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STUDY OF FUNGAL CULTURE AND SENSITIVITY PATTERN IN DERMATOPHYTOSIS IN TERTIARY CARE HOSPITAL

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Abstract

Prospective Observational Study of fungal culture and sensitive pattern in dermatophytes in dermatology department. The objective of the study is to observe the antifungal sensitivity in patients with dermatophytosis and to assess the prevalence of drug resistant species in dermatophytosis. The specimens were collected from the patients who was diagnosed with dermatophytosis at outpatient department of dermatology and cultured for macroscopic and microscopic examinations to identify the species. The isolates were subjected to disk diffusion assay for the study of sensitivity patterns of antifungals. Out of 69 specimens collected 48 yielded growth and 21 samples had no growth of dermatophytes.. Test results of the susceptibility to antifungal drugs were as follows: Ketoconazole: 25 (52.08%) sensitive, 13(27.68%) intermediate, 10 (20.83.6%) resistant. Griseofulvin: 18 (37.5%) resistant, 24 (50%) sensitive, 6 (12.5%) intermediate. Miconazole: 32 (66.66%) sensitive, 12 (25%) intermediate, 4(8.3%) resistant. Terbinafine: 48 (100%) sensitive. Clotrimazole: 39 (81.23%) sensitive, 4 (5.3%) intermediate, 5 (10.41%) resistant .Fluconazole: 38 (79.16%) resistant,8 (16.6%) intermediate, 2 (4.16%) sensitive. from the data, it was revealed that terbinafine and clotrimazole were the most effective antifungal drugs & fluconazole has the least activity and T.rubrum was resistant species in and around Chidambaram. In our study Highest incidence of dermatophytosis was seen in the age group of 31-40 years (30.4%). Dermatophytosis was more common in males (75.4%). Tinea corporis (47.8%) was the most commonly clinically diagnosed. The most commonly isolated species was T.rubrum(45.83%). Study data shows T.rubrum was the drug resistance species (36.36%) and terbinafine was the most sensitive (100%) antifungal agent and fluconazole was the least sensitive (4.16%) antifungal agent. The present study suggests that it would be rational to treat the dermatphytosis patients according to their culture test results and antifungals should be wisely used to prevent the emergence of resistance.

Keywords: Dermatophytosis, Terbinafin, Fluconazole, Clotrimazole.

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INTRODUCTION

Dermatophytes are fungi that invade and multiply within keratinized tissues (skin, hair, and nails) causing infection . Based upon their 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 hair). Based upon the mode of transmission, these are been classified as anthropophillic, zoophilic, and geophilic. Finally, based upon the affected site, these are been classified clinically into tinea capitis (head), tinea faciei (face), tinea barbae (beard), tinea corporis (body), tinea manus (hand), tinea cruris (groin), tinea pedis (foot), and tinea unguium (nail). Other clinical variants include tinea imbricata, tinea pseudoimbricata, and Majocchi granuloma. Pattern of skin diseases vary from country to country. Even in the same country it differs from region to region. India is such a country where wide variations in climate, socio - economic status, religion and customs is quite prevalent in different parts of the country. In developing countries, other than hot and humid climatic conditions, low hygiene, poor access to water, overcrowding contact also plays significant etiological role for dermatophytosis [1-7].

DIAGNOSIS OF DERMATOPHYTOSIS

Laboratory investigations For a laboratory to provide optimal results, quantity and quality of material examined is critical. Scraping should be collected from active margin and transported in a pre sterilized black chart paper which keeps the specimen dry thus, preventing over growth of bacteria contaminants. Following are the various laboratory tests that can be used for confirming a diagnosis of dermatophytosis.

1. Direct microscopic examination: Treatment of skin specimen with 10–20% potassium hydroxide (KOH) is a quick and inexpensive bedside tool to provide evidence of dermatophytic infection. Positive scrapings are characterized by presence of refractile, long, smooth, undulating, branching, and septate hyphal filaments with or without arthroconidiospores. False negative results are seen in 15% cases. Fluorescent staining with optical brighteners (diaminostilbene) is the most sensitive method to microscopically detect fungi in skin scales as well as in specimens from nails and hair. These substances bind to chitin, the main cell wall component of fungi [8].

2. Culture and antifungal sensitivity: Sabouraud dextrose agar (SDA, 4% peptone, 1% glucose, agar, water) is the most commonly used isolation media for dermatophytosis and serves as the medium on which most morphologic descriptions are based. Development of colony takes 7–14

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days. Modified SDA, with addition of gentamicin, chloramphenicol and cycloheximide is more selective for dermatophytes as chloramphenicol inhibits the growth of saprophytic fungus. Dermatophyte test medium is an alternative to isolation media that contain pH indicator phenol red. It is incubated at room temperature for 5–14 days. Dermatophytes utilize the protein resulting in excess ammonium ion and alkaline environment which turn the medium from yellow to bright red [9-14].

Antifungal susceptibility testing

Microdilution method: The broth microdilution assay for antifungal susceptibility testing of dermatophytes has been previously developed as a modification of the Clinical and Laboratory Standards Institute M38-A2 standard method. The final concentrations of terbinafine and itraconazole used is 0.06–32.0 µg/ml and for fluconazole, 0.13–64.0 µg/ml. A standardized inoculum is prepared by counting the microconidia microscopically. Cultures are grown on SDA slants for 7 days at 35°C to produce conidia. Sterile normal saline (85%) is added to the agar slant, and the cultures are gently swabbed with a cotton-tipped applicator to dislodge the conidia from the hyphal mat. The suspension is transferred to a sterile centrifuge tube, and the volume is adjusted to 5 ml with sterile normal saline. The resulting suspension is counted on a hemocytometer and is diluted in RPMI 1640 medium to the desired concentration. Microdilution plates are set up in accordance with the reference method. The microdilution plates are incubated at 35°C and read visually after 4 days of incubation. The minimum inhibitory concentration is defined as the concentration at which the growth of the organism will be inhibited by 80% compared with the growth in the control well.

Minimum fungicidal concentration (MFC) determination: For determination of the MFC, 100-µl aliquots are removed from the assay wells showing no visible growth at the end of incubation and streaked onto SDA plates. The plates are incubated at 30°C for 7 days. The MFC is defined as the lowest drug concentration at which no visible fungal growth or colonies developed.

Dermatophyte identification: This can be based on colony characteristics, microscopic morphology, and physiologic tests. Dermatophytes can be distinguished based upon their morphology of the macroconidia. Few physiological tests are available which help in confirmation of certain species. In addition, special amino acid and vitamin requirements can differentiate Trichohyton species from others. Ability to hydrolyse urea differentiates *T. mentagrophytes* (urease positive) from T. *rubrum* (urease negative) [15-17].

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MATERIALS AND METHODS

This study was conducted at Rajah Muthiah medical college hospital, Annamalai Nagar, TamilNadu, which is 1260 bedded multi-specialty tertiary care teaching hospital from the period of 2016 to 2017. It is a Prospective Observational study. Specimens were collected at outpatient department of dermatology from patients who are diagnosed with dermatophytosis. The specimens are collected in whatmann filter paper and direct microscopic examination was done using KOH mount. The specimens were cultured in Sabouraud's dextrose agar (SDA) with gentamycin and cyclohexamide. The cultures were examined thrice weekly for appearance of growth, Cultures were incubated for 1 month before discarding them as negative. Once the growth is noticed macroscopic and microscopic examinations were carried out and the urease test is performed to identify the species. The isolates are subjected to disk diffusion assay for the study of sensitivity patterns of antifungals using antifungal discs. All the tests were performed according to Esteban et al. . The inoculum was evenly spread on the surface of 10 cm Petri dishes containing Sabouraud dextrose agar medium and exposed to air dry. Then, the antifungal disks were applied to the plates, after which the plates were incubated at 25°C for 5-10 days. After the colonies grew, the zones of inhibition around the disks were measured and recorded. Criteria of susceptibility and resistance of antifungal disks were measured.

Table 1

ANTIFUNGAL DRUGS	POTENCY	ZONE DIAMETER IN mm		
		SENSITIVE	INTERMEDIATE	RESISTANCE
Clotrimazole	10 µg	≥20	19-12	≤11
Fluconazole	25 µg	≥22	21-15	≤14
Griseofulvin	25 µg	≥10	-	- No zone
Ketoconazole	15 µg	≥30	29-23	≤22
Miconazole	10 µg	≥20	19-12	≤11
Terbinafine	30 µg	≥20	19-12	≤11

Required data collected was recorded in a specially designed proforma and the study was ethically approved.

Inclusion Criteria:

• Patients of DVL department who are diagnosed as dermatophytosis.

• Patientsof all age groups.

• Patients of all genders.

Exclusion Criteria:

• Patient who are not willing to participate.

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RESULTS AND DISCUSSION

Table 2: RELATION BETWEEN AGE AND GENDER

GENDER/AGE	0 – 10	11 – 20	21 – 30	31 – 40	41 – 50	≥ 51	TOTAL
MALE	4	7	10	17	9	5	52
FEMALE	1	3	3	4	3	3	17
TOTAL	5	10	13	21	12	8	69

From our study the most affected were male (75.4%) and then female(24.6%) mainly in the age gap of 31-40 years.

Table 3: DISTRIBUTION OF SITE OF INFECTION

SITE OF INFECTION	NO. OF PATIENTS
TRUNK(Tinea corporis)	33
GROIN AREA(Tinea cruris)	22
NAILS(Tinea unguium)	7
FOOT(Tinea pedis)	4
SCALP(Tinea favosa)	3

Among the 69 cases, majority of the specimens collected from trunk (33) followed by groin area (22), nails (7), foot (4) and scalp (3).

Table 4: CULTURE GROWTH

CULTURE GROWTH	NO.OF PATIENTS
POSITIVE	48 (69.6%)
NEGATIVE	21(30.4%)

Out of 69 samples 48 yielded growth and 21 samples had no growth of dermatophytes.

Table 5: DISTRIBUTION OF DERMATOPHYTES:

S.NO.	ORGANISM ISOLATED	NO.OF ISOLATES	%
1	*T.rubrum*	22	45.83%
2	*T.mentagrophytes*	12	25%
3	*T.tonsurans*	5	10.41%
4	*T.violaceum*	2	4.16%
5	*M.gypseum*	3	6.25%
6	*M.ferruginum*	2	4.16%
7	*E.floccosum.*	2	4.16%

Growth in SDA yielded 48 positive cultures. Based on growth in SDA, tween assimilation, urease test specification was done. Out of 48 positive isolates, 22(45.83%) were T.rubrum, 12 (25%) were T.menagrophytes, 5(10.41%) were T.tonsurans, 2(4.16%) were T.violaceum, 3(6.25%) were M.gypseum, 2(4.16%) were M.ferruginum, 2(4.16%) were E.floccosum.

CULTURE SENSITIVITY

All the positive cultures were processed for antifungal susceptibity test using antifungal discs of Terbinafine, Clotrimazole, Ketoconazole, Miconazole, Griseofulvin and Fluconazole. Despite the

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many guidelines that NCCLS(National Committee for Clinical Laboratory Standards) have published for susceptibility tests of moulds, there is no clear method and routine test for the evaluation of dermatophyte antifungal activity.Here is the culture sensitivity pattern of different isolated species noted according to the zone of inhibition around the antifungal discs.

Table 6: CULTURE SENSITIVITY OF *T.rubrum*

DRUGS	SENSITIVITY PATTERNS OF *T.rubrum*		
	SENSITIVE	INTERMEDIATE	RESISTANT
TERBINAFINE	22	-	-
CLOTRIMAZOLE	15	2	5
KETOCONAZOLE	8	6	8
MICONAZOLE	14	4	4
GRISEOFULVIN	11	-	11
FLUCONAZOLE	-	2	20

Table 7: CULTURE SENSITIVITY OF *T.mentagrophytes*

DRUGS	SENSITIVITY PATTERNS OF *T.Mentagrophytes*		
	SENSITIVE	INTERMEDIATE	RESISTANT
TERBINAFINE	12	-	-
CLOTRIMAZOLE	10	2	-
KETOCONAZOLE	8	2	2
MICONAZOLE	9	3	-
GRISEOFULVIN	8	-	4
FLUCONAZOLE	2	2	8

Table 8: CULTURE SENSITIVITY PATTERN OF *T.tonsurans*

DRUGS	SENSITIVITY PATTERNS OF *T.tonsurans*		
	SENSITIVE	INTERMEDIATE	RESISTANT
TERBINAFINE	5	-	-
CLOTRIMAZOLE	5	-	-
KETOCONAZOLE	4	1	-
MICONAZOLE	5	-	-
GRISEOFULVIN	3	-	2
FLUCONAZOLE	-	1	4

Table 9: CULTURE SENSITIVITY PATTERN OF *T.violaceum*

DRUGS	SENSITIVITY PATTERNS OF *T.violaceum*		
	SENSITIVE	INTERMEDIATE	RESISTANT
TERBINAFINE	2	-	-
CLOTRIMAZOLE	2	-	-
KETOCONAZOLE	1	1	-
MICONAZOLE	2	-	-
GRISEOFULVIN	-	2	-
FLUCONAZOLE	-	-	2

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Table 10: CULTURE SENSITIVITY PATTERN OF *M.gypseum*

DRUGS	SENSITIVITY PATTERNS OF *M.gypseum*		
	SENSITIVE	INTERMEDIATE	RESISTANT
TERBINAFINE	3	-	-
CLOTRIMAZOLE	3	-	-
KETOCONAZOLE	2	`1	-
MICONAZOLE	2	1	-
GRISEOFULVIN	2	-	1
FLUCONAZOLE	-	1	2

Table 11: CULTURE SENSITIVITY PATTERN OF *M.ferruginum*

DRUGS	SENSITIVITY PATTERNS OF *M.ferruginum*		
	SENSITIVE	INTERMEDIATE	RESISTANT
TERBINAFINE	2	-	-
CLOTRIMAZOLE	2	-	-
KETOCONAZOLE	1	1	-
MICONAZOLE	-	2	-
GRISEOFULVIN	-	1	1
FLUCONAZOLE	-	-	2

Table 12: CULTURE SENSITIVITY PATTERN OF *E.floccosum*

DRUGS	SENSITIVITY PATTERNS OF *E.floccosum*		
	SENSITIVE	INTERMEDIATE	RESISTANT
TERBINAFINE	2	-	-
CLOTRIMAZOLE	2	-	-
KETOCONAZOLE	1	1	-
MICONAZOLE	-	2	-
GRISEOFULVIN	-	2	-
FLUCONAZOLE	-	1	1

Table 13: DERMATOPHYTES RESISTANCE TO ANTIFUNGALS

ISOLATED SPECIES	PERCENTAGE OF RESISTANCE
T.rubrum	36.36%
T.mentagrophytes	19.44%
T.tonsurans	20%
T.violaceum	16.66%
M.gypseum	16.66%
M.ferruginum	25%
E.floccosum.	8.33%

From the above culture sensitivity pattern tests T.rubrum (36.36%) was found to be the most drug resistant speciesfollowed by M.ferruginum (25%), T.tonsurans (20%), T.mentagrophytes (19.44%), T.violaceum (16.66%), M.gypseum (16.66%), E.floccosum. (8.33%).

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Table 14: ANTIFUNGAL SUSCEPTIBILITY

ANTIFUNGAL AGENT	SUSCEPTIBILITY
TERBINAFINE	100%
CLOTRIMAZOLE	93%
KETOCONAZOLE	57.09%
MICONAZOLE	57.89%
GRISEOFULVIN	34.76%
FLUCONAZOLE	8.38%

Test results of the susceptibility to antifungal drugs were as follows: Ketoconazole: 25 (52.08%) sensitive, 13(27.68%) intermediate, 10 (20.83.6%) resistant. Griseofulvin: 18 (37.5%) resistant, 24 (50%) sensitive, 6 (12.5%) intermediate. Miconazole: 32 (66.66%) sensitive, 12 (25%) intermediate, 4(8.3%) resistant. Terbinafine: 48 (100%) sensitive. Clotrimazole: 39 (81.23%) sensitive, 4 (5.3%) intermediate, 5 (10.41%) resistant. Fluconazole: 38 (79.16%) resistant,8 (16.6%) intermediate, 2 (4.16%) sensitive. From the data, it was revealed that terbinafine and clotrimazole were the most effective antifungal drugs and fluconazole had the poorest activity.

CONCLUSION

The present study shows that, Highest incidence of dermatophytosis was seen in the age group of 31-40 years (30.4%). Dermatophytosis was more common in males (75.4%). Tinea corporis (47.8%) was the most commonly clinically diagnosed, it was presented with hyperpigmentation.The culture positivity was (69.6%).The most commonly isolated species was *T.rubrum (45.83%).*

- In the present study *T.rubrum* (36.36%) was the drug resistance species.
- Our study reveals that Terbinafine (100%) was the most sensitive antifungal agent followed by clotrimazole (81.23%) and Fluconazole (4.16%) was the least sensitive antifungal agent.

Dermatophytes are the most common cause of infectious skin disease. The most challenging task is not the diagnosis but the treatment and patient recovery. Since it requires a long time treatment, the management with appropriate and responsive drug is the need.

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