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Biology

Phenotypic and Molecular Identification of *M.Canis*

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Abstract **Original Research Article**

The current study was conducted to compare morphological and molecular identification methods for the fungus *M.canis* isolated from 42 patients with dermatophytosis who were clinically diagnosed by a dermatologist, after review by a consultant dermatologist and venereologist in Al-Muthanna Governorate at Al-Hussein Teaching Hospital. In the period from January to November 2024, using traditional methods of isolation and laboratory identification, and molecular methods using polymerase chain reaction (PCR) technology.

Keywords: *M. canis*, Dermatophytosis, Morphological identification, Molecular identification, PCR.

INTRODUCTION

Dermatophytes are responsible for various illnesses, including ringworm and ringworm-like infections (Tinea); dermatophytoses is the correct word for a fungus that damagescorneal tissues and produces superficial skin diseases such as skin, hair, and nails. It has three principal species: Trichophyton, Microsporum, and Epidermatophyton. Microsporum sp. This may be a small-spored ectothrix caused by M. audouinii, M. audouinii var. rivalieri, M. canis, M. canis var. distortum, M. equinum or M. ferrugineum. In this type, the hair shaft is invaded in mid-follicle, the intrapilary hyphae continue to grow inwards towards the bulb of the hair. Secondary, extrapilary hyphae burst out and grow in a tortuous manner over the surface of the hair shaft, which is growing outwards continuously. These secondary, extrapilary hyphae segment to produce a mass of small arthroconidia (2–3 µm) diameter (Burns *et al*., 2010). Fluorescence under the Wood's lamp is characteristically present in this type of hair invasion. A similar type of hair invasion occurs with other Microsporum species (e.g. M. gypseum, M. fulvum, M. nanum and M. vanbreuseghemii). The spores, although similarly arranged, are larger, in this case about 5–8 µm. Fluorescence has been reported in some cases (Irene and Richard, 1998; Burns *et al*., 2010). This genus is characterized by fusiform or spindle shaped macroconidia, with thick and rough or spiny walls, the type species of the genus Microsporum, rarely produces macroconidia, but spore production may be increased on yeast extract medium (Kwon-Chung and Bennett, 1992).

Dermatophytosis is mainly limited to the superficial, non-living layers isolated because the fungal agents are unable to penetrate deep tissues or organs of the host. However, this infection also depends on the ability of the fungi to break down stratum corneum, host immune status, and site of infection (Upadhyay *et al*., 2019) Its inability to attack internal tissues is due to several reasons, including: the presence of inhibitory factors in blood serum and body fluids that inhibit the breakdown of keratin and its inability to grow. At temperatures higher than 35°C (Brooks *et al*., 2001,; Matsumoto, 1996), as Immune barriers play a role in limiting their presence to dead keratinized tissue only (Murray *et al*., 1999).This skin fungus grows best in warm, humid environments so it is more common in regions Tropical and subtropical, as in Libya, Iran, Iraq, Turkey and other In warm countries, skin diseases are more common than in countries with cold climates (Gnat *et al*., 2021). Remarkably, enzymes secreted by dermatophytes could underlie fungal survival on the host and development of infection, not only by providing nutrients as the keratin barrier is damaged, but also by modulating the immune response, Depending on the effectiveness of these enzymes, fungal species differ in their preference for tissue type Keratin, as the genus Epidermophyton prefers nail and skin tissues, while the genus Microsporum It prefers skin and hair tissues, and in the Trichophyton genus, it attacks all keratinous

tissues Whether it is skin, hair or nails (Rippon, 1982). As a result of the ability of these fungi to secrete enzymes that are able to decompose and dissolve the keratin layer found in hair, skin, and nails and the stratum corneum Corneum) covering most of the external body parts of these organisms, this gives great importance to these fungi from a medical point of view, as they represent a disease Dermatophytosis in the form of circular spots or inflammatory patches (inflammator patches). Raised and distinct edges, as well as redness, peeling, and itching (Itch) in the affected area of skin, as well as hair loss (Brooks *et al*., 2001) The skin fungus grows in all directions and almost evenly, forming circular spots (Circular lesions on the skin or scalp resemble holes made by moths in clothing. Therefore, this fungal infection was called ringworm (Ajello, 1974).

The Aim of Study: The study aimed to identify the types of skin fungi spread in Al-Muthanna Governorate, including:

1. Collect samples from patients infected with skin fungi in the laboratory.
2. Cultivating the samples on culture media and performing phenotypic and microscopic diagnosis of the fungi present in those media Samples.
3. Conducting molecular diagnosis of some isolated dermatophytes.

MATERIALS AND METHODS

Samples Collection

80 clinical samples from people infected with dermatophytes were collected from the dermatology consultancy in Al-Hussein General Teaching Hospital and some private clinics in Al-Muthanna Governorate for the period from 12/6/2023 to 3/20/2024, The study included collecting samples from the affected areas of the skin, hair, and nails under supervision Directly from the specialist doctor, for all ages and both sexes Skin samples were taken using the scraping method, where the affected area was sterilized with 70% ethyl alcohol, and then the crusts were scraped off from the edge of the infection site using a sharp, sterile blade. As for the skin samples, For the nails, a piece of them was taken using a sharp blade from the affected area with an abnormal shape and color. As for the hair samples, the affected hair was taken using sterile forceps, and the samples were placed in sterile test tubes and brought to the hospital's mycology laboratory for the purpose of examination and cultivation.

Diagnostic Kits

Table 1: The diagnostic kits used in this work with Manufacturing Company and Country

Kits	Manufacturer's/Country
Presto™ Mini gDNA Yeast and mold Kit	Geneaid/ Germany

Culture Media

Culture Media

The culture media to isolate and diagnose fungal isolates attended according to the manufacturer's instructions a, according to the scientific Chapter three Material and Method and then all sterilized using the autoclave at 121Co under pressure 15 lbs / Ing 2 for about 15 minutes. This media has included as follow:

Sabouraud`s Dextrose Agar (SDA)

This medium agar was attended according to the manufacturer's instructions, was prepared by dissolving 65 g of media in 1000 ml of distilled water and was adjusted the pH, then all sterilized in Autoclave and then cooled to 45Codegree (Bailey and Scott, 1974).

Sabouraud`s Dextrose Agar with Chloramphenicol and Cyclohexamid (SDACC)

Sixty-five grams of Sabouraud`s Dextrose Agar was taken and placed it in a Flask and added to it 1000 ml of distilled water then sterilized in autoclave and then cooled to temperature 45Co, before pouring into the dishes, 1000 mg chloramphenicol and 0.5g Cyclohexamid was added to it and then pour into sterilized dishes and remained to cool and then placed in the refrigerator until use (Bailey and Scott, 1974).

Sabouraud's Dextrose Broth (SDB)

This media was attended according to manufactures instruction, represented by dissolving 65 grams of powder Sabouraud's Dextrose Broth equipped from a company Bangalore in 1000 ml of distilled water and then shake well, heated and mediated by oven until boiling. Then add (0.05) g of the chloramphenicol to it after dissolving it in 10 ml ethyl alcohol 95% Then shake well and infertility media by autoclave and then distributed in sterile glass tubes used as a medium to activate fungal isolates (Fadia, 2018).

Isolation and Identification of Dermatophytes

Isolated dermatophytes were diagnosed on the basis of cultural characteristics of developing colonies, such as: Color, size, nature of the culture, reverse side of the dish, and microscopic characteristics such as the shape of the conidia Their size and fungal filaments, as fungi were isolated in 42 samples out of a total of 60 samples, representing 70%. There are 15 different species of Trichophyton, and three species of Epidermophyton about 28 samples belonging to the Microsporum Cains fungus appeared out of 42 isolated Microsporum samples. The results of the current study showed that the isolated species belong to the three genera, and this result is consistent with: The mechanism was found by Saleh (2008) and Naik *et al*., 2019). With it does not agree with the findings of researchers

(Baranova *et al*., (2018), as they isolated the genera Trichophyton and Microsporum.

Direct Examination:

A small piece of the sample was taken with steriled needle and place in it a drop of KOH solution (10%) which carried on a glass slide and covered with a cover slide. Then, it is dried up with a little flam and examined microscopically to observe fungus found in skin, hair peels and nails (Jawetz *et al*., 2015).

The phenotypic Examination of Colonies:

It is one of the important means to differentiate between dermatophytes, the most important things that must be taken into consideration is the number of days it takes to start a fungus growth, as Chapter three Material and Method well as the examination of the colony form flat, high, a thickly, Examination the colour white, black, green, and examine the colour from the reverse and notice the texture Powdery, fluffy, cotton according to classification key (Jawetz *et al*., 2015).

Analysis of Statistics: Statistical analysis was done using the statistical Packag SPSS (Mott *et al*., 1990).

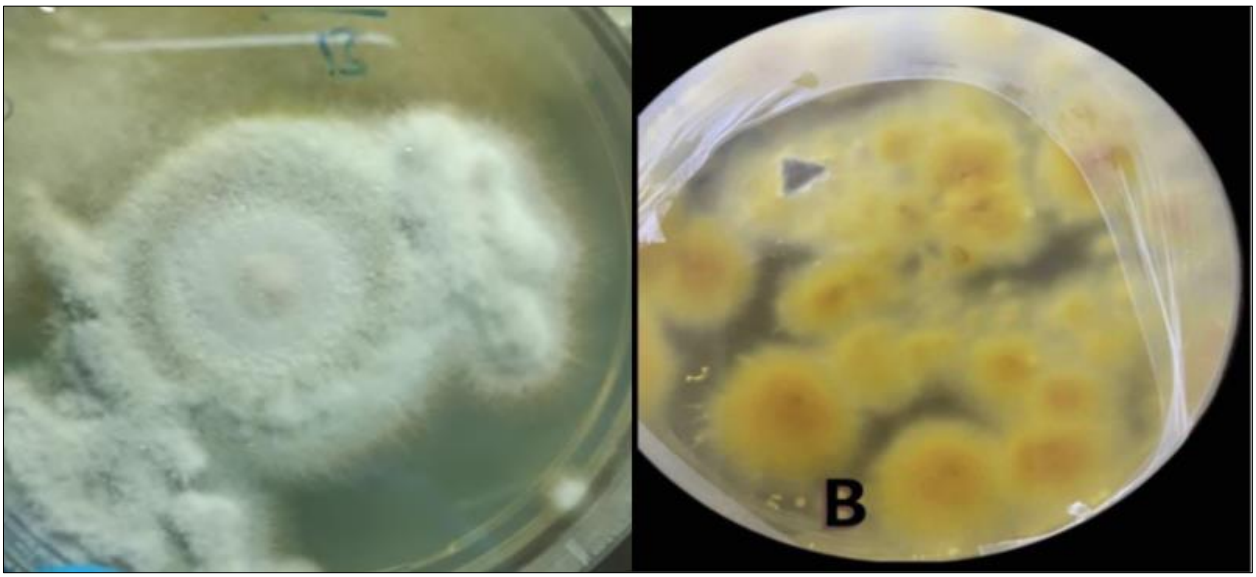

Figure 1: Phenotypic characteristics of *M.canis* and the colony growing on medium of S.D.A.C.C. A – The external appearance from the front, B - the external appearance from the back, temperature 28 ° C, incubation period 10 days

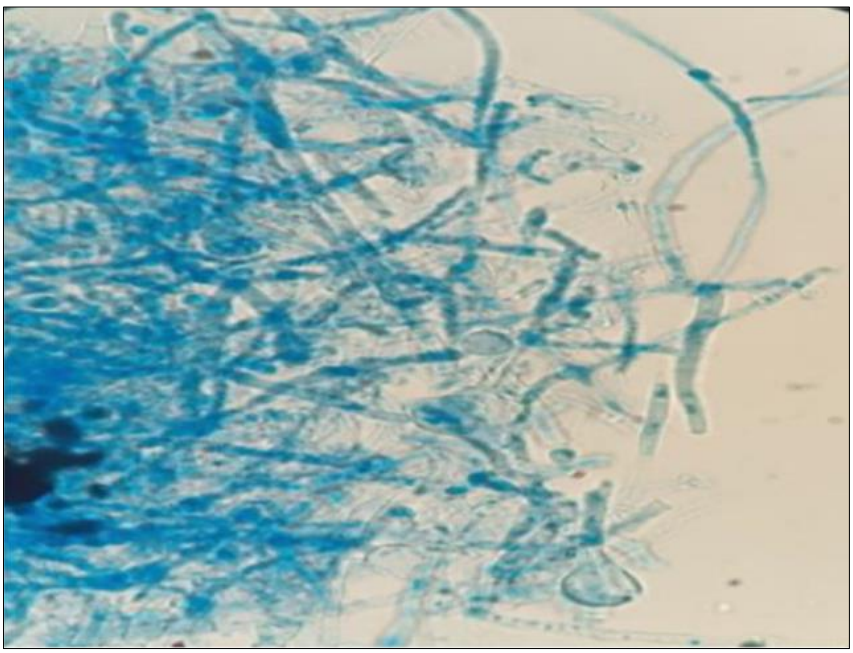

Figure 2: Growth of *M.canis* on SDA medium

ITS- PCR

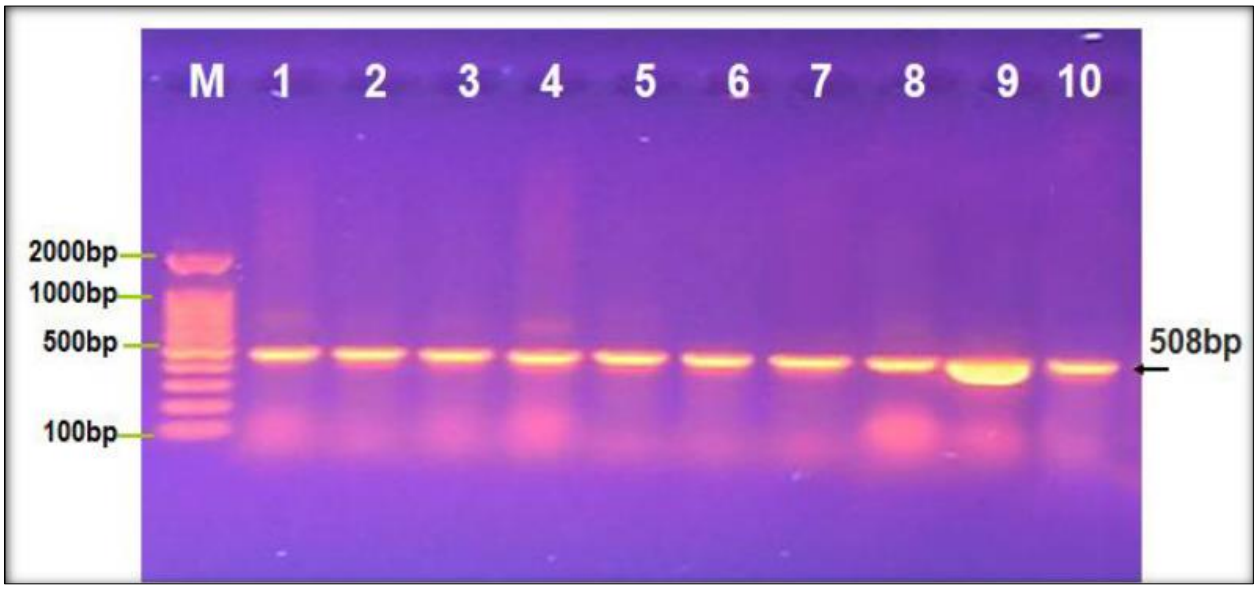

Figure 3: Electrophoresis Using 1% Agarose Gel, which Shows the Results of the Pcr Test for the 18 Ribosomal Rna Gene, which is Used to Diagnose the Fungus Microsporum Cainus, Where M Represents (100-2000bp) and Represents 1-10 Isolates of the Fungus to Examine with a 508pb Result.

RESULTS AND DISCUSSION

Isolation and Identification of Dermatophytes:

During the period of the study between January to November 2024, were examined the occurrence of dermatophytes were investigated among 42 samples skin, hair and nail belong to patients clinically identify by a specialist.

Microsporum Canis:

Colonies of the this species on Sabouraud's dextrose agar with chloramphenicol and cyclohexamid (SDACC), the base emerged rapid with radial grooves, white with cotton texture, and mycelium in the surface after an 8-10 day incubation period, the reverse, on the other hand, turned a dark yellow tint the colony was dark yellow to orange in color (4-13), this result agree with (Lakshmipathy and Kannabiran, 2010).

CONCLUSION

1. A small percentage of males were found to have higher skin fungi than females. And that higher The lowest percentage recorded in the age group 6 months - 9 years, while the lowest percentage recorded in the age group Ages 40-49 and 50 and above.
2. The study showed that Tinea corporis is the most common and widespread form of ringworm 34.8% compared to other different forms.
3. The study showed that infections increase in December by 28.3% and decrease in December The month of Ir.
4. Many characters of many causes and phenolic compounds in egg delays Some fungi tested using GC-Mass technology.
5. The study showed that laboratory fungi are capable of producing some enzymes in proportions Different.
6. Conclusion from the current study that the use of poker methods using PCR technology

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