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Research Article

STUDY OF SUSCEPTIBILITY PATTERN OF NOVEL ANTIFUNGALS ON COMMONLY OCCURRING FUNGAL INFECTIONS IN SOUTHERN REGION OF INDIA

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ABSTRACT

Background: *Fungal infection is one among global public health problem. Many people who are at risk and suffer from fungal disease live in resource limited settings, where the treatment of these infections can be challenging. The increased use of antifungal drugs, often for prolonged periods, has led to acute antifungal resistance among previously suspected strains or species and to the increased incidence of infections with less common species. The objective of this study is to assess the sensitivity pattern of novel antifungal drugs used and also to determine the prevalence rate of various types of fungal infections prevalent in the area.*

Methodology: *It was a prospective observational study conducted in the Department of Dermatology Venereology and Leprosy (DVL), RMMCH, Chidambaram for duration of six months (November 2017- April 2018). The study was approved by Institutional Human Ethics Committee (IHEC). 60 samples were collected from clinically suspected patients diagnosed with dermatophytosis attending the out-patient department of DVL and were processed for susceptibility testing.*

Result: *Among 60 diagnosed patients 49(81.66%) were found to be male and 11(18.33%) were found to be female. Majority of the specimens were collected from the trunk region 33(54.98%). T.mentagrophytes 11(34.37%) was found to be the most isolated culture. T.violaceum (33.33%) and M.gypseum (33.33%) was found to be the most drug resistant species followed by T.mentagrophytes (31.81%). Terbinafine and clotrimazole were found to be the most sensitive antifungal drugs while Fluconzole was found to be the least sensitive drug.*

Conclusion: *Formulation of a protocol for testing and guidelines for antifungal treatment is in need now. It is advisory to conduct research in many centers on antifungal testing to implement a universal guideline for testing and treatment of fungal infections. Proper and effective patient counseling on usage of antifungal drugs may be beneficial to the patients. This aids in combating the ongoing and evolving resistance in future.*

KEYWORDS: *Dermatophytosis, Sensitivity pattern, Prevalence, Resistance.*

INTRODUCTION

The universe of fungi comprises more than 1.5 million species worldwide [11]. Dermatophytes (term derived from Greek words for "skin plant") are contained in the family of arthrodermataceae and are represented ≈ 40 species divided among the three genera: *Epidermophyton, Microsporum, Tricophyton*. Dermatophytes are classified further according to their natural habitats – humans, animals or soil. Their ability to attach and invade keratinized tissues of animals and humans, utilize degradation products as nutritional stores, form the molecular basis for superficial fungal infections of skin, hair, nails are highly studied by the researchers [14].

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Dermatophytes:

Dermatophytes are fungi that invade and multiply within keratinized tissues causing infections. Based upon a genera, Dermatophytes can be classified into three groups *Trichophyton* (which causes infections on skin, hair, and nails), *Epidermophyton* (which causes infections on skin and nails), and *Microsporum* (which causes infections on skin and nails). Based upon the mode of transmission these are been classified as anthropophilic, zoophilic, geophilic. Finally, based upon the affected site, these are been classified clinically to *Tinea*

capitis(head), *Tinea faciei*(face), *Tinea barbae* (beard), *Tinea corporis*(body, *Tinea capitis*(head), *Tinea crusis*(groin), *Tinea pedis*(foot) and *Tinea unguium*(nail). Other clinical variants include *Tinea impercata, Tinea psudoimpericats, Majocchidranuloma.*

Trichophyton:

The genus *Trichophyton* includes 24 species. The colonies formed on agar media is either powdery, velvety or waxy. The predominant spore type is micro conidia with sparse macro conidia. Reverse side pigmentation is characteristic of the species and is used for the identification of species within the genus. The macro conidia are thin walled with smooth surface and variable shape. Whereas in the case with the *Trichophyton* species, they are fastidious in their need a nitrogen source amino acids. *Trichophyton tonsurans* requires ornithine, citrul-line and Arginine whereas *Trichophyton mentagrophytes* requires methionine.

Microsporum:

The genus *Microsporum* includes 16 species. The colony morphology of *Microsporum* species on agar surface is either valvety or powdery with white to brown pigmentation. Both macro and micro conidia produce but the predominant conidial are multi septate with thick wall and rough surface. Rarely some species produce neither micro nor macro conidia. They do not have any special nutritional requirement.

Epidermophyton:

The genus *Epidermophyton* includes two species. The colonies are slow-growing, powdery and unique brownish yellow in colour. This genus is devoid of micro conidia. Macro conidia are abundant and produce in clusters. These macro conidia are thin walled with smooth surface.

Distribution frequency of dermatophyes and dermatophytosis:

All the three genera of Dermatophytes namely *Trichophyton*, *Microsporum* and *Epidermophyton* are worldwide in geographical distribution. The most prominent reason for dermatophytic infections is *Trichophyton* and the next lies the *Epidermophyton* and *Microsporum.* Within the genus *Trichophyton*; *Trichophton rubrum* is the predominant etiological agent accounting for 69.5% followed by *Trichophyton mentagrophytes*, *Trichophyton verrucosum* and *Trichophyton tonsurans*. According to the world health organisation (WHO) survey on the incidence of Dermatophytic infection, about 20% the people of any age can get affected[12]. Among the Tinea infections, the most prominent one is *Tinea corporis* or *Tinea circinata,* then comes the *Tinea cruris*, *Tinea pedis* and *Onychomycosis*. *Tinea corporis* accounts for 70% of the dermatophytic infection.

MATERIALS AND METHODS

Study place: The study was conducted in the department of Dermatology, Venereology and Leprosy (DVL), RMMCH, Annamalai University, Tamil Nadu which is a 1400 bedded super specialty, tertiary care teaching hospital located in rural South India.

Study type: Prospective observational study.

Study period: The study was carried out for duration of 6 months (Nov 2017- April 2018)

Study recruitment procedures: The recruitment of subjects was carried out with the help of physician who had the knowledge of the patient's medical history.

- The subjects selected are the patients who were referred to or those who are admitted in the Department of DVL at RMMCH
- The study procedure was completely explained to the patients and a patient consent form was collected from them.

The patient included in the study were selected based on inclusion and exclusion criteria

Inclusion criteria:

- Patients admitted in DVL wards for the diagnosis of various skin infections.
- Patients across all age groups
- Patients of both the gender

Exclusion criteria:

- Patients who were reluctant to participate in the study.

Study procedure: The specimens collected were cultured using Sabourauds dextrose agar (SDA). Appearance of culture were inspected thrice weekly for growth. The cultures were incubated for almost one month. Once the growth was witnessed, the microscopic examinations were performed.

Disk diffusion assay:

The obtained inoculum was evenly spread on the surface of about 10 cm petridish containing the SDA medium and left air dry. Later, the antifungal discs (drugs incorporated in this study) were applied to the inoculum, after which the plates were let incubated at 25°c for 7 days. Later resultant zones of inhibition were measured and recorded. Data regarding interpretation of susceptibility and resistance pattern of the antifungal drugs were extracted.

Direct microscopy:

KOH mount:

1. 10% KOH for skin scales
2. 20% KOH for hair and nail samples.

Culture:

1. Sabourauds dextrose agar with antibiotics and actidione.
2. Dermatophyte test medium for
   -Macroscopic appearance
   -Microscopic appearance

Collection of samples:

Samples were collected from skin, scalp, hair and nail.

From skin:

The affected area was thoroughly cleaned with 70% alcohol to remove surface contaminants and skin scales were collected from erythematous peripheral growing margins of the lesion with the help of sterile blunt scalpel.

From Scalp:

After cleaning with 70% alcohol, infected hairs were plucked with a pair of sterile surgical forceps. Scales from scalp skin were also collected with the help of blunt scalpel.

From nail:

Nail clipping were taken from proximal part of affected nails and sometimes with surface scrapings also.

Transport of specimen:

Specimens were collected in whatmann filter paper envelope. Collection of samples in filter paper packages reduces humidity, prevents and minimizes multiplication of bacteria. Fungal spores resist drying and remains viable in paper for several weeks.

Direct microscopic examination:

Skin samples were directly placed on a top of 10% KOH on a glass slide, covered with cover slip, gently warmed over Bunsen flame and observed under microscopic after 15 minutes. For hair and nail similar procedure were adopted with 20% KOH after 30 minutes.

Young hyphae of *Dermatophytes* were seen as undulant branch in hyphae, older hyphae were seen as septate or barrel shaped or in the form of arthrospores. Exothrix and Endothrix types of infection were differentiated by the arrangement of arthrospores.

Macroscopic examination of the colonies:

The following things were noted,

1. Rate of growth
2. Colony characteristics
   1. Colour (ex-white, tan, yellow, violet, kakygreen)
   2. Consistency (ex-cottony, fluffy, granular, suede like)
   3. Topography (ex-flat, folded, rugose)
   4. Colony on reverse and its pigmentation (ex-wine red and yellow, reddish brown, yellowish brown)

Test for identification of species among genus *trichophyton:*

Urease test: Christensen's urease agar was inoculated with *Trichophyton* species of Dermatophytes and reaction was observed within 7 days of inoculation. T.mentagrophytes produced urease and changed the colour of the medium pink but T.rubrum did not.

Table No. 1: Criteria for susceptibility and resistance pattern of Antifungal disks

Antifungal drugs	Potency	Zone diameter in mm		
		Sensitive	Intermediate	Resistance
Clotrimazole	10µg	≥20	19-12	≤11
Fluconazole	25µg	≥22	21-15	≤14
Griseofulvin	25 µg	≥10	9- 2	No zone
Ketoconazole	15µg	≥30	29-23	≤22
Miconazole	10µg	≥20	19-12	≤11
Terbinafine	30µg	≥20	19-12	≤11

RESULTS

60 samples were collected from the clinically suspected patients diagnosed with dermatophytosis attending the out-patients Department of DVL during the period of 6 months (Nov 2017- Apr 2018) at RMMCH.

Demographic data's of the patients:

Table No. 2: The age-wise distribution of patients with dermatophytosis

Age group	Number of patients
0-10	2 (3.33%)
11-20	16(26.6%)
21-30	9(15%)
31-40	21(35%)
41-50	5(8.33%)
≥51	7(11.66%)

The most affected age group was seen among the patients of 31-40 years interval (35%) among which 16 were male and 5 were female as shown in (Table 2).

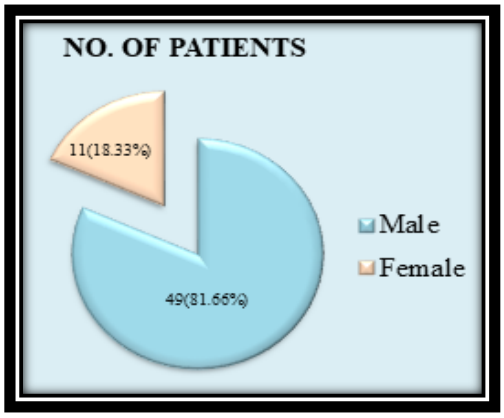

Fig. 1: Gender wise distribution

Among the diagnosed 60 patients with dermatophytosis, 49 were found to be male and 11 were found to be female patients as depicted in (Figure 1).

Table No. 3: KOH mount

KOH MOUNT	NO. OF SAMPLES
POSITIVE	41
NEGATIVE	19

Among the 60 selected samples, 41(68.33%) samples confirmed KOH positive and 19(31.66%) showed KOH negative as shown in (Table 3).

Out of 60 cases, majority of specimens were collected from the Trunk region 33 (54.98%) followed by groin 11(18.33%), foot 9(0.15%), nails 7 (11.66%) as shown in (Table 4).

Out of 60 samples cultured, 32 yielded growth and 28 samples showed no growth of the organism (Table 5).

Growth in SDA yielded 32 positive cultures. Based on growth in SDA, Urease test specification was done. Out of 32 positive isolates T.mentagrophytes 11 (34.37%) was the maximum culture isolated, followed by T.rubrum 9(28.12%), M.gypseum 4(12.5%), T.tonsurans 3(9.37%), E.floccosum 2(6.25%), M.ferruginum 2(6.25%), T.violaceum 1(3.12%) (Table 6).

Our study shows Terbinafine (93.75%) and clotrimazole (68.75%) to be the most sensitive antifungal drugs and fluconazole (12.5%) as the least sensitive drug as shown in (Table 15).

Table No. 4: Distribution of infection site

TYPES OF SKIN CONDITION	NUMBER OF PATIENTS	PERCENTAGE (%)
TRUNK(Tinea corporis)	16	26.66
TRUNK(Tinea versicolor)	10	16.6
NAILS(Tinea unguium)	07	11.66
FOOT (Tinea pedis)	09	0.15
TRUNK (Tinea gladiatorum)	07	11.66
GROIN (Tinea cruris)	11	18.33

Table No. 5: Number of samples showing culture growth

CULTURE GROWTH	NO. OF PATIENTS
Positive	32 (53.33%)
Negative	28 (46.66%)

Table No. 6: Distribution of Dermatophytes

S. NO.	ISOLATED ORGANISM	NUMBER OF ISOLATES	PERCENTAGE (%)
1	T.rubrum	9	28.12
2	T.mentagrophytes	11	34.37
3	T.tonsurans	3	9.37
4	T.violaceum	1	3.12
5	E.floccosum	2	6.25
6	M.gypseum	4	12.5
7	M.ferruginum	2	6.25

Culture sensitivity test through disk diffusion method

Table No. 7: Sensitivity Pattern of T.rubrum 9 (28.125%)

DRUGS	SENSITIVE	INTERMEDIATE	RESISTANT
Terbinafine	8	1	-
Clotrimazole	6	1	2
Ketoconazole	4	3	2
Miconazole	5	2	2
Griseofulvin	6	-	3
Fluconazole	2	-	7

Table No. 8: Sensitivity Pattern of T.mentagrophytes 11 (34.375%)

DRUGS	SENSITIVE	INTERMEDIATE	RESISTANT
Terbinafine	11	-	-
Clotrimazole	7	1	3
Ketoconazole	8	1	2
Miconazole	6	2	3
Griseofulvin	8	-	3
Fluconazole	1	-	10

Table No. 9: Sensitivity Pattern of T.tonsurans 3 (9.375%)

DRUGS	SENSITIVE	INTERMEDIATE	RESISTANT
Terbinafine	3	-	-
Clotrimazole	3	-	-
Ketoconazole	1	2	-
Miconazole	3	-	-
Griseofulvin	2	-	1
Fluconazole	-	-	3

Table No. 10: Sensitivity Pattern of T.violaceum 1 (3.125%)

DRUGS	SENSITIVE	INTERMEDIATE	RESISTANT
Terbinafine	1	-	-
Clotrimazole	1	-	-
Ketoconazole	-	1	-
Miconazole	1	-	-
Griseofulvin	-	-	1
Fluconazole	-	-	1

Table No. 11: Sensitivity Pattern of E.floccosum 2 (6.25%)

DRUGS	SENSITIVE	INTERMEDIATE	RESISTANT
Terbinafine	2	-	-
Clotrimazole	2	-	-
Ketoconazole	1	1	-
Miconazole	2	-	-
Griseofulvin	-	2	-
Fluconazole	-	-	2

Table No. 12: Sensitivity Pattern of M.gypseum 4 (12.5%)

DRUGS	SENSITIVE	INTERMEDIATE	RESISTANT
Terbinafine	3	1	-
Clotrimazole	2	1	1
Ketoconazole	2	1	1
Miconazole	3	-	1
Griseofulvin	2	-	2
Fluconazole	1	-	3

Table No. 13: Sensitivity Pattern of M.ferruginum 2 (6.25%)

DRUGS	SENSITIVE	INTERMEDIATE	RESISTANT
Terbinafine	2	-	-
Clotrimazole	1	1	-
Ketoconazole	1	-	1
Miconazole	1	1	-
Griseofulvin	1	-	1
Fluconazole	-	1	1

Table No. 14: Dermatophytes Resistance to Antifungals

ISOLATED SPECIES	RESISTANCE PERCENTAGE (%)
T.rubrum	29.62
T.mentagrophytes	31.81
T.tonsurans	22.22
T.violaceum	33.33
E.floccosum	16.66
M.gypseum	33.33
M.ferruginum	25

From the above table we can see that T.violaceum (33.33%) and M.gypseum (33.33%) were found to be the most drug resistant species followed by T.mentagrophytes (31.81%), T.rubrum (29.62%), M.ferruginum (25%), T.tonsurans (22.22%) and E.floccosum (16.66%).

Table 15: Antifungal Sensitivity, Intermediate and Resistant percentage

ANTIFUNGAL AGENT	SENSITIVITY (%)	INTERMEDIATE (%)	RESISTANCE (%)
Terbinafine	93.75	6.25	-
Clotrimazole	68.75	12.5	18.75
Ketoconazole	53.12	28.12	18.75
Miconazole	65.62	15.62	18.75
Griseofulvin	59.37	6.25	34.37
Fluconazole	12.5	3.12	84.37

DISCUSSION

Dermatophytic infections are major health problems worldwide, especially in tropical countries like India. Yet, proper methodology for testing and interpreting antifungal susceptibility is lacking in all centers due to unavoidability of universal protocol for antifungal drugs. In this study, which aimed at determining antifungal susceptibility of Dermatophytes isolated from skin, hair and nail specimens we have used disk diffusion method. Dermatophytes comprise a phylogenetically closely related group of genera with numerous species. They focus the particular keratinized tissues and exhibit a wide spectrum of clinical manifestations that ranges from mild to severe.

The present study was conducted in Chidambaram which is situated in eastern coast of South India having hot and humid climate which is more favourable for the development of superficial skin infection.

All age groups and both the sexes were included in the present study. Majority were between the age group of 31-40 (35%) followed by 11-20 (26.6%), 21-30 (15%), ≥51 (11.66%), 41-50 (8.33%) and 0-10 (3.33%). Whereas the study from Uma P et al.[1], from Guntur, Senthamilselvi.G et al [2], from Chennai showed most of the patient belonged to the age group of 21-30 yrs. Dermatophytosis occurs mainly in younger age group and adults. Mostly in daily wage workers and college students. Male were most affected than female 81.66% and 18.33% from the present study. The reason behind higher incidence of infections seen in males could be due to greater physical activity and increased sweating.

Direct microscopy with KOH demonstrated 68.33% cultures to be positive, a study by Belukar et al [3]. showed culture positivity of 71%.

In our study, most common clinically diagnosed case in dermatophytosis were of *Tinea corporis* 16(26.66%). Majority of the samples were collected from the trunk region of patients 33 (54.98%). This result was in accordance with Surendran et al [4] and Bindu et al [5]. and contrary to Sardari et al [6]. and Verma et al [7]. which reports that *Tinea cruris* was the most predominant clinical type.

All the specimens were inoculated in the Sabarouds Dextrose Agar method. Based on growth in SDA urease test specification was done, Out of 32 positive isolates; 9 (28.12%) were T.rubrum, 11(34.37%) were T.mentagrophytes, 3 (9.37%) were T.tonsurans, 1(3.12%) were T. violaceum, 2(6.25%) were E.floccosum, 4(12.5%) were M.gypseum, and 2(6.25%) were M. ferruginum. This results shows that among the isolates T.mentagrophytes was the most common (34.37%) cause of infection. This result was in again accordance with Surendran et al. [4] and also similar to a study from Pondicherry and Calicut; this shows their high relative prevalence in south India.

All the positive cultures were processed for antifungal susceptibility test using Griseofulvin, Ketoconazole, Miconazole, cotrimazole, terbinafine and fluconazole. Universal usage of Fluconazole is due to its low cost and dosage and its widespread availability in all levels of health care centres, which in turn has turned up to increased resistance profile for that drug., the same result is gathered from disk diffusion method when compared where fluconazole if found to be highly resistant.

Among the 32 isolates, 30(93.75%)were sensitive to Terbinafine, 17(53.12%) were sensitive to Ketoconazole, 22(68.75%) were sensitive to clotrimazole, 21(65.62%) were sensitive to Miconazole, 19(59.375%) were sensitive to Griseofulvin, 4(12.5%) were sensitive to Fluconazole. Thus the data shows that Terbinafine were found to be most sensitive and most active antifungal agent tested against Dermatophytes which was well correlated by Ahmed medhathanafy et al [8].and elhamaboualigalehdari et al [9]. Similarly; data shows that Fluconazole has the least antifungal activity, only 12.5% were sensitive. There are many studies indicating that fluconazole has less activity against Dermatophytes, Our data is in agreement with those reports. T.violaceum (33.33%) and M.gypseum (33.33%) were found to be the most drug resistant species in our study.

Over the past few decades, the number of antifungal agents used in clinical practice for the treatment of fungal infection has increased. Nevertheless, not all species have same susceptibility pattern and there is evidence that Dermatophytes have become resistant to certain antimycotics as suggested by Galuppi et al [10].

It is an indisputable fact that there is an increase in the prevalence of dermatophytosis over the past 4–5 years across our country. Comparison of study had done on superficial fungal infections in cities such as Kolkata, Ahmedabad, and Chennai during different time frames have revealed an increasing trend of dermatophytosis.

CONCLUSION

Although usage of antifungal drugs shows promising results in clinical improvements of fungal infections, determination of its susceptibility and formulation of a protocol for testing and guidelines for antifungal treatment is in need now. It is advisory to conduct research in many centers on antifungal testing to implement a universal guideline for testing

and treatment of fungal infections. This aids in combating the ongoing and evolving resistance in future.

Proper and effective patient counseling on usage of antifungal drugs may be beneficial to the patient, Steps taken as preventive measures to elude the fungal diseases should also be taught. We however need larger epidemiological studies to further bolster our nationwide observation of the alarming increase in its incidence as well as the prevalence.

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