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Optimization and Isolation of Dermatophytes from Clinical Samples and *In Vitro* Antifungal Susceptibility Testing By Disc Diffusion Method.

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Research Article

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ABSTRACT

India is a large subcontinent with remarkably varied topography, situated within the tropical and subtropical belts of the world. In the study patients with Tinea infections were examined clinically by dermatologist. Isolation, confirmatory test were done as per the standard procedure, and Antifungal Susceptibility test was done by Disc diffusion method.Other conditions such as seborrheic dermatitis, psoriasis, alopecia areata, folliculitis and pseudopelade may mimic ringworm of head and other tinea must be identified. A total of sixty six patients of dermatophytosis were studied. Males were predominantly affected 51 (77%) cases as compared to females15 (23%) cases. Male to female ratio was 3.4:1. Most common age group affected was 21-30 years with 20 cases (23%). Least common age group affected was 61-70 years with 3 cases (4%).Tinea corporis was more common in the age group 21-30 years with 13 cases (37.14%) and in males with 29 cases (82.85%) than females with 6 cases (17.15%).Tinea unguium was more common in the age group of 31-40 years with 6 cases (37.5%) and in males with 10 cases (62.5%) than females with 6 cases (37.5%).Tinea cruris was more common in the age group 51-60 years with 2 cases (40%) and was more common in males with 5 cases (100%).In tinea pedis, one case was seen in the age group of 11-20 years and the other in the age group of 41-50 and 51-60 years, and was more common in males with 3 cases (100%).Tinea barbae was more common in the age group 21-30 years with 2 cases (66.66%) .Tinea capitis was more common in the age group of 31-40 years with 2 cases (66.66%) and was more common in females with 3 cases (100%). Tinea manuum was more common in the age group of 31-40 years and in males with 1 case (100%). In males, commonest infection was T. corporis while in female commonest infection was T.corporis.rate of direct microscopy and culture (78.79%). About 89.47% of the dermatophytes grew faster in DTM with compare to SDA, so the growth rate of dermatophyte is better in DTM. A total of thirty five species of dermatophytes were isolated and identified. *T.rubrum* 15(42.85%) is commonest among other isolates.Ketoconazole showed best susceptibility i.e 26 (74.28%). The present study suggests that every patient of tinea infection should be properly studied for mycological examination and should be treated accordingly. This study revealed that Ketaconazole highest susceptibility.DTM is better medium than SDA.

INTRODUCTION

The dermatophytes are a group of fungi that invade the superficial layer of the epidermis and degrade the keratinized tissues of skin, hair, and nails in living animals including man [1,2]. Infections caused by these fungi are also known by the names "Tinea" and "Ringworm." It is important to emphasize that "ringworm" is not caused by a worm, but rather by a type of fungus called 'Dermatophyte' [3]. The species of dermatophytes are differentiated by Microconidia & Macroconidia. Clinically, ringworm can be classified depending on the site involved. These include Tinea capitis (scalp), Tinea corporis (non-hairy skin of the body), Tinea cruris (groin), Tinea pedis (foot) or athlete's foot and Tinea barbae or barber's itch (bearded areas of the face and neck). Favus is a chronic

type of ringworm involving the hair follicles [4]. The diseases caused by non-dermatophytic fungi infecting skin are called as dermatomycoses whereas hair and nail are known as piedra and onychomycoses.An example of a very common dermatophyte infection is athlete's foot, which is also called tinea pedis. Another common dermatophyte infection affecting the groin area is jock itch, also known as tinea cruris [5].

The organisms are transmitted by either direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair brushes, clothing, furniture, theatre seats, caps, bed linens, towels, hotel rugs, and locker room floors. Depending on the species the organism may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a pre-existing injury to the skin such as scars, burns, marching, excessive temperature and humidity.

Depending on their habitat, dermatophytes are described as anthropophilic (human), zoophilic (animal) or geophilic (soil). Anthropophilic dermatophytes are the most common sources of Tinea infections [6,7,8,9,10,11,12] *Trichophyton rubrum*and *Trichophyton tonsurans* are two common dermatophytes. T. rubrum found in face, trunk, beard area, nails, feet and groin area infection. T.tonsurans found in endothrix and black dot infection. These two species are usually transmitted from person to person. Another common dermatophyte is *Microsporum canis*, which is transmitted from animals such as cats and dogs to humans [13,14,15,16,17,18,19,20,21]. Dermatophytes like to live on moist areas of the skin, such as places where there are skin folds. The dermatophyteinfection that affects the scalp and hair is known as tinea capitis. It is especially common among school-aged children. For reasons that are not well understood, tinea capitis does not usually occur after puberty. In the recent times few cases of subcutaneous and deep fungal infections have been reported to be caused by dermatophytes. It has been noted that dermatophyte infections are more common in adolescents and adults [3].

Cutaneous dermatophyte infections are common in the general population with up to 20% of people being infected at any time. However, adults are generally less susceptible to skin infection than are children owing to the fungistatic properties of fatty acids in the sebum. Most of these infections are not life threatening, but they can cause morbidity in immunocompromised, diabetic patients, people who use communal baths, and people who are involved in contact sports such as wrestling.[2] Outbreaks of infections can occur in schools, households and institutional settings. Such infections can spread usually through direct contact with an infected person or animal, clothing, bedding and towels can also become contaminated and spread the infection. Dermatophyte infections can affect the skin on almost any area of the body, such as the scalp, legs, arms, feet, groin and nails. These infections are usually itchy, redness, scaling, or fissuring of the skin, or a ring with irregular borders and a cleared central area may occur. If the infection involves the scalp, an area of hair loss may result. More aggressive infections may lead to an abscess or cellulitis. Areas infected by dermatophytes may become secondarily infected by bacteria. Symptoms typically appear between 4 and 14 days following exposure [3]. Studies regarding use of culture media which are optimally suited for isolation of dermatophytes are very limited in Navi Mumbai.

The present study addresses the two points (1) Selection of an optimal medium for conidial formation by dermatophytes and (2) the validations of the method with a large number of dermatophytes. Dermatophytosis, mycotic infection caused by dermatophytes are commonly related in tropical countries and represent an important public health problem yet unresolved. Though there are several antifungal drugs used to treat dermatophytosis, some infections respond well to topical antifungal therapy, whereas others like tinea capitis, tinea unguium (nail infection), and more extensive or severe types may require systemic therapy. Occasionally, in some cases, antifungal therapy is a failure because of resistance to theantifungal drugs by the fungi. Although recent antifungal agents have high success rate in treating these conditions, lack of clinical response may occur in 20%.

Antifungal susceptibility testing is performed to provide information to allow clinicians to select appropriate antifungal agents useful for treating a particular fungal infection.[7]For a definitive therapy also it is essential to evaluate the resistant dermatophytes using a standardize, simple and reproducible in vitroassay to determine the antifungal activity of drugs against isolates. In vitroantifungal susceptibility tests are now mainly used for epidemiological surveys, determination of the degree of antifungal activity, and the prediction of clinical outcome based upon an optimization of antifungal therapy [5]. A few antifungal agents are available and licensed for use in veterinary practice or human being treatment. The use of systemic drugs is limited to treat man or animal due to their high toxicity and problems of residues in products intended for human consumption.Different treatments have been recommended to control dermatophytes [6]. Several methods have been developed for testing antifungal agents against this group of pathogens. Multicenter studies to develop a standardized antifungal susceptibility assay were initiated by the Clinical and Laboratory Standards Institute (CLSI, formerly 'National Committee for Clinical Laboratory Standards', NCCLS) in1983. Dilution tests are widely used in macro- and micro-assays, but these methods are difficult to be used in most laboratories. Recently, studies were done to establish a simple method to solve this problem. The agar-based disk diffusion (DD) susceptibility method for dermatophytes is simple, inexpensive, and does not require specialized equipment. The disk diffusion method has a good correlation with the reference dilution assay. The main aim of this study is to determine *in vitro* activity of four antifungal drugs that are most commonly used to treat dermatophytosis; Miconazole (MIZ), Clotrimazole (CTZ), Fluconazole (FLZ) and Ketoconazole (KTZ). Hence the present study is being undertaken to evaluate the optimum method for rapid isolation of dermatophytes and to study its resistance to antifungal drugs [5].

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MATERIAL AND METHOD

The infected skin, hair and nail sample were collected, KOH mount of the samples were prepared and cultured on SDA+CC and DTM medium for isolation, after isolation slide culture and urease test is done for the identification of the dermatophytes.

Selection of cases

Inclusion criteria

Patients of all age groups and of both sexes, attending Skin and Venereology outpatient department of MGM hospital, Kamothe, Navi Mumbai were taken for the study.

Exclusion criteria

Patients already under antifungal treatment were excluded from the study group.After the detailed history, clinical examination of patient was made in good light which included site of lesion, number of lesions, types, presence of inflammatory margin, etc.

Specimen collection

The affected area was cleaned with 70% ethyl alcohol, skin scales, crusts and pieces of nail were collected in clean black paper and in the case of hair collected on clean white paper packets.Skin specimen was collected by scraping across the inflamed margin of lesion into the apparently healthy tissue.Nail specimen was collected by taking clippings of the infected part and scrapings beneath the nail. Hair specimen was collected by plucking with epilating forceps along with the base of the hair shaft around the follicle.

Direct microscopic examination

KOH mount

a) Emulsify the specimen in a drop of 10% or 40% KOH on a microscopic slide with help of a straight wire.
b) Apply gentle heat by passing the slide over a Bunsen flame for 3-4 times.
c) Cover the smear with cover slip.
d) Leave it for 10-15 minutes. But in the case of hair or nail wait for overnight.
e) Examine the slide under low power (10X) and high power (40X) magnification.
f) Examine the slide for 15-20 minutes for demonstration of shining fungal elements.

Culture

After direct microscopic examination, irrespective of demonstration of fungal elements, the specimen was inoculated onto two sets of test tubes, one containing Sabouraud's dextrose agar with 0.05% chloramphenicol and 0.5% cycloheximide and the other to Dermatophyte test medium.

Sabouraud's dextrose agar with chloramphenicol and cycloheximide

The standard medium for growing dermatophyte is Sabouraud's dextrose agar containing chloramphenicol and cycloheximide, which inhibit bacteria and saprophytic fungi respectively. The cycloheximide (Actidione) in a concentration of 0.1 to 0.4 mg per ml suppresses the growth of most saprophytic fungi such as Scytalidium, Hendersonula, Aspergillus, Candida species without deterring the growth of dermatophytes.

Dermatophyte test medium

Specimen from skin, hair or nail were inoculated directly onto DTM and incubated at room temperature with the cap of the culture tube loose. Dermatophytes change the medium from yellow to red within 14 days. Care must be taken in specimen collection and interpretation of result, as many contaminants and other fungi increase the number of false positive changes in color. DTM does not interfere with macroscopic morphology and microscopic characteristics of the dermatophytes, but it cannot be used to study pigment production because of the intense red color of the indicator.

Macroscopic examination of culture

The growth on Sabouraud's dextrose agar was examined to study the colony morphology based on following characteristics.

Colony characters on obverse

The colour (white, pearl, ivory) and consistency (cottony, velvety, fluffy, suede).

Colony characters on the reverse

Presence or absence of pigment, whether diffusing or not.

Microscopic examination of culture

Tease mount

The tease mount was observed under low and high power objective of microscope, for the presence of hyphae, macroconidia, microconidia and other accessory structures of vegetative hyphae and the characters of each was noted (Fig 1, 14-19). Place a drop of lactophenol cotton blue on a clean glass slide.Remove a small portion of the colony and the supporting agar at a point between the centre and periphery and place it in the drop of LPCB.With a needle, tease the fungal culture first and spread in the LPCB and cover with cover slip.Examine microscopically after giving sufficient time for the structure to take up the stain, usually 30 minutes. Examine the slide under low power (10X) and high power (40X) magnification.

Slide culture of fungal isolates

From the petri dish containing Potato dextrose agar cut out 1 cm square block of agar for each slide culture to be inoculated.With a flat side of a sterile bacteriological loop, or with a spatula, place an agar block in the centre of the slide in the slide culture set up.With a probe, inoculated around the periphery of the agar block, three to four fragments of the mould to be cultured.With forceps, the tips of which have been flamed, place the cover slip on the agar block.With a pipette, thoroughly moisten, but not to excess, the filter paper with sterile distilled water.Incubate the slide culture at room temperature.Remove the slide culture from the petri dish and dry the bottom of the slide with a tissue.When growth appears beneath the cover slip, take a slide place a drop of LPCB on it, and place the cover slip removed from the block on the LPCB.Place the slide on the microscope stage and examine. The aerial hyphae including the conidiophores will be seen to grow along the undersurface of the cover slip (Fig 2).

Urease test

Pick up the growth of fungi from culture tube.Inoculated Christensen's urea agar slope with this fungal growth.Incubate the tube at room temperature for 2-4 days. Observe any change of colour in the inoculated medium (Fig 3).

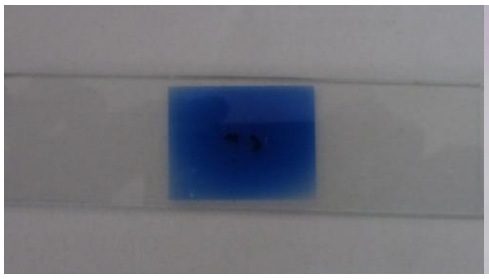

Figure 1: Lactophenol cotton blue mounts

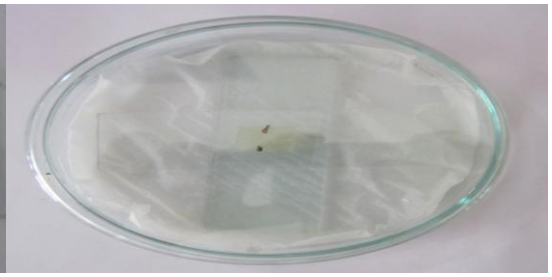

Figure 2: Slide culture on potato dextrose agar

Figure 3: Urease test showing positive & negative reactions

Antifungal Susceptibility test

Anti-fungal susceptibility test will be carried out as per the methodology described by CLSI guide lines (Clinical and Laboratory Standards Institute, formerly 'National Committee for Clinical Laboratory Standards', NCCLS). Agar based disc diffusion susceptibility method was performed using the antifungal agents such as Griseofulvin, Miconazole, Terbinafine, Clotrimazole,

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Fluconazole and Ketoconazole.Sixty –six sample of skin, hair or nail from clinically suspected case of dermatophytosis attending O.P.D at MGM hospital, Kamothe, Navi Mumbai.Commercially available discs from *HiMedia* Laboratories, preloaded with fluconazole (25µ g/disk), Clotrimazole (10µg/disk), ketoconazole (15 µg/disk), and Miconazole (10µg/disk) will be used.The isolates were transferred from distilled water stocks and sub-cultured to potato dextrose agar (Hi Media, India) to enhance sporulation. Seven day-old cultures were covered with 1ml distilled water and the colonies probed with the tip of a sterile Pasteur pipette to obtain a mixture of mycelium and conidia. The suspensions were transferred to sterile tubes and allowed to sediment for 30 minutes. The inocula will be evenly spread on the surface of Petri dishes containing Sabouraud dextrose agar, Mueller-Hinton (MH) agar medium. Then, the antifungal disks were applied to the plates, after which the plates were incubated at 25°C for 5-10 days. After the incubation colonies grow and the zones of inhibition around the disks were measured and recorded.

RESULTS

A total of sixty six patients with dermatophytosis were studied over a period of one year. Following are demographic details of the study group (Table 1)

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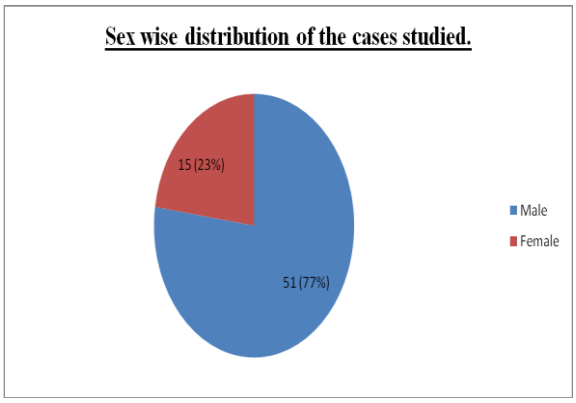
Table 1: Sex wise distribution in the study group

	Male	Female	Total	M: F ratio
Number of cases	51	15	66	3.4:1
Percentage	77%	23%	100%	



Males were predominantly affected 51 cases (77%) as compared to female's 15 cases 23%. Male to female ratio was 3.4:1.

Table 2: Age wise distribution in the study group

Age in years	Number of cases	Percentage
≥ 10	02	03%
11 – 20	06	09%
21 – 30	20	30%
31 – 40	15	23%
41 – 50	11	17%
51 – 60	09	14%
61 – 70	03	04%
Total	66	100%

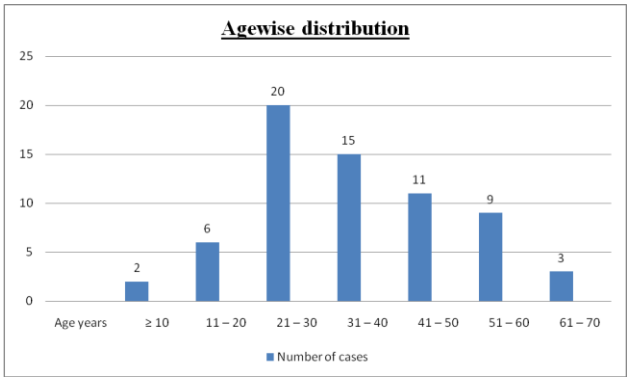


Figure 4: Age wise Distribution

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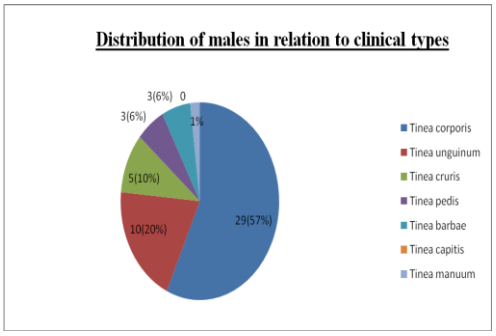
Total number of 66 cases was distributed between the ranges of 2-70 years.Mean age was 33.15 years. Most common age group affected was 21-30 years with 20 cases (23%) followed by 31-40 years with 15 cases (17%) and 41-50 years with 11 cases (9%). Least common age group affected was 61-70 years with 3 cases (4%) followed by 51-60 years with 9 cases (14%) (Fig 4, Table 2).

Table 3: Age and sex wise distribution in relation to clinical types

Clinical types	Age group in years							Sex		Total
	≥ 10	11-20	21-30	31-40	41-50	51-60	61-70	Male	Female	
Tinea corporis	1 2.85%	2 5.71%	13 37.14%	5 14.28%	8 22.85%	5 14.28%	1 2.85%	29 82.85%	6 17.15%	35 53%
Tinea unguium	1 6.25%	2 12.5%	4 25%	6 37.5%	_	1 6.25%	2 12.5%	10 62.5%	6 37.5%	16 24%
Tinea cruris	_	1 20%	_	1 20%	1 20%	2 40%	_	5 100%	_	5 7.5%
Tinea pedis	_	1 33.33%	_	_	1 33.33%	1 33.33%	_	3 100%	_	3 4.5%
Tinea barbae	_	_	2 66.66%	_	1 33.33%	_	_	3 100%	_	3 4.5%
Tinea capitis	_	_	1 33.33%	2 66.66%	_	_	_	_	3 100%	3 4.5%
Tinea manuum	_	_	_	1 100%	_	_	_	1 100%	_	1 2%
Total	2 3%	6 9%	20 30%	15 23%	11 17%	9 14%	3 4%	51 77%	15 23%	66 100%



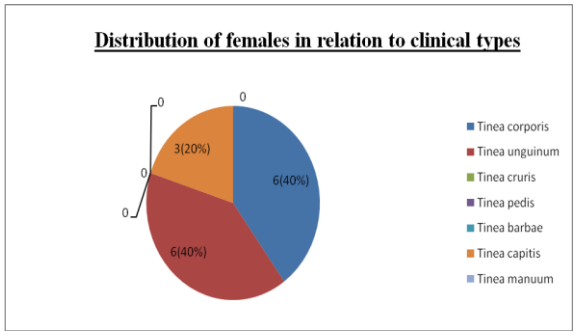


Figure 5: Males In relation to clinical types **Figure 6: Females In relation to clinical types**

Tinea corporis was more common in the age group 21-30 years with 13 cases (37.14%) and in males with 29 cases (82.85%) than females with 6 cases (17.15%) (Fig 5, 6 & Table 3). *Tinea unguium* was more common in the age group of 31-40 years with 6 cases (37.5%) and in males with 10 cases (62.5%) than females with 6 cases (37.5%). *Tinea cruris* was more common in the age group 51-60 years with 2 cases (40%) and was more common in males with 5 cases (100%).

In *tinea pedis*, one case was seen in the age group of 11-20 years and the otherin the age group of 41-50 and 51-60 years, and was more common in males with 3 cases (100%). *Tinea barbae* was more common in the age group 21-30 years with 2 cases (66.66%) and was more common in males with 3 cases (100%). *Tinea capitis* was more common in the age group of 31-40 years with 2 cases (66.66%) and was more common in females with 3 cases (100%). *Tinea manuum* was more common in the age group of 31-40 years and inmales with 1 case (100%) (Table 3).

In males, commonest infection was *T. corporis* followed by *T. unguium* (Fig 10), *T. cruris* (Fig 6), *T. pedis* (Fig 7), *T. barbae* (Fig 9) and *T. manuum* (Fig 8).

In females, commonest infection was *T. corporis* (Fig 5) followed by *T. unguium* (Fig 10) and *T. capitis* (Fig 4) and *T Imbricate* (Fig 11).

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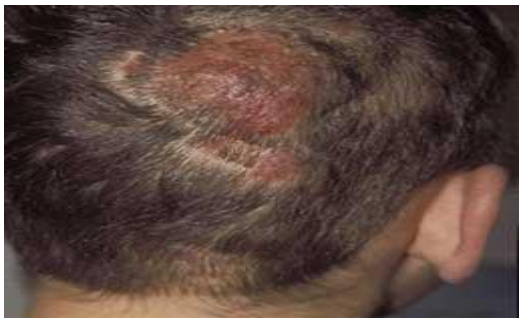

Figure 4: *Tinea capitis* showing circular lesion

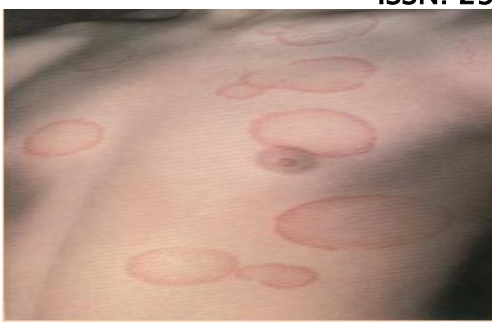

Figure 5: *Tinea corporis* showing circular lesions on the chest

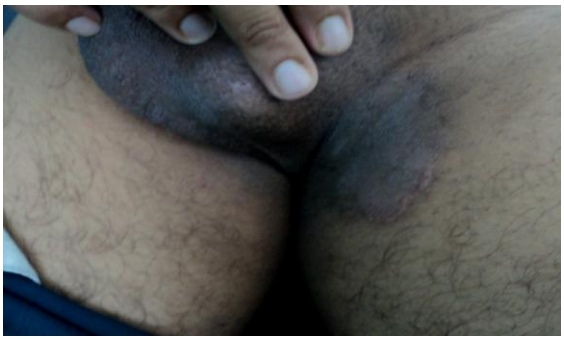

Figure 6: *Tinea cruris* showing lesions over the groin region

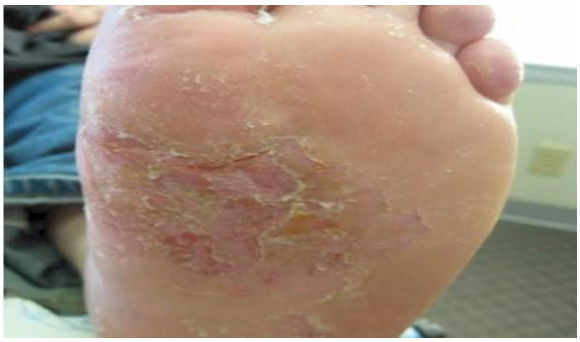

Figure 7: *Tinea pedis* showing lesions on the dorsum of foot

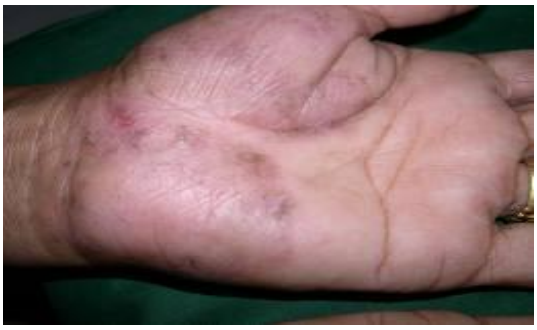

Figure 8: *Tinea manuum*

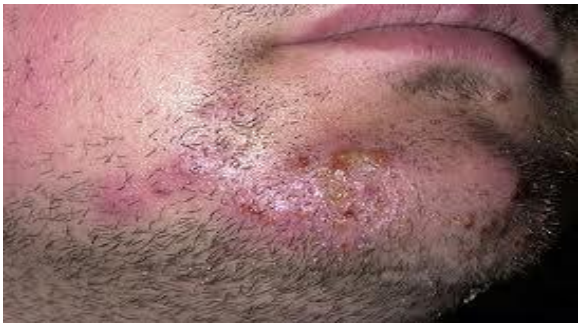

Figure 9: *Tinea barbae*

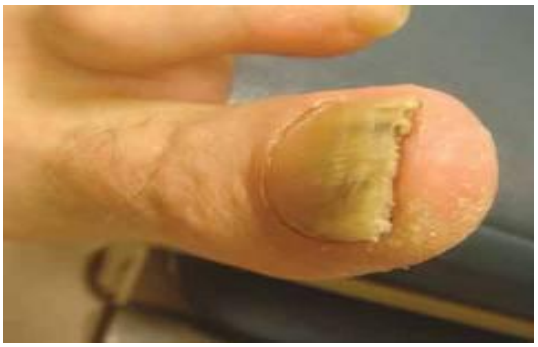

Figure 10: *Tinea unguium* on big toe

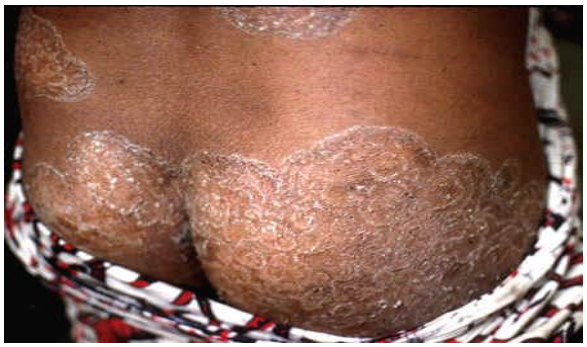

Figure 11: *Tinea imbricate*

Table 4: Shows correlation between findings of KOH mount and culture

	Total KOH and/or Culture +ve	KOH +ve Culture +ve	KOH +ve Culture -ve	KOH -ve Culture +ve	KOH -ve Culture -ve
Number of cases	52	35	13	4	14
Percentage	78.79	53.03%	19.70%	6.06%	21.21%

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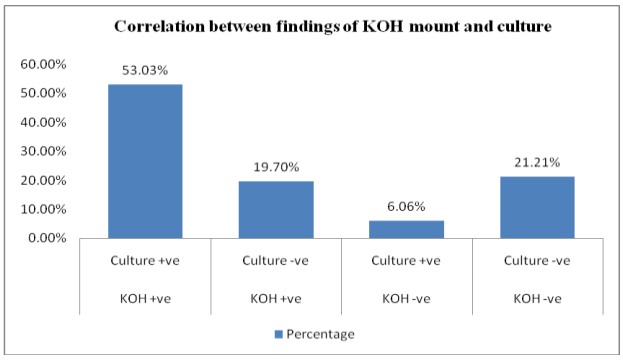

About 66 clinically suspected cases of dermatophytosis; fungi were demonstrated in 52 cases (78.79%) either by direct microscopy and/or culture. Thirty-five cases (53.03%) were positive by both microscopy and culture. 13 cases (19.70%) were positive by microscopy and negative by culture. 4 cases (6.06%) werenegative by microscopy but culture positive. 14 cases (21.21%) were negativeboth by microscopy and culture (Table 4).

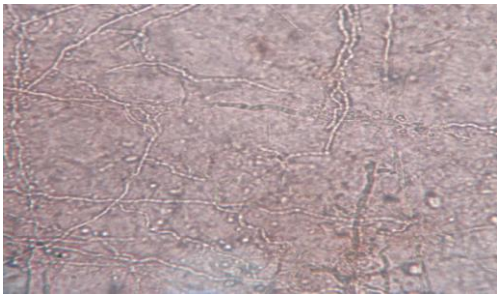

Figure 12: Refractile fungal hyphae seen in KOH mount (40X)

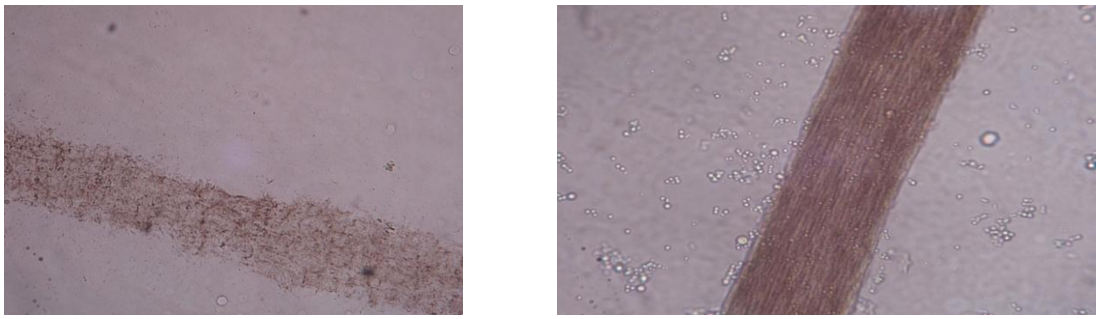

Figure 13: KOH mount of hair (40X)

Table 5: Show dermatophytes isolated in relation to clinical types

Clinical types	Number of clinically suspected cases	Total % isolates in each clinical category	*T. rubrum*	*T. mentagrophytes*	*T. tonsurans*	Fungi other than Dermatophytes
Tinea corporis	35	21 60%	9 42.86%	7 33.33%	5 23.81%	–
Tinea unguium	16	10 62.5%	3 30%	3 30%	–	4 40%
Tinea cruris	5	2 40%	2 100%	–	–	–
Tinea pedis	3	1 33.33%	–	1 100%	–	–
Tinea barbae	3	2 66.67%	1 50%	–	1 50%	–
Tinea capitis	3	3 100%	–	2 66.67%	1 33.33%	–
Tinea manuum	1	0	–	–	–	–
Total	66	39 59.09%	15 38.46%	13 33.33%	07 17.95%	04 10.26%

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In *tinea corporis*, out of 21 culture isolates, *T. rubrum* was the commonest isolate with 9 cases (42.86%) followed by *T. mentagrophytes* 7 (33.33%) and *T. tonsurans* 5 (23.81%) .In tinea unguium, out of 10 culture isolates, *T. rubrum* and *T. mentagrophytes* was isolated in 3 cases each (30%). Four isolates (40%) obtained from the nail sample were fungi other than dermatophytes, which were identified as *Aspergillus spp.* and *Penicillium spp.* In tinea cruris, all the 2 culture isolates were *T. rubrum* (100%). In *tinea pedis*, *T. mentagrophytes* with 1 case (100%) was isolated. In *tinea barbae*, out of 2 culture isolates, *T. rubrum* and *T. tonsurans* was the isolated in 1 case each (50%). In *tinea capitis*, out of 3 culture isolates, *T. mentagrophytes* was the isolates with 2 cases (66.67%) followed by *T. tonsurans* 1 (33.33%) (Table 5). In *tinea manuum*, nodermatophyte was isolated (Fig 12, 13). Overall out of the 39 culture isolates, *T. rubrum* was the most common isolate with 15 cases (38.46%) followed by *T. mentagrophytes* 13 (33.33%), and *T. tonsurans* 07 (17.95%). Four isolates (10.26%) obtained from the nail sample were fungi other than dermatophytes, which were identified as *Aspergillus spp.* and *Penicillium spp.*

Figure 14: Culture of *T. rubrum* in obverse show fluffy white growth and reverse show wine red pigmentation.

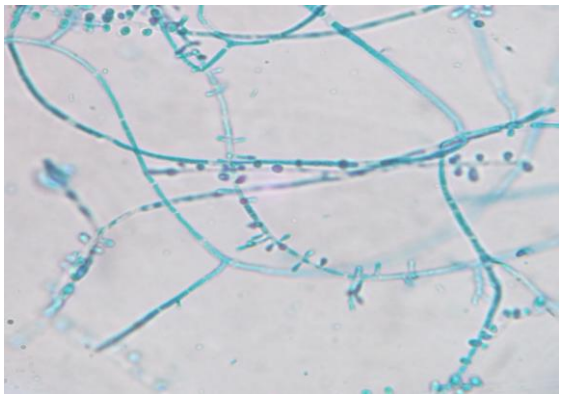

Figure 15: Microconidia arranged in birds-on-a-fence seen in Lactophenol cotton blue mount (40X)

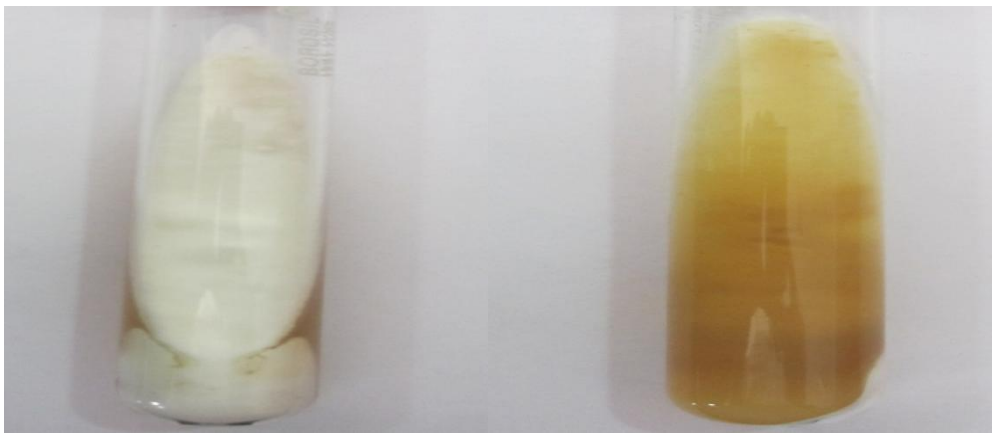

Figure 16: Culture of *T. mentagrophytes* in obverse show buff and powdery white growth and reverse show no pigmentation

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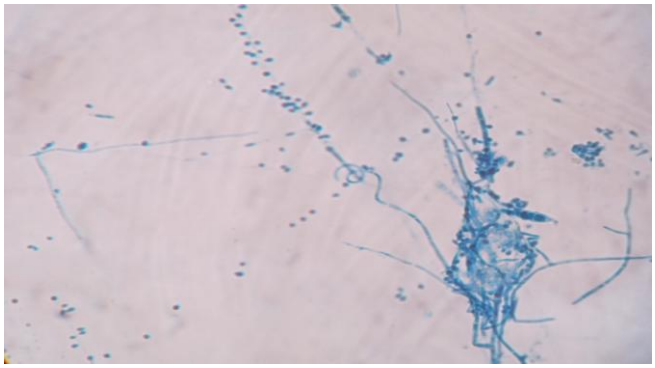

Figure 17: In lactophenol cotton blue spiral hyphae, microconidia and macroconidia seen (40X)

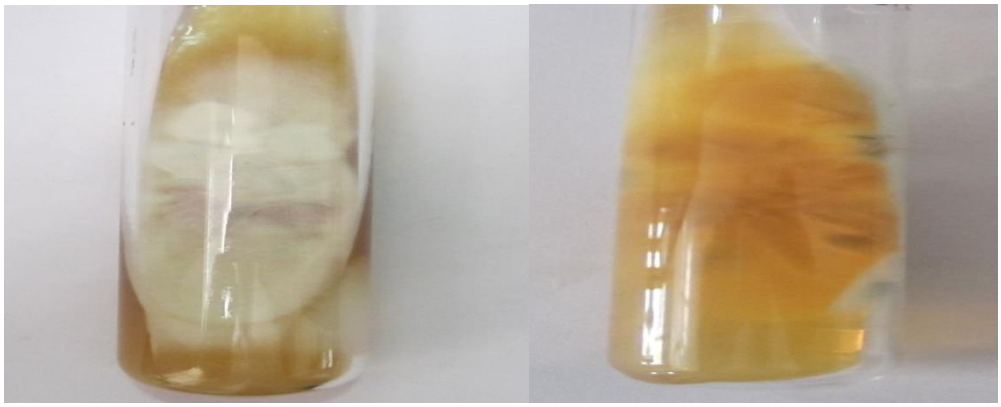

Figure 18: Culture of *T. tonsurans* in obverse show white powdery with flat and folded edges growth and reverse show no pigmentation

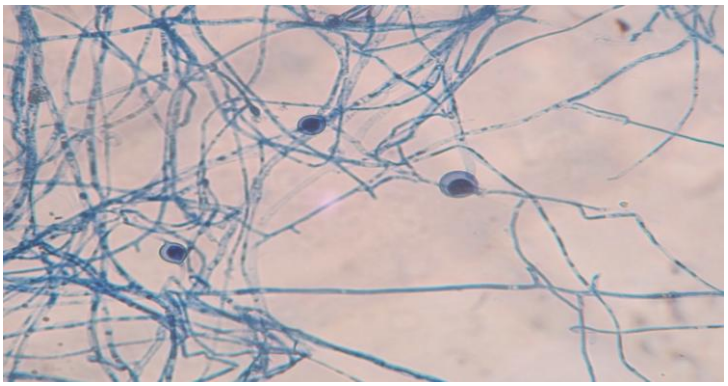

Figure 19:- In lactophenol cotton blue tear drop microconidia and terminal chlamydospores Seen (40X)

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Table6: Comparison of growth rate on SDA & DTM

Sample number	Growth on DTM	Groth on SDA
My-17	3rd day	5th day
My-20	3rd day	5th day
My-25	3rd day	5th day
My-26	3rd day	6th day
My-31	3rd day	5th day
My-32	4th day	5th day
My-34	5th day	5th day
My-35	5th day	5th day
My-39	5th day	7th day
My-41	5th day	7th day
My-45	6th day	6th day
My-46	6th day	7th day
My-47	5th day	7th day
My-50	4th day	6th day
My-51	4th day	6th day
My-52	3th day	3rd day
My-53	5th day	3rd day
My-65	5th day	4th day
My-68	3rd day	4th day

Comparison of growth rate on SDA & DTM

	Growth on DTM			Growth on SDA	
Day	No. of samples	Percentage (%)	Day	No. of samples	Percentage (%)
3rd	7	36.84%	3rd	2	10.53%
4th	3	15.79%	4th	2	10.53%
5th	7	36.84%	5th	7	36.84%
6th	2	10.53%	6th	4	21.05%
7th	0	0%	7th	4	21.05%

Table 7: Criteria of susceptibility and resistance of antifungal disks

Antifungal drugs	Potency	Zone diameter in mm		
		Sensitive	Intermediate	Resistant
Clotrimazole	10 µg	≥20	19-21	≤11
Fluconazole	25 µg	≥22	21-15	≤14
Ketoconazole	15 µg	≥30	29-23	≤22
Miconazole	10 µg	≥20	19-12	11

A total of thirty five species of dermatophytes were isolated and identified.The isolates belong to three species as follows: *T.rubrum* 15(42.85%), *T. mentagrophytes* 13(37.15 %), T*. tonsurans* 7(20%). Test results of the susceptibility to antifungal drugs were as follows: Ketoconazole: 26 (74.28%) susceptible, 4(11.42%) intermediate, 5 (14.28%) resistant. Miconazole: 30 (85.71%) sensitive, 5 (14.28%) intermediate. Clotrimazole: 34 (97.5%) susceptible, 1 (2.5%) intermediate. Fluconazole: 33 (94.28%) resistant, 2 (5.72%) intermediate. Regarding the data, it was revealed that clotrimazole were the most effective antifungal drugs and fluconazole had the poorest activity (Table 7 & Fig 20).

Table 8: Sex distribution in various studies

Name of the author	Place	Year	Male to Female ratio
Present study	Navi Mumbai	2013	3.4:1
Huda MM et al.	Assam	1995	1.86:1
Karmakar S et al.	Rajasthan	1995	2:1
Bindu V et al.	Calicut	2002	2.06:1
Grover S	Bangalore	2003	1.63:1
Singh S et al.	Gujarat	2003	1.57:1
Cordeiro RA et al.	Brazil	2005	0.31:1
Nada H et al.	Saudi Arabia	2005	0.69:1

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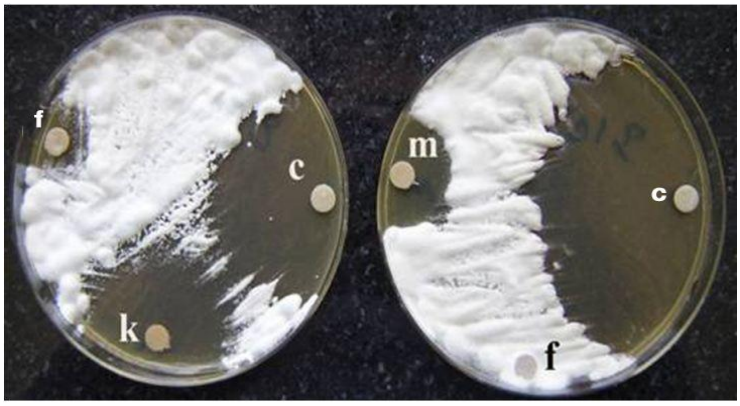

Figure 20: Sensitivity *T.mentagrophytes* to tested antifungal drugs
K, ketoconazole; C, clotrimazole; M, miconazole; F, fluconazole

In the present study, males (77%) were more commonly affected than females (23%). Male to female ratio was 3.4:1, which is comparable with other studies doneby Huda MM [29,30], Karmakar S [31], Bindu V [35], Grover S [36], Singh S [37], whereas Cordeiro RA [54] andNada H [55] reported that females were more commonly affected than males, with male tofemale ratio being 0.31:1 and 0.69:1 respectively [32, 33]

Male predominance may be due to increased outdoor physical activities andincreased opportunity for exposure to infection than females. Also in rural India, males may visit the hospital to a greater extent than females who may not be very open for hospital visit for dermatological infections especially in rural areas (Table 8).

Table 9: Age distribution as found in various studies (in percentage)

Name of the author	Place	Year	Commonest age group (percentage)
Present study	Navi Mumbai	2013	21-30 years (28%)
Karmakar S et al.	Rajasthan	1995	0-30 years (64%)
Mishra M et al.	Sambalpur	1998	15-35 years (30%)
Bokhari MA et al.	Lahore	1999	20-40 years (36%)
Singh S et al.	Baroda	2003	16-45 yeas (31.36%)
Sen SS et al.	Assam	2006	21-30 years (44%)
Veer P et al.	Aurangabad	2007	31-40 years (39.4%)

The present study shows that dermatophytosis was more common in the age group of 21-30 years (30%) followed by 31-40 years (23%), which is comparable with other studies done byMishra M [34], Sen SS [39,40]. However Veer P hasreported that the most common age group affected was 31-40 years followed by 41-50 years (Table 9).The highest incidence in young adults aged 21-30 years may be due toincreased physical activity and increased opportunity for exposure.

Tinea corporis

In the present study, tinea corporis was the commonest clinical type encountered (53%) followed by tinea unguium (24%) and the commonest age group affected was 21-30 years (30%). Males were predominantly affected with male to female ratio being 3.4:1, which is comparable with other studies done by Bindu V(54.6%) [35], Singh S (58.8%) [37], Sen SS (48%) [40] and Venkatesan G (64.8%) [41,42,43].

Tinea unguium

In the present study, onychomycosis was second commonest clinical type and more common in males. Male to female ratio was 1.6:1, which is comparable with other studies done by Grover S [36] and Vijaya D[38]; whereas Cordeiro RA [54] and Nada H [55] in their study reported that female's were commonly affected than males, with male to female ratio being 0.31:1 and 0.69:1 respectively [44,45,46,47,48,49,50.51,52].

Tinea cruris

In the present study, tinea cruris showed prevalence of (7.5%) and commonest age group affected was 51-60 years (40%). Males (100%) were more commonly affected than females, which is comparable withother studies done by Siddappa K [22], Mishra M [34] and Sen SS [40].

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Tinea pedis

In the present study, out of 66 cases, *tinea pedis* was seen in 4.5% cases, which is comparable with the study done by Siddappa K[22], whereas Chimelli PAV [53] in their study on dermatophytosis, reported *tinea pedis* in 9.9% cases respectively.

Tinea barbae

In the present study, out of 66 cases, *tinea barbae* was seen in 4.5% cases, which is comparable with the study done by Singh S [37], Sen SS [40] and Keyvan Pakshir [56,57,58,59,60].

Tinea capitis

In the present study, out of 66 cases, *tinea capitis* was seen in 4.5% cases, more common age group of 31-40 years (66.66%), which iscomparable with other studies done by Siddappa K [22], Kumar AG [23], Reddy BSN [25] and Kalla G [26,27,28].

Tinea manuum

In the present study, out of 66 cases of dermatophytosis tinea manuum was 1 case (2%), which is comparable with other studies done by Siddappa K (1.53%)[22] andChimelli PAV (1.9%) [53].

Table 10: Comparison of KOH and culture findings with other studies (in percentage)

Author name, yearand place	Total KOH or Culture +ve	KOH +ve Culture +ve	KOH +ve Culture -ve	KOH -ve Culture +ve	KOH -ve Culture -ve
Present study, 2013,Navi Mumbai	78.79%	53.03%	19.70%	6.06%	21.21%
Huda MM et al.,1995, Assam	92.85%	57.14%	1.19%	34.52%	7.15%
Singh S et al.,2003, Baroda	66.16%	43.65%	18.66%	3.85%	33.84%
Nada H et al.,2005, SaudiArabia	74.08%	46.30%	20.37%	7.41%	25.93%
Karmakar S et al.,1995, Rajasthan	88.40%	39.2%	46.8%	2.4%	11.6%

In the present study, out of 66 clinically diagnosed cases of dermatophytosis, 52 cases (78.79%) were positive for fungi, either by KOH and/or culture. 35 cases (53.03%) were positive by both KOH and culture, 13 cases (19.70%) were positive by KOH and negative by culture, 4 cases (6.06%) were negative by KOH but culture positive, 14 cases (21.21%) were negative by both KOH and culture, which iscomparable with other studies done bySingh S [37] and Nada H [55].This variation could be due to non-viability of fungal elements in some cases, inadequacy in sampling due to very small lesions and non-reported partial treatment with antifungal agents Table 10.

Table 11: Dermatophytes isolated in various studies (in percentage)

Name of the author, yearand place	T. rubrum	T. mentagrophyte	T. tonsurans	Fungi other than Dermatophytes
Present study, 2013, Navi Mumbai	38.46	33.33	17.95	10.26
Siddappa K et al., 1982, Davangere	81.82	1.54	_	_
Ranganathan S et al.,1995, Madras	52.2	29.35	_	_
Bindu V et al., 2002,Calicut	66.2	25	5.9	_
Venkatesan G et al.,2007, Chennai	73.3	19.7	_	_
Fathi HI et al., 2000, Iraq	20.9	16.2	10.5	_

In the present study, *T. rubrum* 15(38.46%) was the commonest aetiologicalagent in majority of clinical types followed by *T. mentagrophytes* 13 (33.33%), *T. tonsurans* 07 (17.95%) and fungi other than Dermatophytes 04 (10.26%).

Growth of total ninteen (19) samples were compared on SDA & DTM, 89.47% of the Dermatophytes grew faster in DTM with compare to SDA, so the growth rate of dermatophyte is better in DTM, around 89.47% of Dermatophytes grew upto 5th day on DTM, while 57.9% of Dermatophytes only grew upto 5thday Table 11 [67,68,69,70,71].

Antifungal susceptibility testing

Development and standardization of antifungal susceptibility tests have shown remarkable progress in the field of medical mycology. Despite the many guide lines that NCCLS have published for susceptibility tests of moulds (M-27A, M-28A), there is no clear method and routine test for the evaluation of dermatophyte antifungal activity. The agar diffusion method is a practical, agar-based method which enables the determination of the activity of various antifungal drugs against various fungal genera and species. Broth macro- and micro-dilution assays can be used to determine antifungal susceptibility of dermatophytes, but these methods are

expensive and require specific media and equipment such as RPMI, MOPS buffer, and micro plate trays. The standard disk diffusion assay constitutes a good model to be used for investigational purposes to test other fungal genera and drugs as well. This assay can be adapted for routine diagnosis in the laboratory and for assessment of dermatophyte resistance against antifungal drugs. Some studies have focused on the comparison of the disk diffusion method with the reference micro-dilution method. These studies suggest that disk diffusion is a reproducible method which in general shows good correlation with the reference method for micro-dilution antifungal susceptibility test. Other studies such as the one done by Singh *et al* could not find a significant correlation between micro-dilution and disk diffusion methods, probably due to their use of Dermasel agar medium. This medium is unacceptable for antifungal susceptibility testing. In the present study, clotrimazole, and miconazole had large inhibition zones around the disks; clotrimazole had the best activity against the isolates. Clotrimazole is one of the oldest antifungal drugs formulated as a topical for use against dermatophytosis. Although clotrimazole is effective against most cases of dermatophytosis, it is not suitable for severe infections involving hair and nail, which need additional systemic therapy. In this study fluconazole had poor activity on isolates tested. In most isolates, no inhibition zones were observed around the disks. There are many studies indicating that fluconazole had less activity against dermatophytes.

This is perhaps because fluconazole is a triazole, and Sabouraud dextrose agar has components that can interfere with the test. Moreover, *in vitro* determination of antifungal activity of fluconazole against dermatophytes has variable results because of the use of different methods and media. The other antifungal drugs used in this study include ketoconazole that showed good activity.

CONCLUSION

Dermatophyte infections are very common in our country where hot and humid climate in association with poor hygienic conditions play an important role in the growth of these fungi. There is varying difference in isolation of different species from southern and northern part of India. Sixty-six clinically diagnosed cases of dermatophytosis were studied. *Tinea corporis* 35 (53%) was the commonest clinical type followed by *tinea unguium* 16 (24%), *tinea cruris* 5 (7.5%), *tinea pedis* 3 (4.5%), *tinea barbae* 3 (4.5%), *tinea capitis* 3 (4.5%) and *tinea manuum* 1 (2%). All cases were from the skin and venerology OPD of MGM Hospital, Kamothe, Navi Mumbai. Out ofsixty six samples studied, 13 cases (19.70%) were positive by KOH mount. Out of sixty six samples studied, 4 cases (6.06%) were positive by culture. Commonest age group affected was 21-30 years. Male to female ratio was 3.4:1. Fungi were demonstrated in 78.89% cases, either by direct microscopy and culture. Of the dermatophytes isolated, *T. rubrum* 15 (38.46%) was the commonest followed by *T. mentagrophyte*s 13 (33.33%) and *T. tonsurans* 7 (17.95%). In the 4 cases (10.26%) of *tinea unguium* fungi other than dermatophytes were isolated. By and large Trichophyton species forms the commonest aetiological agent of dermatophytosis.T. Rubrum was the commonest isolate in *tinea corporis, tineaunguium, tinea cruris and tinea barbae.T. Mentagrophytes* was second commonest isolate in *tinea corporis, tineaunguium, tinea pedis and tinea capitis. T. tonsurans* was third commonest isolate in *tinea corporis, tinea barbae and tinea capitis.* It was demonstrated thatclotrimazole was the best antimycoticagent against dermatophytes followed by miconazole and ketoconazole. Clotrimazole, miconazole and ketoconazole had large inhibition zones around the disks; clotrimazole had the best activity against the fungal isolates.

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