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ISOLATION, IDENTIFICATION & ANTIMYCOTIC SENSITIVITY OF DERMATOPHYTES BY DISC DISFUSION METHOD

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ABSTRACT

Introduction: - Dermatophytosis is a very common superficial fungal skin infection. The clinical presentation, through very typical of ringworm infection, is often confused with other skin disorder, making laboratory diagnosis and confirmation necessary. The present study was undertaken to isolate, identify and to check the Antimycotic susceptibility pattern of aetiological agents of Dermatophytosis. **Method & Material:-** skin scrapping were inoculated on SDA and isolates were identified by macroscopic(Colony observe, Colony reverse & onset of sporulation and pigment production) & microscopic (LCB) observation. Antimycotic susceptibility test was done by method as described by CLSI Guideline 2014[5]. **Result:-** A total of 50 clinically diagnosed randomly selected patients of Dermatophytosis attending the outpatient department of skin, MDM Hospital associated with Dr. S. N. Medical College, Jodhpur were studied. Dermatophytosis was more common in the age group of 11-20 years (26%) and in males (62%). Most of the patients belonged to urban area (80%). Fungi were isolated in 23 cases (46%). Most common clinical type was tinea corporis (30%) followed by tinea cruris (28%). Most common aetiological agent was *T.verrucosum* (54%) followed by *T. tonsurans* (10%), *T. rubrum* (6%), *E. floccosum* (2%).

KEYWORDS:- 1. Dermatophytosis 2. Disc Disfusion Method 3. Antimycotic Sensitivity.

INTRODUCTION

Superficial fungal infections are the most common skin diseases, affecting millions of people throughout the world. The dermatophytes are by far the most significant fungi because of their widespread involvement of population at large and their prevalence all over the world. The estimated lifetime risk of acquiring a dermatophyte infection is between 10- 20 %.[1]

Dermatophytes are assuming greater significance both in developed and developing countries particularly due to the advent of immunosuppressive drugs and disease.[2] Hot and humid climate in tropical and subtropical countries like India makes Dermatophytosis a very common superficial fungal skin infection.[3]

Although Dermatophytosis does not cause mortality, it does cause morbidity and poises a major public health problem and also is of cosmetic importance.[4]

METHOD AND MATERIAL

This study was conducted at Microbiology Laboratory in Dr. S.N. Medical College to isolate dermatophytes from clinical samples and to find out Antimycotic sensitivity pattern of dermatophytes.

The present study was conducted on 6 species of various Dermatophytes and 1pathogenic mold strains of pathogenic species comprising of *Microsporum ferrugineum* (6), *Trichophyton rubrum* (3), *Trichophyton tonsurans* (5), *Trichophyton verrucosum* (7), *Epidermophyton floccosum* (1) and *fusarium* mold species (1).

Dermatophytes were isolated from various clinical samples like tinea Cruris, tinea corporis, tinea mannum, tinea pedis, tinea faciei from MDM Hospital associated to Dr. Sampurnanand Medical Collage, Jodhpur.

Collection of Sample

The infected area of patient is clear with the help of spirit and sterile gauge then sterile swab were rotted several times on the surface of the infected area.

Inoculation of the sample on Sabouroud dextrose Agar

The media is inoculated with the material and incubated in BOD incubator (at room temperature i.e., 22-33 °) for minimum of four weeks. In majority of dermatophytes, growth and sporulation occurs in 7 days. In some it may take longer time. T. verrucosum and some strains of T. tonsurans grow better at 37ºC. At the onset of sporulation and pigment production, growth is examined by lactophenol cotton blue preparation (LPCB).

Dermatophytes are identified based on the following features

1) **Colony observe**

   The color (white, pearl, ivory etc), texture of the surface (glabrous or waxy, powdery, granular, suede-like velvety, downy or fluffy).

2) **Colony reverse**

   Presence or absence of the pigment, whether diffusing into the medium or not and topography (flat, raised, heaped) and rate of growth.

3) **Microscopic morphology**

studied in tease-mount method.

Tease-mounts (Lactophenol Cotton Blue)

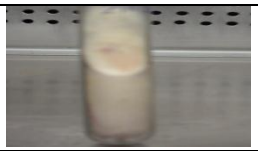
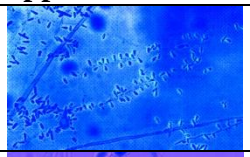
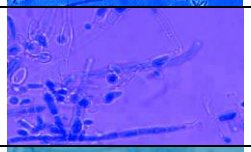
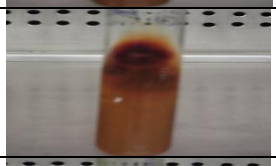
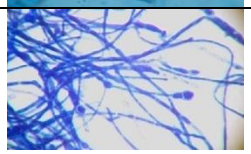
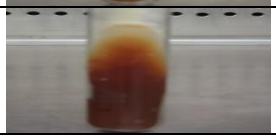
Cultures were examined microscopically by removing a portion of aerial mycelium with a spud. The material was placed on a glass slide in a drop of Lacto phenol cotton blue and the matted mycelium was gently teased with a pair teasing needles. A cover-slip was then placed and excess of stain removed with blotting paper. The morphology was then observed under microscope.

Advantage of slide culture over tease mount technique is that it allows the study of microscopic features of fungus preserving the continuity between the hyphae, microconidia. Macroconidia.

Depending in the morphological characters dermatophytes are classified into three genera:

1) Microsporum
2) Trichophyton
3) Epidermophyton

Table No. 1: Macroscopic (on Sabouroud Dextrose Agar) and Microscopic characteristics of Dermatophytes

S.No.	Species	Colony appearance		Microscopic appearance
		Front	Reverse	
1	*T. rubrum*			
2	*T. tonsurans*			
3	*T. verrucosum*			
4	*M. ferrugineum*			
5	*E. floccosum*			
6	*Fusarium mold*			

Antimycotic susceptibility Test

Antimycotic susceptibility test was done by method as described by CLSI Guideline 2014.[5]

- The plate was prepared with Sabouroud dextrose agar for growing different dermatophytes for carrying out susceptibility of antifungal discs.
- Pure culture was used as inoculums. Taking 3-4 colonies and transferring them into about 5 ml of suitable broth, such as Sabouroud Dextrose Broth. This would incubate at 25-30°C for 2-8 hours till light-to-moderate turbidity develops.
- Colony takes from culture tube and inoculates in Sabouroud dextrose broth and gently shakes with the help of vortex mixture for 5-8 minutes.
- After this when media turns turbid. Inoculate on the SDA plates and keep it for 5minutes then discard it from plates.
- The discs were applied using aseptic technique. The discs were placed in the centers of at least 24 mm apart.

- Immediately incubate the plates at 25-30º C and examine after 14-19 hours or later if necessary. The zones showing complete inhibition and the diameters of the zones are recorded to the nearest millimeter.

Figure showing : Antimycotic sensitivity Trichophyton rubrum

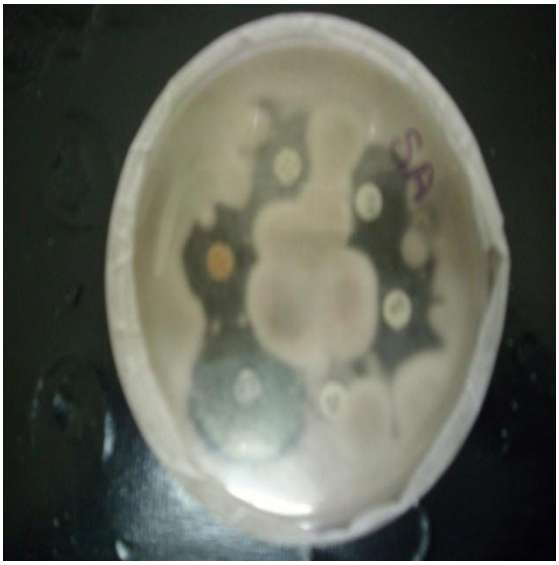


Figure Showing: Antimycotic sensitivity of of Trichophyton verrucosum

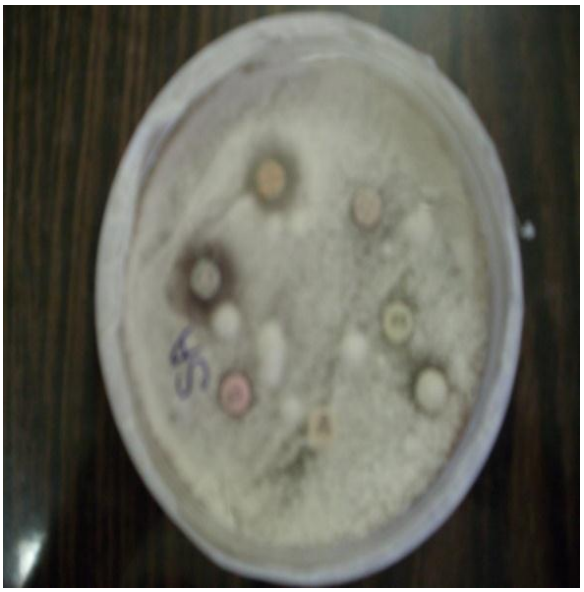


Figure Showing: Antimycotic sensitivity of Microsporum ferrugineum

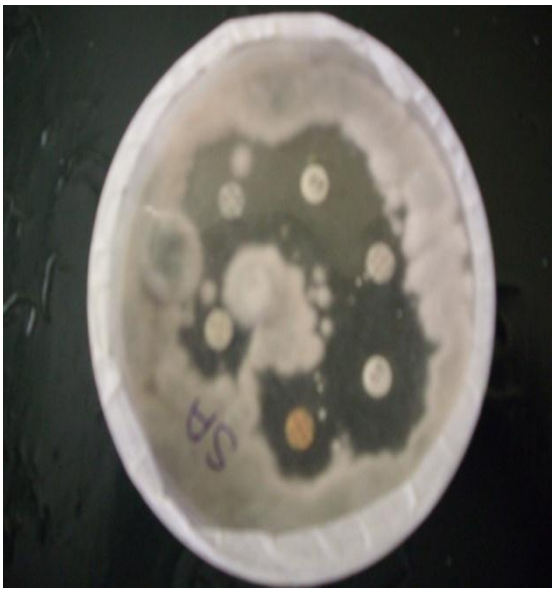


Figure Showing: Antimycotic sensitivity of Epidermophyton floccosum

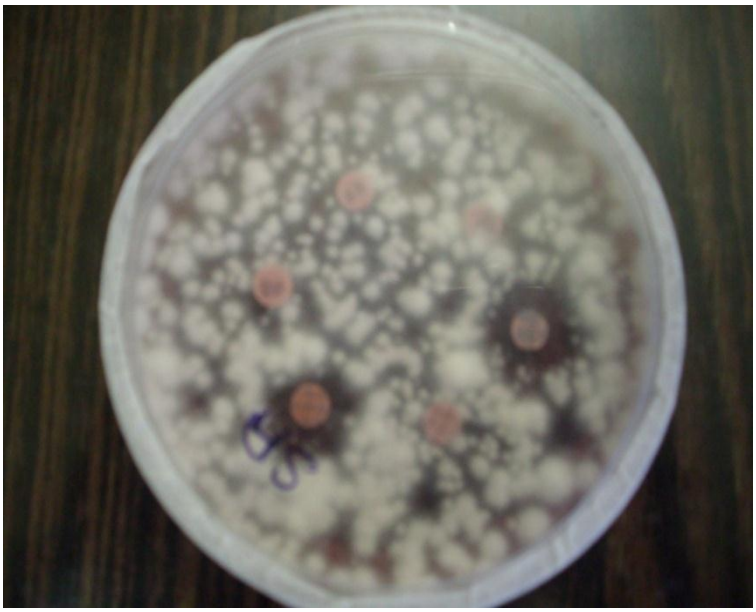


Figure Showing: Antimycotic sensitivity of fusarium mold

TABLES AND OBSERVATIONS

This study was carried out at Microbiology Laboratory of Dr. S.N. Medical College, Jodhpur (Rajasthan) to study "Antimycotic Sensitivity of Dermatophytes by Disc Diffusion Method".

Table No. 2: ISOLATION OF VARIOUS SPECIES OF DERMATOPHYTES

S. No.	Source	Total No. of Sample	Growth of Sample		Isolated dermatophytes
			Positive	Negative	
1	Tinea cruris	14	9	5	*M.ferrugineum*(3), *T.rubrum*(2), *T.tonsurans*(1),T. *verrucosum*(3)
2	Tinea corporis	15	5	10	*T.tonsurans* (3), *T.rubrum* (1), *M. ferrugineum* (1)
3	Tinea capitis	1	1	0	*Fusarium* mold (1)
4	Tinea manuum	10	3	7	*E.floccosum*(1),*M. ferrugineum*(1),*T. tonsurans* (1)
5	Tinea faciei	5	2	3	*M.ferrugineum*(1),*T. verrucosum* (1)
6	Tinea pedis	5	3	2	*T. verrucosum* (3)

From the different source, dermatophytes were isolated in which no. of *Microsporum ferrugineum is* 6, *Trichophyton rubrum* is 3, *Trichophyton tonsurans* is 5, *Trichophyton verrucosum* is 7, *Epidermophyton floccosum* is 1 and *fusarium* mold is 1.

SENSETIVITY OF DIFFERENT SPECIES OF DERMATOPHYTES TO VARIOUS DRUGS

Table No 3: Percentage of isolates Sensitive to Various Drugs by Drug Diffusion Method-

S. No.	Species	Total no. of isolated dermatophytes	Percentage of isolates Sensitive to Various Drugs					
			Amphotericin B (Ap B)	Clotrimazole (Cc)	Nystatin (Ns)	Fluconazole (Fu)	Ketoconazole (Kt)	Itraconazole (It)
1	*M. ferrugineum*	6	33.33%	0%	50%	16%	0%	16%
2	*T. rubrum*	3	66.66%	33.33%	100%	0%	33.33%	66.66%
3	*T. tonsurans*	5	0%	100%	100%	0%	80%	40%
4	*T. verrucosum*	7	16%	16%	66.66%	16%	0%	0%
5	*E. floccosum*	1	100%	0%	100%	0%	100%	100%
6	*Fusarium mold*	1	100%	0%	0%	0%	0%	0%

Table shows that *M. ferrugineum* is sensitive to Clotrimazole (50 %), followed by Amphotericin B (33.3%).

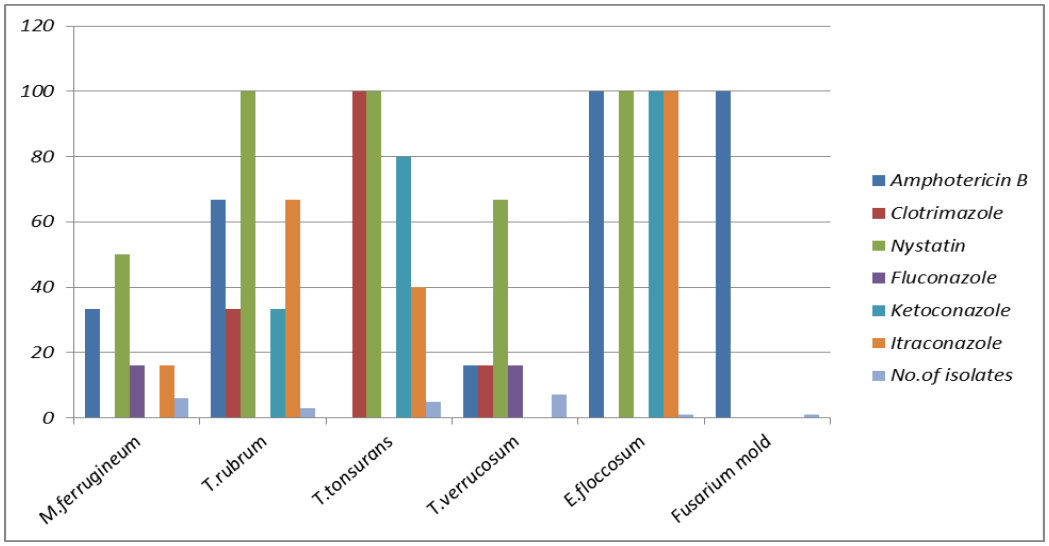
T.rubrum is completely sensitive to Nystatin (100%), followed by Amphotericin (66.6%), Itraconazole (66.6%), Clotrimazole (33.33%) and Itraconazole (33.33%).

T. tonsurans is completely sensitive to Clotrimazole (100%) and Nystatin (100%) followed by Ketoconazole (80%) and Itraconazole (40%).

T. verrucosum is sensitive to Nystatin (66.66%) and less sensitive to Clotrimazole (16%) and Fluconazole (16%).

E. floccosum is highly sensitive to Amphotericin B (100%), Nystatin (100%), and Ketoconazole (100%) and Itraconazole (100%).

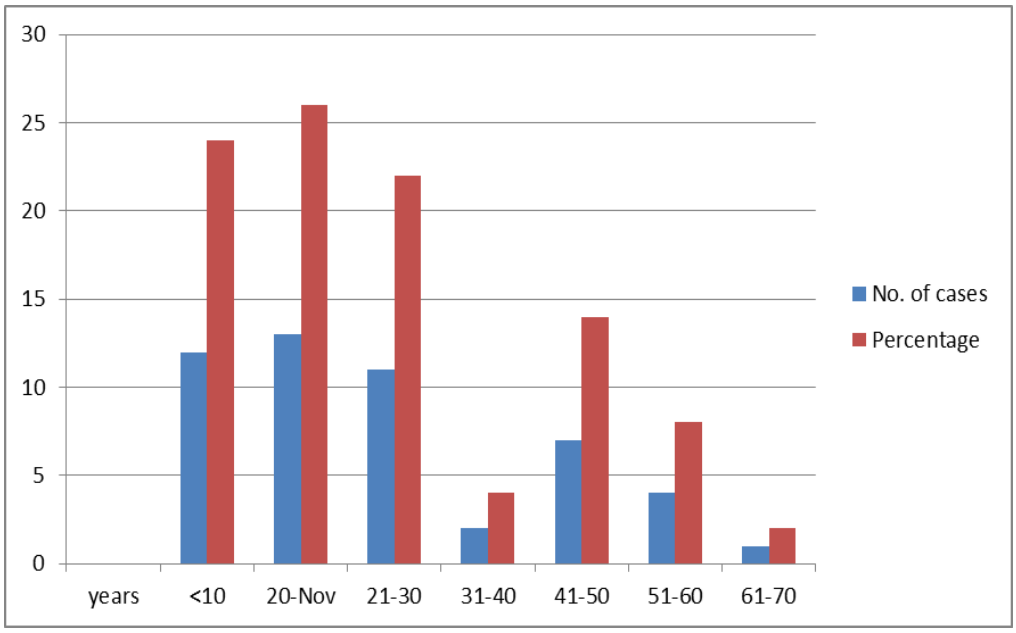
Fusarium mold is highly sensitive to Amphotericin B (100%).



Graph No. 1: % of isolates Sensitive to Various Drugs by Drug Diffusion Method

Table No.4: Age distribution of Dermatophytosis in the study group-

Age (years)	No. of cases	Percentage
<10	12	24
11-20	13	26
21-30	11	22
31-40	2	4
41-50	7	14
51-60	4	8
61-70	1	2
Total	**50**	**100**



Graph No. 2: Age distribution of Dermatophytosis in the study group

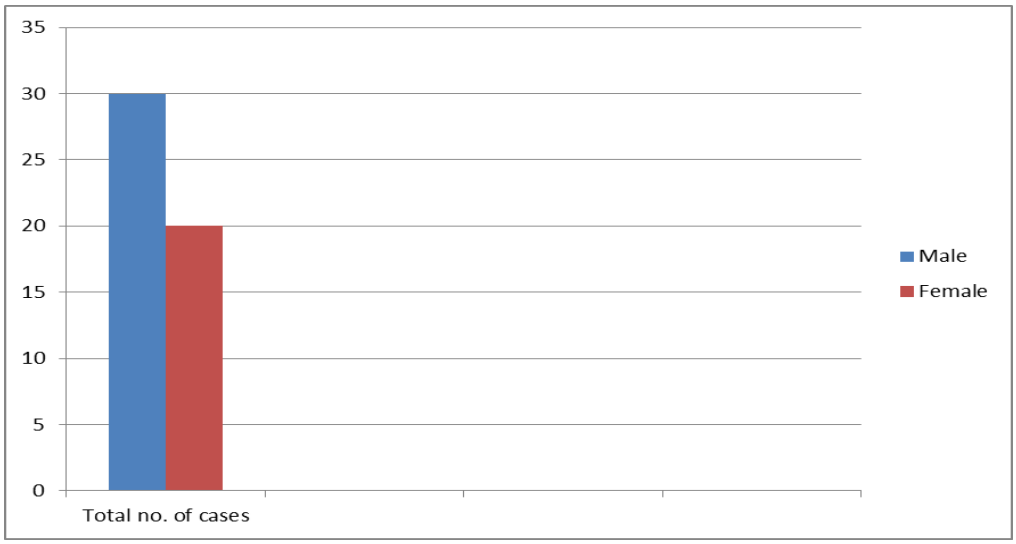
Total no. of 50 cases was distributed between the ranges of 2-70 years. Mean age group was 26.7 years.

Most common age group affected was 11-20 years with 13 cases (26 %) followed by 21-30 (22%), <10 (22%) and 41-50 (14%), 51-60 (12%). Least common age group affected was 31-40 years with 2 cases (4%) followed by 61-70 years with 1 case (2%).

Table No. 5: Sex wise distribution in the study group-

	Male	Female	Total	M:F ratio
No. of cases	30	20	50	1.5:1
Percentage	60	40	100	

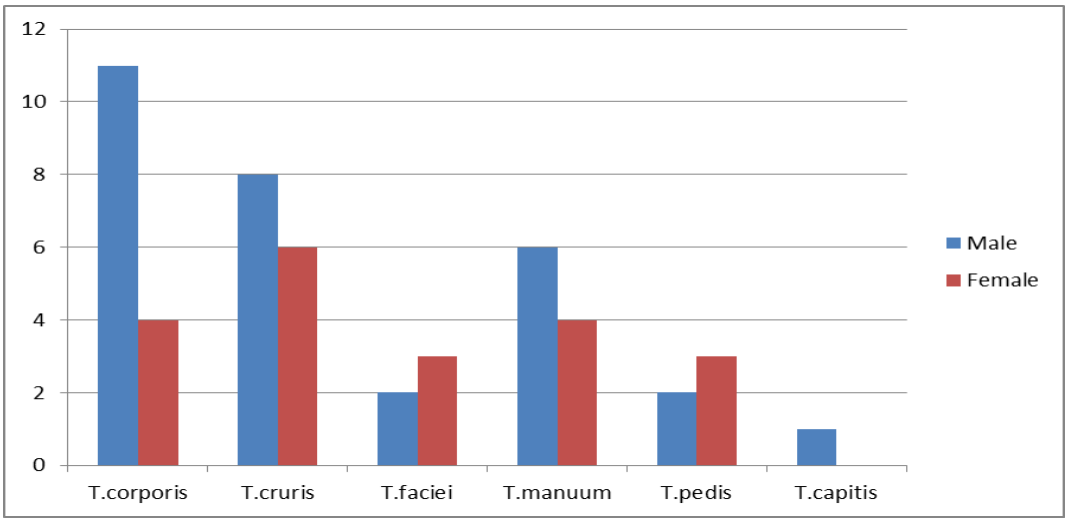
Out of 50 cases, the males were more commonly affected with 30 cases (60%), than females with 20 cases (40%). Male to female ratio was 1.5:10.



Graph No. 3: Sex wise distribution in the study group

Table No 6: Age and Sex wise distribution in relation to clinical types-

S. No.	Clinical Types	Age group in years							Sex		Total	%
		<10	11-20	21-30	31-40	41-50	51-60	61-70	Male	Female		
1	T.corporis	3	4	2	1	2	3	-	11	4	15	30
2	T.cruris	3	-	5	1	4	1	-	8	6	14	28
3	T.faciei	2	3	-	-	-	-	-	2	3	5	10
4	T.manuum	3	5	2	-	-	-	-	6	4	10	20
5	T.pedis	-	1	2	-	1	-	1	2	3	5	10
6	T.capitis	1	-	-	-	-	-	-	1	-	1	2



Graph No. 4 Age and Sex wise distribution in relation to clinical types

RESULT

In this study total 50 samples collected from patients of Skin Department of MDM hospital associated with Dr. S.N. Medical College, Jodhpur, were cultured.

Out of 50 cases, the males were more commonly affected with 30 cases (60%), than females with 20 cases (40%). Male to female ratio was 1.5:1 Total no. of 50 cases was distributed between the ranges of 2-70 years. Mean age group was 25.7 years. Most common age group affected was 11-20 years with 13 cases (26 %) followed by 21-30 (22%), <10 (22%) and 41-50 (12%), 51-60 (12%)

From different 50 No. of clinical samples. In 23 samples fungal growth appear of which 5 species of dermatophytes and 1 Fusarium mold species were isolated. Out of 22 dermatophytes 6 sp. of M. ferrugineum, 3 sp. of T. rubrum, 5 sp. of T. tonsurans, 7 sp. of T. verrucosum, 1 sp. of E. floccosum were identified.

M. ferrugineum is sensitive to Clotrimazole (50 %), followed by Amphotericin B (33.3%). *T.rubrum* is completely sensitive to Nystatin (100%), followed by Amphotericin (66.6%), Itraconazole (66.6%), Clotrimazole (33.33%) and Itraconazole (33.33%). *T. tonsurans* is completely sensitive to Clotrimazole (100%) and Nystatin (100%) followed by Ketoconazole (80%) and Itraconazole (40%). *T. verrucosum* is sensitive to Nystatin (66.66%) and less sensitive to Clotrimazole (16%) and Fluconazole (16%). *E. floccosum* is highly sensitive to Amphotericin B (100%), Nystatin (100%), and Ketoconazole (100%) and Itraconazole (100%). *Fusarium* mold is highly sensitive to Amphotericin B (100%).

DISCUSSION

In the present study, 50 clinically diagnosed cases of Dermatophytosis attending skin department of MDM hospital, associated with Dr. S.N. Medical Collage Jodhpur, were studied. The present study shows that Dermatophytosis was more common in the age group of 11-20 years (28%) followed by 21-30 years (18%), which is comparable with other studies done by Madhuri JT, Sen SS, Mishra M, whereas Veer P has reported that the most common age group affected was 31-40 years followed by 41-50 years,[6-9] The highest incident in young adults aged 21-30 years may be due to increased physical activity and increased opportunity for exposure.

Age distribution as found in various studies (in percentage)

Name of the author, year and place	Commonest age group (percentage)
Karmakar S et al., 1995, Rajasthan	0-30 years (64%)
Mishra M et al.,1998, Sambalpur	15-35 years (30%)
Bokhari MA et al., 1999, Lahore	20-40 years (36%)
Madhuri JT et al., 2002, Vishakhapatnam	21-30-years (33.33%)
Singh S et al., 2003, Gujarat	16-30 years (31.36%)
Sen SS et al., 2006, Guhawati	21-30 years (44%)
Veer P et al., 2007, Aurangabad	31-40 years (39.4%)
Present study, 2012, Jodhpur	11-20 years (26%)

In the present study, males (62%) were more commonly affected than females (38%). Male to female ratio was 1.63:1, which is comparable with other studies done by Huda MM, Karmakar S, Grover S, whereas Cordeiro RA and Nada H reported that females were more commonly affected, with male to female ratio being 0.31:1 and 0.69:1 respectively.[4, 10-13] Male's predominance may be due to increased outdoor physical activities and increased opportunity for exposure to infection than females.

Sex distribution as found in various studies

Name of the author, year and place	Male to Female ratio
Huda MM et al., 1995, Assam	1.86:1
Karmakar S et al., 1995, Rajasthan	2:1
Grover S et al., 2003, Banglore	1.63:1
Singh S et al., 2003, Gujarat	1.57:1
Cordeiro RA et al., 2005, Brazil	0.21:1
Nada H et al., 2005, Saudi Arabia	0.69:1
Present study, 2012, Jodhpur	1.5:1

In the present study, tinea corporis was the second commonest clinical type (28%) and the commonest age group affected was 21-30 years (35.71%).Male to female ratio being 1.3:1.

Males (57%) were more commonly affected than females (42%), which is comparable with other studies done by Siddappa K, Mishra M and Sen SS.[6, 8, 14]

In the present study, tinea corporis was the commonest clinical type (30%) followed by tinea cruris and commonest age group affected was 11-20 years (28.57%). Male were predominantly affected with male to female ratio being 1.33:1, which is comparable with other studies done by Ellabib (45.9%), Singh s, Sen Ss (48%) and Venketsan G (64.8%). [3, 5 , 15, 16]

In the present study, tinea capitis was more seen in males (100% than females (0%), which is comparable with other studies done by Siddappa K (77.78%), Kumar AG (78%), Reddy BSN (73.5%) and Kalla G (85.2%).[17 – 19]

In the present study, out of 50 cases, tinea pedis was seen in 10% cases, which is comparable with the study done by Siddappa K, whereas Chemelli PAV and Ellabib MS in their on Dermatophytosis, reported tinea pedis in 9.95 and 8.1% cases respectively.[16]

In the present study, out of 50 cases of Dermatophytosis tinea manuum was 20%, which is comparable with other studies done by Siddappa K (1.53%) and Chemelli PAS (1.9%).[14, 20]

In the present study, tinea faciei was seen in 105 cases, which is comparable with other studies done by Huda MM (1%) and Mishra M (0.32%) whereas Karmakar S has reported tinea faciei in 6% cases.[4, 6, 10]

In the present study, *T. verrucosum* (54%) was the commonest aetiological agent in majority of clinical types followed by *T. tonsurans* (10%), *T. rubrum* (6%), *and E. floccosum* (2%).[5, 10, 14, 21, 22]

Dermatophytes isolation as found in various studies (percentage)

Name of author, year and place	T. rubrum	T. tonsurans	E. floccosum	T. verrucosum
Siddappa K et al., 1982, Davangere	81.82	-	9.09	-
Rangnathan S et al., 1995, Madras	52.2	-	6.11	-
Venketsan G et al., 2007, Chennai	73.3		4.2	-
Fathi HJ et al., 200 Iraq	20.9	10.5		36.2

Karmakar S et al., 1995, Rajasthan	42.3	-	-	-
Present study, 2012, Jodhpur	6	10	2	54

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