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A STUDY OF PHENOTYPIC CHARACTERISTICS AND THE EFFECT OF SOME ANTIFUNGALS AGAINST THE FUNGUS TRICHOPHYTON MENTAGROPHYTES THAT CAUSES TINEA CORPORIS IN KIRKUK CITY

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Abstract

This study was conducted in the graduate studies laboratory in the College of Science at the University of Kirkuk for the duration 7/11/2021 to 5/25/2022 with the goal of investigating dermatomycosis fungi in the human body (tinea corporis) and the incidence of Trichophyton mentagrophytes. a hundred and twenty samples were accrued from sufferers attending a dermatology consultation at Azadi Teaching Hospital and some personal clinics in the city of Kirkuk between (1 - 60)age groups and for each genders. The samples included taken from areas of the pores and skin in quite a number parts of the physique and under the direct supervision of the professional doctor. Skin samples were taken scraping method, The affected area was sterilized with ethyl alcohol at a awareness of 70%, and then the scales were scraped from the side of the fungus-infected focal point using a sharp sterilized blade, and the samples were recognized by means of direct microscopy the use of koh at a attention of 10%, and the closing part of the pathological samples was once cultivated) on dishes containing saproid dextrose agar (SDA) medium to which cycloheximide was added. To forestall the increase of saprophytic fungi and chloramphenicol to forestall the growth of bacteria, the occlusion was checked continuously to note fungal growth. Direct microscopic examination of fungi confirmed wonderful results, with an contamination fee of 66.66% of the complete one hundred twenty samples, whilst laboratory subculture confirmed high quality results, with a rate of 49.16% of the total of one hundred twenty samples. The results of the phenotypic examinations of the remoted dermatophytes confirmed (they belong to both genes, Trichophyton and Microsporum). All samples were recognized by using common techniques the usage of unique way of life media and based on the phenotypic characteristics of the colonies in addition to the microscopic characteristics. The outcomes of the current study showed that T.mentagrophytes is greater frequent among patients suffering from tinea corporis than other types of skin fungi, with a rate of (9.2%). And the percentage of infection with skin fungi differed according to the region of residence, as it used to be extra common in the rural area (62.5%) and the lowest number of infections in the town (37.5%). A study used to be conducted of the effect of antifungals nystatin, amphotricin B, ketoconazole, fluconazole and clotrimazole in opposition to T.mentagrophytes, as the effects confirmed that the antifungal ketoconazole had higher inhibition on T.mentagrophytes compared to different antifungals and the least antifungal nystatin inhibited the fungus by means of a small percentage.

Keywords: Dermatophytes , Trichophyton mentagrophytes ,Antifungals .

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Introduction

Fungal infections ensuing from dermatophytes fungi are known by a number of names, such as ringworm, tinea, or dermatophytosis, which are extraordinarily contagious skin diseases for humans and animals (pal, 2017). 20-25% of pores and skin infections (Sahoo and Mahjan, 2016). Skin fungi want keratin to grow and they infect (areas wealthy in keratin because the enzyme keratinase is secreted such as the skin, nails and hair, and the contamination happens either through direct contact with anthropophilic people, zoophilic animals, or geophlic soil, or the contamination is in a roundabout way via clothes and tools infected with the fungus) farag et al., 2018), these dermatophytes purpose severe infection of the host, and include three genera, trichophyton, epidermophyton, and microsporium, and these genera cause infections of the skin, hair, and nails (Reddy, 2017). Fungal infections are chronic due to their gradual growth, and these infections are (the most important cause) of life-threatening infections for human beings with susceptible immunity. The title of skin fungi depends on the vicinity of the infection in the body, or ringworm, which means ringworms, due to the fact the contamination often takes the form of a ring with (inflammation). Edges and a clear core of regular pores and skin with the presence of fungal elements in an lively country at the side of the lesion, and it is preferable to take a sample from it upon microscopic prognosis (Al-janabi, 2014). Trichophyton mentagrophytes is a frequent dermatophyte fungus that causes skin diseases in humans and other animals (Quiñones et al., 2016). It (belongs to the genus Trichophyton, of the household Arthrodermataceae, of the order Onygenales, of the type of Eurotiomycetes, of the phylum Ascomycota. The high frequency and incidence of fungal infections. Several antifungal capsules had been used, but the fungi showed resistance to antifungal antifungals. Most of the antifungals did not be triumphant in treating several cases (Hilmar et al., 2014). The danger of antifungal resistance (and the challenges concerned in the improvement of antifungal drugs) is due to the reality that all (fungi, humans, and animals are eukaryotic organisms) that made researchers take advantage of alternative remedies (Jamiu et al., 2021).

Materials and Methods

Sample Collection

This learn about was conducted in the laboratories of the Department (Life Sciences / College of Science / University of Kirkuk. 120 clinical samples have been gathered from human beings infected with pores and skin fungi from a dermatology advisor in (Azadi Teaching Hospital and Health Center in Kirkuk Tasfirat Prison) and some personal clinics for a length between 7/11/2021 And till 25/5/2022, when samples had been taken from areas of the pores and skin in a range of components of the body, under the direct supervision of the professional doctor, for a while between(1 - 60)age groups and for each genders. Skin samples had been scraped the usage of scrapping method, as the contaminated place was sterilized with ethyl alcohol at a concentration of 70%, and then the crusts had been scraped from the part of the fungus-infected focus the usage of a sharp sterilized blade, and transferred to the laboratory (fungi in the College of Science / University of Kirkuk) for the purpose of examination and cultivation, and a questionnaire used to be allocated for each patient containing some scientific and

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private statistics about the auditors. . Diagnosis of isolated samples by direct method and culture of samples according to a researcher(Ellis,1994).

Test for sensitivity of t.mentagrophytes to antifungals: 1- Prepare medium dishes (SDA) containing chloromphenicol and cycloheximide. 2- Take 1 cm of the fungal tissue of an active colony (and add it to 3 ml of distilled water or sterile normal saline, mix the sample well until the suspension is formed) of the isolated fungi. 3- Put the suspension of fungi on medium dishes (SDA) and leave the dish for 10 minutes until it absorbs moisture. 4- Take with tweezers the anti-fungal tablets (clotrimazole, amphotericin B, ketoconazole, fluconazole, nystatin), and place them on the center of the inoculated SDA, so that each tablet is 2.5 cm away from the center of the plate and from the edge. 5- The dishes were placed in an incubator at a temperature of 28 0 C for a period of 5-10 days, and the results were read by measuring the inhibition zone around the antifungal tablets (Al-Bajilan, 2016).

Results and Discussion

patient samples were collected from clinically diagnosed infections by specialized doctors for patients with dermatophytes and some private clinics for the period between 7/11/2021 to 25/5/2022 for age groups between (1-60) age groups and both genders. The results of direct microscopic examination appeared. The presence of 80 positive skin samples with a percentage of 66.66% of the total samples, and 40 negative skin samples for examination, i.e. 33.3%, while the results of laboratory culture on the medium of SDA showed the presence of 59 positive samples and (1- negative samples with a percentage of(49.16)% and 50.84%, respectively. In Table 60)., the reason for the appearance of negative samples for microscopic examination may be an error in the sampling process or the method of storing the sample during its transportation to the laboratory, or due to taking a small amount of the sample so that it is not sufficient to give a positive result, in addition to the presence of saprophytic fungi. Saprophytic fungi that grow with dermatophytes at the site of infection that may compete with them for nutrients and prevent dermatophytes from growing on the SDA medium (Habeb, 2016) .

Table 1. Represents the infection of dermatophytes based on direct microscopic examination and laboratory culture.

Total summation	Negative	sample	Positive Sample		Test type
100 %	33.3 %	40	66.7 %	80	**Direct microscopy**
100 %	50.84 %	61	49.16 %	59	**Laboratory culture**

The relationship of infection with skin fungi and the vicinity of residence:

The results of the study confirmed that the proportion of (infection with skin fungal infections) differed (according to the residential location (rural, city) as proven in Table 2), the place the perfect infection rate was in the rural areas via 62.5% (75 isolates), and the lowest contamination fee in the urban areas by 62.5%. 37.5% ((45 isolates), the cause for the increase in the incidence of contamination in rural areas is due to the deteriorating health condition ensuing from lack of attention and awareness, or it

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may additionally be the purpose for human contact with zoophilic contaminated animals and therefore transferring it to humans due to the fact the genus T.metagrophytes is an animal-loving fungus or Human contact with soil contaminated with scales fallen from infected animals, or contact with soil containing geophilic fungi (Al-naeemi, 2007). This result consents with (Muhammad, 2015), which found the infection charge in rural areas 61.11% and urban areas 38.89%, and this learn about does now not agree with (Mustafa, 2009), which found the infection rate in rural areas 64.8% and city areas 35.2%. City areas compared to the countryside because of the (large population density) in the town of Kirkuk and the common interaction and friction between people. Also, this end result does no longer agree) with the findings of (Hamad,2008) and (Al- Taie, 2001) the place they determined that there used to be no difference) between the wide variety of injuries In the geographical region and the city, the cause (the difference in the effects is the occurrence of infection between people who live in the countryside and the city is the country of continuous contact between them and the animals that stay with them, as in Picture No. 1.

Table 2. Represents a relationship between infection with skin fungi and the area of residence (city, countryside)

percentage	The number of isolates	Housing area
62.5 %	75	countryside
37.5 %	45	city
100 %	120	The total

B A C

Picture No. 1 represents people infected with skin fungi, according to housing, with Tinea corporis. A- A 32-year-old man with a fungus in his foot while traveling in Kirkuk. b- A 45-year-old female infected with her hand in the villages of Laylan sub-district (Rural area). c- A 29-year-old girl infected with a fungus in her hand in the center of the governorate.

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Study the effect of antifungals on T.mentagrophytes.

The current study was tested for the sensitivity of T. mentagrophytes towards some types of antifungals, namely nystatin, amphotricin B, ketoconazole, fluconazole, and clotrimazole using antibiotic discs. The results of the study showed the inhibitory effect of five types of antifungal against T.mentagrophytes, where the effect of ketoconazole had the highest inhibitory activity against T.mentagrophytes, as the average diameter of inhibition (for three iterations) was 5.73 mm. This is a result consistent with (Al-Zubaidi, 2019), where he found the highest inhibitory activity against T.mentagrophytes during his study, the diameter of inhibition of the fungus was 15 mm, and also consistent with (dos santos et al., 2021) As for the anti-clotrimazole, the anti-clotrimazole showed inhibitory effectiveness against the fungus T.mentagrophytes, as it reached an average of The diameter of inhibition was 3.19 mm. Fluconazole also showed inhibitory activity against T.mentagrophytes, as the average diameter of inhibition was 2.33 mm, followed by amphotricin b. The average diameter of its inhibitory effectiveness against T.mentagrophytes was 1.23, while the anti-nystatin had the least inhibitory effect on the fungus, as it amounted to 1.23 mm. The average diameter of its inhibitory activity is 0.8333 mm, which is consistent with (Al-Zubaidi, 2019), where during his study he found an average diameter of 5 mm for the anti-fungus T.mentagrophytes. This result does not agree with (Al-Khazali, 2021), which found during her study that the anti-nystatin that is resistant to the fungus T.mentagrophytes.

Table 3 represents the inhibitory activity of some antifungal against T.mentagrophytes (the unit of measure is mm).

Zone inhibition rate MIC	inhibition zone 3	inhibition zone 2	inhibition zone 1	concentration	Antifungal
0.8333	0 mm	1.5	1	100I.U	**Nystatin**
1.2333	1 mm	1.5	1.2	20 ml	**Amphotricin B**
5.7333	6 mm	5.85	5.35	10 ml	**Ketoconazole**
2.3333	2.75 mm	2	2.25	25 ml	**Fluconazole**
3.1900	3 mm	3.12	3.45	50 ml	**Clotrimazole**

The antifungal ketoconazole showed the best possible inhibition exercise for T.mentagrophytes, with a mean of 5.7333, accompanied via the anti-clotrimazole, with an common of 3.1900mm, observed by using the anti-fluconazole, with an common of 2.333mm, followed through the anti-amphotricin b, with an common of 1.2333mm, and the anti-fungal nystatin with the lowest inhibitory undertaking (for the fungus). The arithmetic mean was once 0.76376mm, as proven in Table 4 The distinction between the results of the contemporary find out about and the outcomes of other research in terms of sensitivity and resistance to the difference in the environmental area and the immoderate use of antibiotics and antibiotics, as properly as relies upon on healthy and unhealthy personal practices (aslam, 2016).

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Table 4: Average inhibition diameters of some antifungals on T.mentagrophytes, using the results of statistical analysis.

Descriptives						
inhibition zone						
	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Nystatin100I.U	3	d 0.8333	0.76376	0.44096	-1.0640	2.7306
Amphotricin B 20 ml	3	d 1.2333	0.25166	0.14530	0.6082	1.8585
Ketoconazole 10 ml	3	a 5.7333	0.34034	0.19650	4.8879	6.5788
Fluconazole25 ml	3	c 2.3333	0.38188	0.22048	1.3847	3.2820
Clotrimazole50 ml	3	b 3.1900	0.23302	0.13454	2.6111	3.7689

The letter difference consequences indicated that there is a huge distinction between antibodies using Dunkin's a couple of range check at the stage of 5%. The purpose for the resistance of T.mentagrophytes to the used antibiotic is that it has obtained (resistance due to the repeated and indiscriminate use of pills via the affected person except consulting the specialist doctor, which leads to the pathogen acquiring defense mechanisms and virulence factors that lead to the pathogen's resistance to the antibiotic used (Alexander et al., 2013) , as the repeated and random use (led to the pathogens overcoming) the effect of the antibiotics and gaining resistance against the used treatment, or it can also have an effect on the consequences of the antifungal sensitivity test in accordance to the dimension of the fungal inoculum, the incubation period, the temperature of the medium (used in the test) (Berkow et al., 2020) . Mechanisms of innate drug resistance consist of alteration and overexpression of the drug target and development of other pathways for targeted erogosterol production (Jamiu et al., 2021). Also, lengthy publicity to the concentrations used towards dermatophytes of the azoles group led to cure failure and continual infections (Roana, 2021), Can be explained changes in drug sensitivity over time and their variations from one species to any other and from one species to some other and within one species through the capability of fungi (dermatophytes) to undergo the toxic results of antibiotics and their capacity to enhance resistance mechanisms. The antifungal sensitivity test plays a quintessential position in testing the fantastic antifungal for the remedy of fungal infections (Gandhi et al., 2015), which leads to a discount in morbidity and mortality costs among patients, that the frequent antifungal test must be carefully after conducting a diagnostic take a look at The fungal and confirmatory test for the diagnosis of fungi to recognize their sensitivity (Mahboob et al., 2019). The antibiotic disc occurrence test is a widely used take a look at that is fast, inexpensive, requires (few equipment) and is additionally effortless to interpret (results as in Figure 2), and is an ideal take a look at technique for fungal isolates (berkow et al. .,2020).

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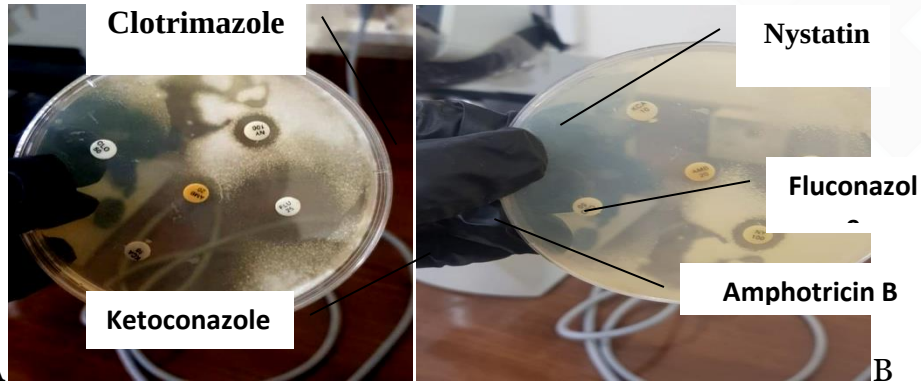

Figure 2 represents the effect of an antifungal susceptibility to T.mentagrophytes. A- Represents the front side of a dish showing the inhibition zones. B- Represents the back side of the dish.

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