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ASSESSING THE ANTIFUNGAL SUSCEPTIBILITY PATTERN AND RISK INSIGHTS OF DERMATOPHYTOSES IN A TERTIARY CARE HOSPITAL IN WESTERN TAMIL NADU

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ABSTRACT

Background: Though dermatophytoses is considered to be a trivial superficial fungal infection globally, it is a universal intractable infection deleteriously affecting the eminence of life through communal dishonour and distressing the day-to-day behaviour. It can be acquired by direct contact with soil, animals or humans infected with fungal spores. The pre disposing factors include humid skin and tight ill fitting under garments. The psychosomatic effects of the disease are vastly significant and because of its high morbidity, it provides monetary burden to the affected population in provisions of loss of working hours and treatment. Due to risk factors, ineffective treatment and emerging drug resistance, there has been an increase in the incidence and non-responsiveness of dermatophytes to conventional antifungal agents which suggest the need of antifungal sensitivity testing. **Objective:** To analyse in detail about the risk factors of dermatophytoses and their antifungal susceptibility pattern from newly diagnosed patients with dermatophytoses attending the Dermatology Out Patient Department in a tertiary care hospital. **Materials and Methods:** This cross-sectional study was conducted in 100 newly diagnosed patients with suspected dermatophytoses attending the Dermatology OPD in a tertiary care hospital. A detailed history, clinical examination and specimen collection for mycological examination for identification and speciation of dermatophyte was done. In vitro antifungal susceptibility testing was performed on dermatophyte species isolated from culture as per CLSI-M38A2 guidelines with micro broth dilution method. **Results:** Totally 100 newly diagnosed patients with dermatophytoses were included in this study. Tinea corporis was the most common type observed. KOH positivity was seen in 62 samples and culture positivity was found in 43 samples. The most common species isolated was Trichophyton rubrum (22 isolates) followed by Trichophyton mentagrophytes (10 isolates). It was more prevalent in agriculturers (33%) followed by outdoor labourers (22%). Risk factors like Close family contacts mainly affected (34%) followed by hyperhidrosis (23%). Majority of affected patients having history of wearing Synthetic garments and tight fitting garments like leggings &jeans (34%) followed by not wiping body after bath (21%) and sharing of fomites (21%), The Minimum inhibitory concentration (MIC) of griseofulvin, fluconazole, ketoconazole, itraconazole, terbinafine and sertaconazole were compared. Three isolates of Trichophyton rubrum were found to be resistant in which itraconazole (1 isolate), terbinafine (1 isolate), fluconazole and ketoconazole (1 isolate). Sertaconazole was the most efficient drug among the tested anti fungal. Griseofulvin was also an effective antifungal drug.

Conclusion: Inadequate, irregular, in appropriate application, irrational use of antifungal drugs and self medication has led to the emergence of resistant strains which cause poor treatment outcome. Thus, it is very essential to perform antifungal susceptibility testing to check for resistance pattern of antifungal agents.
Keywords: CLSI (Clinical and Laboratory Standards Institute), Antifungal agent, Minimum Inhibitory Concentration (MIC), micro broth dilution, antifungal resistance.

INTRODUCTION

Dermatophytoses or ring worm is the most common superficial mycoses affecting keratinized structures (skin, hair, nails) caused by a group of keratinophylic fungi called dermatophytes. It is classified into three genera namely Trichophyton, Microsporum, Epidermophyton. The severity of infection depends on the infecting fungi, immune status of the host and the site of lesion. In humans, the estimated risk of acquiring superficial mycoses in life time is among 20% to 25%. The various species and strain of dermatophytes determine the type and severity of host response. Patients live in crowded conditions, poor hygiene, co-morbidities, contact with pet animals and close contact with family members or friends having similar skin and soft tissue infection, immunocompromised individuals are at higher risk of acquiring these types of fungal infections.

It is a condition if not diagnosed and treated early; it may lead to disfigurement of the areas involved. In order to prevent the morbidity and recurrence, early diagnosis of the condition is required. The duration of the treatment rests on the site involved for skin lesions 1 to 2 weeks, for hair infection 6 weeks, for finger nail infection 6 months and for toe nail infection 1 year.

Henceforth the antifungal resistance is an emerging problem; there is an alarming in the incidence of chronic and recurrent dermatophytoses in India. The introduction of the modern treatment modalities and aggressive use of more chemotherapeutic agents result in promptly expanding chemically induced immune suppression. These patients are more prone for severe fungal infections. The inadequate and inappropriate usage of antifungal agents and over the counter topical corticosteroids and host factors such as non-compliance and immuno suppression result in emergence of resistance to antifungal agents and also in recurrence.

Relapses and recurrences are more common in people visiting communal places like salons, spas, gyms and using common toilets in spite of proper treatment. The management of recurrent and chronic dermatophytosis has become a great therapeutic challenge for the dermatologists. The topical application of corticosteroid ointments or creams also leads to further misdiagnosis,[1] and mismanagement. So newer antifungal agents with broad spectrum and different target of action are being developed. An ideal antifungal agent should have a broad spectrum of activity, it should be effective invivo and there should not be drug resistance.

The standard invitro antifungal susceptibility testing affords a consistent and reproducible data that may predict the clinical consequence and treatment response when used in conjunction with patient's risk factors. The changing trend in dermatophyte infection presents as chronic, treatment nonresponsive and recurrent cases. The primary objectives of the study are as follows

- To isolate and identify the different species of dermatophytes by conventional mycological laboratory techniques.
- To study the antifungal susceptibility pattern by microbroth dilution method as per CLSI-38 A2 guidelines.
- To correlate the outcome in relation to a various array of clinical presentation and risk factors associated with the disease.

Hence, the present study is conducted with an aim to isolate, speciate dermatophytes to reveal the changing trend in the prevalence and also to ensure their antifungal susceptibility.

MATERIALS AND METHODS

Ethical Consideration

This hospital based cross sectional study was conducted in 100 clinically diagnosed new patients with dermatophytoses in a tertiary care hospital for a period of one year in the Department of Microbiology in collaboration with Dermatology department after obtaining the Institutional Ethical Committee approval. An Informed written consent was obtained from the patients satisfying the inclusion criteria.

Inclusion Criteria

Patients of all age group irrespective of sex with clinical features suggestive of dermatophytosis attending the Dermatology Out Patient Department who were newly diagnosed/Patients with co-morbid systemic illness

Exclusion Criteria

Patients who are on oral, topical, systemic antifungal therapy/Defaulter/Patients who did not provide informed consent / Patients with other bacterial and fungal infections in the skin folds, hair and nails.

Questionnaire related with History

All relevant details like distribution of lesion, history of similar complaints in the past, personal history, detailed history of similar illness in family members, treatment history, history of contact with animals or

soil, pets at home & all information about general health and treatment history for co-morbid systemic illness like Diabetes, Tuberculosis, Neoplasms, HIV, surgeries, etc were elicited and recorded.[2]

Specimen collection and processing

Based on the clinical features, respective samples like skin scrapings, epilated hair and nail clippings were collected aseptically by using blunt scalpel, forceps and nail clipper or tweezers respectively. Specimens were processed in the Microbiology Diagnostic laboratory for direct microscopic examination with 10% to 40% Potassium hydroxide (KOH mount) and irrespective of KOH positivity all specimens are inoculated in duplicate sets of Modified Sabourard Dextrose Agar with Cycloheximide & Chloramphenicol and Dermatophyte Test Medium for fungal culture as per recommended standard laboratory protocols and one set was incubated at 25°C and other set at 37°C for 3 to 4 weeks. Identification of Species was done macroscopically with reference to rate of growth, texture, colony topography & pigment production. For further species identification, the colony was teased and Lacto Phenol Cotton Blue mount was made to demonstrate macroconidia, microconidia and special hyphae (Figure 1 a-f). Sub culture in Potato dextrose agar (Figure 1g), slide culture (Figure 1h), hair perforation test, urea hydrolysis test were performed whenever necessary.

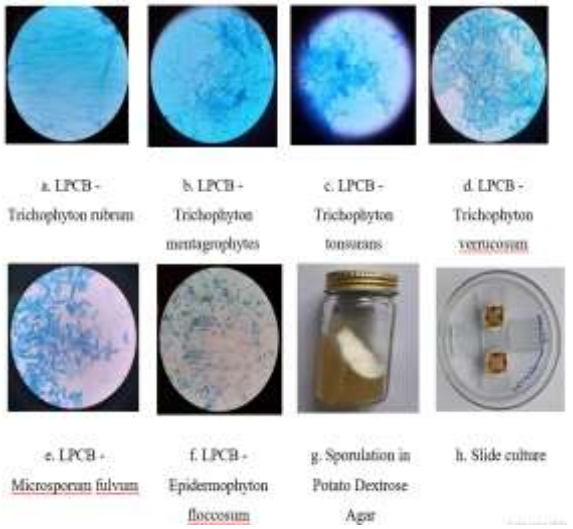

Figure 1: Conventional mycological techniques used for identification of species

Antifungal Susceptibility Testing

Antifungal susceptibility testing was performed by microbroth dilution method as per (Clinical and Laboratory Standards Institute) CLSI-38 A2 guidelines to determine the minimum inhibitory concentration by which the in vivo effectiveness of antifungal drugs can be evaluated. Moreover, the development of drug resistance can also be assessed. It depends on the following parameters pH of the medium, inoculum size of the isolate, medium, time and temperature for incubation & invitro and invivo correlation.[3]

The amount of antifungal powder (mg) and volume of diluents (ml) needed for preparation of Drug stock solutions are derived by means of either of the formulae (Equation 1 and 2).

$$\text{Weight (mg) of drug} = \frac{\text{Volume (ml)} \times \text{Concentration (µg/ml)}}{\text{Assay potency (µg/mg)}} \quad \text{--- (1)}$$

$$\text{Volume of Diluents (ml)} = \frac{\text{Weight (mg)} \times \text{Assay potency (µg/mg)}}{\text{Concentration (µg/ml)}} \quad \text{--- (2)}$$

Number of Concentrations Tested

Ketoconazole 0.0313 to 16µg/ml, Itraconazole 0.0313 to 16µg/ml, Griseofulvin 0.0313 to 16µg/ml, Fluconazole 0.0125 to 64µg/ml, Terbinafine 0.0313 to 16µg/ml & Sertaconazole 0.0313 to 16µg/ml [4].

Medium used: commercially available Rosewell Park Memorial Institute (RPMI) 1640 with glutamine without bicarbonate. **Buffer:** MOPS (3-N-Morpholino prophane sulfonic acid).

Preparation of Stock Solution of Antifungal Drugs

Dimethyl sulfoxide (DMSO) was used as solvent for water insoluble drugs like Ketoconazole, Itraconazole, Terbinafine, Griseofulvin, Sertaconazole & distilled water was used as solvent for water soluble drug like Fluconazole. The antifungal stock solutions were prepared 100times more than the highest concentration to be tested (i-e) 1600 µg/ml is prepared for Ketoconazole, Itraconazole, Griseofulvin, Terbinafine & Sertaconazole and 6400 µg/ml for Fluconazole.

The appropriate amount of drug was weighed by the analytical balance for the requirement of 10ml stock solution. Then the drug was dissolved in 10ml of DMSO and aliquot into 5 × 2 ml snap cap tubes and stored at -70°C. Then a series of dilutions at 100 times the final concentration was prepared from the antifungal stock solution in the same solvent. Each intermediate solution was then further diluted to final strength in the test medium.

Preparation of Stock Solution and Series of Dilutions of Water Insoluble Drugs

The water insoluble drugs like Ketoconazole, Itraconazole, Griseofulvin, Terbinafine & Sertaconazole were dissolved in DMSO. 1 to 10 test tubes in 2 rows were kept. Initial drug concentration of 1600 µg /ml (STOCK) was kept in Tube 1. Intermediate drug solution concentrations were prepared from 1600 µg /ml to 3.13 µg/ml (1-10 test tubes in first row) Then the final drug solution concentrations were prepared by diluting the prepared intermediate drug concentrations in supplemented RPMI-1640 medium from 32 µg/ml to 0.0625 µg/ml (1-10 test tubes in row 2).

Preparation of Stock Solution and Series of Dilutions of Water Soluble Drugs

Stock solution in a concentration of 6400µg/ml was prepared by dissolving the water soluble drug Fluconazole in distilled water (initial concentration of the drug in tube1).Then, a series of intermediate drug solution concentrations were prepared with RPMI medium between 640 µg/ml to 1.25 µg /ml (1-10 test

tubes in Row 1).Then the final concentration of drug solution was prepared from the intermediate drug solution concentration by diluting in supplemented RPMI-1640 medium from 128µg/ml to 0.25 µg/ml (1-10 test tubes in row 2).

Inoculum Preparation

Dermatophytes grown on Potato Dextrose Agar (PDA) slants at 25°C were used preferably 3 to 10 days old culture with development of sufficient conidia.3ml of sterile distilled water was instilled into the agar slant. The surface of the slant was gently flushed with a sterile Pasteur pipette to obtain the conidial suspension. The agar slant was allowed to stand for 5 to 10 min for heavy particles to settle down. Then the conidial suspension was transferred gently to a fresh test tube without disturbing and adjusted to 0.5 McFarland Turbidity standard.100µl of conidial suspension was added to 5ml of RPMI-1640 medium with the dilution of 1:50 to get final concentration of $0.4\text{-}0.5\times10^4$.

Addition of Inoculum to Microtitre Plate

0.1 ml of final concentration drug solution (water insoluble or water soluble) from row 2 tubes were added to corresponding wells in microtitre plate followed by 0.1 ml of inoculam to respective wells from 1-10.Then the final concentration of water insoluble drug (Ketoconazole, Itraconazole, Griseofulvin, Terbinafine & Sertaconazole) and water soluble drug((Fluconazole) were modified between 16 µg/ml(1st well) to 0.0313 µg/ml (10th well) and 64µg/ml(1st well) to 0.125 µg/ml (10th well) respectively.

Controls

Growth control containing 0.1 ml supplemented RPMI-1640 and 0.1 ml of the inoculums (11th well), and Drug control containing 100µl supplemented RPMI-1640 and 100µl of working solution of antifungal agent without inoculums (Negative control) (12th well).

Incubation

All microdilution trays were incubated at room temperature without agitation till growth was detected.

Figure 2: AFST-Terbinafine

Interpretation

Reading was taken after visible growth at growth control wells after incubation at room temperature. The Minimum Inhibitory Concentration was determined by the first well where turbidity is reduced to at least 80% (Figure 2).

Minimum inhibitory concentration (MIC) is the lowest concentration of an antifungal agent that significantly inhibits growth of fungal growth as detected visually. In conventional microdilution method, the growth in each MIC well is compared with that of the growth control with the help of reading mirror. Each microdilution well was specified with a numerical score as follows;

- 0 - Optically clear or absence of growth (CLSI-M-38A2)
- 1 - Approximately 25% of the growth control or slight growth
- 2 - Approximately 50% of the growth control or prominent reduction in growth
- 3 - Approximately as of the growth medium or slight reduction in the growth (75% of growth control)
- 4 - No reduction in the growth

Clinical Significance

Since there are no breakpoints established for antifungal agents against dermatophytes. the interpretive criteria was assessed according to breakpoints established in CLSI-M38-A2 reference documents for Trichophyton mentagrophytes MRL 1957 ATCC MYA-4439, Trichophyton rubrum MRL666ATCC MYA-4438.

General guidelines for Non-defined Drugs or Species (µg/ml)

For Fluconazole, isolates with MIC 64≤µg/ml are categorized as susceptible and ≥64µg/ml as resistant. For Ketoconazole, Itraconazole, Griseofulvin, terbinafine and Sertaconazole, isolates with MIC≤1µg/ml are considered as susceptible and≥1µg/ml are resistant.

Statistical Data Analysis

Data entry was made in the Excel software and analysis was done with SPSS26 computer package by using chi square test and independent sample 't'- test. P value of < 0.05 was considered as statistically significant.

RESULTS

The above study was conducted in a tertiary care hospital with 100 samples including skin scraping (72), epilated hair (12) and nail clipping (16) of clinically diagnosed new dermatophytoses patients. Among 72 skin scraping specimens (n=72), Tinea corporis was the principal dermatophytic lesion accounted for 43 cases followed by tinea cruris 24 cases in this study. tinea pedis and tinea manuum were seen in 2 (2.8%) patients each. Tinea faciei was seen in 1 (1.4%) case. The epilated hair (n=12)

specimens, 6 (50%) were from tinea capitis, 6 (50%) were from tinea barbae. The nail clippings (16) were from Tinea unguium. The direct microscopic examination (KOH) was positive for fungal elements in 62 (62%) samples and was negative for fungal elements in 38(38%) samples. The total number of samples which showed growth in culture was 43 (43%) and the total number of samples which showed no growth in cultures was 57 (57%). Among study population, out of 43 cultures isolated, 30 (30%) of them were isolated from the skin scrapings, 7 (16.28%) of them were from nail clippings and 6 (13.95%) of them were from the epilated hair samples.

In our study among study population, majority of cases were reported in occupation related to agriculturers 33 (33%) followed by outdoor labourers 22 (22%), students 15 (15%), 15patients (15%) working as office employees and 15 patients (15%) housewives (Figure 3). The p-value by Chi-square test was 0.015 which was found to be statistically significant.

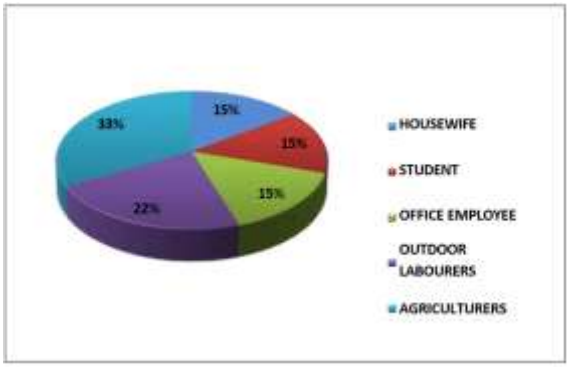

Figure 3: Occupation wise Distribution of Dermatophytosis Among Study Population (n=100)

In association with risk factors, in our study 34 patients (34%) were reported in close family contacts followed by hyperhidrosis in 23patients (23%), 18patients (18%) hostellers, 15patients (15%) had contact with soil and 9patients (9%) had pets at home, 1(1%) patient with history of handling of animal. [Table 1]

Table 1: Distribution of dermatophytosis in association with risk factors (n=100)

Risk factors	Number of persons(n)	Percentage (%)
CLOSE FAMILY CONTACTS	34	34%
HYPERHIDROSIS	23	23%
HOSTELERS	18	18%
PETS AT HOME	9	9%
ANIMAL HANDLING	1	1%
CONTACT WITH SOIL	15	15%
TOTAL	100	100%

In association with personal history, Among the study population,21 patients (21%) gave history of wearing clothes immediately after bath without wiping, of which 7 (7%) were females & 14 (14%) were males, 21 patients (21%) gave history of sharing of the fomites like towel, bed linen, dresses, of which 4 (4%) were females & 17 (17%) were males, 34 patients (34%) were frequently using synthetic garments like synthetic sarees, leggings, jeans, 68 of which 18 (18%) were females & 16 (16%)were males. 4 patients (4%) were using waistband, of which all the 4 (4%) were males. The most widespread individual factor attributing to the disease is the usage of synthetic garments reported with total of 34patients (34%).12 patients (12%) observed with history of ill fitting/ wet under garments of which 3 (3%) were females & 9 (9%) were males and 8 patients (8%) were shoe socks wearers, of which 4 (4%) were females & 4 (4%) were males. [Table 2]

Table 2: Distribution of dermatophytosis in association with personal history (n=100)

Personal history	Total number of Persons affected	Percentage (%)	Number of males Affected	Percentage (%)	Number of females Affected	Percentage (%)
NOT WIPING BODY AFTER BATH	21	21%	14	14%	7	7%
SHARING OF FOMITES	21	21%	17	17%	4	4%
USE OF SYNTHETIC GARMENTS / LEGGING/JEANS	34	34%	16	16%	18	18%
USE OF WAIST BAND	4	4%	4	4%	0	0%
ILL FITTING / WET UNDER GARMENTS	12	12%	9	9%	3	3%
SHOE SOCKS WEARER	8	8%	4	4%	4	4%

In association with family history, in our present study among study population, family members of 33 patients (100%) were also affected by dermatophytoses of which 10 patients (30%) were males and 23 (70%) patients were females. Among study population, 10 patients were associated with diabetes mellitus, 5 patients with hypertension, 4 patients both hypertension and diabetes mellitus,7 patients with bronchial asthma, 2 patients with Psoriasis, 3 patients with polyarthritis and 69 patients did not have any associated co-morbid conditions.

Table 3: Distribution of dermatophyte species isolated from the clinical specimens (skin scrapings, nail clippings, epilated hair samples) (n=43)

Fungal isolates	Tinea corporis	Tinea cruris	Tinea manuum	Tinea pedis	Tinea faciei	Tinea capitis	Tinea barbae	Tinea unguium
Trichophyton Rubrum	11 (64.7%)	3 (30%)	1 (100%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)	5 (71.42%)
Trichophyton Mentagrophytes	3 (17.6%)	3 (30%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	2 (100%)	1 (14.29%)
Trichophyton tonsurans	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0
Trichophyton verrucosum	1 (5.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0
Microsporum Fulvum	2 (11.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0
Epidermophyton floccosum	0 (0%)	4 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (14.29%)
Total	17 (56.8%)	10 (33.3%)	1 (3.3%)	1 (3.3%)	1 (3.3%)	4 (66.7%)	2 (33.3%)	7 (100%)

The p-value by Chi-square test was <0.001 which was found to be statistically significant. In Tinea corporis, Trichophyton rubrum 11/17 (64.7%) was the predominant isolates followed by Trichophyton mentagrophytes 3/17 (17.6%), Microsporum fulvum 2/17(11.8%), Trichophyton verrucosum 1/17 (5.9%) In Tinea cruris, Epidermophyton floccosum 4/10 (40%) was the predominant isolates followed by Trichophyton rubrum3/10 (30%) and Trichophyton mentagrophytes3/10 (30%). One case 1/1 (100%) of Trichophyton rubrum was isolated from Tinea manuum, Tinea pedis and Tinea faciei. Out of 4 dermatophytes isolated from Tinea capitis, 2/4 (50%) were Trichophyton tonsurans and 1/4(25%) was Trichophyton mentagrophytes and 1/4 (25%) was Microsporum fulvum. The 2 isolates of Tinea barbae (100%) were Trichophyton mentagrophytes. Trichophyton mentagrophytes 3/6(50%) was the predominant isolate in hair sample followed by Trichophyton tonsurans 2/6 (33.33%) and Microsporum fulvum 1/6 (16.67%). Out of the 7 isolated dermatophytes in Tinea unguium, Trichophyton rubrum was the predominant isolate 5/7(71.42%), followed by Trichophyton mentagrophytes 1/ 7 (14.29%) and Epidermophyton floccosum 1/7 (14.29%). [Table3]

Table 4: Minimal inhibitory concentration of the drugs fluconazole, ketoconazole, griseofulvin, itraconazole, terbinafine and sertaconazole to the isolated dermatophytes (n=43)

FUNGAL ISOLATES	Fluconazole		Ketoconazole		Griseofulvin		Itraconazole		Terbinafine		Sertaconazole	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
T.rubrum	2	16	0.0625	0.5	0.0625	0.5	0.0625	0.5	0.0625	0.25	0.0313	0.125
T.mentagrophyte	4	16	0.125	0.5	0.0625	0.25	0.0625	0.5	0.0625	0.25	0.0313	0.125
T.tonsurans	8	16	0.125	0.25	0.125	0.25	0.0625	0.125	0.0625	0.125	0.0313	0.0625
T.verrucosum	8	8	0.125	0.125	0.0625	0.0625	0.125	0.125	0.125	0.125	0.0313	0.0625
M. fulvum	2	4	0.0625	0.125	0.0625	0.125	0.0625	0.125	0.0625	0.125	0.0313	0.0625
E.floccosum	4	8	0.0625	0.25	0.125	0.5	0.0625	0.25	0.0625	0.125	0.0313	0.0625

Griseofulvin

MIC 50 and MIC 90 of Griseofulvin for the species isolated in this study Trichophyton rubrum was 0.0625 µg/ml and 0.5 µg/ml respectively. Trichophyton mentagrophytes was 0.0625 µg/ml and 0.25 µg/ml respectively. Trichophyton tonsurans was 0.125 µg/ml and 0.25 µg/ml respectively. Trichophyton verrucosum was 0.0625 µg/ ml and 0.0625 µg/ml respectively. Microsporum fulvum was 0.0625 µg/ml and 0.125 µg/ml respectively. Epidermophyton floccosum was 0.125 µg/ml and 0.5 µg/ml respectively. All isolates were sensitive to the drug griseofulvin. [Table 4]

Ketoconazole

MIC 50 and MIC 90 of Ketoconazole for the species isolated in this study are Trichophyton rubrum was 0.0625 µg/ml and 0.5 µg/ml respectively. Trichophyton mentagrophytes was 0.125 µg/ml and 0.5 µg/ml respectively. Trichophyton tonsurans was 0.125 µg/ml and 0.25 µg/ml respectively. Trichophyton verrucosum was 0.125 µg/ml and 0.125 µg/ml respectively. Microsporum fulvum was 0.0625 µg/ml and 0.125 µg/ml respectively. Epidermophyton floccosum was 0.0625 µg/ml and 0.25 µg/ml respectively. All isolates were sensitive to Ketoconazole except one strain of Trichophyton rubrum. The MIC of resistant strain was 4µg/ml.

Fluconazole

MIC 50 and MIC 90 of Fluconazole for the species isolated in this study are Trichophyton rubrum was 2 µg/ml and 16 µg/ml respectively. Trichophyton mentagrophytes was 4 µg/ml and 16 µg/ml respectively. Trichophyton tonsurans was 8 µg/ml and 16 µg/ml respectively. Trichophyton verrucosum was 8 µg/ml and 8µg/ml respectively. Microsporum fulvum was 2 µg/ml and 4 µg/ml respectively Epidermophyton floccosum was 4 µg/ml and 8 µg/ml respectively. All isolates were sensitive to the drug Fluconazole except one strain of Trichophyton rubrum. 79 * MIC of resistant strain 64µg/ml.

Itraconazole

MIC 50 and MIC 90 of Itraconazole for the species isolated in this study are Trichophyton rubrum was 0.0625 µg/ml and 0.5 µg/ml respectively. Trichophyton mentagrophytes was 0.0625 µg/ml and 0.5 µg/ml respectively. Trichophyton tonsurans was 0.0625 µg/ml and 0.125 µg/ml respectively. Trichophyton verrucosum was 0.125 µg/ml and 0.125 µg/ml respectively. Microsporum fulvum was 0.0625 µg/ml and 0.125 µg/ml respectively. Epidermophyton floccosum was 0.0625 µg/ml and 0.25 µg/ml respectively. All isolates were sensitive to Itraconazole except for one strain of Trichophyton rubrum. MIC of resistant strain of Trichophyton rubrum is 2 µg/ml

Terbinafine

MIC 50 and MIC 90 of Terbinafine for the species isolated in this study are Trichophyton rubrum was 0.0625 µg/ml and 0.25µg/ml respectively. Trichophyton mentagrophytes was 0.0625 µg/ml and 0.25 µg/ml respectively. Trichophyton tonsurans was 0.0625µg/ml and 0.125µg/ml respectively. Trichophyton verrucosum was 0.125µg/ml and 0.125µg/ml respectively. Microsporum fulvum was 0.0625µg/ml and 0.125µg/ml respectively. Epidermophyton floccosum was 0.0625µg/ml and 0.125µg/ml respectively. All isolates were sensitive to Terbinafine except one strain of Trichophyton rubrum. MIC of resistant strain was 4µg /ml.

Sertaconazole

MIC 50 and MIC 90 of Sertaconazole for the species isolated in this study are Trichophyton rubrum was 0.0313µg/ml and 0.125µg/ml respectively. Trichophyton mentagrophytes was 0.0313µg/ml and 0.125µg/ml respectively. Trichophyton tonsurans was 0.0313µg/ml and 0.0625µg/ml respectively. Trichophyton verrucosum was 0.0313µg/ml and 0.0625µg/ml respectively. 80 Microsporum fulvum was 0.0313 µg/ml and 0.0625µg/ml respectively. Epidermophyton floccosum was 0.0313µg/ml and 0.0625µg/ml respectively. All isolates were sensitive to the drug Sertaconazole. [Figure 4]

DISCUSSION

Dermatophytosis is a significant public health problem in tropical and subtropical countries like India, yet remains unresolved. With regard to the increasing trend of antifungal resistant dermatophytes, the requirement of rapid and precise identification of causative fungi and antifungal susceptibility testing become vital. So, this hospital based cross-sectional study was conducted in the Department of Microbiology in a tertiary care hospital for a period of one year. The study included a total of 100 patients attending the Dermatology Outpatient Department who were newly diagnosed clinically as a case of dermatophytosis. Skin scrapings, nail clippings and epilated hair samples were collected and processed. The fungi were isolated, speciated and the anti-fungal susceptibility patterns were determined

Collection of specimens

In this study, Majority 72 of samples was skin scrapings, 16 nail clippings and 12epilated hair specimens. These findings are in accordance with the studies done by Dr.Raghavendra Rao M et al,[5] in which Skin 73.33%, Hair 16.66% &Nail 10% and Kumaran et al,[6] in which Skin scraping 54%, Hair samples 39% & Nail clippings 7% reported. The collection of specimen among study population was found to be statistically significant (p value< 0.001).

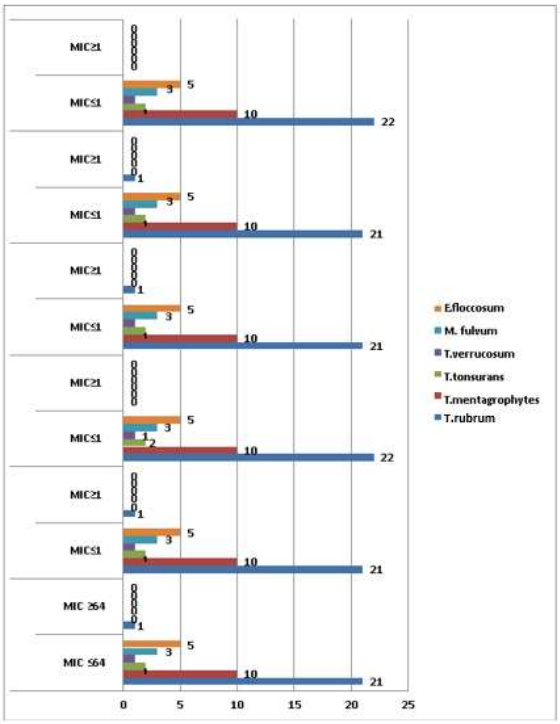

Figure 4: comparison of antifungal susceptibility (Sensitive, Resistant) pattern of the drugs fluconazole, ketoconazole, griseofulvin, itraconazole, terbinafine and sertaconazole from below upwards to the isolated dermatophytes (n=43)

Clinical types of dermatophytosis

In our study, among the skin lesions, tinea corporis was the predominant clinical presentation which occurred in 43 (59.7%) patients followed by tinea cruris in 24 patients (33.3%), tinea manuum in 2 patients (2.8%), tinea pedis in 2(2.8%)patients and tinea faciei only 1 patient (1.4%). The higher incidence of tinea corporis followed by tinea cruris was probably due to its symptomatic (pruiritic) nature which leads the patient to seek medical advice. It was observed that most of the patients with tinea infection were involved in exhausting physical work and prolonged exposure to sun that result in profuse sweating. The tight fittings and synthetic clothing may also provide damp. sweaty and warm skin conditions that favour dermatophytic infection.

The most common site of Tinea corporis involved in females was the waist area due to the patterns of clothing i.e. sarees and salwars which were worn by

the women act as predisposing factors because of friction, excessive sweating, collection of dust and fungal spores at belt line. A low incidence of tinea pedis in 2(2.8%) patients correlate with the study of Venkatesan et al,[7] with the incidence of 4patients (5.6%) in Chennai. The predominance of Tinea pedis in office employees & in western countries could be due of the habitual use of occlusive footwears like socks &shoes, resulting in clamminess and warmness of the feet thereby facilitate the dermatophyte growth. In the present study, among 12 hair samples Tinea capitis was detected in 6 and Tinea barbae in 6 patients. Tinea capitis is less common in India than in other countries, this may be attributable to the topical application of hair oil by Indians which has been revealed to have an inhibitory consequence on dermatophytic infection of scalp. These findings are in accordance with the studies done by Sudha M et al, Vijayakumar Ramaraj et al,[8] and Srinivasan Balakumar et al.[9]

Co-morbid conditions

The Co-existing diseases may be a co-incidence or play a role as aggravating factor in Tinea infections. In this study, Diabetes mellitus was the most common association seen in 10 patients followed by systemic hypertension was observed in 5 patients, both Diabetes mellitus and Systemic hypertension in 4 patients, Psoariasis was found in 2 patients &Bronchial asthma in 2 patients. This is concordance with the studies done by Vignesh D et al (2015) in Kanchipuram in which DM accounts for20%&16% Hypertension, Dr.Bindu Mitruka et al,[10] in which 14%Atopy, 9%DM, 1.3%Psoriasis, 1.3%Pulm.TB &0.7% SLE, Lakshmi Vasantha Poluri et al,[11] in which 8.06% diabetics, 6.45% anaemic,3.23% atopy &1.61%HIV Positive and B.Janardhan et al,[12] in which DM 22%,HT 9%,Atopy 2% &HIV 2% reported . Associated co-morbid conditions among study population was found to be statistically significant (p value< 0.001).

Occupation

Occupational conditions like wet humid environment, occlusive clothing and footwear, contact with soil and animal can determine the clinical types of tinea. In the present study, 33 patients were engaged in occupations like agriculturer and 22 patients were outdoor labourer, 15 patients were students, 15patients were office employees and 15 patients were housewives. The highest prevalence in agriculturers coupled with the fact that host susceptibility which may be enhanced by moisture, warmth, specific skin chemistry due to prolonged exposure to sun, perspiration, age, heavy exposure to hot and humid climates, crowded living conditions and unhygienic conditions associated with poverty. Their constant handling of hay, clay & soil in the field, bare foot walking, working with unprotected hands in the fields, gardens with manure and decaying organic matter in the soil will add further risk of getting these fungal infections.

Housewives have a predilection towards dermatophytosis as they have a propensity to sweat enormously owing to their household and kitchen works. The amplified occurrence of dermatophytosis in housewives and outdoor labourers can be explained by their characteristic pattern of vocation whereas the office employees and students are very prone for dermatophytic infections owing to their custom of wearing ill fittig tight synthetic garments, shoe & socks, occlusive footwear and prolonged working hours. These findings correlate with the studies done by Dr.Bindu Mitruka et al,[10] in which Farmers 52 cases, Labourers 26 cases, Housewives 21cases, Students 19 cases,Business class 9 cases, Army 6 cases & Servicemen 3 cases, Singh S et al,[13] in which Agricultural workers &Manual labourers 44%,Household workers10%,Students10% &Professionals 7% , B.Janardhan et al,[12] in which Active workers 41%, Sedentary workers 26%, Housewives 17% & Students 14% and Vignesh D et al in Kanchipuram in which Labourers 40% ,Students 22% followed by Housewives reported. The occupation among study population was found to be statistically significant (p value 0.015).

Risk factors

Most common risk factor involved in this study was close family contacts (34 patients), followed by 23 patients with history suggestive of hyperhidrosis, 18 patients were hostellers, 15 patients had contact with soil and 9 patients had a history of pets at home &1 patient with handling of animal. Transmission of fungal spores in family members can be due to direct contact or through fomites or denovo infectivity which can be explained by the reality that 21 patients in this study had habit of sharing of fomites such as towel, comb, soaps, footwears, bed linens among their family members which is a universal carry out across our nation that harbor the fungal spores capable of transmitting the disease among the family members. These findings are in accordance with the studies done by Mahajan et al,[14] in which Family history 30.9%, Hyperhidrosis 2.2%, Lakshmi Vasantha Poluri et al,[11] in which 32.25%Contact with soil reported and Monika Kucheria et al,[15] in which 33% 86 accounts for % Family history. The risk factors among study population was found to be statistically significant (p value< 0.001).

Personal History

In the present study usage of synthetic garments, leggings and jeans were observed in 34 patients of which 16 were males and 18 were females followed by 21patients with history of sharing of fomites , 21patients did not have the practice of wiping body appropriately following bath which was substantiated by the study of Krishnendu Das,[16] et al. Similar findings were observed in the studies of Dr. Bindu Mitruka et al,[10] in which Occlusive clothing 19cases,Wet occupation 13 cases &occlusive footwear 9 cases reported and Lakshmi Vasantha Poluri et al,[11] in which 40.32% accounts for Occlusive or synthetic dressing

Fungal isolates

Most of the isolates were from skin scrapings (30%) followed by 7% from nail clippings and the least 6%

from epilated hair samples. All the three genera of dermatophytes such as Trichophyton 35(81%), Microsporum 3 (7%) and Epidermophyton 5 (12%) had been isolated as the causal agent in the present study. Out of 43 isolates of dermatophytes, 35(81%) isolates belonged to the Genus Trichophyton of which Trichophyton rubrum was the predominant isolate 23 (65.7%). The high preponderance of Trichophyton rubrum was explained by the persistent characteristic pattern of infection and the variation of dermatophyte to skin of humanbeings. Out of 23 isolates of Trichophyton rubrum, 12 (52.1%) isolated from tinea corporis, 3 isolates(13%)from tinea cruris,one isolate (4.3%)from tinea pedis, only one isolate (4.3%) from tinea mannum, one isolate (4.3%) from tinea faciei and 5(21.7%) from tinea unguium followed by Trichophyton mentagrophytes 10(23%), Trichophyton tonsurans 2 (4.7%), Trichophyton verrucosum 1(2.3%), Microsporum fulvum 3(7%) and Epidermophyton floccosum 5(11.6%) which could be due to the interaction of patients with domestic animals and soil. These findings were substantiated by the studies of Sudha M et al, Vijayakumar Ramaraj et al,[8] and Srinivasan Balakumar et al.[9]

Antifungal susceptibility testing

In the present study the Minimum Inhibitory Concentration range, Minimum Inhibitory Concentration 50 and Minimum Inhibitory Concentration 90 and antifungal susceptibility pattern for the drugs Griseofulvin, Ketoconazole, Fluconazole, Itraconazole, Terbinafine and Sertaconazole were determined. Even though Norries et al and C.J.Jessup,[17] et al studies established the inoculums size, optimum condition, optimum medium for conidial formation, incubation time duration and end point determination but standard reference method for antifungal susceptibility testing of dermatophytic infection is lacking. In our study, all isolates were sensitive to Griseofulvin, Fluconazole, Terbinafine, Itraconazole and Ketoconazole except three isolates of Trichophyton rubrum. Out of three resistant isolates, one (Trichophyton rubrum) was resistant to Ketoconazole &Fluconazole, another (Trichophyton rubrum) to Terbinafine, the another one (Trichophyton rubrum) to Itraconazole. Colin.S.osborne et al had found that some strains of Trichophyton rubrum,[18] isolated showed intrinsic resistance to Terbinafine which on prolonged exposure to the drug can raise the MIC values. Mukharjee et al first confirmed the Terbinafine resistance in superficial fungal infections. Triazole antifungal resistance is more or less similar to studies done by Dominique and Pramod. Cervelatti EP et al and Fachin AL et al had detailed the involvement of ATP Binding Cassette transporter gene in the development of resistance to azoles in Trichophyton rubrum. These findings are substantiated by the studies of Prabhat Kiran Khatri et al in which the Antifungal Resistance pattern of T.rubrum is 38.46% Fluconazole, 30.77% Terbinafine, 38.46% Clotrimazole, 15.38% Ketoconazole. The repeated exposure to azoles group of antifungal may be responsible for the development of resistance in dermatophytes. T.rubrum can develop resistance to azoles and Terbinafine after prolonged exposure to sub-inhibitory concentration of these drugs leading to treatment failures and persistence and chronicity of infections. The suboptimal quality of many antifungal brands in India may further worsen the situation. In India, inadequate treatment regimen or discontinuation of medication due to cost of treatment, difficulties in eliminating predisposing factors and sources of reinfection may play a vital role in antifungal resistance.

Antifungal resistance

Since last few years, there has been a lot of change in the climate, life style and attitude towards health in general population. The hot & humid climate, working conditions and tight fitting clothes such as jeans, leggings &synthetic garments provide a moist and occlusive milieu where dermatophytes thrive. The attitudinal changes such as self medications for unrealistic demand of quick relief, reluctance to seek expert opinion, self medication and non compliance further augment the emergence of antifungal resistance. Self medications such as prolonged application of steroid based creams results in increased prevalence of recalcitrant clinical variants. As long as these factors persist in an individual, the chances of recurrent or persistent infection will preponderate in the community. Inappropriate selection of antifungal agents in addition to inadequate dose and duration of treatment can result in partial response or rapid recurrence of infection and also facilitate the development of drug resistance. Inappropriate application method of topical antifungal preparations may result in prolonged or recurrent infections. The risk of fungi causing deep fungal infections will develop resistance to systemic antifungal agents like itraconazole if it is used rationally in the treatment of dermatophytosis.

The rule of two in the treatment

The topical antifungal agents used in the treatment of dermatophytosis supposed to be applied 2centimeter outside the periphery of the lesion, twice a day for 2 weeks (skin lesion) and bath twice daily.

Prevention of dermatophytosis

Tinea corporis and tinea cruris can be transmitted with contaminated clothing, towel and bed linen hence washed separately, disinfected regularly and drying the cloth inside out. Avoidance of use of tight fitting inner wear, non absorbent clothes and prolonged exposure to wet cloth and weight reduction can avoid the occurrence of tinea cruris. To avoid the occurrence of tinea pedis and onychomycosis protective footwear can be worn when using public facilities. To avoid recurrence, measures should be taken to reduce foot moisture, like drying foot after baths and applying antifungal powder. For zoophilic infections, the source of infection must be traced and treated. Removal of waistband and wristband were must. Summerbell and Weitzman detailed the

preventive measures like good sanitation and use of fungicidal sprays. Disinfection of clothes could be best done by washing in hot water at 60°C, drying in sunlight is considered to be the most effective physical disinfectant for dermatophytes. In the absence of sunlight, ironing the clothes would be beneficial.

CONCLUSION

Apart from urbanization, poverty, overcrowding, non compliance and constitutional factors and co morbid conditions such as diabetes, atopy and immunosuppressive medications that lead to immunosuppression of the host, the unchecked availability of irrational and inexpensive corticosteroid antifungal antibacterial combination sold over the counter is the main cause of concern in India. Fixed drug formulations with topical corticosteroids are used erratically by the patients only for symptom relief. They are influenced by the advertisements promoting the creams claiming dramatic cure of superficial fungal infections. People stop using them when the intense itching and redness of lesion mitigated and once again begin to apply when the symptoms reappear. These medications are being prescribed rampantly by alternative medicine practitioners and general physicians or self medicated,[19] by the patients. Inappropriate selection of antifungal agents in addition to inadequate dose and duration of therapy, inappropriate application methods of topical preparations,[20] and inefficacy of the drug can result in partial response or emergence of resistant recalcitrant clinical variants which cause poor treatment outcome.

Thus, it is important to test for antifungal susceptibility to check the resistance pattern of antifungal agents. Since KOH mount is simple and adequate to screen dermatophytosis, medical and paramedical personal may be trained in the same in resource limited settings for practical purposes. The uses of less efficacious molecules result in clinical failure and also drug resistance. Hence, the quality controls of these drugs are also a real concern.

What we observed in the society is just the tip of iceberg and many hidden cases still in the community that account for relapse and ongoing transmission of dermatophytoses. Hence, there is an impending need for inclusion of this dermatophytic infection in National Public Health Programme in India.

The need for this moment to rectify this problem is comprehensive information, communication activities, education as well as stringent drug controlling laws. Because of laxity in implementation of drug laws there is occurrence of prescription sharing within the family and medication abuse and its consequential side effects. We need to execute an effective strategy to educate the population regarding predisposing factors, adverse effects of over the counter drugs, necessitate expert consultation and importance of subsequent expert's opinion on management of Dermatophytoses. It is also essential to educate the general physicians about the ill effects of steroid combinations and adequate dosage, duration and proper application method of topical preparations of appropriate antifungal agents. The judicious use of newer antifungal drugs, antifungal stewardship, emphasizing the patient's compliance and prescribing without oral or topical corticosteroids are the adjunctive steps to offer cure to the patient.

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