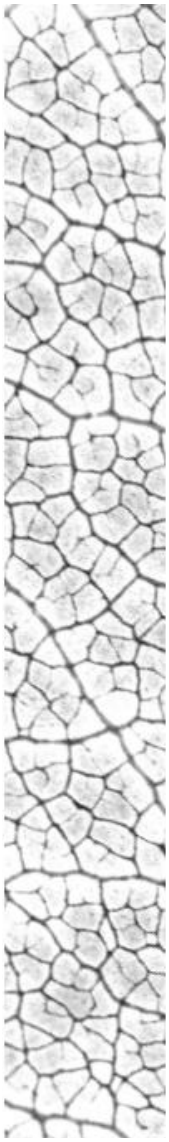

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Microbial study of Trichophyton rubrum isolated from various Tinea infections

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Abstract
Dermatophytes infections, especially those affecting the keratinized tissues, are a major concern worldwide and are increasing on a global scale. Dermatophytosis is an infection of the skin, hair, and nails as a result of colonization of the Keratin layers in the body. The factors causing dermatomycosis are classified in three distorted genera, Epidermophyton, Microsporum, and Trichophyton. This study was aimed to isolate and identify Trichiphyton rubrum from different Tinea infection using morphological features including scanning electron microscope examination and to evaluate its sensitivity towards several antifungal drugs. Seventy-three Tinea infections specimens were included in this study. All the clinical specimens were cultured onto sabouraud dextrose agar plates and potato dextrose agar plates and identified using cultural and microscopic features. The positive specimens for Trichiphyton rubrum were tested for susceptibility towards different antifungal drugs. The results showed that from all the clinical specimens (73), included in this study 25 (34.2%) were positive for Trichiphyton rubrum. Tinea corporis was the most common clinical type of Trichiphyton rubrum infection with incidence of 9 (36%). All of the Trichiphyton rubrum isolates were sensitive to Nystatin (NS 100 IU), Amphotericin-B (AP 100 IU) and Itraconazole (IT 10µg), and resistant to Clotrimazole (CC 10µg), Ketoconazole (KT 10µg), and Fluconazole (FLC 10µg). In Conclusion Tinea corporis is the most dermatophytosis caused by Trichiphyton rubrum and Nystatin is the most effective antifungal drug towered it.

Keywords: Trichiphyton rubrum, Tinea Infections, dermatophytes

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INTRODUCTION

Dermatophytosis are serious concerns around the world and grow on a global scale. These infections affect the skin, hair, and nails as a result of colonization of the Keratin layers in the body, and are caused by skin fungi that are distributed between three genera, namely Trichophyton, Microsporum and Epidermophyton (Emmons et al. 1977, Gurgel et al. 2005). Dermatophytosis can rarely be caused by Candida sp. and by non-dermatophytes fungi such as species belong to Fusarium, Scopulariopsis and Aspergillus (Pinto et al. 2006, Naveed et al. 2009). Dermatophytosis is prevalent in warm and developing countries where hot and humid weather is favorable for this infection (Rippon 1988).

Fungi belong to the genera Epidermophyton, Microsporum, and Trichophyton. Lacking sexual reproduction (Fungi Imperfecti). The description of the genera basically follows the Emmons classification scheme (Emmons 1934) based on alien morphology and conidia formation and is updated after the discovery of new species (Matsumoto and Ajello1987, Ajello 1968).

Dermatophytes fungi are distributed geographically throughout the world. The predominant cause of dermatomycosis is Trichophyton followed by Epidermophyton and Microsporum. Trichophyton rubrum is the most responsible species within the genus Trichophyton, it is responsible for 69.5% of the infections followed by T. mentagrophytes, T. verrucosum and T. tonsurans (Chen and Friedlander, 2001, Coloe and Baird, 2010) According to a World Health Organization (WHO) survey of the occurrence of skin mycosis, about 20% of people worldwide suffer from skin infections (Marques et al. 2000). Sickness does not relieve people of any age. (Vander et al. 2003) Among the most common infections are ringworm, followed by ringworm, tinea pedis, and onychomycosis. Tinea corporis accounts for about 70% of the skin infection (Pal 2007).

The source of the infection is always external. Cats and dogs can become infected with skin (animal) fungi, geological skin fungi (soil), and human (human) skin

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fungi. Direct contact with the patient and indirect contact with contaminated fomites act as a vehicle for the transmission of cutaneous fungi (Pal 2007). The genus Trichophyton includes 24 species. The dominant conidia are microconidia with scattered macroconidia (Jagdish 1995). Reverse side of the colony pigmentation is a characteristic of the species and is used to identify the species within the genus (Wagner and Sohnle 1995; Goa, 2015). Macroconidia have thin walls, with a smooth surface and variable shape (Philpot 1977).

Skin fungi can enter the host body through affected skin, scars and burns. Infection occurs through joints or conidia (Weitzman and Summerbell 1995). The pathogen invades the non-living upper keratin layer of the skin, the stratum corneum, produces exoenzyme keratinase and stimulates an inflammatory reaction at the site of infection (Wawrzkiewicz et al 1991, Muhsin et al. 1997]. The usual signs of inflammatory reactions such as redness, swelling (induration), heat, and alopecia (hair loss) appear at the site of the injury. This mo.vement of the organism away from the site of infection produces the classic annular lesion (Dahl 1994).

Infections caused by the skin fungus are usually referred to as a tinea or ringworm due to the characteristic annular lesions (White et al. 2008). Depending on the site of the injury, tinea infections are referred to as tinea capitis (infection of the scalp), tinea corporis (infect the non-hairy area of the body), tinea pedis (infect the foot or athlete's foot), and tinea ungium (infect the nail or onychomycosis) tinea mannum (infect the hands), tinea barbae (barber's itch); and tinea cruris (infect the groin or jock itch (Woodfolk 2005).

There are two methods of treatment that include topical or systemic anti-fungal agents for a fungal skin infection. To achieve a definitive and successful drug treatment for mycosis, there is an essential need for accurate identification of the pathogen at the species level (Ameen 2010, Vander et al. 2003) . According to various reports, several types of ringworm appear insufficient response to topical medication. Consequently, there are many types of ringworm that must be treated with systemic anti-fungal drugs. Amphotericin B, azole antifungal agents including clotrimazole, fluconazole, itraconazole, ketoconazole, miconazole, voriconazole, grizeofulvin, and terbinafine are predominant good options with effective antifungal activities (Kaushik et al. 2015, Fernández et al. 2001).

This study was aimed to isolate and identify Trichiphyton rubrum from different Tinea infection using morphological features including scanning electron microscope examination and to evaluate its sensitivity towards several antifungal drugs.

MATERIALS AND METHODS

Clinical isolates

Seventy three Tinea infections specimens were obtained from patients attending at Al-Sader Medical city in Al-Najaf , Al-Hussien Teaching hospital in Al-Muthana, Al-Sadar and Al-Zahraoi Teaching hospital in Messan in Iraq, and from students in students dorm at the university of Kufa campus, for the period between October 2018 and January 2019 clinical isolates were obtained by scraping the lesion of infection with sterile surgical blade at antiseptic condition, all the clinical isolates were examined for Trichophyton spp.

Culture of Clinical Specimens

All the clinical specimens were inoculated onto sabouraud dextrose agar plates and potato dextrose agar plates (HIMEDIA, India). The inoculated plates were incubated at 25 °C and held for up to two weeks. Colonies exhibiting morphologies consistent with those of Trichophyton rubrum were examined microscopically for characteristic patterns of macroconidia and microconidia.

Light Microscope Examination

A small portion of the fungus colony was placed on clean slide containing a drop of water, then covered with a coverslip and examined under 10X and 40X for characteristic patterns of septate hyphae, macroconidia and microconidia.

Scanning Electron Microscope (SEM) Examination

A small portion of the fungus colony was dried with air dryer and sputtered coated with Gold and fixed on the SEM stub then examined for characteristic patterns of septate hyphae, macroconidia and microconidia. The images were acquired with FEI Quanta 450 Scanning Electron Microscope (Netherland).

ANTIFUNGAL SENSITIVITY TEST

Six types of antifungal drug discs were used in current study: fluconazole (FLC 10µg), ketoconazole (KT 10µg), itraconazole (IT 10µg), clotrimazole (CC 10µg), nystatin (NS 100 IU) and amphotericin-B (AP 100 IU).

Disk diffusion testing was performed as described by CLSI document M44-A (CLSI 2004) as follows:

A. Inoculum suspensions: fungal inoculums suspensions were prepared in a turbidity adjusted to match a 0.5 McFarland density standard. This suspension was used to directly inoculate agar plates in the disc diffusion method.

B. Agar plates preparation: Mueller-Hinton agar supplemented with 2% glucose was dispensed in 90-mm diameter Petri dishes (20 ml medium/plate).

C. Inoculation and incubation: The agar surface was inoculated by using a cotton swab dipped in the inoculum suspension, rotated several times to remove

Table 1. Standard values of growth inhibition zones diameter [mm] of antifungal drugs used in this study based on NCCLS, M44-A2 (CLSI 2004)

Antifungal disk	Symbol	Potency	Zone diameter in mm		
			Sensitive(S)	Intermediate (I)	Resistance (R)
Amphotericin-B	AP	100 IU	≥ 15	14 – 10	< 10
Clotrimazole	CC	10µg	≥ 20	19 – 12	≤ 11
Fluconazole	FLC	10µg	≥ 19	18 – 15	≤ 14
Itraconazole	IT	10µg	≥ 23	22 – 14	< 13
Ketoconazole	KT	10µg	≥ 28	27 – 21	≤ 20
Nystatin	NS	100 IU	≥15	14 – 10	No zone

*Significant at the alpha level (p < 0.05)

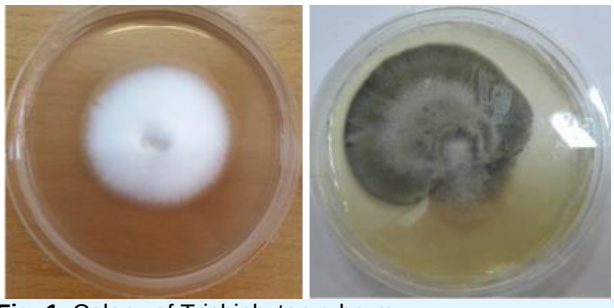

Fig. 1. Colony of Trichiphyton rubrum

excess fluid from the swab by pressing firmly against the inside wall above the fluid level. The dried surface of the agar was streaked by the swap according to the standard method ensuring an even distribution of the inoculums. The plates were left opened for 15 – 20 minutes, allowing excess moisture to be absorbed. The antibiotic disks were placed onto the surfaces of the plates by sterile forceps, and the plates were incubated at 25°C and observed after 72h.

D. Interpretation of zone size: the diameter of inhibition zone for individual antifungal drug was translated in terms of sensitive and resistant categories referring to an interpretation chart of CLSI document M44-A (CLSI 2004) as shown in **Table 1**. Inhibition zone diameters were measured in millimeters at the transition point at which growth decreased at 72 h. For azoles the inhibition zone was measured up to colonies of normal size. And for Polyenes the clear zone with no visible growth was measured.

Statistical Analysis

Statistical analysis was performed by a chi squared test with P-values of P ≤ 0.05 using Statistical Package for Social Sciences (SPSS).

RESULTS

Morphological examination of suspected isolates showed that their colonies appeared on SDA and PDA as a white-to-green, velvet colony (**Fig. 1**). These characteristics come according to those to which they belong *Trichiphyton rubrum* (Reiss et al 2012).

Light microscopic examination and scanning electron microscopic SEM examination of the slides prepared from the isolation colony showed the septate hyphae

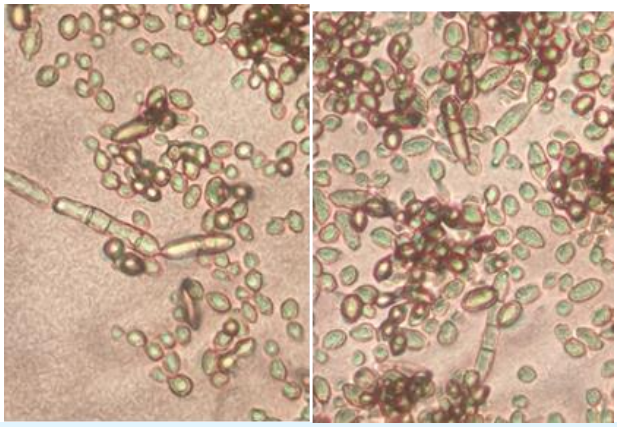

Fig. 2. The microscopic of slides of Trichiphyton rubrum 40X

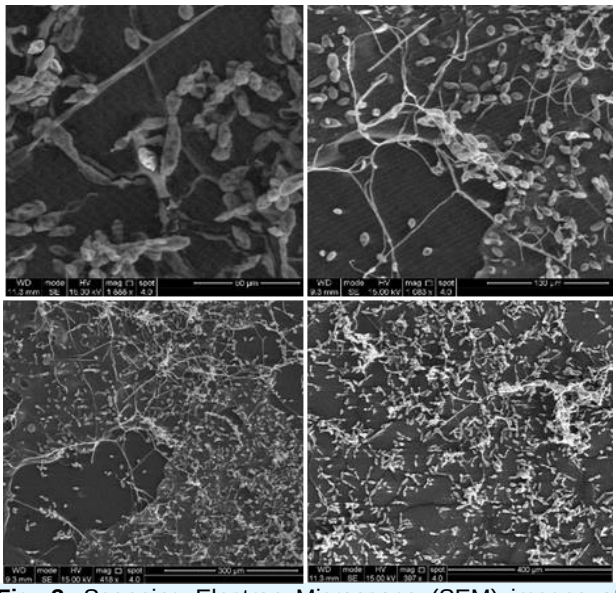

Fig. 3. Scanning Electron Microscope (SEM) images of Trichiphyton rubrum

and the spherical microconidia and the elongated soft wall macroconidia these properties come according to those that belong to Trichiphyton rubrum (Reiss et al 2012) (**Figs. 2** and **3**).

From all the clinical specimens (73), included in this study 25 (34.2%) were culture positive and slide positive (**Fig. 2**) for Trichiphyton rubrum. Out of 25 cases included in our study male were 16 (64%) and females were 9 (36%).

Tinea corporis was the most common clinical type of Trichiphyton rubrum infection with incidence of 9 (36%) followed by Tinea pedis 6 (24%), Tinea Barbae 5 (20%), Tinea manum 4 (16%) Tinea capitis was the least common clinical type of Trichiphyton rubrum infection encountered in our study seen in 1 (4%) case (**Table 2**) (**Fig. 4**).

The results in the current study revealed that all of the Trichiphyton rubrum isolates were sensitive to Nystatin (NS 100 IU), Amphotericin-B (AP 100 IU) and

Table 2. Number of Trichiphyton rubrum isolates in relation to Tinea types

Clinical types	Trichiphyton rubrum N (%)
Tinea corporis	9 (36%)
Tinea pedis	6 (24%)
Tinea Barbae	5 (20%)
Tinea manum	4 (16%)
Tinea capitis	1 (4%)
Total	25 (100%)

*Significant at the alpha level (p < 0.05)

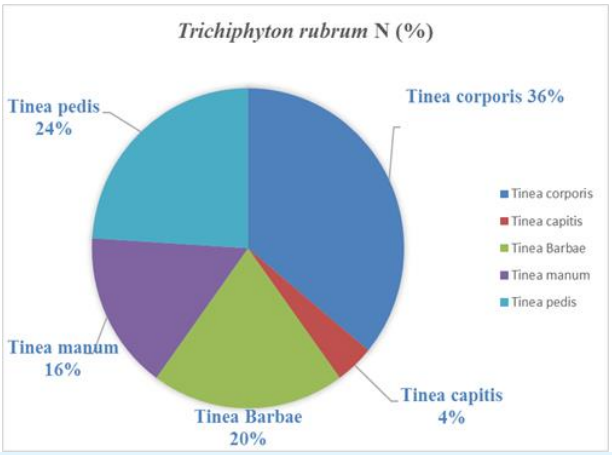

Fig. 4. Number of Trichiphyton rubrum isolates in relation to Tinea types

Itraconazole (IT 10µg), and resistant to Clotrimazole (CC 10µg), Ketoconazole (KT 10µg), and Fluconazole (FLC 10µg) (**Table 3**, **Fig. 5**), the statistical analysis (p≤ 0.05) showed a significant differences among the tested isolates after compared with the standard zone.

DISCUSSION

The present study demonstrates the clinical manifestations of dermatophytosis caused by Trichophyton rubrum, and their susceptibility patterns. It was done at the microbiology laboratory in faculty of science – University of Kufa in Najaf Governorate, Iraq.

The results showed that males (64%) are more affected than females (36%), and are similar to studies conducted by Anupama (Anupama 2017), Singh et al. (Singh and Beena 2003). The increased prevalence in males may be due to occupational risks related to the nature of their work and an increased risk of infection. In this study, tinea corporis was the most common clinical type in 36% of patients, which can be compared to studies by Noronha et al. (Noronha et al. 2016) and Surendran et al. (Surendran et al. 2014). Tinea pedis was the second most common clinical type in the current study, as it was seen in 24% of patients, which is consistent with Findoh et al. (Lyngdoh et al 2013).

Table 3. The diameter of growth inhibition zone of Trichophyton rubrum toward different antifungal drug disks.

Antifungal disks	NS	KT	AP	FLC	CC	IT
Growth inhibition zone diameter (mm)*	34 (S)	12 (R)	22 (S)	0 (R)	0 (R)	28 (S)

*Significant at the alpha level (p < 0.05).
** Significant at the alpha level (p < 0.05)
N.S. Non-significant

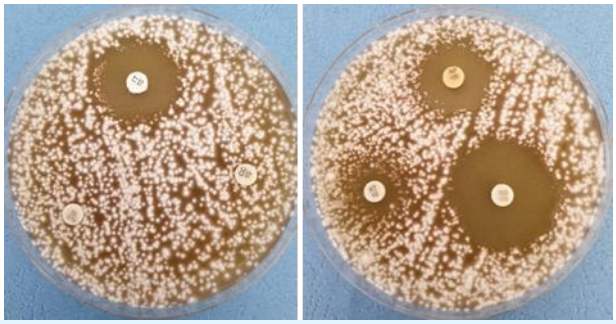

Fig. 5. Growth inhibition zone of Trichophyton rubrum toward different antifungal drug disks

The results of the current study showed that all Trichiphyton rubrum isolates were sensitive to Nystatin, Amphotericin-B and Itraconazole, and are resistant to Clotrimazole, Ketoconazole and Fluconazole. These results are agreed with the results of Sinha and Sardana (Sinha and Sardana 2018) which found that amphotericin-B and nystatin were better than ketoconazole and fluconazole against all the tested skin fungi, and Fernandez-Torres et al. (Fernández-Torres 2001) those who compared ten antifungal drugs versus 508 skin fungus isolates found that amphotericin-B was superior to fluconazole. Coelho et al. (Coelho et al. 2008) comparison of in-vitro antifungal sensitivity from T. rubrum and T. tonurans to Amphotericin-B, fluconazole, terbinafine, itraconazole, and griseofulvin. They found that AMB was the most superior drug.

CONCLUSIONS

Tinea corporis is the most dermatophytosis caused by Trichiphyton rubrum. Nystatin is the most effective antifungal drug towered Trichiphyton rubrum followed by Amphotericin-B and Itraconazole.

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