



Comparison of Two Conventional Methods for Identification of Dermatophyte Fungi

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Received in:1 /Mach /2018, Accepted in:9/May/2018

Abstract

The current study is the identification and isolation dermatophyte species in clinical isolates by both Sabouraud's Dextrose Agar (SDA) and on Dermatophyte Test Medium (DTM). Clinical specimens of hair, nails and skin scales were collected from patients with dermatophytosis and submitted to direct microscopic examination after immersion in 20% of potassium hydroxide solution. The clinical specimens were cultured on SDA containing chloramphenicol and cycloheximide, and on DTM. *Tinea corporis* showed the highest prevalent dermatophyte infection among patients (26.7%), followed by *Tinea pedis* (23.3%), whereas *Tinea manuum* exhibited the lowest fungal infection (6.7 %). Rural areas revealed the highest prevalence of dermatophyte infection (70.0 %) in comparison to 30.0% in urban areas. Based on the conventional laboratory methods, 30 clinical isolates of dermatophytes showed positive cultures which belong to three genera (*Trichophyton*, *Microsporum* and *Epidermophyton*). *Trichophyton mentagrophytes* was the most common species (21.7%) isolated among 30 positive dermatophytes, followed by *Epidermophyton floccosum* (17.4%), then *Trichophyton bulbosum* and *Trichophyton tonsurans* (13.0%).

Keywords: Dermatophytosis, Sabouraud's Dextrose Agar, Dermatophyte Test Media.

Introduction

Dermatophytes referred as a unique group of superficial keratinophilic and filamentous fungi which have the ability to invade keratinized tissue of the skin, hair and nail in human and animals causing dermatophytosis or tinea (ring worm) [1]. Dermatophytosis manifests as significant cause of hair and nail loss, inflammation, pustules, itching, and scaling. Dermatophyte fungi belonging to three genera; *Trichophyton*, *Microsporum*, and *Epidermophyton* [2]. The infection pathways for dermatophytes are thus either directly (by skin contact from one person to another) or indirectly (most commonly by walking barefoot on surfaces that have been contaminated with infectious material from the skin, floors, rugs, etc.) [3]. Based upon the affected site, dermatophytes have been classified clinically into Tinea capitis (head), Tinea faciei (face), Tinea barbae (beard), Tinea corporis (body), Tinea manuum (hand), Tinea cruris (groin), Tinea pedis (foot), and Tinea unguium (nail) [4]. Direct microscopy on clinical specimens is a fast screening method for fungal structures, but it lacks specificity. Thus, culture is important for both a therapeutic and epidemiological standpoint [5]. Specimens of skin scraping, nails, and hair fragments (irrespective of the negative or positive direct microscopy results) were cultured Sabouraud's Dextrose Agar (SDA) supplemented with cycloheximide to inhibit the growth of non-dermatophytes molds and chloramphenicol to reduce contamination with bacteria [6]. For identification of cultured dermatophytes, different morphological characteristics and structures such as colony pigmentation, texture, growth rate, presence of microconidia and/or macroconidia, spirals, pectinate branches, pedicels and nodular organs are used [7]. All specimens were also cultured on Dermatophyte Test Medium (DTM), which is used for presumptive identification and it is based on a color change in the medium due to the production of alkali metabolites by dermatophyte growth result in color change to red in its constituent phenol red indicator [8]. The identification of dermatophytes to the genus level as well as to the species level guide to appropriate treatment, provide knowledge of likely source of infection and risk of transmission, and finally report data for epidemiological studies [9]. The aim of this study is the identification and isolation dermatophyte species in clinical isolates by both Sabouraud's Dextrose Agar (SDA) and on Dermatophyte Test Medium (DTM).

Materials and Methods

Subjects: A total of 115 clinical specimens (skin scrapings, nails and hair clippings) were collected from patients with dermatophytosis (irrespective to the age and gender) attending the Dermatology Department of either Al-Kadhimiya Teaching Hospital/Baghdad or General Hospital Azizia/Wasit, 73 rural and 42 urban, during the period from December 2016 to June 2017. However, a proper explanation of the study was addressed to the patients and the consent was taken from them before collection of the sample.

Materials and Methods: Skin specimen was obtained by scraping the active margin of lesion with a scalpel blade after cleaning the surface with 70% isopropyl alcohol. The clinical specimens were examined by direct microscopic examination after treating with 20% potassium hydroxide Solution. Also, the clinical specimens were inoculated on Sabouraud's Dextrose Agar (SDA) containing chloramphenicol (0.04 g/l) and cycloheximide (0.5 g/l), and on Dermatophyte Test Medium (DTM). Cultures were incubated at 28°C with daily observation for a period of 4-5 weeks before they were considered negative. Identification of the growth was depended on the morphological features of colony growth (the color, reverse of the colony and pigments that produced). DTM is based on color change in the medium [10]. To study the characteristics of isolated fungi and classified them accurately (by investigating the size, shape and arrangement of micro- and macroconidia, and the hyphal structures) a direct microscopical examination of fungal growth treated with lactophenol cotton blue was performed. [6].

Results

Figures (1) and (2) showed the culture results of SDA, which were positive in 55 (47.8%) and negative in 60 (52.2%) of 115 cases. Among the 55, culture positive isolates, 30 (26.0%) dermatophytes, 17(14.8%) *Aspergillus spp.* and 8 (7.0%) *Candida spp.* were isolated.

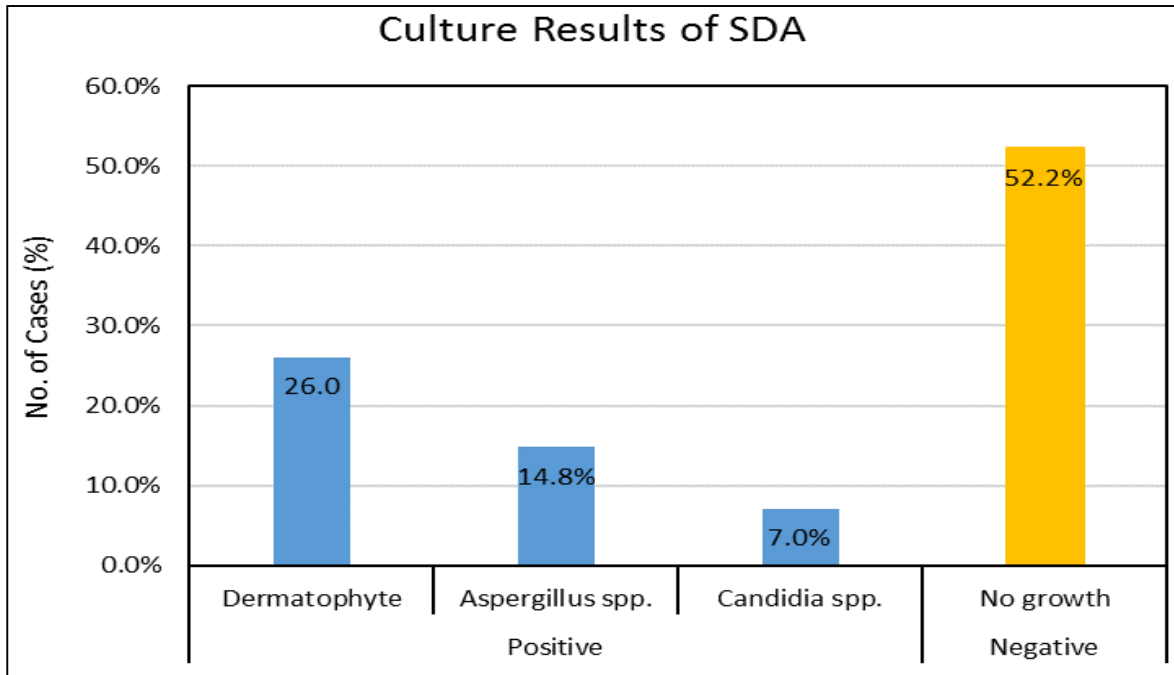


Figure (1): Distribution of clinically diagnosed patients according to Sabouraud Dextrose Agar (SDA) culture results.



Figure (2): Sabouraud Dextrose Agar. (A) *Trichophyton spp.*, (B) *Microsporum spp.*

Figures (3) and (4) demonstrated the culture results of DTM, which revealed that 30 (26.1%) of 115 cases were positive (red color change) for dermatophytes in DTM, and 85 (73.9%) were negative (no color change).

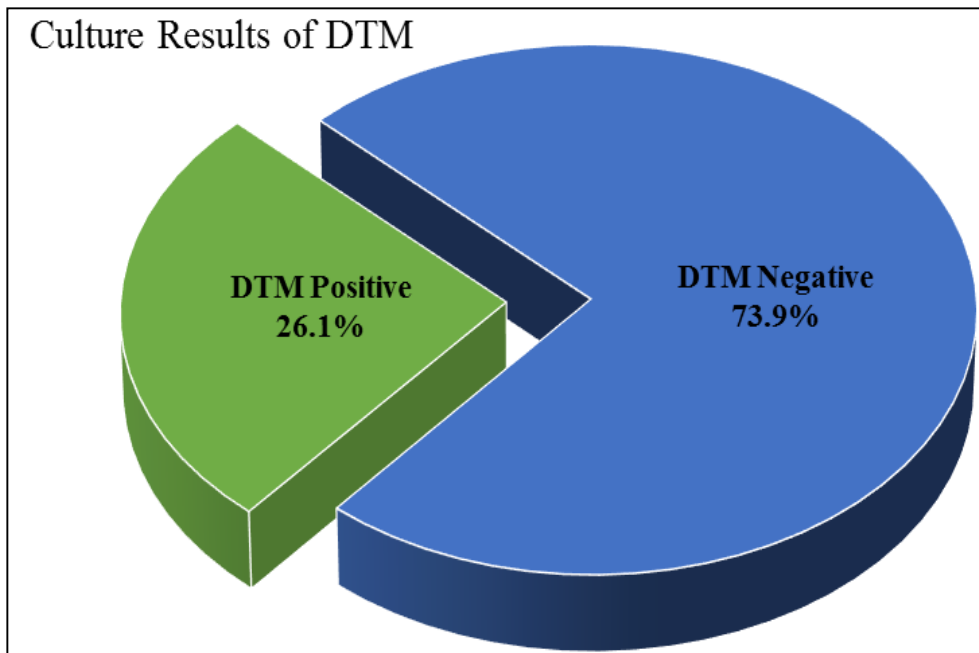


Figure (3): Distribution of Dermatophyte Test Medium (DTM) culture results.

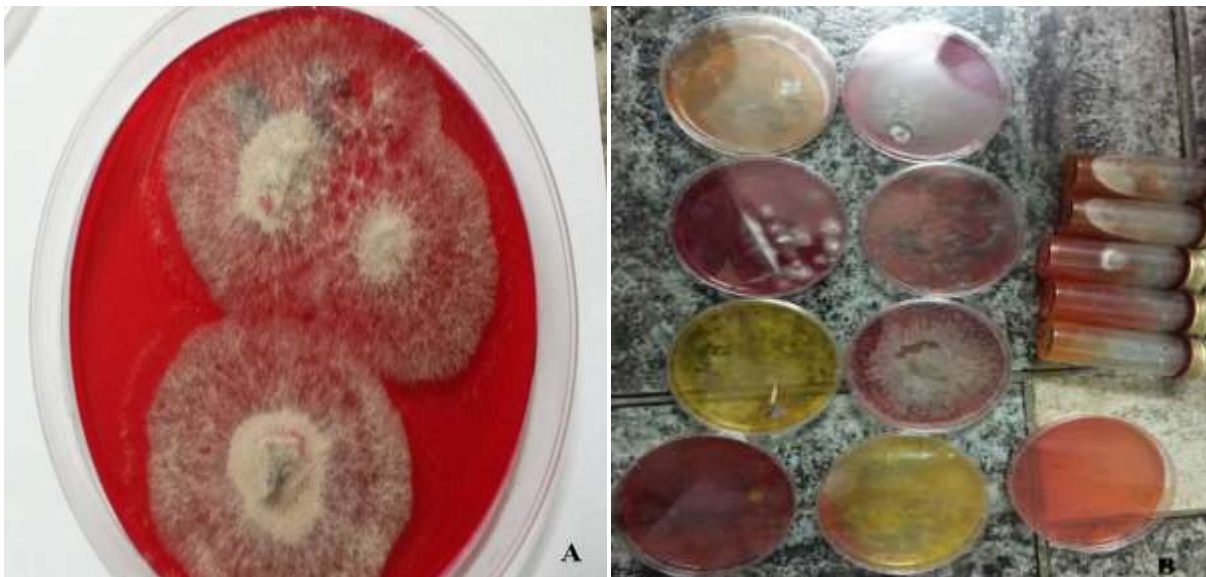


Figure (4): Dermatophyte Test Medium (DTM). A: *Microsporum spp.* on DTM (red color medium). B: DTM with positive (red color) and negative (no color change) results.

Figure (5) exhibited that the most common clinical type of dermatophytes was *Tinea corporis* in 26.7% of 30 cultured-positive dermatophyte cases, followed by *Tinea pedis* (23.3%) and *Tinea capitis* (20.0%). whereas *Tinea manuum* showed the least cases (6.7 %).

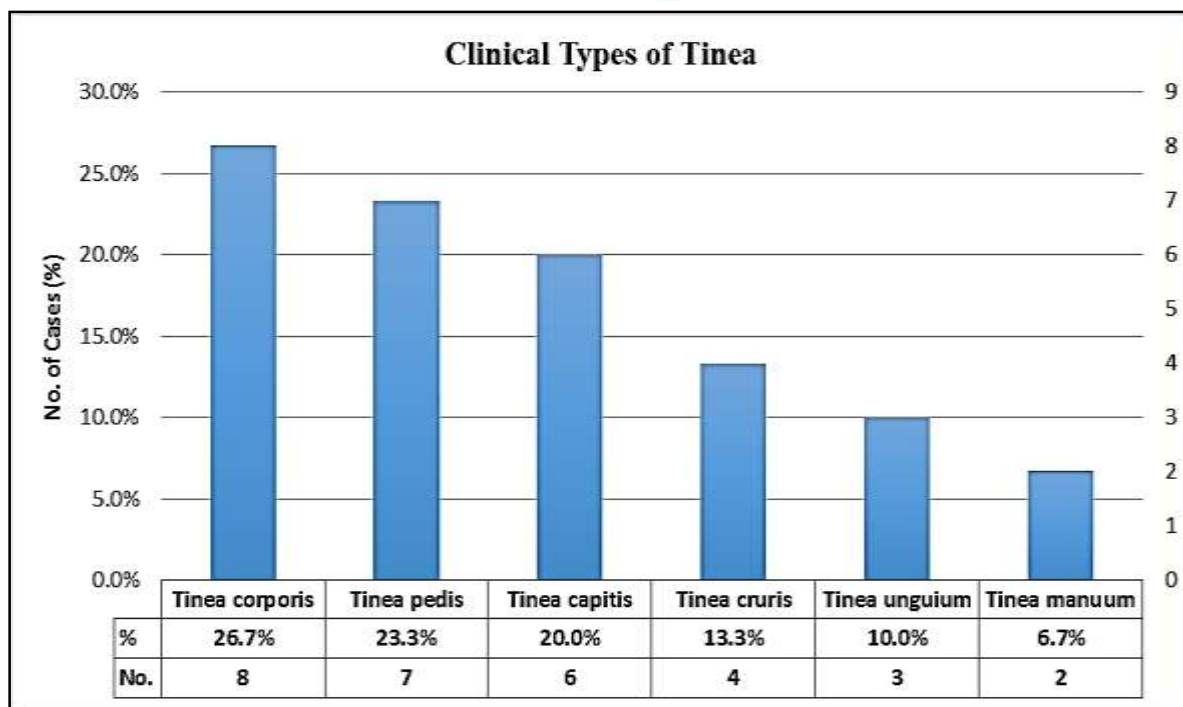


Figure (5): Distribution of clinical types of Tinea.

Table (1) exhibited that the dermatophyte infections were more common in rural (70.0%) than in urban (30.0%) areas of 30 cultured-positive dermatophytes, and Tinea corporis was the most prevalent clinical type (16.7%), whereas Tinea manuum was the lowest type (6.7%).

Table (1): Distribution of clinical type of Tinea according to residence.

Clinical Types of Tinea		Residence		Total
		Rural	Urban	
Tinea corporis	No.	5	3	8
	%	16.7%	10.0%	26.7%
Tinea pedis	No.	5	2	7
	%	16.7%	6.7%	23.3%
Tinea capitis	No.	4	2	6
	%	13.3%	6.7%	20.0%
Tinea cruris	No.	3	1	4
	%	10.0%	3.3%	13.3%
Tinea unguium	No.	2	1	3
	%	6.7%	3.3%	10.0%
Tinea manuum	No.	2	0	2
	%	6.7%	.0%	6.7%
Total	No.	21	9	30
	%	70.0%	30.0%	100.0%

Figure (6) manifested the distribution of dermatophyte species based on culture results, the most common etiologic agent of dermatophytosis was *Trichophyton mentagrophytes* (21.7%), followed by *Epidermophyton floccosum* (17.4%), then *Trichophyton bulbosum*, *Trichophyton tonsurans* (13.0%), and *Trichophyton rubrum* (8.7%). Whereas *Microsporum canis*, *Trichophyton interdigitale* and *Trichophyton verrucosum* showed the lowest percentage (4.3%).

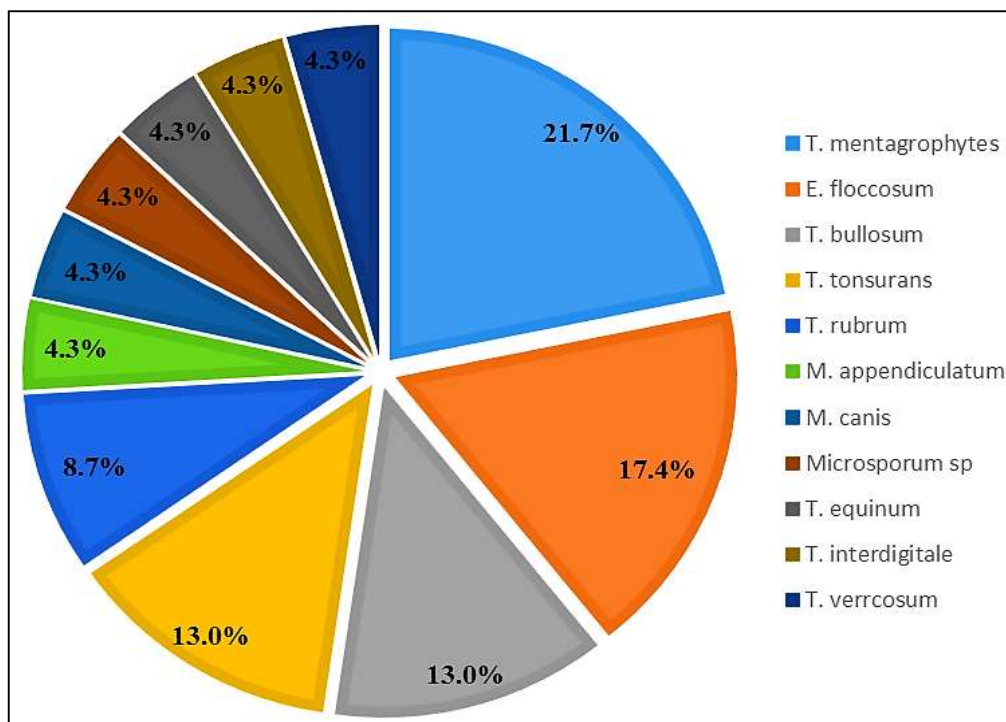


Figure (6): Distribution of dermatophyte species according to culture results

Discussion

Culture on Sabouraud's Dextrose Agar (SDA), required for species identification, was positive in 47.8% and negative in 52.2% of 115 cases. Among the culture positive isolates, dermatophytes (26.0%), *Aspergillus spp.* (14.8%), and *Candida spp.* (7.0%) were isolated as demonstrated in Figures (1) and (2). These results were in agreement with Khadka *et al.* (2016) who reported that among 111 clinical dermatophyte specimens 64% were dermatophytes on SDA, whereas 14.4% and 7.2% were non-dermatophytes (*Aspergillus spp.* and *Candida spp.*, respectively) on SDA [11]. The failure of growth on SDA, in the current study, was consistent with a study by Habeb *et al.* (2016), which found that among 220 clinical specimens of dermatophytosis 64% had no growth on SDA cultures [12]. This is probably due to many reasons such as the use of antifungal agent before specimen collection, clinical misdiagnosis, error in the sampling process or inadequate specimen [13]. In addition, the presence of saprophytic fungi which accompanied with the dermatophytes at the infected site, possibly compete with it and prevent the dermatophytes from growing in the agar medium [12]. Hayette and Sacheli (2015) reported that the most common cause of negative culture was belonged to inappropriate topical use of corticosteroid drugs, which had been taken randomly by the patients [14]. In the present study, the 115 clinical dermatophyte specimens were also cultured on Dermatophyte Test Medium (DTM), as differential and selective media for dermatophytes, which revealed that 30 (26.1%) were positive (red color change) for dermatophytes, and 85 (73.9%) were negative (no color change) as illustrated in Figures (3) and (4). The results of the current study were in agreement with Singh *et al.* [10] study in India, which revealed that DTM is a good reliable medium for the selective primary

isolation and early detection of dermatophytes from clinical specimens, which is superior to SDA in dermatophytes isolation. Figure (5) exhibited that the most common clinical types of dermatophyte was *Tinea corporis* in 26.7% of cases, followed by *Tinea pedis* (23.3%) and *Tinea capitis* (20.0%); whereas *Tinea cruris*, *Tinea unguium* and *Tinea manuum* showed the least cases (13.3 %), (10.0 %) and (6.7 %) respectively. The results of the current study were consistent with the findings by Narain and Gupta (2016), which found that *Tinea corporis* constitute the highest percentage (44.5%) among 36 cultured positive dermatophytes [15]. Najem *et al.* (2016) study in Al-Nassiriyah city was also consistent with the current results as they mentioned that the *Tinea corporis*, *Tinea capitis*, and *Tinea pedis* were the most prevalent in 110 positive dermatophyte specimens [16]. On the other hand, the findings of Teklebirhan and Bitew (2015) were inconsistent with the current results, which revealed that *Tinea unguium* constitutes the most prevalent one (51%) in 130 cultured positive dermatophytes [17]. The reason of the high prevalence of *Tinea corporis*, in the present study, may be due to various exposed parts of the body are affected as a result of exhaustive physical work and prolonged exposure to sun leading to excessive sweating. Additionally, the tight fitting and synthetic clothing, provide damp, sweaty and warm skin conditions that enhance dermatophyte infection [18]. Concerning to *Tinea pedis*, as the second clinical manifestation, may be due to wearing of socks and shoes for a long period providing damp conditions especially in inter-digital space [19]. Table (1) exhibited that the dermatophyte infections were more common in rural (70.0%) than in urban (30.0%) areas among 30 cultured positive dermatophytes. *Tinea corporis*, *Tinea pedis* and *Tinea capitis* were the most common *Tinea* infections in rural (16.7%, 16.7%, and 13.3% respectively) than in urban areas (10.0%, 6.7%, and 6.7% respectively) compared to other types of *Tinea*. The reasons of these results probably that the most of rural areas are inhabited by the families with animal husbandry such as canines and cattle, which are the source of *Tinea* infections [20]. Also, the low levels of education and sharing personal belongings such as combs, clothes, towels and shaving tools, which is common between family members in low socioeconomic levels, as well as, the overcrowding of population with poor hygiene may leading to contact between infected individuals and healthy [21]. Similarly, Hasan and Al-Shibli (2016) as well as Abed Ali *et al.* (2017) also demonstrated that the frequency of dermatophyte infections was higher in rural (58% and 52% respectively) than in urban areas (42% and 48% respectively) [22, 23]. On the other hand, Al-Hmadani *et al.* (2014) findings were antithetical to the results of the present study as they found that the *Tinea* infections were more common in urban areas (76%) than in rural areas (24%) in Al-Najaf province [24].

As shown in Figure (6), *T. mentagrophytes* (21.7%) was the most common fungal pathogen isolated from all clinical types of dermatophytosis. This may be due to variation in environmental condition and geographical distribution. Also, because *T. mentagrophytes* have greater capacity to infect the hard keratin. The results agreed with Nasimuddin *et al.* (2014) who mentioned that *T. mentagrophytes* (38.75%) was the most common isolate from the clinical samples [25], but inconsistent with Madhavi *et al* (2011) study [26] that revealed that *T. rubrum* was the commonest etiological agent (51.72%) of dermatophytosis. *Epidermophyton floccosum* (17.4%) was the second isolate from the clinical samples of dermatophytosis, while *M. canis*, *T. interdigitale*, and *T. verurcosum* all showed the lowest percentage (4.3%). *Trichophyton tonsurans* and *T. rubrum* constitute 13.0% and 8.7% (respectively) of clinical positive dermatophytes.

Trichophyton bullosum (13.0%) was also isolated from Iraqi patients with *Tinea corporis* (Figure 6). The species is a zoophilic dermatophyte, which was isolated for the first time in 1933 by Lebasque during a survey of the occurrence of equine dermatophytosis in different countries [27]. In France, Sitterle *et al.* (2012) reported the first human case of

dermatophytosis caused by *T. bullosum* in a 21-year-old male who had a skin lesion located on his forearm, phenotypically; *T. bullosum* may be misidentified as *T. verrucosum* [28].

Microsporum appendiculatum represented about 4.3% of dermatophyte infection in the current study in a patient with Tinea capitis, as mentioned in Figure (6). Appendaged strains should be referred to *M. gypseum*, and that *M. appendiculatum* should be reduced to synonymy [29]. *Trichophyton equinum* constituted about 4.3% of dermatophytosis in the current study in a patient with Onychomycosis. *T. equinum* is closely related to the most widespread anthropophilic agent *T. tonsurans* that both have abundant microconidia staining deeply with lactophenol blue and balloon forms of chlamydoconidia-like cells [30]. *Microsporum sp* comprised 4.3% of dermatophytes, in this study, in a patient with Tinea corporis. It is closely similar to *M. mirabile*, and a new dermatophyte species *M. cookie* clade [31].

In conclusion, DTM is a good reliable medium for the selective primary isolation and early detection of dermatophytes from clinical specimens, which is superior to SDA in dermatophytes isolation. Tinea corporis is the most common clinical type of dermatophytosis, and *T. mentagrophytes* is the most prevalent etiological agent of dermatophyte infections.

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