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Isolation and Identification of Certain Types of Skin Fungi and Their Ability to Produce Virulence Factors

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Abstract

The current study examined the isolation and diagnosis of dermatological fungi from patients reviewed for the dermatology consultation of Tikrit Teaching Hospital and diagnosed by dermatologists and for the period from 1/4/2022 to 15/10/2023 and the detection of the ability of some of the isolated dermatological fungi to produce some of the important virulent factors for incidence electrosporum. Epidermatophyton, where Trichophyton was the most frequent sex and 65 isolated spots belonging to several species, the sex Microsoft recorded 13 isolations while the sex Epidermatophyton recorded two isolations.

Five clinical forms emerged: Tinea Corporis, Tinea capitis, Tinea Pedis, Tinea Manus and Tinea Faciei, with the highest Tinea Corporis infection rate at 32.5%, while the lowest Tinea Faciei infection rate at 8.75%.

Through the study, the susceptibility of isolated fungi to the production of hemolysin and phospholipase enzymes was identified. The most productive Dermatophyte of the phospholipase enzyme are subordinate to the fungal species T.rubrum, T.mentagrophytes, T.tonsurans and 21 isolates. 17, 8 each, while M.canis and the lowest number of 5 isolates are recorded, M.gypsum and Epidermatophyton flocosum have no susceptibility to the production of phospholipes. As the results of this study have shown, all isolated Dermatophyte have the potential to produce hemolysin enzyme and the isotropic skin fungus events. The production of hemolysin by fungi plays an important role in the balance between the host's cellular immunity and the susceptibility of mushrooms to reduce the host's immune response.

Based on the foregoing, the study aims to: Isolate and diagnose types of Dermatophyte causing skin injuries as well Detect their susceptibility to the production of certain virulence agents in vitro.

Keywords: Dermatophytes, Hemolysin, phosholipease, Tinea uagium, Tinea capitis, Tinea pedis, Tinea corporis, Tinea cruris.

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Introduction

Dermatophyte

Views on the pathogenicity of these microorganisms have changed since the Greeks of antiquity first identified fungus. For people with strong immune systems, it is currently known that they are frequently non-pathogenic (1). Because of population density, poor health conditions, and malnutrition, between 20% and 50% of people worldwide suffer from fungal skin diseases, which are thought to be among the most serious health issues that some people face. This is especially the case in developing nations (2). It is a group of filamentous fungi that cause superficial diseases of human beings and that interact with the host thus causing some of the clinical changesThe reason for their ability to produce many of these digestive and analyzed enzymes of keratin and the host's protein materials in keratin-rich areas such as skin, hair and nails It also causes superficial infections called Ring Worm, or Tinea Faciei, which means bitter worms. This name is also given to skin injuries, which start as a small rash and then spread as a loop. These fungi live in wet places of the body. They are also found on the coupled layer. These mucous fungi do not affect some of the endosomes (3)

These Dermatophyte are classified into three major collections classified based on their presence in the environment: Anthropophilic human fungi, Geophilic fungi and Zoophilic animal-loving fungi (4).

Clinical Forms of dermatophytes

Depending on the location of the fungal injury in the body, these filamentary skin injuries were clinically classified into several forms: Tinea, Corporis, Tinea Capitis Tinea Pedis, and Tinea Manus, Tinea Faciei,

The ability of dermal fungi to cause disease depends on many factors, including environmental conditions, pathogen's fertility, host immunity, chronic diseases, especially diabetes, lymphoma and others. (5)

There are several ways of spreading superficial Dermatophytes injuries, including contact with infected animals or through infected people. Although the infection is curable, its susceptibility to widespread spread and its therapeutic cost as well as its impact on the psyche of the infected person as it affects the beauty of the infected person is a major public health problem worldwide (6).

Fungi Dermatophytes has three genera: Microsporum, Trichophyton and Epidermophyton (7).

In recent years, this gradual increase in the prevalence of fungal infection, in addition to its resistance to antifungal drugs, has affected a large proportion of the world's total population, 20_25% of which are Dermatomycosis and Candidiasis (8).

What enables these Dermatophyte from this widespread spread is that they produce a variety of ferocity enzymes such as Keratinase enzyme, Proteas anrem and Phospholipase lipase elastase and produce these enzymes depending on the area of presence of fungi and therefore have an important role in disease events on host tissue 9).

These enzymes are a major ferocity factor for conjunction invasion, so the strategy created by Dermatophyte to escape or how to discourage the host's defences (10) must be identified.

Virulence can be defined as a measure of pathogenicity or the severity of damage it causes to the breadwinner. No pathogen can cause pathogenicity unless Virulent is a factor. Virulent is associated with genes that may be lost or acquired during their evolutionary development. This means that virulence factors may be in the form of cellular compositions or may thus affect the form of enzymes or even other toxins Skin, scars or burns through Arthrospores or Conidia.

The pathogen invades the Keratinase and other enzymes that are important for breaking down the surface layer of the skin. This activates the host's Keratinase cells and is followed by an inflammatory response mediated by a host group of defenses that are representative of fungal immunity and acquired immunity It causes sensory reactions and thus the immune response in the form of a rash is far from the affected skin (11).

Materials and Methods

Preparation of Culture media:

Dextrose Dextrose Agar Sabourauds

This medium was attended according to the recommendations of the manufacturer Himedia by dissolving 65 g of SDA powder in 1000 ml of sterile distilled water and then adding to this powder 0.05 g of chloramphenicol antibiotic to prevent any growth of opportunistic fungi and after sterilization pour this mixture into 9 mm diameter sterile plastic dishes and this medium is important for isolating Dermatophyte(12).

Yeast Medium Agar extract:

The solution attended by dissolving 10g of yeast extract yeast extract in 100ml sterile distilled water and then sterilized the solution by filtration method and using precise special filtration paper with diameter (0.45) micron (13).

Potassium Hydroxide Solution KOH

Attended this solution by dissolving 10g of potassium hydroxide solution KOH in 100 ml of sterile distilled water and the importance of this solution in the microscopic examination of fungal compositions.

Medium Sheep Blood Agar

This medium was present by dissolving 65gm of SDA powder in 1000ml of distilled water and infertilizing this medium with thresholds. It was left to cool at 45 mM and then added 70ml of sheep's blood. 30 g of glucose sugar was added and this medium was important to detect the susceptibility of Dermatophyte in the production of the hemolicin enzyme, which is 14.

Nutrient agar fortified with lecithin.

This medium of material came from 20 grams of Nutreint Agar as well as 1gm of sodium chloride and in addition to 0.05 of calcium chloride and 8% of sterile egg yolks. Egg yolks were prepared as powder. A fresh egg was taken and glue was separated from the slippery by a medical injection To mix them well and then place this mixture in the centrifuge and rpm 500 capacity for 15 minutes and then take the deposit and dilute with 100 ml of sterile distilled water and add to the center of the sterile test pH5 this medium is important because it is used to detect the ability of fungi to secrete the Phospholipase enzyme and then after planting the dishes incubated for four days, after which the colony's diameter was measured (15).

Collection Samples.

100 clinical samples of people with dermal fungi were collected from dermal consultation at Tikrit Teaching Hospital and some private clinics in Salah al-Din governorate for 1/4/2022 to 15/10/2023. The study included the collection of samples from the affected areas of skin, hair and nails and directly supervised by the competent physician and both sexes Sterile tongs and then placed samples in sterile test tubes and brought to laboratories in Faculty of Science Life Sciences/Tikrit University for examination and cultivation.

Direct microscopy

Part of the sample taken from the skin, hair or nails was taken and then placed on a clean glass chip containing a drop of potassium hydroxide KOH with a concentration of 10% to clarify the sample and then placed the glass chip cover on it and quietly heated it over the flame two or three times and then left the chip for 20-30 minutes in order to become the soft specimen and then examined the Under the Greater Force X 100 to observe the presence of fungi structures such as Conidia and fungal threads (16).

Culturtion of Specimens

Part of the clinical specimens not treated with potassium hydroxide were taken using sterile tongs and grown on the saproids dextrose SDA that contain chloromphenicol. The dishes were incubated at 28 ° C for four weeks and then examined the developing dishes to observe any fungal growth.

Phenomenal and microscopic examination of fungus synthesis.

To study the phenotype and exact diagnosis of isolated Dermatophyte, I examined the dishes after four weeks of cuddling. The phenotype of the samples was examined depending on the exterior form of the fungal colony, as well as the colour and tissue of the fungal colony, in addition to examining the opposite side of the colony and the colony diameter Chlamydospores. Thus, glass slides were made by taking part of the developing fungal colony and placing them on a sterile glass slice containing a drop of blue lactophenol and examining them under the light microscope using 40X forces (17).

Detection of the susceptibility of certain fungi to phospholipase secretion

Dishes in Fig. (1) containing amidst modified nourishing thoughts and supported by sythine have been vaccinated with fungi to be tested by transporting part of this fungal colony in the form of a 7mm disc using the flyne piercer to the center of the dishes, considering the leaving of an unvaccinated dish to be used as a control sample and repetitive reality for each case. The dishes were then embraced at a temperature of 28 ° C. The fungal growth was observed and the effectiveness of the enzyme was observed for two weeks. The dishes were then examined to detect the enzymatic effectiveness. The appearance of sediment around the fungal colonies is evidence of the fungi's production of Phospholipase (18).

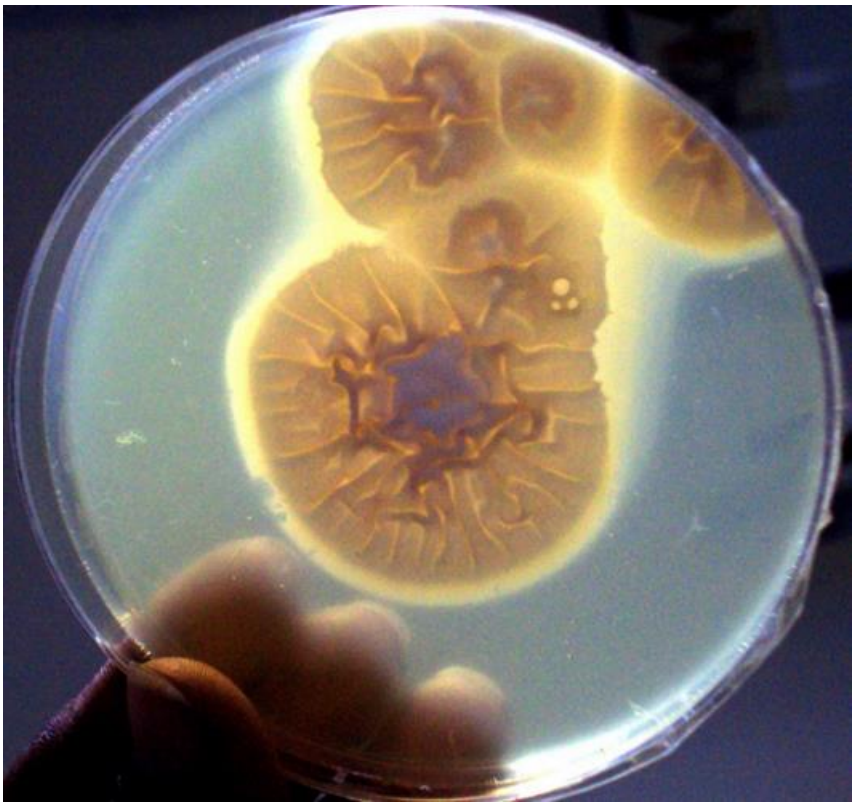

Figure (1): Test production of phospholipase enzyme from fung T.rubrum

Detection of the susceptibility of certain fungi to the secretion of Hemolysin enzyme the sheep's blood was used to detect the ability of dermal fungi to analyze blood by placing a 7 mm dial from the edge of the fungal colony and taken seven days old and planted on the medium saproids Dextrose Agar SDA for thoughtful fungi, and then I put on the middle of Acar the blood of sheep and incubated these dishes for

1-5 days at a temperature of 37c and the appearance of transparent halos around the place of transplantation is evidence of the production of this enzyme 19 (Fig. 2).

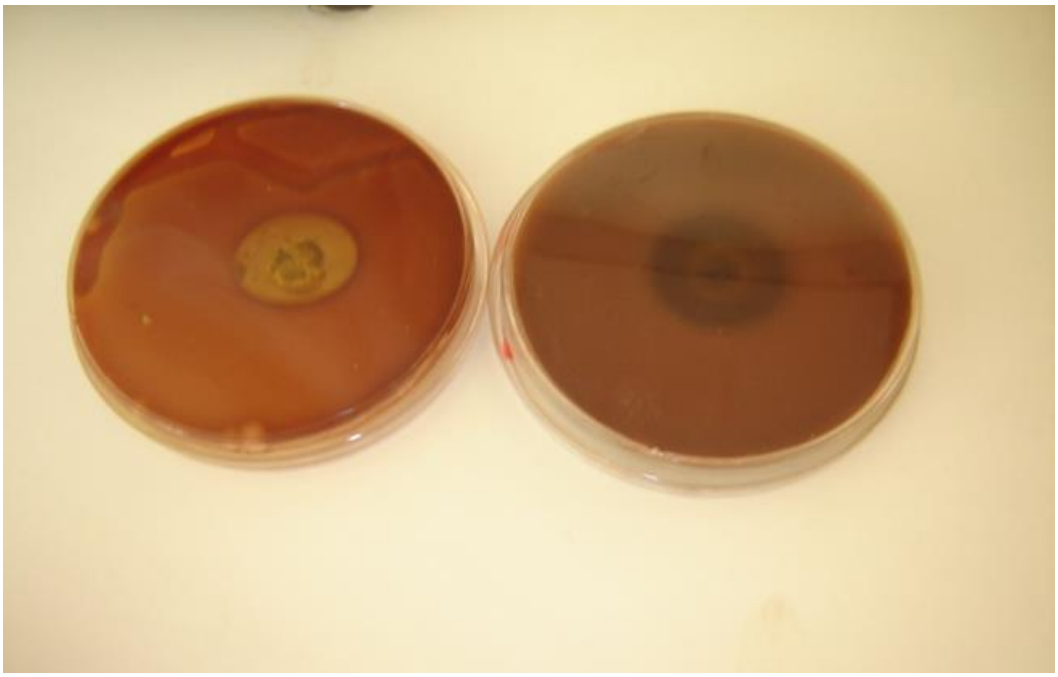

Figure (2): Test production of hemolysin enzyme from fungus T.rubrum

Conclusions and discussion

Phenomenal and microscopic examination of isolated fungi

Samples are diagnosed depending on the characteristics and appearance qualities of the developing fungal colonies on the saproide transplant medium Dextrose Agar SDA and for the period of (3-14 days) are the nature and form of the innate colonies, as well as the colour of the fungal colony, its strength, the produced tinctures, the form of the opposite entity, as well as its microscopic properties, including the fungal compositions produced such as large conides and small Conedes, in terms of the shape, size, number, wall thickness, connectors and Hyphae (20).

Isolate surface dermatological fungi

The results of the current study show that out of a total of 100 samples of people with dermal fungi, the number of positive samples was 80 sample and 80%, while the number of negative samples was 20 sample and 20%.

The result was an approach to what came up with a 21, with the 79% infection in Tikrit and in a study conducted by Tikrit. (22) The rate of 80% infections in Karbala City is inconsistent with 23 Since the infection rate was 58% in Kirkuk City, the high incidence of these fungi may be due to the duration of the collection of samples, which is a suitable condition, as well as the temperatures suitable for the growth of fungi, and the samples taken are sufficient for the appearance of fungal growth.

As for negative outcomes, it may be due to the fact that the infected person has consistently and indiscriminately taken antifungal antiretroviral. Hence, these antibodies significantly affect fungal development or because the pathogen may not only be fungal but may be bacterial and due to similar symptoms to other non-fungal diseases.

Clinical patterns of skin injuries

Fungal isolates (Table 1) were collected from different patterns of tinea and depending on the locations of the injury. The highest incidence of each of the body's contaminants and tinea capitis was found in 26 isolations of the corporis tinea s and 17 isolations of the tinea capitis each with 32.5% and 21.25%

followed by the tinea of the foot and hand with 17.5% and 13.75% respectively, while the lowest types of tinea s were 10% and 5% respectively.

Table (1): Clinical forms of skin injuries and their distribution between males and females

Tinea type	Number in males	Number in females	Total number	Total percentage
Tinea corporis	16	10	26	32.5%
Tinea Capitis	13	8	21	26.25%
Tinea Pedis	9	6	15	18.75%
Tinea Manus	6	5	11	13.75%
Tinea Faciei	3	4	7	8.75%
total	47	33	80	100%

The results obtained were an approach to the achievement of (21) where the highest rate of tinea corporis injury was 32.2%. (23) The highest rate tinea corporis injury was 46.55% and the lowest rate of tinea Faciei was 5.17%, while (22) cases of tinea corporis injury were 41.98%, while the lowest rate was 5.34% for tinea Cruris.

The high incidence of infections in Tinea corporis can be attributed to a low standard of living, as well as the possibility of disease transmission through contact with and use of contaminated instruments of the infected person, climatic conditions, moisture and persistent sweating have a significant role in fungal events as well as the weakness of the immune system, which is a major factor in fungal injury events.

In general, the diversity of these clinical patterns may be due to varying factors that cause them from one region to another in the world, in addition to social status, weak economic situation, population density and poor health conditions. All these factors will increase the prevalence of skin injuries in many developing countries (24).

The results of this study showed that the highest injuries were in males, with 47 infected, the highest incidence of Tinea Corporis 32.5%, followed by Tinea Capitis 21.25%, while the number of infections in females was 33, with the highest incidence of Tinea Corporis was 32.5%, while Tinea Capitis was 21.25% while the Tinea faciei was least 5%.

The results of the current study show that the incidence in the Tinea Corporis in males is higher than the incidence in females and this is consistent with the study (25) who recorded that the highest incidence is male while not consistent with (21) who recorded that the highest incidence of female infections may be due to the high incidence of Tinea Corporis in both sexes because the main pathogen is human and the direct transmission of fungal infections through contact or indirect methods such as the common use of contaminated instruments such as clothing, towels and coverings contaminated with fungal crusts.

Some cases of fungal infection may be due to the prevalence of wet and hot climates. The cause of the spread of dermal infection is severe itchiness, which leads the patient to seek early medical advice as well as environmental conditions such as hot and humid weather, which provides the environment for the growth and increase of Dermatophyte on the skin (26).

Insulated Dermatophyte types and their ability to produce phospholipase and hemolaysin enzymes Results from a phenomenal diagnosis showed that the number of Dermatophyte causing skin injuries isolated from different locations is 80 isolation of the three Genus Trychophyton and Microsporum, Epidermatophyton since the genus Trichophyton was the most frequent and the reality of 65 isolation belonging to several species while the genus Microsporum was recorded as isolated 13.

The results of this study (Table 2) are agreeing with the study of 23 enabling the isolation of the three genus while not agreeing with the study of Zubaidi (21) Mohammed (27) who did not isolate the order Epidermatophyton. In this study we notice the dominance of the fungal species T.rubrum and the reality of 27 isolation which is a human-loving species compared to the Zoophilic Anthropophilic human fungi of T.mentagrophytes and the reality of 21 isolation and these results are incompatible with the study (22) (23) which recorded the highest incidence of animal-loving species.

The prevalence of the human-loving species is due to the fact that this infection is transmitted through direct solicitation between infected people in crowded areas, especially markets, schools and sports halls, and as happens through indirect solicitation through the use of the infected person's tools such as clothing and others. (28) The largest proportion of isolated fungi is Trichophyton, type T.rubrum, 15 isolation of the total number and type of fungus T.mentagrophytes, and 8 isolation.

Table (2): Number and percentage of Dermatophyte and their susceptibility to the production of phospholipase enzyme and hemolaysin enzyme.

Fungi Species	Isolates number	Percentage	Number of isolates productive for phospholipase enzyme	Number of unproductive isolations	Number of isolates productive for hemolaysin enzyme	Number of unproductive isolations
T.rubrum	27	33.75	21	6	25	2
T.mentagrophytes	21	26.25	17	4	21	0
T.tonsurans	17	21.25	8	11	10	7
M.canis	9	11.25	5	2	7	2
M.gypsum	4	5	0	0	2	2
Epidermatophyton flocosum	2	2.5	0	0	1	3
Total	80	100	51	23	66	16

The difference in the results of the current and previous studies in isolated species is due to the size of the sample and to the environmental nature of the area from which the samples were collected and may be the result of mobility and travel, as some species are widespread globally and others are confined to specific areas. In addition, living standards and healthy habits play a major role in the emergence of new species and the isolation of animal-loving species may be due to the breeding of certain animals affected by skin fungels or through their presence 29.

The table shows that the highest Dermatophyte produced for phospholipase are T.rubrum, T.mentagrophytes, T.tonsurans with 21,17, 8 number of isolates, While M.canis has the lowest number of 5 isolates, M.gypsum and Epidermatophyton flocosum have no susceptibility to phospholipase production.

Pathogenic fungus cells possess many means to help them invade analyzed enzyme cells and tissues that break down or disrupt the vital components of the host cells' cell membrane and thus turn off the work of the membrane or disrupt cellular biological functions because most biosphere components are made up of fat and proteins by doing so, enzymes take such vital ingredients as a powerful target for attacking cells. Pathogenic fungi release analyzed enzymes that are an important part of pathogenic events in their habitats and that are one of the most important virulent factors. Among these enzymes is the phospholipise enzyme, which analyzes exploded fats So phospholipase enzyme belongs to hydrolysis enzymes Group III so that exploded chlorine fat makes up the bulk of the cellular casing ingredients. (30).

The results of this study showed that all isolated Dermatophyte have the potential to produce hemolysin enzyme since the fungal specie T.rubrum was one of the most productive of the enzyme, since the number of isolates produced for the hemolysin enzyme was 25 and followed by the fungal species T .mentagrophytes, T.tonsurans, M.canzum with 21,10,10,7,2 isolates, the lowest of the fungal isolates produced by the hemolysin enzyme is the fungal specie Epidermatophyton flocosum and only one isolation spot.

The results of this study do not correspond to another study (31) of Dermatophyte isolated from various skin injuries which recorded that the fungal species Trichophyton. rubrum is the highest degradable blood at 65.1% while the lowest degradable blood is for type T. mentagrophytes, Microsporum canis who registered 7.6% while the fungal type Trichophyton .violaceum recorded its degradability at 3%, and the enzyme on hemolacin produced has an effect.

This difference between the results of the current study and other studies may be due to the size of the isolates studied and the medium used to detect blood degradation and the genetic difference between innate isolates and strains belonging to the same mushrooms. As we note from these findings, isolated Dermatophyte have the potential to degrade blood. This is another virulent factor that helps them to cause fungal injury (32).

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