geographical areas in agreement with this study results, as in Nablus (Ali-Shtayeh *et al.*, 1998) tinea capitis was the predominant clinical manifestation accounted for 39.6% of dermatophytosis infections. In Tunis tinea capitis accounted for 69.4% of dermatophytic infections and it was predominant clinical manifestation (Sharma *et al.*, 2007), and in Malta also it was the predominant clinical manifestation (Vella *et al.*, 2003). However in KSA, tinea capitis accounted for 47.7% and as in this study, *M.canis* was the



commonest causative agent responsible for 46.9% of ringworm infections (Venugopal & Venugopal 1992).

**Table (3.1): Details of patients with reference to clinical manifestation and sex.**

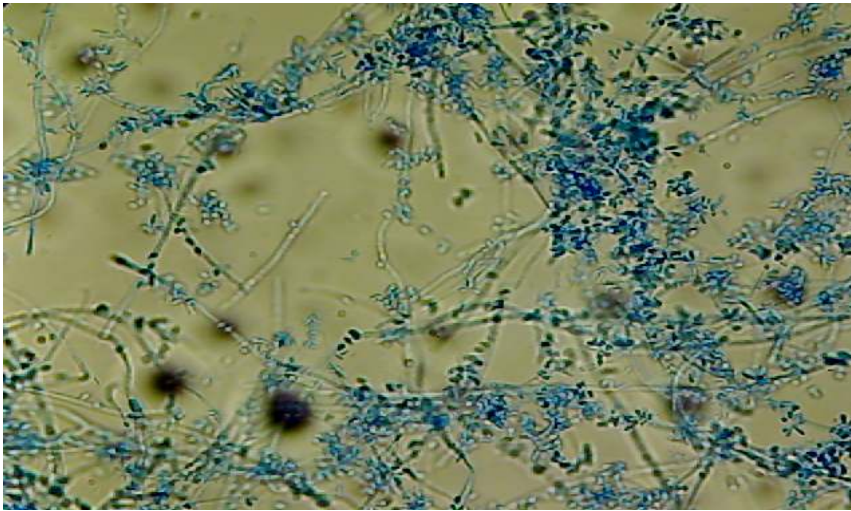
Clinical Manifestation	Total # of patients (n/%)	Sex	
		M	F
Tinea capitis	52 (27.7)	31 (60)	21 (40)
Tinea corporis	12 (6.4)	5 (41.7)	7 (58.3)
Tinea cruris	38 (20.2%)	34 (89)	4 (11)
Tinea nail	42 (22.3)	30 (71)	12 (29)
Tinea pedis	44 (23.4)	37 (84)	7(16)
<b>Total</b>	<b>188(100)</b>	<b>137 (72.9)</b>	<b>51 (27.1)</b>

Initial microbiological observation at the collection spot revealed the presence of fungal hyphae by direct microscopy, KOH mount in 60% cases. Also after culture on SDA media the spores were check under direct microscopy and Figure 3.1 is an example for macroconidia from *Microsporum canis* isolate under direct microscopy (400 x) after 10 days cultured. *Microsporum canis* Macroconidia were spindle-shaped with 5-15 cells, verrucose, thick-walled and often have a terminal knob, 35-110 x 12-25  $\mu\text{m}$ .



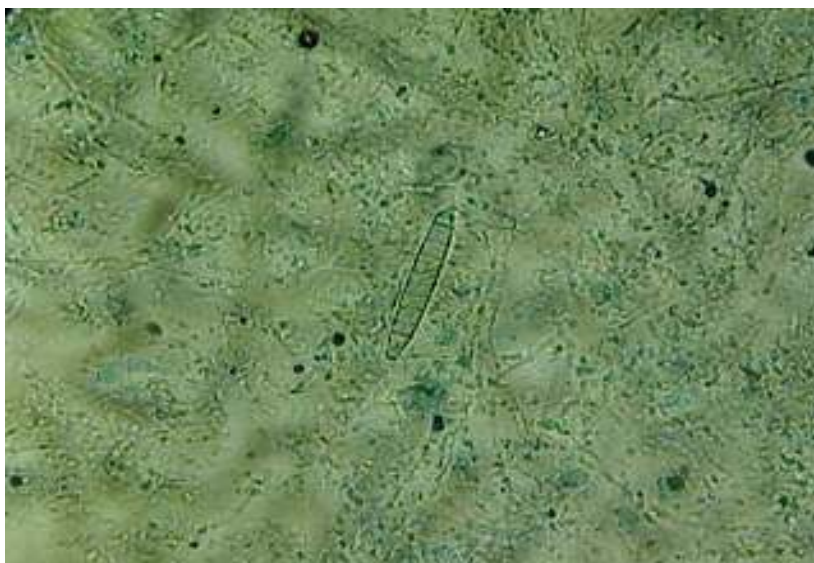
**Figure (3.1): Macroconidia of *Microsporum canis* under direct microscopy (400 x)**

Figure 3.2 is an example for microconidia from *Trichophyton mentagrophytes* isolate under direct microscopy (400 x) after 10 days cultured. Numerous single-celled microconidia were formed. Microconidia were hyaline, smooth-walled, and were predominantly spherical to subspherical in shape; however occasional clavate to pyriform forms may occur, and thin-walled, clavate- shaped, multi celled macroconidia were present.



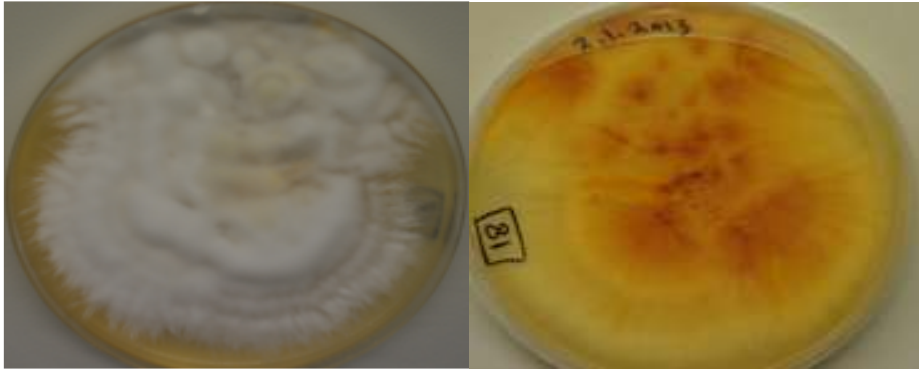
**Figure (3.2): Microconidia of *T. mentagrophytes* under direct microscopy (400 x)**

Figure 3.3 is an example for microconidia and macroconidia from *Trichophyton rubrum* isolate under direct microscopy (400 x) after 10 days cultured. Most cultures showed scanty to moderate numbers of slender clavate to pyriform microconidia with or without moderate to abundant numbers of thin-walled, cigar-shaped macroconidia.



**Figure (3.3): Microconidia of *T. mentagrophytes* under direct microscopy (400 x)**

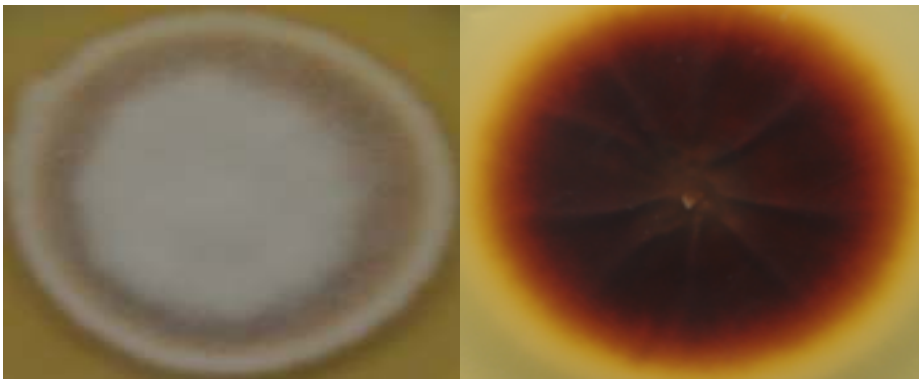
The morphology identification of dermatophytic fungi showed that *Microsporum canis* colonies on (SDA) which were flat, spreading, and white to cream-coloured, with a dense cottony surface which may show some radial grooves. Colonies were a bright golden yellow to brownish yellow reverse pigment, but non-pigmented strains may also occur (Figure 3.4). *Trichophyton mentagrophytes* colonies on (SDA) were flat to heaped, white to cream in colour, with a powdery to granular surface. Some cultures showed central folding or develop raised central tufts or pleomorphic suede-like to downy areas. Reverse pigmentation was a yellow-brown to reddish-brown colour or without reverse pigment (Figure 3.5). *Trichophyton rubrum* colonies on (SDA) were flat to slightly raised center, white to cream, suede-like to downy, with a yellow-brown to wine-red reverse (Figure 3. 6).



**Figure (3.4): Both sides of primary culture of *Microsporium canis***



**Figure (3. 5): Both sides of primary culture of *Trichophyton mentagrophytes***



**Figure (3. 6): Both sides of primary culture of *Trichophyton rubrum***

Among all the pathogens which were identified *Microsporium canis* (75.4%) was the predominant pathogen followed by *Trichophyton rubrum* (22.5%) then *Trichophyton mentagrophytes* (2.1%).(Table 3. 2).

**Table (3.2): Prevalence pattern of dermatophytic fungi (n, %).**

Clinical Manifestation	Total # of positive samples (n/%)	Dermatophytic fungi		
		<i>M. canis</i>	<i>T. rubrum</i>	<i>T. mentagrophytes</i>
Tinea Cruris	36 (26.1%)	24(66.7%)	11(30.6%)	1(2.7%)
Tinea Nail	21 (15.2%)	14(66.7%)	7(33.3%)	0
Tinea Pedis	32 (23.2%)	21(65.6%)	10(31.3%)	1(3.1%)
Tinea Capitis	40 (29%)	40(100%)	0	0
Tinea Corporis	9 (6.5%)	5(55.6%)	3(33.3%)	1(11.1)
Total	138(100%)	104(75.4%)	31(22.5%)	3(2.1%)

Two main dermatophytes genera were isolated in this study (Table 3.3). There was a statistically significant difference between the two genera; the most common isolated species of dermatophytes was *Microsporum canis* (75.36%), followed by *T. rubrum*, and *T. mentagrophytes* (24.64%).

**Table (3.3): Frequency of causative agents causing dermatophytic infections isolated from positive cultures**

Pathogen isolate	Frequency	Percentage
<i>Microsporum canis</i>	104	75.36%
<i>Trichophyton rubrum</i> & <i>Trichophyton mentagrophytes</i>	34	24.64%
Total	138	100.00%

The multifocal infection distribution of dermatophytosis according to the etiological agent and infection type of collected sample are presented in Table 3.4. Thirty eight samples (59.4%) were positive from 64 multifocal infection cases taken from different samples of the same patient. The frequency rate of positive samples which is mostly in the form of tinea cruris (86.8%) was higher than tinea pedis (84.2%) then tinea nail (63.2%), tinea capitis (13.2%) and tinea corporis (5.3%). Tinea cruris was also the

dominant clinical manifestation in Iran and that result is conducted with this study result (Lari *et al.*, 2003).

*Microsporum canis* was the most prevalent etiological agent, 12 patients have positive isolate from three multifocal infection (tinea cruris, tinea nail and tinea pedis), 3 patients have positive isolate from two multifocal infection sites ( tinea cruris and tinea pedis), 3 patients have positive isolate from two multifocal infection (tinea cruris and tinea capitis), 2 patients have positive isolate from three multifocal infection sites (tinea cruris, tinea pedis and tinea capitis), 2 patients have positive isolate from two multifocal infection sites (tinea pedis and tinea nail), and one patient has two multifocal infection sites (tinea cruris and tinea corporis).

Also, it can be seen that there is a tight correlation between tinea cruris, tinea pedis and tinea nail, there is 12 cases with positive results on the three tineas, and that is due to tinea nail because it serves as a reservoir for the infection. This data agreed with Roujeau *et al.*, 2004 results which found that the onychomycosis can serve as a reservoir for dermatophytes, which can re-infect the skin. So in all dermatophytes cases the clinical specimen from the foot and / or nail should be taken to test it also, and to start the treatment from it to prevent the re-infection again. The presence of tinea pedis and/or onychomycosis is also a significant risk factor for acute bacterial cellulitis of the leg as well as diabetes-related foot and leg

complications in at-risk individuals (Gupta & Humke, 2000; Roujeau *et al.*, 2004)

On the other hand *Trichophyton rubrum* positive isolates were from 6 patients have three multifocal infection (tinea cruris, tinea nail and tinea pedis), 4 patients have positive isolate from two multifocal infection sites (tinea cruris and tinea pedis), 3 patients from two multifocal infection (tinea nail and tinea pedis), one patient has two multifocal infection (tinea cruris and tinea nail), one patient has two multifocal infection (tinea cruris and tinea corporis).

**Table (3.4): Prevalence pattern of dermatophytic fungi in multifocal infections (n, %).**

Dermatophytes Specis	Multifocal infections 38 were positive from 64(59.4%)				
	T. Cruris	T. Pedis	T. Nail	T. Corporis	T.Capitis
<i>M. canis</i>	12	12	12		
	3	3			
		2	2		
	1			1	
	2	2			2
	3				3
<b>Subtotal</b>	<b>21</b>	<b>19</b>	<b>14</b>	<b>1</b>	<b>5</b>
<i>T. rubrum</i>	1		1		
		3	3		
	6	6	6		
	4	4			
	1			1	
<b>Subtotal</b>	<b>12</b>	<b>13</b>	<b>10</b>	<b>1</b>	<b>0</b>
<b>Total</b>	<b>33(86.8%)</b>	<b>32(84.2%)</b>	<b>24(63.2%)</b>	<b>2(5.3%)</b>	<b>5(13.2%)</b>

In this study the results show a huge change of the dermatophytes spectrum during the last 28 years. The change in the spectrum of dermatophytes was in the dominant cuasative agent *Microsporium canis* (zoophilic dermatophytes) which has shifted to in the head instead of

*Trichophyton violaceum* (anthropophilic dermatophyte), also *Trichophyton rubrum* (anthropophilic dermatophytes) and *Trichophyton mentagrophytes* and the absent of the other dermatophytes types specially *Trichophyton violaceum* which was the dominant one in the past.

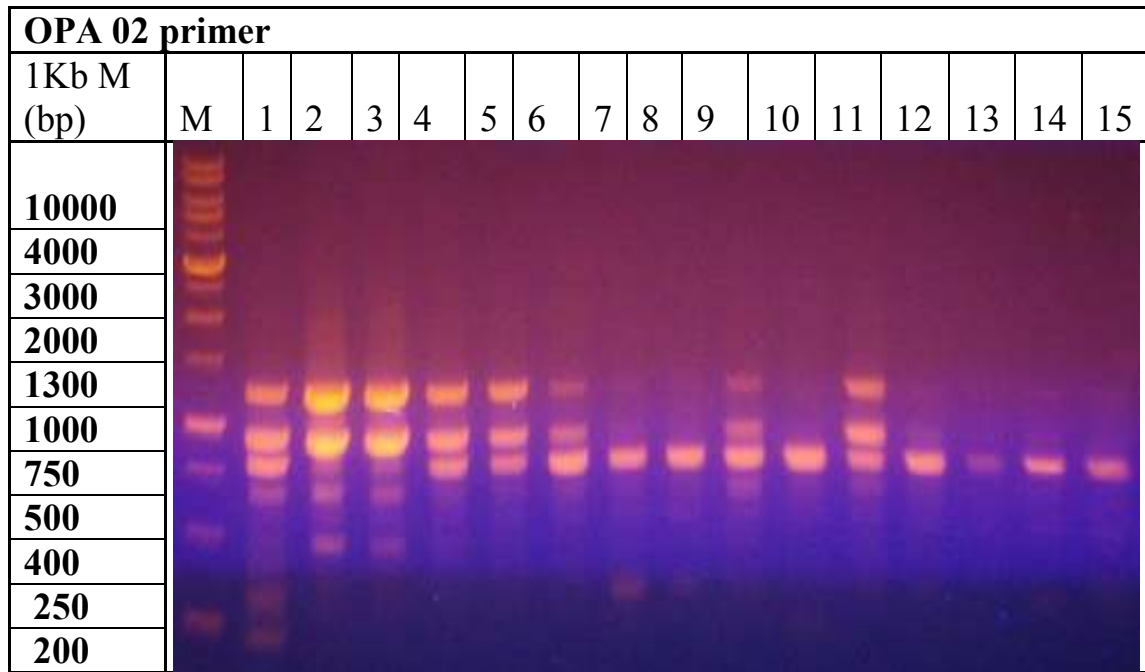
This change could be due to several factors as activity related to human and animals hygiene and interaction between human and human, animal and soil, and changing population over the times (Yehia *et al.*, 2010).

### **3.2 Genotypical Pattern of Dermatophytes Strains by using RAPD Analysis Primers**

#### **3.2.1 RAPD-PCR amplification**

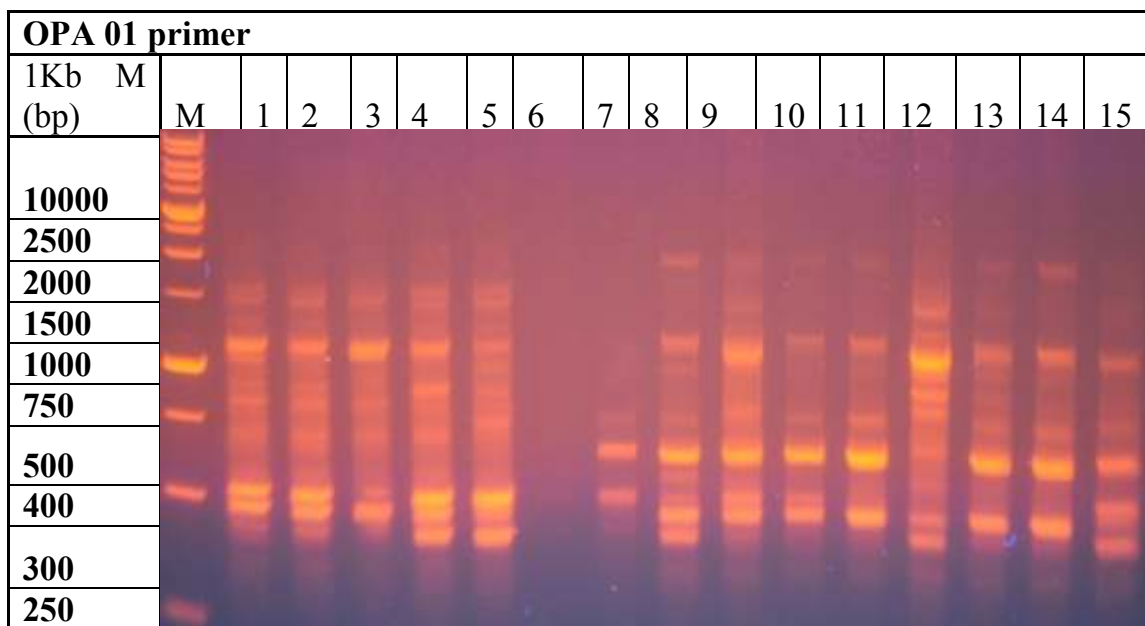
All examined isolates produced distinct banding pattern with all primers. Among the *Microsporum canis* isolates, three genotypes were distinguished and designated A, B and C, which were characteristic for all the isolates of *Microsporum canis*. For example the three types which were produced by OPA 02 primer, which yielded up from 11 to 5 bands, ranging from approximately 250 to 2500 bp in length (Figure 3.1). Comparable result was obtained by Shehata *et al.* (2008) obtained similar profiles with *Microsporum canis* isolates , with up to 11 bands ranging from 600 bp to 2500 bp.



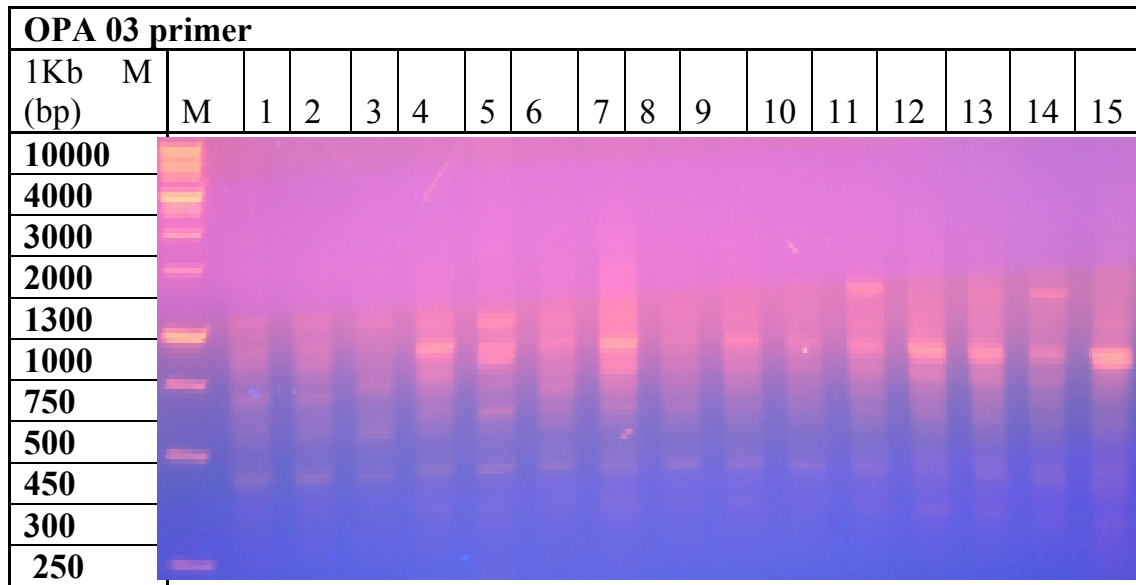


**Figure (3.7):** Example of RAPD banding patterns generated in first 15 isolates of *M.canis* using OPA02

In other hand OPA 01 primer produced bands , which yielded up to 13 bands, ranging from approximately 400 to 2700 bp in length (Figure 3.8), and OPA 03 primer produced 10 bands , ranging from approximately 300 to 1500 bp in length (Figure 3.9).

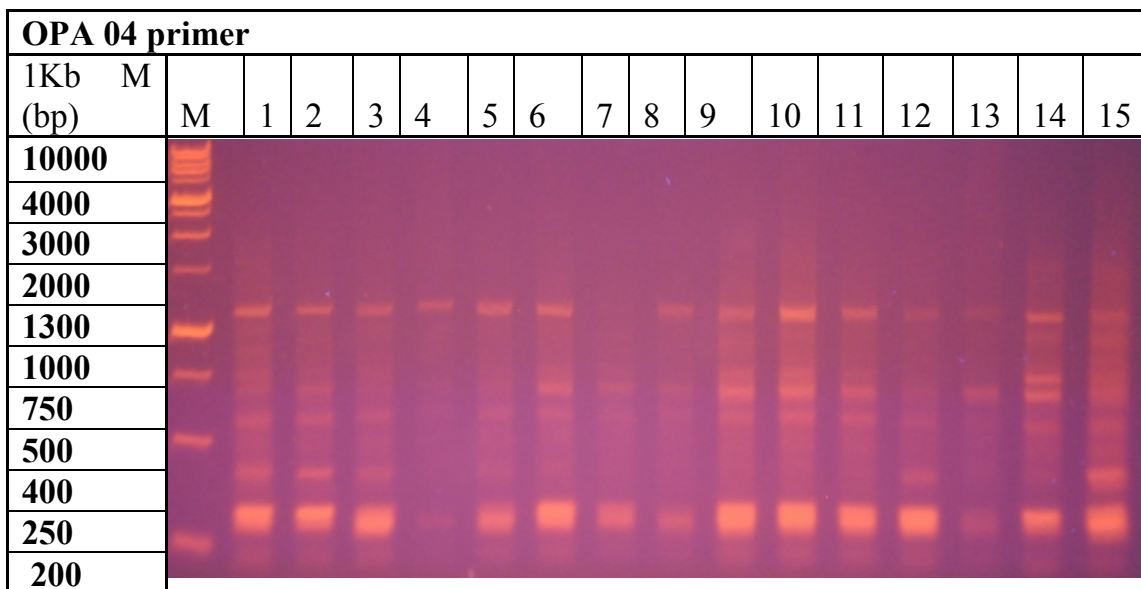


**Figure (3.8):** Example of RAPD banding patterns generated in first 15 isolates of *M.canis* using OPA01

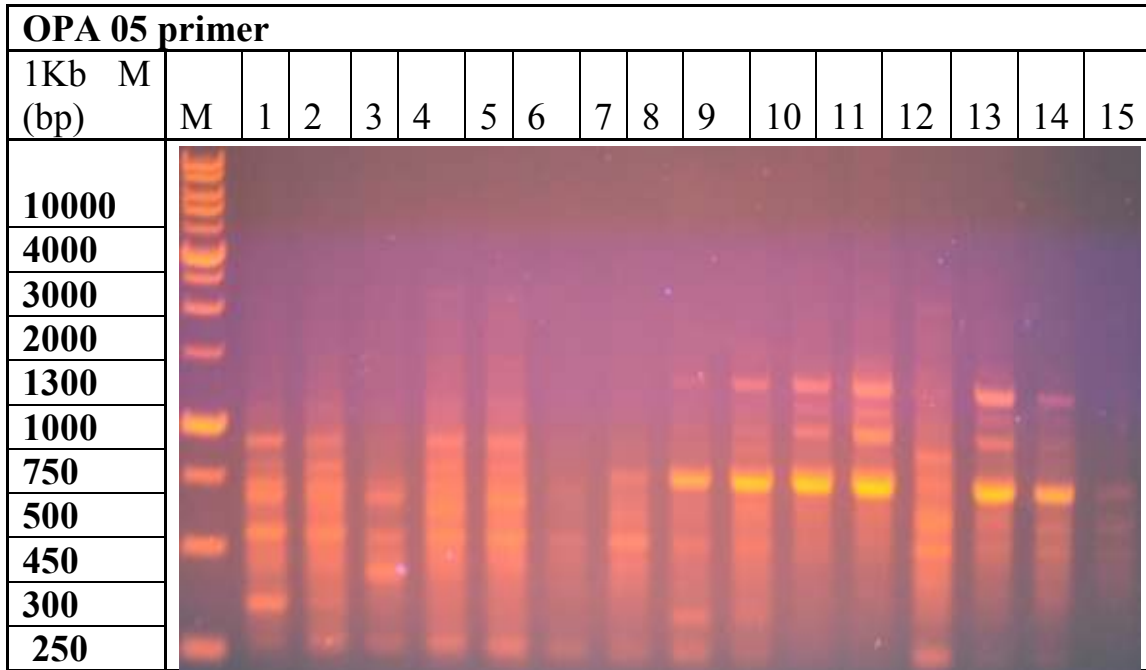


**Figure (3.9): Example of RAPD banding patterns generated in first 15 isolates of *M.canis* using OPA03**

OPA 04 primer produced several bands, which yielded up to 7 bands, ranging from approximately 300 to 1200 bp in length (Figure 3.10), and OPA 05 primer was yielded up to 11 bands , ranging from approximately 250 to 1500 bp in length (Figure 3.11).



**Figure3.10: Example of RAPD banding patterns generated in first 15 isolates of *M.canis* using OPA04**



**Figure (3.11): Example of RAPD banding patterns generated in first 15 isolates of *M.canis* using OPA05**

### 3.2.2 RAPD Data analysis

Dermatomycosis induced by *Microsporum canis* has become a serious problem in recent years. Epidemiological studies conducted in Poland (Mochizuki *et al.*, 2003; Yazdanparast *et al.*, 2003) revealed a low level of intraspecies polymorphisms within *Microsporum canis* isolates. In this study three types of *Microsporum canis* was revealed. The dendrogram and the similarity matrix bellow shows that there are three genotypes of *Microsporum canis*, two of *Trichophyton rubrum* and one for the *Trichophyton mentagrophytes*. The similarity matrix resulting from the combined RAPD-DNA markers data were performed to generate correct relationships based on large and different genome regions (Table 3.6).

The *Microsporum canis* dendrogram part include three clusters, the first containing two subclusters 18 isolates, while the second cluster

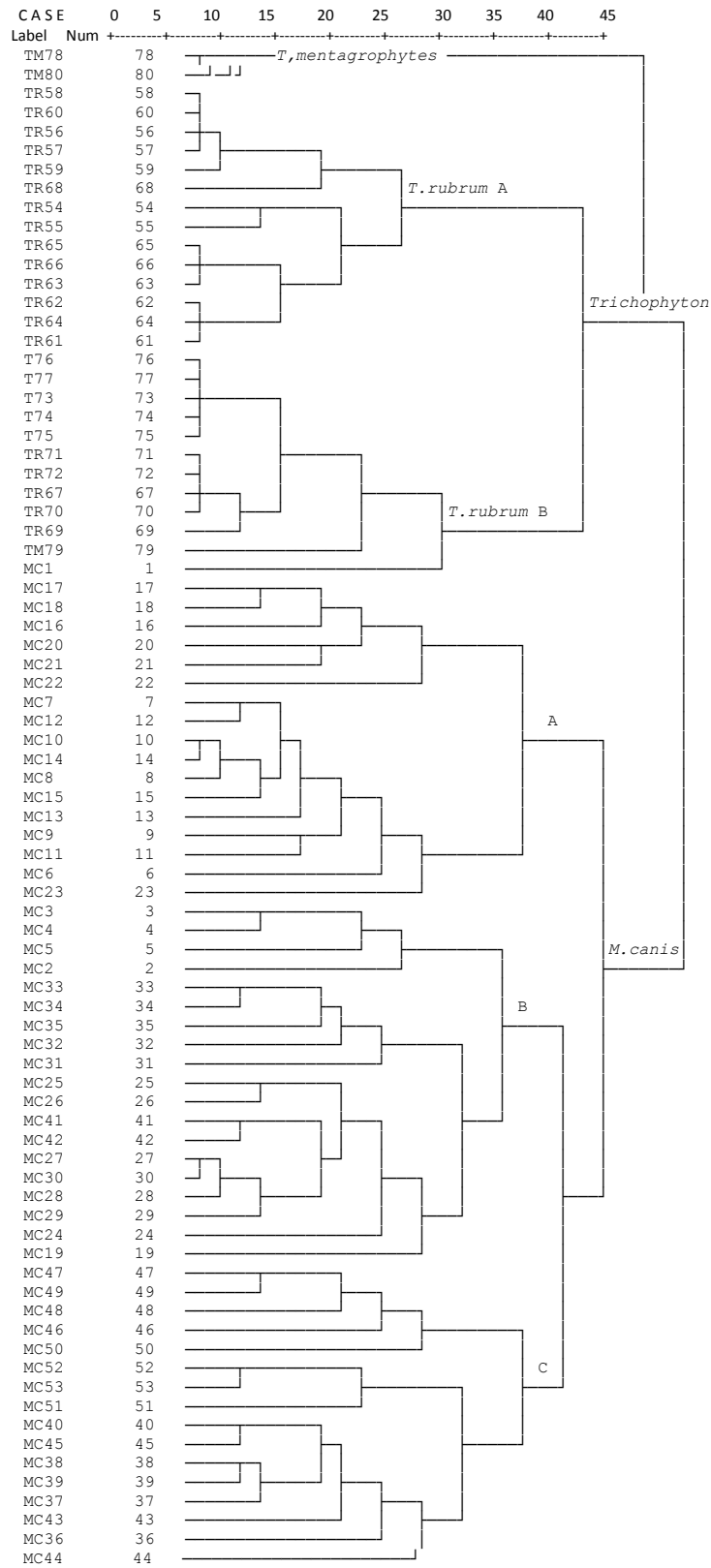
contains two subclusters 35 isolates. The first subcluster contains and the second is divided into two main groups which contain, in the first group and in the last group. And according to *T. rubrum* there were two clusters also and the *T. mentagrophytes* has one cluster (Figure 3.12).

This result gives evidence that the use of molecular tools for dermatophytes diagnosis is most easily, faster and it has sufficient discrimination power to distinguish closely related species and strains. Molecular tools give appropriate species and strain typing in dermatophytes samples which were tested. This type of analysis has been performed for many of the dermatophytes (Jackson *et al.*, 1999; Jackson *et al.*, 2000; Gupta *et al.*, 2001; Gaedigk *et al.*, 2003; Yazdanparast *et al.*, 2003; Rad *et al.*, 2005; Sugita *et al.*, 2006).

### Table (3.5): Similarity matrix

[illegible]

## Rescaled Distance Cluster Combine



**Figure (3.12): Dendrogram using Average Linkage (Within Group)**

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## APPENDIX

### Media and Stains

#### Sabourad's dextrose agar

##### Formula / Liter

Enzymatic Digest of Casein .....	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Dextrose.....	40 g
Agar .....	15 g
Final pH: 5.6 ± 0.2 at 25°C	
Chloramphenicol.....	0.05 mg
Cyclohexamide .....	0.5 mg

##### Preparation

Sixty five grams of the medium were suspended in one liter of purified water.

And heated with frequent agitation and boiled for one minute to completely dissolve the medium. After that media autoclaved at 121°C for 15 minutes and mixed well before pouring.

#### Sabourad's dextrose Broth preparation

Half gram of yeast extract, one gram peptone, two grams of dextrose were dissolved in 100ml distilled water then autoclaved. Chloramphenicol (50µg /ml) was added in 2 ml of absolute alcohol to SD broth.

#### Lactophenol-Cotton Blue Stain

Distilled water.....	20ml
Lactic acid .....	20ml
Phenol crystals.....	20g
Aniline blue( cotton blue,Pirrier's blue).....	0.05g
Glycerol.....	40ml

**Preparation method**

Phenol was dissolved in lactic acid; glycerol and water were added and mixed gently with heating until completely dissolved then aniline blue was added.

**Potassium hydroxide (KOH) Solution**

KOH crystals.....10g  
 Glycerin.....10ml  
 Distilled water.....80ml

**Preparation method**

KOH crystals were dissolved in glycerin and mixed well with distilled water.

**70% Ethanol**

Thirty milliliters distilled water was added to 70 ml of pure ethanol (100%) to prepared 100 ml.

**1X TAE running buffer**

Tris-HCl.....242g  
 Glacial acetic acid.....57.1ml  
 500mM EDTA (pH 8.0).....100ml

**Preparation method**

TAE buffer was prepared as a 50X stock solution. A 50X stock solution was prepared by dissolving 242g Tris-HCl base in water, 57.1mL glacial acetic acid, and 100mL of 500mM EDTA (pH 8.0) solution were adding, and the final volume was brined up to 1 liter. This stock solution was diluted 50:1 with water to make a 1X TAE working solution.

**Lysis buffer**

400mM Tris-HCl [pH8.0]

60mM EDTA [pH8.0]

150mM NaCl

SDS 1%

**Potassium acetate [pH8.0]**

5M potassium acetate.....	60 ml
Glacial acetic acid.....	11.5ml
Distilled water.....	28ml

**Preparation method**

Sixty milliliters from 5M potassium acetate was mixed with glacial acetic acid and water to prepare 100ml.

جامعة النجاح الوطنية  
كلية الدراسات العليا

## وبائية الأمراض الجلدية في فلسطين: دراسة للتحديث

إعداد

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قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا جامعة النجاح الوطنية، نابلس ، فلسطين.

2013م

ب

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### الملخص

**خلفية:** الفطريات الجلدية هي مجموعة من القوالب الفطرية ذات طرز شكلية وفسولوجية مرتبطة مع بعضها البعض، تسبب التهابات واضحة المعالم (القوباء الحلقية)، لديهم خاصيتان تعتبران من الخصائص الهامة بالنسبة للفطريات وهما: أن هذه المجموعة من الفطريات لديها القدرة على الارتباط وهضم الكيراتين والاستفادة منه باعتباره الركيزة الأساسية للغذاء لذلك يمكن لبعضها أن يغزو الأنسجة المحتوية على الكيراتين في جسم الكائن الحي مسببة له القوباء أو الأمراض الجلدية.

**الأهداف:** تم تصميم هذه الدراسة لتحديد وبائية الفطريات الجلدية (Dermatophytes) ، بما في ذلك انتشارها ووجود العوامل المسببة للفطريات الجلدية للمرضى في فلسطين؛ والكشف عن أية تغييرات في العوامل المسببة خلال الـ 28 عاما الماضية؛ كما وتهدف هذه الدراسة أيضا، إلى دراسة العوامل المؤثرة في انتشار المرض مثل الظروف الاجتماعية والعمر والتعامل مع الحيوانات وغيرها من العوامل المسببة للمرض؛ و دراسة العلاقة بين التهابات متعددة البؤر وهي سعة القدم، سعة الأظافر و سعة الفخذين في الشخص الواحد وإذا ما كان المسبب واحدا في كل الحالات، كما وتهدف هذه الدراسة أيضا إلى تقييم العلاقة المحتملة بين الأنماط الجينية المختلفة بين السلالات المختلفة المعزولة من الفطريات الجلدية التي تصيب الإنسان في فلسطين. ومعرفة مدى إمكانية استخدام الطريقة الجزيئية لتشخيص الفطريات الجلدية تشخيصا سريعا ودقيقا ومن أهداف هذه الدراسة أيضا دراسة التنوع الجيني بين الأنواع والأصناف المختلفة وداخل النوع والصنف الواحد خاصة من الفطريات الجلدية المعزولة وهي



, *Trichophyton rubrum* and *Trichophyton Microsporum canis*,  
*mentagrophytes* الدراسة على المستوى الجزيئي كانت باستخدام تقنية التضخيم العشوائي من  
 الحمض النووي متعدد الأشكال (Random Amplification of Polymorphic DNA  
 (RAPD) للتحقيق في العلاقات الوراثية بين السلالات المختلفة وكيف يمكن استخدامها كأداة  
 جزيئية للتشخيص السريع للأمراض الجلدية المختلفة.

**النتائج:** في هذه الدراسة، كان العدد الإجمالي للعينات الذي فحص 220 من 188 مريض، 137  
 من الذكور (72.9%) و 51 من الإناث (27.1%). سعة الرأس (27.7%) هو مظهر من مظاهر  
 السريرية الغالبة تليها سعة القدم (23.4%)، سعة الأظافر (22.3%)، سعة الفخذين (20.2%)  
 وسعة الجسم (6.4%). وكان العامل المسبب السائد هو *Microsporum canis* 104 عينات من  
 138 (75.36%) وأظهرت النتائج أن جميع السلالات التي تم تحليلها باستخدام RAPD أنها  
 أساسا تعود لثلاث أنماط جينية متصلة وراثيا وجينيا معا. يليه العامل المسبب الثاني وهو  
*Trichophyton rubrum* يتكون من نمطين جينيين وأخيرا *Trichophyton*  
*mentagrophytes* يتكون من نمط جيني واحد. وبالاعتماد على تحليل البيانات والنتائج وجد  
 أن ثمة علاقة وثيقة تربط بين النمط الجيني والنمط الشكلي لكل سلالة من السلالات الفلسطينية  
 المعزولة.

**الاستنتاجات:** أظهرت النتائج أن العامل المسبب السائد للإصابة الفطرية الجلدية كان  
*Microsporum canis*. وهذا تغير كبير في مسبب الإصابة المرضية؛ ويمكن أن يكون هذا  
 التغير نتيجة لعدة عوامل مثل التغير في النشاط الذي يمارسه الإنسان مؤخرا والتفاعل بين  
 الإنسان والحيوان والتربة وما يتعلق بالإنسان والنظافة، وتغيير عدد سكان والهجرة في الثلاث  
 عقود الماضية. كما وأوصت الدراسة باتخاذ التدابير الوقائية اللازمة لتجنب مسبب المرض  
 والتوجه للطبيب مباشرة فور الإصابة بالمرض.