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Detection and Molecular Study of Dermatophytes sp. Isolated from Tattoo Patients in Diyala Governorate

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A B S T R A C T

Dermatophytes that may affect up to 20% of the global population. Fungal contamination during tattooing is thought to be due to the presence of fungal pathogens in the tattoo needle. The study aims to detect and molecular study to Dermatophytes sp. That isolated from people with tattoos in Diyala governorate of Iraq. Across sectional study was done in the period from 1st October 2020 until 15th February 2021 in Baquba teaching hospital (Consulting clinic).The study including 100 patients (43 were male and 57 female) aged from (10-65 years old). After the preparation of samples and detection of *subtilisin-like protease* gene by PCR, the teqnition sent the sample that has this gene to sequencing. The results showed the frequency of fungi species as follows *T.mentagrophytes* formed (9.0%), *T.rubrum* formed(3.0%), *T.nigra* (3.0%), *M.audouinii* (3.0%), *M.gypseum* (3.0%), other growth (29.0%), no growth (50.0%), with highly significant($P<0.05$). Depending on the age groups, it was found *T.mentagrophytes* constituted the highest incidence of fungal infection in age groups (21-30 years old) followed by (31-40 years old) and (41-50 years old) with percentages (33.3%,22.2%, and 22.2%) respectively. The fungal species *M.gypseum*, *T. nigra*, and *T. rubrum* were found to have the highest incidence of infection in age groups (31-40) with (33.3%) for fungi infections (*M.gypseum, T. nigra, and T. rubrum*). As well, Other growth and No growth constituted the highest incidence rate for the same age group (44.8% and 38.0%) respectively. The differences between age groups and inbred races were significant ($p <0.05$). Depending on the gender, *T. rubrum*, no growth, and *T.mentagrophytes* were found, which constituted the highest rates of infection in males (66.70%, 56.00%, and 34.50%) respectively. In females, *M.audouinii*, *T. rubrum*, and *T.mentagrophytes* formed the highest rates of infection (100.00%, 100.00%, and 77.80%) respectively. The differences between fungal infection and gender were not significant ($p> 0.5$). Depending on the housing, *T. nigra*, no growth, and other growth were found, it highest rates of infection were found in people living in rural areas (66.70%, 40.00%, and 44.40%) respectively. As for the people who live in the city, *T. nigra*, Other growth, and *M.gypseum* formed the highest rates of infection (100.00%, 75.90%, and 66.70%) respectively. The differences between fungal infection and habitation were not significant ($p> 0.5$). Depending on education level, *M.gypseum*, *T. nigra, and M.audouinii* were found, it highest rates of infection among people were those who graduated from secondary school with (66.70%). Other growth and no growth also constituted a high rate of infection among secondary school graduates, with a percentage (58.60% and 46.00%) respectively. The differences between fungal infection and education level were not significant ($p> 0.5$). The results of the sequence were 96.22% similar to the *T. rubrum* standard and record in the Gene bank of NCBI.

Introduction

Dermatophyte infections are one of the earliest known infections of mankind and are very common throughout the world (Shahid and khan, 2016). The infections caused by these fungi are usually named after the infected part of the body rather than the infecting organism. Fungal infections can cause illness in immunosuppressed persons, mainly those suffering from transplants, chemotherapy. And human immunodeficiency virus-positive patients (Rouzaud *et al.,* 2015). The greatest valuable dermatophytes isolated in Europe are *T.rubrum*, *M.canis*, *T.mentagrophyte* var. granulasum, and *T.verrucosum*. Numerous of these are supposed to have expanse from the Mediterranean Countries. Further dermatophytes such as *M.audouinii*. *T.soudenense* and *T.violaceum* are endemic in Africa and Asia is now hardly ever isolated in Europe. In Asia *T.rubrum* and *T.mentagrophyte* are the greatest usually isolated pathogens, causing *Tinea pedis* and *Tinea unguium* (Menon and Routray, 2015). Dermatophytes are referred to as a unique group of superficial keratinophilic and filamentous fungi which can invade keratinized tissue of the skin, hair, and nail in humans and animals leading to dermatophytosis or tinea (ringworm) Dermatophytes are pathogenic fungi that can attack keratinized forms and infect the skin, hair, and nails of animals and humans (Al-Khafajii, 2014; Xiao *et al.,* 2015).

Dermatophytosis is described either by the unusual range of skin surface parts affected by the infection or by the uncommon amount of affected locations. However, the infection remains confined to the epidermis or associated keratinized structures such as nails (Mansouri *et al.,* 2012).

They are based on keratin as a source of nutrients and cause its hydrolysis by releasing several enzymes such as keratinase as a proteolytic enzyme of keratin protein which is very hard and strong (Sharma, and Swati, 2012). Overall, these fungi live in the old keratinized layer, the upper layer of skin cells in humid parts of the body, low standards of living along high humidity environments are contributing to the increased prevalence of these fungal infections (Blutfield *et al.,* 2015).

Dermatophytes infections are triggered by 40 fungal species which are classified into 3 genera; *Trichophyton*, *Microsporum*, and *Epidermophyton* (Abdeen and El-Diasty, 2015). According to the anamorphic state, there are two species of *Epidermophyton*, 25 species of *Trichophyton*, and 18 species of *Microsporum* (Mucoma, 2000; Sharma, and Swati, 2012). Fungal contamination during tattooing is thought to be due to the presence of fungal pathogens in the tattoo needle. Cases of superficial tinea infection infected with *Trichophyton rubrum* and *Epidermophyton floccosum* have been reported associated with tattooing (Temiz and Özlü, 2021). Literature reports suggest the occurrence of *Tinea spp.* infection within a month of tattoo placement (Yadav *et al.,* 2018).

Materials and Methods

A cross-sectional study was conducted on 100 patients (43 were male and 57 female) tattooing and infected with dermatophytes in Dermatology unite at Consulting clinic in Baquba Teaching Hospital in Diyala province according to (Appendix 2) from 1st October 2020 until 15th February 2021.

The clinical specimens collected were epidermal scales and hair. The scales were scrapped from near the advancing edges of the lesions and take the hair infected after disinfecting the lesions with 70% alcohol. The sample was put in the tube with swab media and transport to the laboratory to culture on Sabouraud's dextrose agar containing chloramphenicol (0.05%) with and cycloheximide (0.5%) and incubated at 28°C for 2 to 4 weeks (Garg, 2009). The macroscopic or morphologic study of dermatophytes, after that, was carried out by placing a small part of the fungal colony on a clean glass slide with a drop of Lacto phenol Cotton Blue (LPCB) and mixed gently then covered with a coverslip and pressed to flatten and spread the

tissue. The slide was examined microscopically under X10 than under X40 power to see fungal hyphae, arthrospores, chlamydospores, micro and macroconidia which an important character for the diagnosis of fungal species. Biochemical Test Urease Test when a small amount of the fungal colony was inoculated into the ureaes agar for testing the ability of the fungus to release urea enzyme then incubated at 25 C for 48-72 hour The changing of the plate color from yellow to pink indicate a positive result.

Genomic DNA was isolated from fungal growth according to the protocol of Geneaid Extraction. After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria. PCR product was sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email then analyzed using genius software.

Antifungal agents were tested in duplicate against all dermatophyte isolates according to the manufacturer's instructions. The isolates were sub-cultured on Sabouraud's dextrose agar and potato dextrose agar. Isolates were incubated at 28±1°C. Inoculum suspensions were prepared from fresh, mature (7-10 days old) cultures grown on Sabouraud's dextrose agar and potato dextrose agar.

The colonies were flooding with 5 ml of 0.85 % sterile normal saline and harvested by gently scraping the surface of the fungal colonies. The suspension was mixed for 15 seconds with a vortex, and then the suspension was filtered through sterile filter paper to remove the majority of hypha and collected in a sterile tube.

The suspension was inoculated on two MHE agar surfaces (90 mm diameter) and air-dried for 10 min. Fluconazole and Caspofungin disk were then placed on a plate individually. The plates were incubated at 28±1C°, and then the area of inhibition was noticed after 3-7 days.

Statistical Analysis

All data were analyzed using the Statistical Analysis Program (SAS) - 2012, version 22, number, percentage, and chi-square were used to test the effect of different factors in the study. For a significant comparison of the percentage (0.05 and 0.01 probability) in this study.

Results and Discussion

Among the 100 patients, the numbers of fungi species that isolated from patients with dermatophytosis following aspects show that the no growth of fungi, other growth, and *T. mentagrophytes* growth formed the highest percentage in patients (50.0%, 29.0%, and 9.0%) respectively, while the other fungal types formed the least types in patients (3.0%) for each fungal species. The differences between the fungal growth species were highly significant ($p < 0.05$) (Fig- 1)

The results of Personal characters with fungi species showed age groups and fungal infections, *T.mentagrophytes* was found to have the highest incidence of infection in age groups (21-30, 31-40, and 41-50), with (33.3%, 22.2%, and 22.2%) respectively. The fungal species *M.gypseum, T. nigra,* and *T. rubrum* were found to have the highest incidence of infection in age groups (31-40) with (33.3%) for fungi infections (*M.gypseum, T. nigra,* and *T. rubrum*). As well, other growth and No growth constituted the highest incidence rate for the same age group (44.8% and 38.0%) respectively. The differences between age groups and inbred races were significant ($p < 0.05$). (Table 1)

When comparing fungal infections with sex, *T. rubrum,* no growth, and *T.mentagrophytes* were found, it constituted the highest rates of infection in males (66.70%, 56.00%, and 34.50%) respectively. In females, *M.audouinii, T. rubrum,* and *T.mentagrophytes* formed the highest rates of infection (100.00%, 100.00%, and (77.80%) respectively. The differences between fungal infection and sex were not significant ($p > 0.5$).

(Table1). When comparing fungal infections with housing, *T. nigra*, no growth, and other growth were found, it highest rates of infection were found in people living in rural areas (66.70%, 40.00%, and 44.40%) respectively. As for the people who live in the city, *T. nigra*, Other growth, and *M.gypseum* formed the highest rates of infection (100.00%, 75.90%, and 66.70%) respectively. The differences between fungal infection and habitation were not significant (p> 0.5) (Table 1).

When comparing fungal infections with education, *M.gypseum, T. nigra*, and *M.audouinii* were found, it highest rates of infection among people were those who graduated from secondary school with (66.70%). Other growth and no growth also constituted a high rate of infection among secondary school graduates, with a percentage (58.60% and 46.00%) respectively. The differences between fungal infection and education level were not significant (p> 0.5) (Table1).

The Molecular Identification of seven isolates of Dermatophytes showed results assessing and estimating the DNA extraction with an optimum concentration range from (7-14) ng/ml (Table 2).

Detection of *subtilisin-like protease* gene to this isolates revealed 2/7 isolates were classified as *T. rubrum* with amplicon size equal 623 bp after band electrophoresis and UV-trans illuminated of the product as shown in (figure 3).

20 ml of PCR products of *Trichophyton rubrum* and 2o ml of (subtilisin-like protease gene were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email then analyzed using generous software multiple alignments were performed using the NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&BLAST_SPEC=&LINK_LOC=blasttab&LAST_PAGE=blastx). The isolate of *Trichophyton rubrum* from the clinical specimen of human dermatophyte in Diyala province recorded in Gene bank-NCBI and have a symbol code (No.LC633537). (Fig 4). Long-term immunosuppressed patients are often young adults who may wish to consider tattooing. It is well recognized that immunosuppressed patients are at increased risk of infection including cutaneous mycobacterial infections. They, therefore, represent a group that is at a potentially higher risk of tattoo-related complications and warrant special consideration (Wilson *et al.*, 2018). Immunosuppression may be associated with the appearance of tinea over a longer time frame (e.g., several years) (Gathings *et al.*, 2018). Tinea is known to be spread by direct contact, and a likely source of infection can usually be identified. Fungal infections at tattoo sites have been described, including a case of fungal eye infection thought to be related to a tattoo (Kluger and Saarinen, 2014). Skin fungal infections are caused mainly by dermatophytes such as *Trichophyton, Microsporum*, and *Epidermophyton* that can invade the stratum corneum and keratinized tissues. Skin fungal infections are less frequently caused by non dermatophyte fungi (e.g., *Malassezia* in tinea *versicolor*) (Kim *et al.*, 2015). Dermatological examination of tattooed specimens revealed a circular, erythematous, scaly plaque, with centrifugal growth and central resolution, presenting an active, raised, erythematous, vesiculopustular edge, giving the appearance of Tinea corporis (OanIJě and Irimie, 2016).

Tinea incognito occurs when superficial fungal infections fail to demonstrate typical clinical features in the setting of immune suppression caused by topical or systemic steroids (Gathings *et al.*, 2018). Tinea on tattoos is rarely reported. Fungal infections can be explained by the skin break created by the needle during tattooing resulting in an impaired skin barrier. Fungal infections are not related to a lack of hygiene on the part of the tattooist, but rather to contamination during the healing phase (Schwob and Kluger, 2020).

Fungal contamination during tattooing is thought to be due to the presence of fungal pathogens in the tattoo needle. Reported some Cases of superficial

tinea infection infected with *Trichophyton rubrum* and *Epidermophyton floccosum* that associated with tattooing (Kazandjieva and Tsankov, 2007). It has also been defined this infection as a tattoo complication (Temiz and Özlü, 2021). Severe systemic mycoses can be transmitted rarely by tattooing such as *Candida endophthalmitis* (Khunger *et al.,* 2015). *Purpureocillium platinum* is an emerging pathogenic mold among immunocompromised hosts that causes cutaneous infections related to skin breakdown. This mold is a tattoo-related skin infection about kidney transplant recipients recently treated for acute cellular rejection presented with skin papules overlying a tattoo (Trinh and Angarone, 2017).

They concluded inappropriate hygiene measures in tattoo parlors and non-medical wound care are major risk factors for tattoo-related infections (Dieckmann *et al.*, 2016). The prevalence of *T.mentagrophytes* in tattoo people (Chi *et al.,* 2005). These results are compatible with our results. (De Cuyper, 2008) mentioned positivity of *T.mentagrophytes* and *Microsporum gypseum* in peoples with tattooing. where these studies are similar to our results, that indicate the prevalence of *Microsporum gypseum* in tattoos people with needle injection (Cullen, 2018). Other study showed the positivity of *T. rubrum* and *M.audouinii* in people with tattooing (Temiz and Özlü, 2021). where these results agreed with our study.

Tinea corporis was the most commonly diagnosed clinical condition in teaching hospitals in India followed by *Tinea cruris*. *T. mentagrophytes* was implicated as the predominating species (Kalita *et al.*, 2019). Fungal infections caused by *M. canis*, followed by *M. gypseum* and *M. hominis*, involving skin and its appendages, represent one of the most common diseases worldwide and a recalcitrant problem in dermatology that demands appropriate diagnostic and treatment strategies (Skerlev and Miklić, 2010).

The presence of fungal species pathogens is an indication that hairdressing and beauty salons could be contributing to the spread of infection within visitors' salons (Enemuor *et al.,* 2013). Fungal isolates consist of these potential pathogens showed in this study that fomites used in beauty salons harbor significantly high microbial load including microorganisms of possible public health significance (Stanley *et al.*, 2019). Nho *et al.,* (2020) indicate that commercial tattoo and permanent makeup inks on the US market surveyed contain a wide range of micro-organisms.

Depending on the comparison between age groups and fungal infections, *T.mentagrophyte* was found to have the highest incidence of infection in age groups (21-30, 31-40, and 41-50), with (33.3%, 22.2%, and 22.2%) respectively. The fungal species *M.gypseum, T. nigra, and T. rubrum* were found to have the highest incidence of infection in age groups (31-40) with (33.3%) for fungi infections (*M.gypesum, T. nigra, and T. rubrum*). As well, other growth and No growth constituted the highest incidence rate for the same age group (44.8% and 38.0%) respectively. The differences between age groups and inbred races were significant ($p<0.05$). The prevalences of skin fungal infection in men and women were similar (7.01% vs. 6.26%). It is interesting to note that adults from the 50–79-year age group showed a higher prevalence than children and young adults (Kim *et al.,* 2015). Acne, eczema, and fungal infection were the most common skin diseases identified. The findings also indicate that more respondents between 15-19 years old were more prone to skin diseases (Kawshar and Rajesh, 2013). Reporters inveterate the spread of *T. rubrum* as the predominant dermatophyte in our area and reported an increase in non-endemic anthropophilic dermatophytes in the last few years, especially in the African and Hindustani population up to 15 years of age (Antuori *et al.,* 2019). The prevalence of fungal infections with age in tattooed persons is due to the weakening of the immune status with advancing age, and consequently, the microbes can invade the body and cause disease. Results of the conducted study indicate that skin fungal infections (*T.mentagrophytes and M.gypseum)* prevalence has been showing high frequency in females and age

periods (31-40) years and these results are compatible with our study (Otašević *et al.,* 2019). *T. tonsurans* was the most common pathogen in *Tinea capitis*, whereas *T. mentagrophytes* was the most common pathogen in *Tinea corporis*. The prevalence of dermatophytes was higher in *Tinea capitis* 46/72 (63.8%) and *T. tonsurans* 29/72 (40%) was the dominant-isolated dermatophyte with age periods (1-14 years) (Araya *et al.,* 2020). These results are not compatible with our study about the relation of fungal infection with age periods. Since dermatophytosis has a high prevalence, affecting both children and adults particularly in tropical climates (Sanguino *et al.,* 2019). The prevalence of skin fungal infection differs by social, geographic, economical status, and living environment (Kim *et al.,* 2015). In 39% of patients, *M. gypsum* infection was diagnosed in children younger than 9 years (Dolenc-Voljč and Gasparič, 2017). *Tinea faciei* shows a complex spectrum of differential diagnosis and age-related variations concerning other superficial dermatophytoses. *M. canis* is the main organism responsible for children's infections residing in Cagliari, the capitol city of Sardinia, Italy (Atzori *et al.,* 2012).

When comparing fungal infections with sex, *T. rubrum*, no growth, and *T.mentagrophytes* were found, it constituted the highest rates of infection in males (66.70%, 56.00%, and 34.50%) respectively. In females, *M.audouinii*, *T. rubrum*, and *T.mentagrophytes* formed the highest rates of infection (100.00%, 100.00%, and 77.80%) respectively. The differences between fungal infection and sex were not significant ($p > 0.5$).

Tinea capitis is uncommon among infants, as shown in Tunisia, where a 12-year retrospective study showed that only 4% of infants were diagnosed with *Tinea capitis* (Coulibaly *et al.,* 2018). There was no difference between females and males in pain intensity during tattooing. Directly after the procedure, however, pain intensity was higher in women when compared to men. The most important factors increasing pain were time, bleeding, and the level of stress (Witkoİ and Hartman-Petrycka, 2020). In our study, the prevalence of fungal infections in women is higher than in tattooed men, due to the weakening of the immune status of women compared to men, and also because the incidence of tattoos in women is higher than in men. In particular, the prevalence of *Tinea capitis* was 3 to 5 times higher in boys than in girls in Central Nigeria (Coulibaly *et al.,* 2018). And these results are not compatible with our study that showed high-frequency *Tinea capitis* in women than men.

This study showed that the percentage of males who treated tattoos was (72.7%), while females were (27.3%), age group (26-40) years recorded the highest percentage of males and females who treated tattoo with (75%) and (33.33%) respectively (Lateef *et al.,* 2020).

When comparing fungal infections with housing, *T. nigra*, no growth, and other growth were found, it highest rates of infection were found in people living in rural areas (66.70%, 40.00%, and 44.40%) respectively. As for the people who live in the city, *T. nigra*, Other growth, and *M.gypseum* formed the highest rates of infection (100.00%, 75.90%, and 66.70%) respectively. The differences between fungal infection and habitation were not significant ($p > 0.5$).

According to a few studies, the low socioeconomic level was associated with an increased risk of *Tinea capitis*, which is likely linked to poor hygiene conditions (Coulibaly *et al.,* 2018). Moreover, the prevalence of *Tinea capitis* was higher in schools serving populations of low socioeconomic levels (Hogewoning *et al.,* 2013). Such as, in Ghana, 10% of the pupils of low socioeconomic level and 5% of middle or high socioeconomic-level students presented with *Tinea capitis*. Cross-sectional population-based studies performed in Ghana, Gabon, and Rwanda showed that among schoolchildren, the highest prevalence of *Tinea capitis* was observed in rural areas than urban (Coulibaly *et al.,* 2018).

Table.1 Number of personal characters with fungi species

		T.mentagrophytes	*M.gypseu m*	*T. nigra*	*M.audouinii*	*T.rubrum*	Other growth	No	Total
Age ≤10	n	2	0	0	0	0	0	0	**2**
	%	22.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**2.0%**
11-20	n	0	1	0	0	1	3	3	**8**
	%	0.0%	33.3%	0.0%	0.0%	33.3%	10.3%	6.0%	**8.0%**
21-30	n	3	1	0	1	1	9	26	**41**
	%	33.3%	33.3%	0.0%	33.3%	33.3%	31.0%	52.0%	**41.0%**
31-40	n	2	1	1	2	1	13	19	**39**
	%	22.2%	33.3%	33.3%	66.7%	33.3%	44.8%	38.0%	**39.0%**
41-50	n	2	0	0	0	0	4	2	**8**
	%	22.2%	0.0%	0.0%	0.0%	0.0%	13.8%	4.0%	**8.0%**
>50	n	0	0	2	0	0	0	0	**2**
	%	0.0%	0.0%	66.7%	0.0%	0.0%	0.0%	0.0%	**2.0%**
P value					0.02*				
Gender Male	n	2	1	2	0	0	10	28	**43**
	%	22.20%	33.30%	66.70%	0.00%	0.00%	34.50%	56.00%	**43.00%**
Female	n	7	2	1	3	3	19	22	**57**
	%	77.80%	66.70%	33.30%	100.00%	100.00%	65.50%	44.00%	**57.00%**
P value					0.121				
Living Rural	n	4	1	0	1	2	7	20	**35**
	%	44.40%	33.30%	0.00%	33.30%	66.70%	24.10%	40.00%	**35.00%**
City	5	5	2	3	2	1	22	30	**65**
	%	55.60%	66.70%	100.00%	66.70%	33.30%	75.90%	60.00%	**65.00%**
P value					0.51				
Education Primary	n	6	1	1	1	1	6	15	**31**
	%	66.70%	33.30%	33.30%	33.30%	33.30%	20.70%	30.00%	**31.00%**
Secondary	n	2	2	2	2	1	17	23	**49**
	%	22.20%	66.70%	66.70%	66.70%	33.30%	58.60%	46.00%	**49.00%**
College	n	1	0	0	0	1	6	12	**20**
	%	11.10%	0.00%	0.00%	0.00%	33.30%	20.70%	24.00%	**20.00%**
P-value					**0.71**				

Table.2 Values of DNA concentration of a selected sample of DNA extracted

Sample	Conc.
01	10
02	9
03	11
04	8
05	12
06	7
07	14

Table.3 Antifungal sensitivity

Name of isolate	Caspofungin	Fluconazole
T. rubrum	**R**	**R**
T.mentagrophytes	**R**	**R**
M.audouinii	**R**	**R**
	R=Resistance to antifungal	

Three of the isolates explained the resistance to the Fluconazole and caspofungin. (Table 3)

Fig.1 Frequency and Percentages of Fungi Species Infection

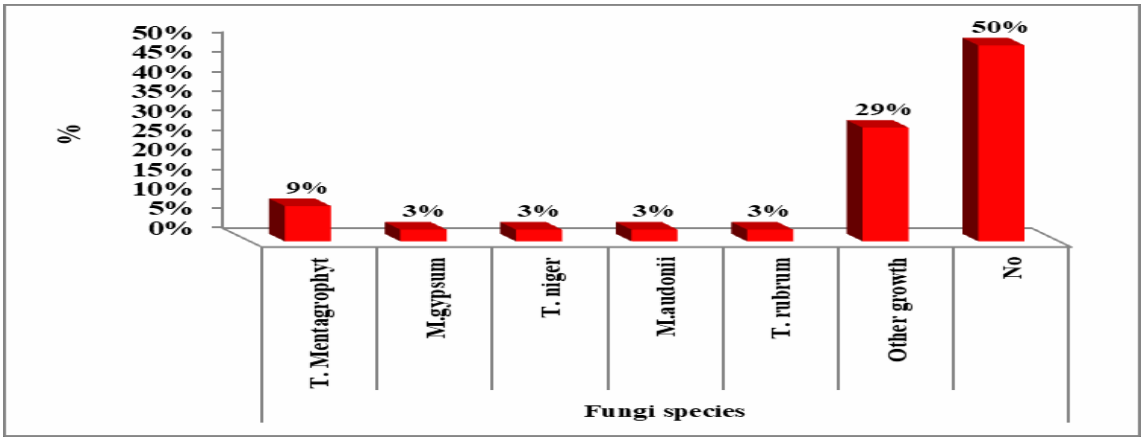


Fig.2 Examination of Fungus (A) growth of fungus, (B) examination by microscope 40x, (C) biochemical tests, (D) microscopic examination by 10x

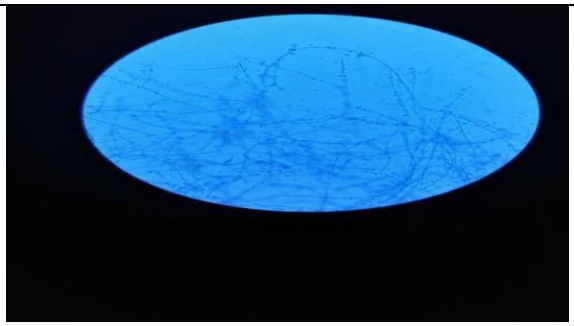
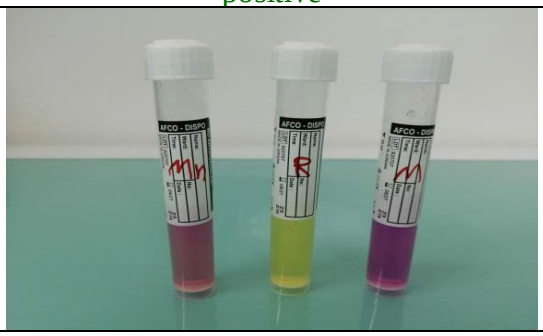
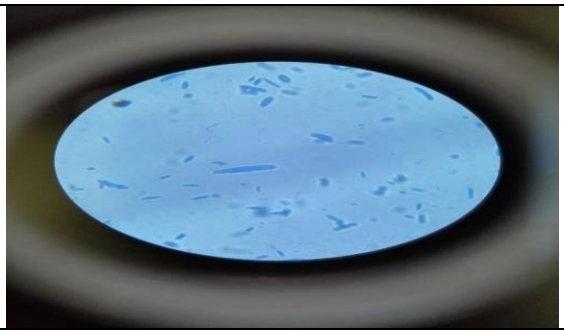
A *T.rubrum* growth on PDA Surface white color and like cotton	**B** Microscopy examination 40x *T.rubrum*
	
C Biochemical test *T. rubrum* negative to urease test, *T. mentagrophytes* positive, *M.audouinii* positive	D Microscopy examination 10x *T.rubrum*
	

Fig.3 The amplification of subtilisin-like protease gene region of *Trichophyton* ssp. samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker. Lanes 1-4 resemble 623bp PCR products.

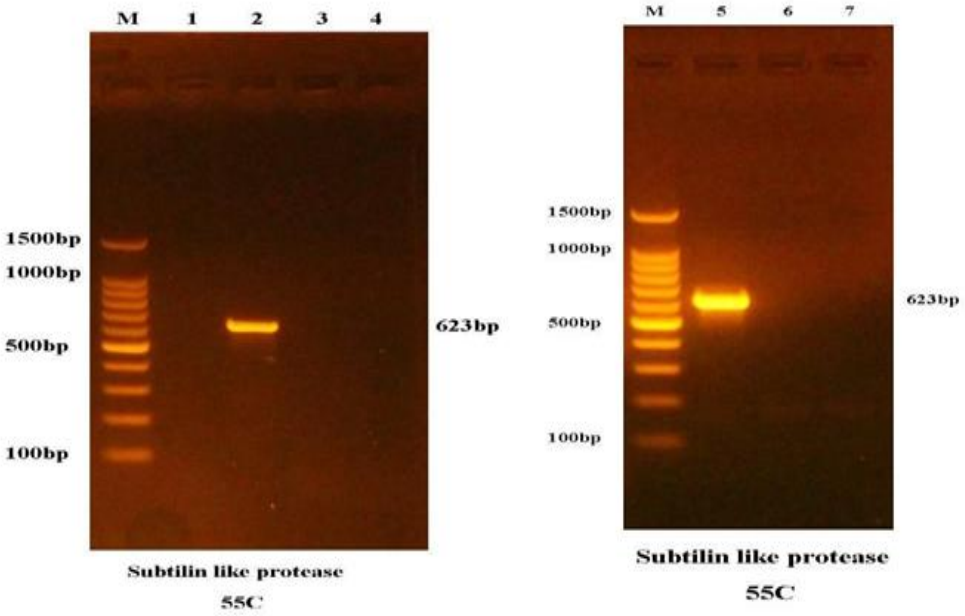
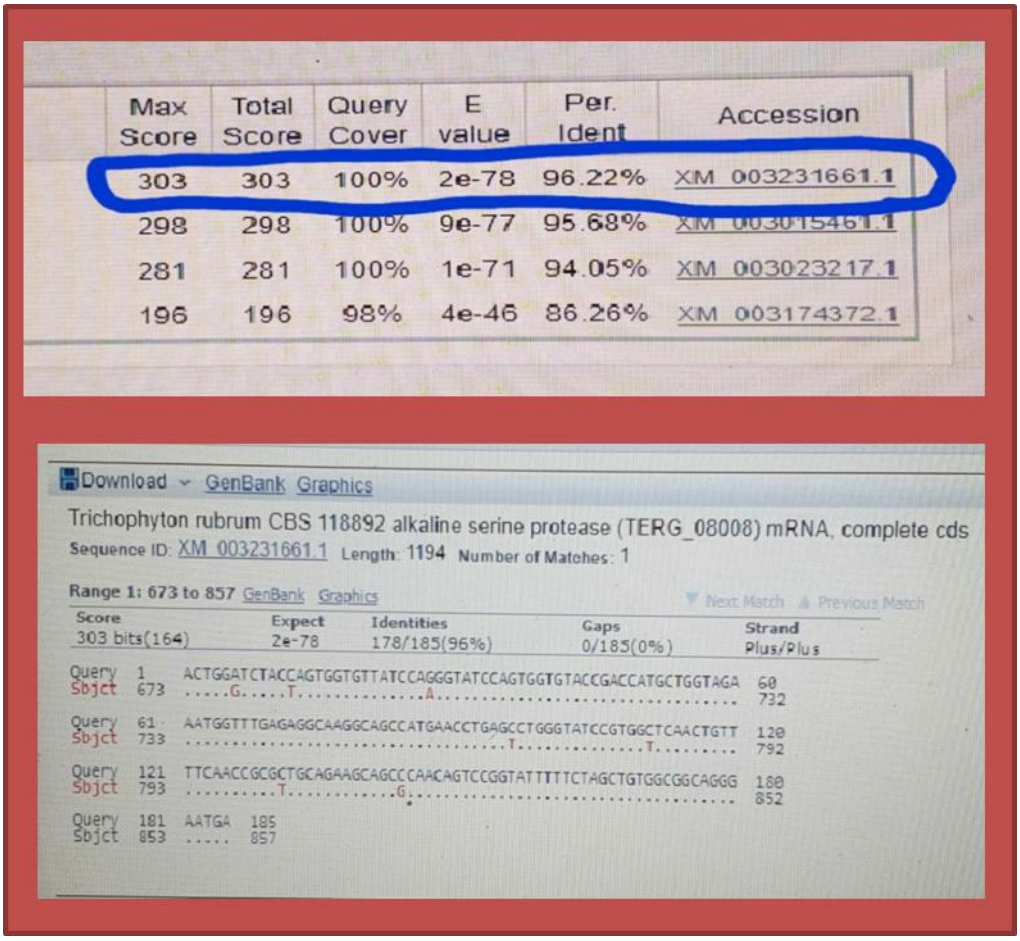


Fig.4 Sequence of *T.rubrum*



According to the current study, the prevalence of fungal infections in the city more than in the countryside is because most of the tattoo users are from the educated classes that live in the city, and therefore we notice the spread of tattoos, most of them in the city dwellers. Women with tattoos reported a greater willingness to engage in uncommitted sexual relations, as well as the higher endorsement of egalitarianism and sensation-seeking, relative to non-tattooed women. Among tattooed women alone, several personalities and tattooing variables predicted sexual openness. Findings suggesting body tattooing as an indicator of sexual openness are critically discussed about contemporary stereotypes surrounding femininity and sexuality (Skoda *et al.*, 2020). When comparing fungal infections with education, *M.gypesum*, *T. nigra*, and *M.audouinii* were found, it highest rates of infection among people were those who graduated from secondary school with (66.70%). Other growth and no growth also constituted a high rate of infection among secondary school graduates, with a percentage (58.60% and 46.00%) respectively. The differences between fungal infection and education level were not significant ($p > 0.5$).

Various causes such as environment, overcrowding, and poor living conditions are major factors, and not only adolescents or old age group but also the entire population between 21 and 50 years of age were found to be suffering more commonly from eczema and infective dermatoses (Jain *et al.*, 2016). The prevalence of skin fungal infection *Tinea capitis*, *Tinea corporis Tinea faciei* and *Tinea pedis* in primary school children, male (56%) and female

(44%) with age periods 10-14 years (Ogbu *et al.,* 2015). In our study the increase in the prevalence of fungal infections in the secondary stage of education In this study because the population is in the stage of adolescence and also because of the lack of cultural awareness and the attraction of societal cultures that are directed towards beauty and fashion. *Microsporum audouinii*, an anthropophilic dermatophyte fungus that has been rarely reported since the middle of the last century, is re-emerging in several European countries. Unlike other species that cause ringworm, it can cause epidemics of *Tinea capitis* in schools (Lozano-Masdemont *et al.,* 2019).

Recurrent dermatophytosis refers to the recurrence of the dermatophyte infection within a few weeks, after full completion of treatment (Dogra and Uprety, 2016). The results of the current study showed the resistance of *T. rubrum, T. mentagrophytes, M.audouinii* to the antifungal fluconazole and caspofungin.This results similarity to Amin *et al.,* (2017); Pakshir *et al.,* (2009) and (Hryncewicz-GwóŹdŹ *et al.,* 2013; Bao *et al.,* 2013) and disagree with (Sinha and Sardana, 2018). Resistance of dermatophytes and other pathogenic mycoses increased alarmingly, due to elevated numbers of persons with HIV. The long-living of these patients with antifungal agent treatment leads to the emergence of resistant strains of pathogenic microbes including dermatophytes (Ghannoum, 2016).

Dermatophytes are fungi that live on the skin. The physiological and morphological changes that have been observed in dermatophytes complicate or obstruct the diagnosis method. This discrepancy may be attributable to differences in culture methodology, incubation temperature, and pharmaceutical usage (Brilhante *et al.,* 2005). *Trichophyton rubrum* is the most commonly observed dermatophyte isolated from humans in European countries. This species is a prevalence of approximately 80 % (Monod *et al.,* 2002). In the current study, the genomic DNA was isolated from fungal growth according to the protocol of Geneaid Extraction. The PCR product of the positive isolates to the *Subtilisin-like protease* gene region was sent for Sangar sequencing using ABI3730XL. Resulted of PCR products ranging from 673-857 bp. The results are compared by using nucleotide blast (method of alignments explorer clusterin Microsoft. The gene sequence of the local strain of *T. rubrum* showed (96.22%) similarity to the *T. rubrum* standard strain CBS 118892. The results in the current study agree with Jousson *et al.,* (2004) and Al-Masaoodi *et al.,* (2020) In terms of where the sample was taken, the type of Dermatophytes, and the medium of development.

In this study, we conclude that the Dermatophytosis in females is more than in males. The sequence of isolated *Trichophyton rubrum* was the new sequence, the similarity was (96.22%).*T. mentagrophyte* was more infected species in patients male and female. Resistance to antifungal was noticed in Caspofungin and Fluconazole.

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