PCR test in detecting dermatophyte species among a sample of tinea patients attending to Imamein Kadhimein Medical City

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Abstract

Background: Dermatophytosis is a common infection caused by superficial filament fungi that affects the skin, nails, and hair. It holds global importance, especially for countrieslocated in tropical and subtropical regions.

Objective : identify of dermatophytes species by Conventional PCR method using ITS1 and ITS4 primer.

Methods: cross-sectional study conducted at the dermatology clinic at Imamein Kadhimein Medical City in Baghdad between 22th /January /2024 to 29 /July/2024, the specimens were obtained from all participants, and a potassium hydroxide (KOH) test, cultured on Sabouraud's dextrose agar and polymerase chain reaction (PCR) analysis was utilized to for identification and identification of the dermatophyte species.

Results: The age distribution reveals a mean age of 31.39 ± 16.5, with the 20–29 age group appeared more group affected with dermatophytosis counting 24.0%, where the 60–69 age group exhibits the lowest frequency at 6.9%. Residence of urban dwellers (66.6%), the students (30.6%) and housewives (29.1%) being the most impacted. where T. corporis has the highest percentage, about 33.4% of cases, followed by 16.3% of T. cruris, then T. capitis count 12.9% of cases, positively rate was in microscopic examination (KOH), 66.9% in culture 87.7%, *Trichophyton mentagrophytes* accounted for 22.2% of all dermatophyte cases, followed by *Microsporum canis* (19.8%), and *Trichophyton rubrum* (17.8%) and PCR test was performed on 207 samples; 88.4% of the samples were positive. Conclusion: Tinea corporis predominant type and 20-29 age group more affected group.

Conclusions : Tinea corporis and tinea cruris are most common clinical types of dermatophytosis while *Trichophyton mentagrophytes* the most common species affect younger age group (20–29 years) with 26.6 % relapse rate.

Key words: **Dermatophytosis , Conventional PCR, *Trichophyton Mentagrophytes*.**

Introduction:

Dermatophytosis, a common infection caused by superficial filament fungi that affects the skin, nails, and hair, holds global importance, especially for countries located in tropical and subtropical regions. According to the World Health Organization (WHO), dermatophyte infections affect 25% of the world's population, and asymptomatic carriers estimate 30 to 70% of adults [1]. Certain fungal species are found all over the world, while others are restricted to specific continents or geographical areas; dermatophyte infections are more common in relatively hot, humid, and rainy regions, such as tropical regions; the sex or gender of the host influences the incidence rate of dermatophytosis, due to

differences in the host's immune state, personal behavior, awareness level, and early detection and treatment measures [2] [3]. The incidence and causative agent of dermatophyte infections in the Middle East differ from one area to another. According to a study conducted in Iraq, the most common dermatophytes were *Trichophyton rubrum*, followed by *Trichophyton mentagrophytes* and *Microsporum canis*. Tinea corporis was the most common type of dermatophytosis, followed by tinea capitis and tinea pedis [4]. Classification of etiologic agents of dermatophytosis into three genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*. A few types of dermatophytes, primarily the geophilic and zoophilic species of Trichophyton and Microsporum [5]. These microorganisms typically cause cutaneous infections that only affect the non-living, cornified layers of skin. Fungi can penetrate deeper tissues in chronic situations, especially when infected with other organisms. The dermatophytes are often incapable of penetrating the host's deeper tissues or organs. Because of their ring-like appearance, dermatophyte diseases are commonly referred to as ringworm infections [6]. The routine methods for identifying dermatophyte species are to examine the colony (pigmentation of the surface as well as size, texture, and rate of growth), microscopic morphology (size and form of macroconidia, microconidia, and pectinate branches), and biochemical tests are additional aspects of identification. Molecular techniques are helpful analytical tools for assessing the structure and function of microorganisms. DNA analysis has been conducted at various resolution levels for whole communities, bacterial, fungal, and yeast isolates, as well as clones of specific genes [7]. Since 1985, the polymerase chain reaction (PCR) has been the most used technique for amplifying nucleic acids for characterizing of microorganisms and recently has had an important role in the detection and identification of various microorganisms as an alternative to culture-based methods [8].Although the fact that dermatophytosis is not fatal, treating it can be extremely expensive, requiring millions of dollars each year, especially if the initial course of treatment is insufficient and the illness recurs. Furthermore, dermatophytosis can show clinically similarly to dermatitis, which could result in patients receiving improper treatment lead to development the resistance. Even though dermatophytosis only affects the skin, it can have an effect on the appearance of aesthetic issues, which lowers quality of life [9].

Materials and methods

The present study was carried out at Imamein Kadhimein Medical City in Baghdad, involving 350 patients who presented with dermatophytosis at the dermatology clinic between 22th /January /2024 to

29 /July/2024. The clinical identification of tinea patient was performed by a specialized dermatologist utilizing a structured questionnaire aimed at obtaining sociodemographic characteristics, medical history, clinical features of the disease, and treatment details. Specimens were obtained from all participants. Each sample was direct examined by microscope. A part of the sample was placed on a sterile microscope slide with a few drops of 20% (KOH). Hair and skin samples were examined after 15-20 minutes and nail samples were examined after 30 minutes. then positive specimens cultured on Sabouraud dextrose agar (SDA) with cycloheximide (500 µg/ml) and chloramphenicol (40 µg/ml) and dermatophyte test medium (DTM) at 28°C for 2-4 weeks with regular examination. DNA extraction performed for positive dermatophytes' cultures using Fungi Genomic DNA Extraction kit by bead bashing method, then DNAs were amplified by polymerase chain reaction (PCR) using universal primers; a forward primer (ITS1F: 5′-TCCGTAGGTGAACCTGCGG-3′) and a reverse primer (ITS4 R: 5′-TCCTCCGCTTATTGATATGC-3′). The PCR amplification in a total volume of 25µl consisting of 5µl DNA, 12 µl Taq PCR PreMix 2µl of each primer and 5µlof nuclease- free distilled water. Thermal cycling conditions were applied as: Denaturation at 95 °C for 5 min, followed by 45 cycles of 96 °C for 20s, 52°C for 30s and 72 °C for 30s with final incubation at 72 °C for 5 min. Extracted DNAs were electrophoretically on 2% agarose gel to determine DNA bands through which observed by UV transilluminator.

Results

The age distribution reveals a mean age of 31.39 ± 16.5, with the 20–29 age group constituting the greatest percent at 24.0% while the 60–69 age group exhibits the lowest frequency at 6.9%. Data on marital status indicates that 65.0% of participants are married. Residence data shows a predominance of urban dwellers (66.6%), related occupation, the students (30.6%) and housewives (29.1%) being the most impacted. As illustrated in Table (1)

Table 1: Distribution of demographic characteristic among tinea patients

		No.	**%**
Age (years)	<10years	30	8.6
	10---19	65	18.6

	20---29	84	24.0
	30---39	66	18.9
	40---49	44	12.6
	50---59	37	10.6
	60---69years	24	6.9
	Mean ± SD	**31.39 ±16.5**	
Marital status	Unmarried	112	35.0
	Married	208	65.0
Residence	Urban	233	66.6
	Rural	117	33.4
Occupation	Student	104	30.6
	Housewife	99	29.1
	Employee	37	10.9
	Worker	70	20.6
	Retired	10	2.9
	Military	20	5.9

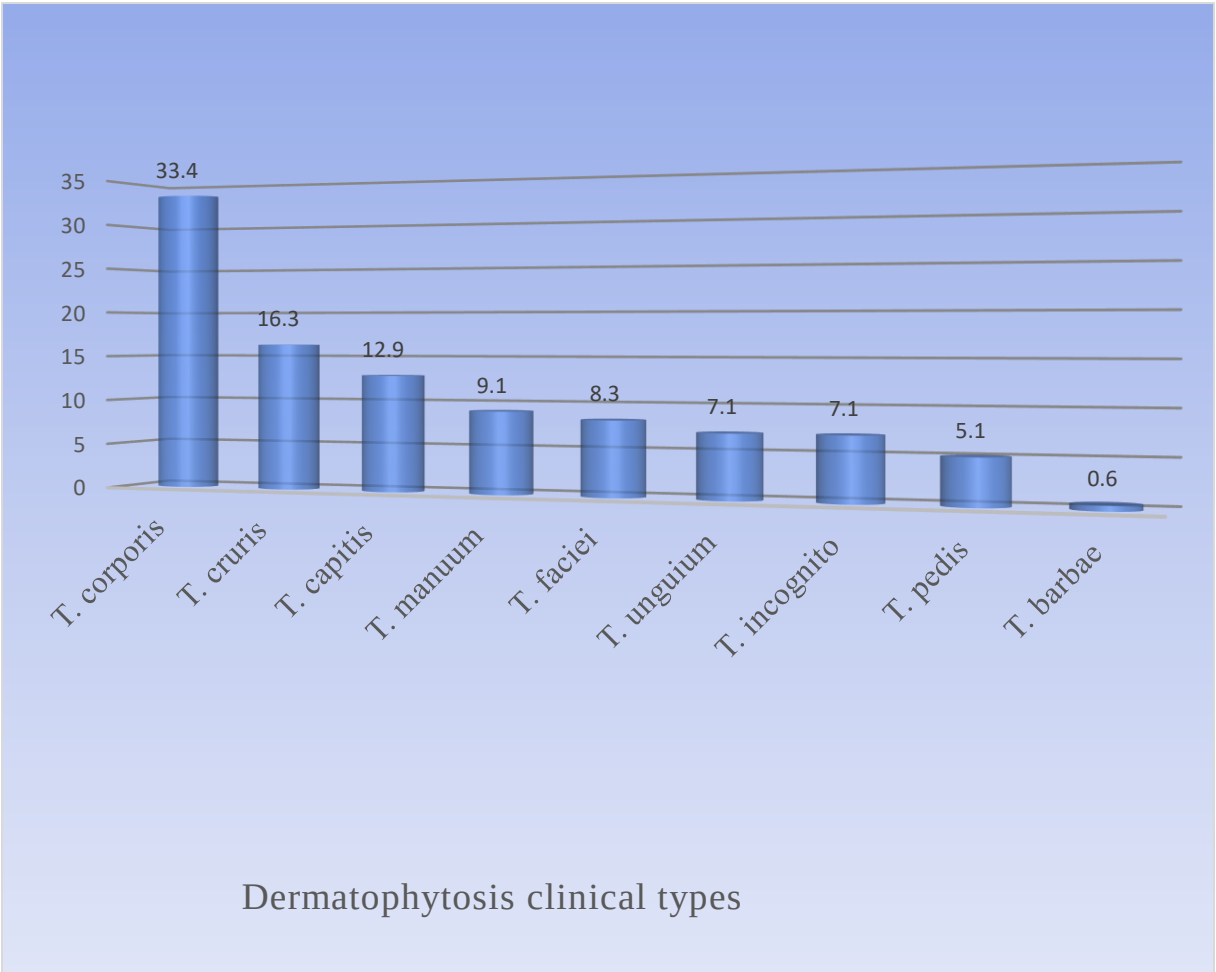


Figure 1: Distribution of clinical types of dermatophytosis

The clinical type of dermatophytosis, where T. corporis has the highest percentage, about 33.4% of cases, while T. barbae represents the lowest percent, 0.6% of cases.

Table 2 shows the medical history of patients with dermatophytosis. It reveals that 10% of patients have been diagnosed with the condition, 4.9% have symptoms of illness, and roughly 26.3% of patients have recurrent infections. Additionally, 19.2% of patients are taking steroid medications, 3% are taking immune-suppressing medications, and 2.9% have received chemotherapy as table 2

Table 2: Distribution of patients according medical history

Medical history		**No.**	**%**
Past history of dermatophytosis	Yes	35	10.0
	No	315	90.0
Type of dermatophytosis	T. corporis	23	65.7
	T. unguium	1	2.9
	T. faciei	2	5.7
	T. cruris	8	22.9
	T. manuum	1	2.9
Recurrence of infection	Yes	92	26.3
	No	258	73.7
Drug history	Steroid	67	19.2
	Immune-suppressing agents	11	3.0
	Chemotherapy	10	2.9
	No	262	74.9

In table 3 Association between the clinical types of dermatophytosis and acute and chronic infections. T. corporis is most common type had acute infection at 34.6%. T. cruris acute infections are 15.5% of cases of chronic infections. While T. capitis is 13.9% of acute infections. Conversely, T. Pedis, only 3% of cases are acute infections and 11.9% is a comparable percentage of chronic infections.

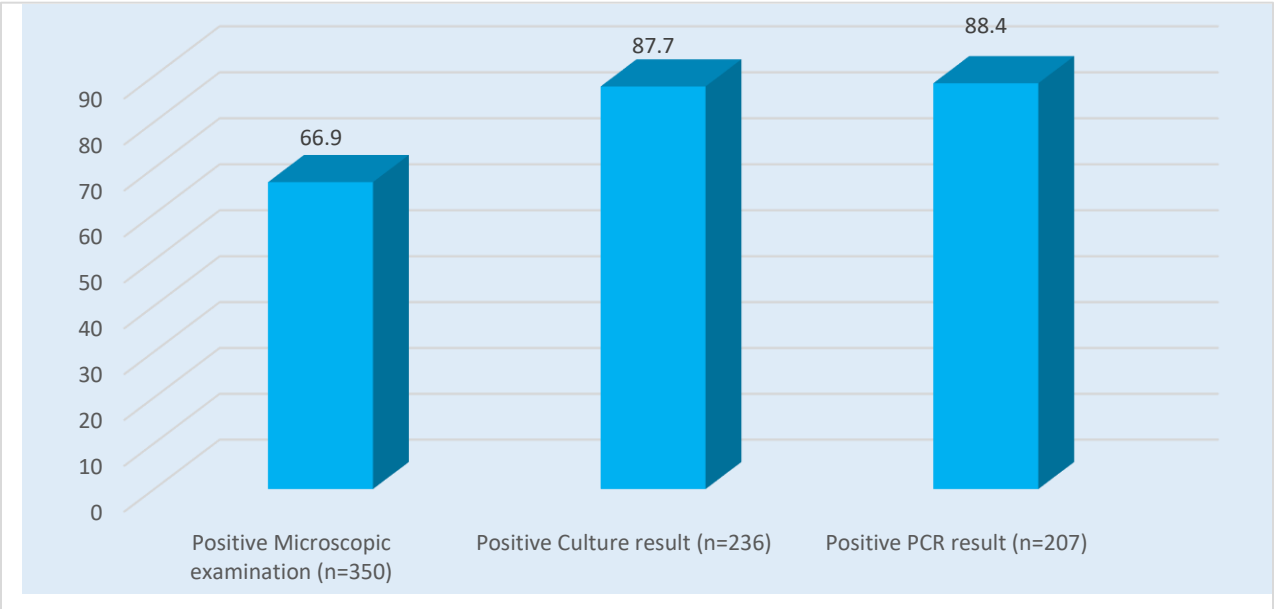
Table 3: Association between the clinical types of dermatophytosis and types of infection

Clinical type of dermatophytosis	Type of infection			
	Acute		Chronic	
	No.	%	No.	%
T. corporis	92	34.6	25	29.8
T. unguium	15	5.3	10	13.1
T. pedis	8	3.0	10	11.9
T. capitis	37	13.9	8	9.5
T. faciei	27	10.1	2	2.4
T. cruris	44	16.5	13	15.5
T. manuum	30	11.3	2	2.4
T. barbae	2	0.8	-	0.0
T. incognito	12	4.5	13	15.4
Total	**267**	**100**	**83**	**100**
P value	0.0001*			

Table 4 show *Trichophyton mentagrophytes* accounted for 22.2% of all dermatophyte cases, followed by *Microsporum canis* (19.8%), and *Trichophyton rubrum* (17.8%) (n = 207). *Trichophyton verrucosum* had the lowest percentage (1.9%; n = 207) of cases.

Table 4: Distribution of dermatophytes species

species	No.	%
Trichophyton mentagrophytes	46	22.2
Microsporum canis	41	19.8
Trichophyton rubrum	37	17.8
Microsporum ferrugineum	32	15.4
Microsporum audounii	21	10.1
Trichophyton tonsurans	14	6.6
Epidermophyton floccosum	7	3.38
Microsporum gypseum	5	2.4
Trichophyton verrucosum	4	1.9
total	**207**	**100**



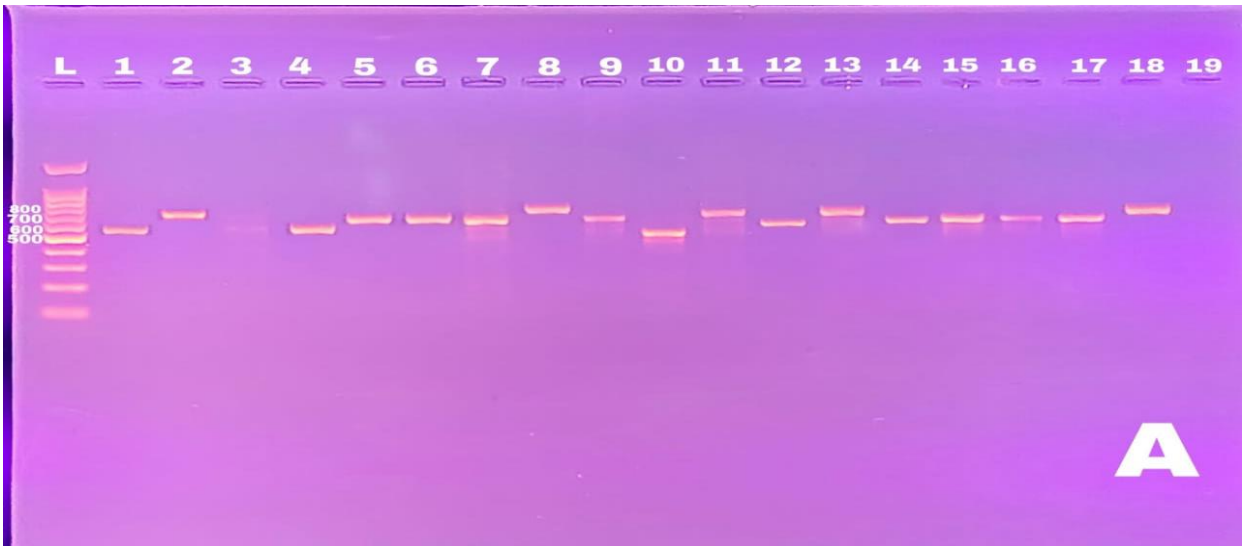
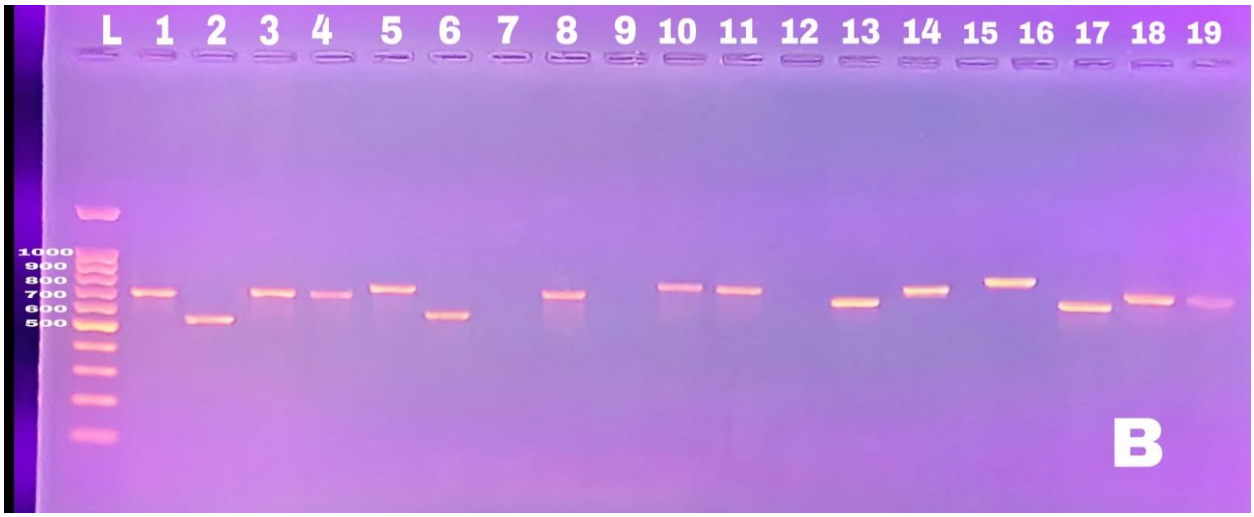


Figure 2 show positively rate in the diagnostic examination the diagnostic laboratory results, of the



350 samples that examined microscopic examination (KOH), 66.9% were positive and 87.7% (n = 236) were positive in the culture count. A PCR test was performed on 207 samples; 88.4% of the samples were positive. As figure 2

Figure 3: PCR product of ITS region for dermatophytes species, electrophoresis 2% agarose gel 70 volt/cm^2 for 1:2 hours with UV visualization,(A;B) lane L : DNA ladder 100bp , lane (1-19) PCR product with band size 550-740 bp.

Discussion :

This study was conducted on (350) patients diagnosed dermatophytosis. Most individuals were in with mean age of patient and standard division was (31.39 ± 16.5). The distribution of dermatophytosis among age groups shows widespread was in the third decade of life the 20–29 (24%), agreement with findings of many studies in India [10] ,[11], who reported the 21–30 age group exhibited the highest

frequency, while in Erbile [12] study revealed the 31–40 age group most group affected. The students were the most affected group (30.6%), followed by housewives (29.1%) and workers (20.6%). This aligns with Dalei study [13] who reported that unemployed people (students and homemakers) were predominantly affected, followed by workers. Similarly, other studies such as [14] in Iraq,[11] in India and [15] in Nigeria also identified students as a high-risk group, with39.9%, 37.3% and 38.1% of cases, respectively. Multiple studies have consistently shown that students are the most impacted group, which suggests that there are specific factors that increase their susceptibility. These factors could include close physical contact in public places like classrooms and hostels, the communal facilities like gyms and sports equipment, and possibly a lack of knowledge or emphasis on personal cleanliness among this age group[16],[17].

Dermatophytosis was more widespread in urban areas (66.6%) than rural areas (33.4%), which is agreement with findings from Al-Yasiri study [18] who reported higher percentage of 64.71%. Where tinea corporis, tinea capitis, tinea cruris and tinea unguium more common in urban residences compared with rural are counting 69.2%, 71.1%,68.4%, 68% respectively. This finding compatible with Ahmed [14] in Diyala governate. Similarly, Najem [19] research from the Iraqi provinces of Al-Nassiriyah revealed urban area have high percentage infection in urban populations around 86.36%. Conversely, Musa study [20] found that the frequency was higher in the Anbar Governorate's rural districts (66.8%). The disparities between urban and rural prevalence rates can be attributed to various factors. Higher population densities, more human interaction, and living circumstances that support fungal development are all common in urban settings. The risk may be further increased by socioeconomic variables, such as limited access to public health resources and healthcare in urban areas. However, rural communities confront distinct dangers, such as agricultural activities and direct contact with animals, which may increase infection rates in some situations[21], [22].Tinea corporis was predominant clinical type (33.4%), followed by tinea cruris (16.3%) and tinea capitis (12.9%). The findings are consistent with Ansari study [23] in southern Iran and [11] in Northwest India where the most common type was tinea corporis, which was followed by tinea cruris and tinea capitis in comparable percentages. Similarly, Naseri study [24] in Mashhad, Iran, revealed a close occurrence between tinea corporis and tinea capitis. In a contrast study conducted by Mohammed [25] in Baghdad, tinea capitis was the predominant clinical type, accounting for 47.5% of cases, followed by other types of dermatophytosis. This result can be explained by the study's smaller sample size and emphasis on younger participants, especially those under 10 years of age. The recurrence rate of dermatophytosis at 26%. Similarly, [6] in Himalayan state and the multicentric study [26]in India observed recurrence rates of 34.3% and 21.95%, respectively. The KOH positivity rate was 66.9% in suspected cases. This finding is agreement with Salahudeen [27], and Girgis [28] studies reported KOH positivity rates of 66.36% and 71.7%, respectively, closely aligning with Siddiqui study [5], who reported a KOH positivity rate of 60.8%. Where culture growth was 87% of isolates was positivity , this results are agreement with the findings of Musa and khalaf [20], who reported a culture positivity rate of 85%. closely followed by Agarwal [11] and Al-Hmadani [29] studies, at 80% and 78.7%, respectively. The most commonly isolated dermatophyte, though its

prevalence varies, is. The present study identified it in 22.2% of isolates was *T. mentagrophytes*, which is agreement with Aref study [30], who report 29%, indicating the species' predominance, whereas Shukla [31] and Jebara [32] studies both reported significantly higher percentages. The PCR positivity rate was approximately 88.4% among isolates from culture that were negative, which was 11.6%; this finding is agree with Aho-Laukkanen [33] and Sharquieand Jabbar [34] studies who had a positivity rate of 87.6%,90% respectively, and closely from Sakshi study [35] who had 94.6% positives among isolates. The PCR products of the ITS1 and ITS4 regions were 740 bp for *M. canis* and *Epidermophyton floccosum*, 690 bp for *T. mentagrophytes* and *T. rubrum*, 650 bp for *M. audounii*, and 550 bp for *T. tonsurans* .this results agree with several studies use its primers in identification of dermatophytes species [36, 37, 7]

Conclusion:

Tinea corporis and tinea cruris are most common clinical types of dermatophytosis while *Trichophyton mentagrophytes* the most common species affect younger age group (20–29 years) with 26.6 % relapse rate.

Recommendations

Providing molecular technologies in screening programs at health institutions to enable early detection and prompt intervention.

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