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RESEARCH ARTICLE

MYCOLOGICAL AND CLINICAL PROFILE ALONG WITH ANTIFUNGAL SUSCEPTIBILITY PATTERN OF DERMATOPHYTOSIS IN A TERTIARY CARE HOSPITAL FROM WESTERN INDIA

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Abstract

Introduction: Dermatophytosis is a common superficial mycosis causing significant cutaneous morbidity. In recent times, the prevalence of dermatophytosis is increasing. The dermatophyte infections spread easily and rapidly especially in low socioeconomic classes and thus warrant early therapy.Dermatophyte infections are commonly treated by topical antifungal drugs like clotrimazole, terbinafine, ketoconazole. But severe and chronic form of dermatophytosis requires treatment with systemic antifungal drugs like itraconazole, griseofulvin and terbinafine.There is emergence of antifungal resistant strains due to incongruous use of antifungals and poor antifungal policy. There are limited studies related to antifungal susceptibility testing (AFST). So present study was undertaken to determine mycological, clinical profile and antifungal Susceptibility testing ofdermatophytosis.

Material & Methods: A prospective study was conducted on patients with superficial fungal infections over a period of 11 months (October 2018 to August 2019). Various samples like skin scrapings, scales, hair and nail clippings were processed by standard fungal culture methods. AFST was performed by using E-test strips (HiMedia) of fluconazole, itraconazole and terbinafine on Sabourauds dextrose agar plates and interpreted according to CLSI (M38A).

Results: A total of 25 (23.8%) dermatophytes were isolated from 105 (skin 57, Nail 41, scales 11, Hair 6) samples. Out of 25 culture positive patients, 18 presented as tineacorporis, 3 as tineacruris, 3 onchomycosis, 1 each as tineacapitis&tinea incognito. *T. tonsurans* was most common dermatophyte 40% (N10), followed by *T. rubrum* 36% (N9), *T. mentagrophytes* 12% (N3) and M. canis 8% (N2) and T. megninii 4% (N1). AFST of all 25 isolates revealed that 21 isolates were sensitive to itraconazole (0.023 to 0.75 mcg/ml) wheras a single isolate of *T. rubrum* and *T. tonsurans* each were resistant. Two isolates of *T.tonsurans* showed lower MICs for itraconazole (0.023mcg/ml). For terbinafine (0.002-0.008mcg/ml), 14 isolates (56%) showed resistance with MICs >32 mcg/ml. For fluconazole (range 0.5-4 mcg/ml) only 3 isolates showed MIC in range while22 were resistant MICs >256mcg/ml. The results were communicated with dermatologists and appropriate changes were made in patient therapy.

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Conclusion: The emergence of resistant dermatophytesemphasises the need of antifungal drug susceptibility tests, antifungal stewardship and strong antifungal policy to enable the clinician to start suitable antifungals to avoid antifungal resistance and treatment failure.

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Introduction:-

Dermatophytosis is a common superficial mycosis causing significant cutaneous morbidity.[1] In recent times, the prevalence of dermatophytosis is increasing.[2,3,4] There is emergence of antifungal resistant strains due to incongruous use of antifungals and poor antifungal policy.[5]CLSI recommends Broth Microdilution method for AFST of filamentous fungi which is difficult to implement in day to day practice. 'E test' is a very simple, effective and reproducible method to test antifungal susceptibility of dermatophytes.There are limited studies related to antifungal susceptibility testing (AFST). The present study was conducted to determine profile and AFST of dermatophytosis.

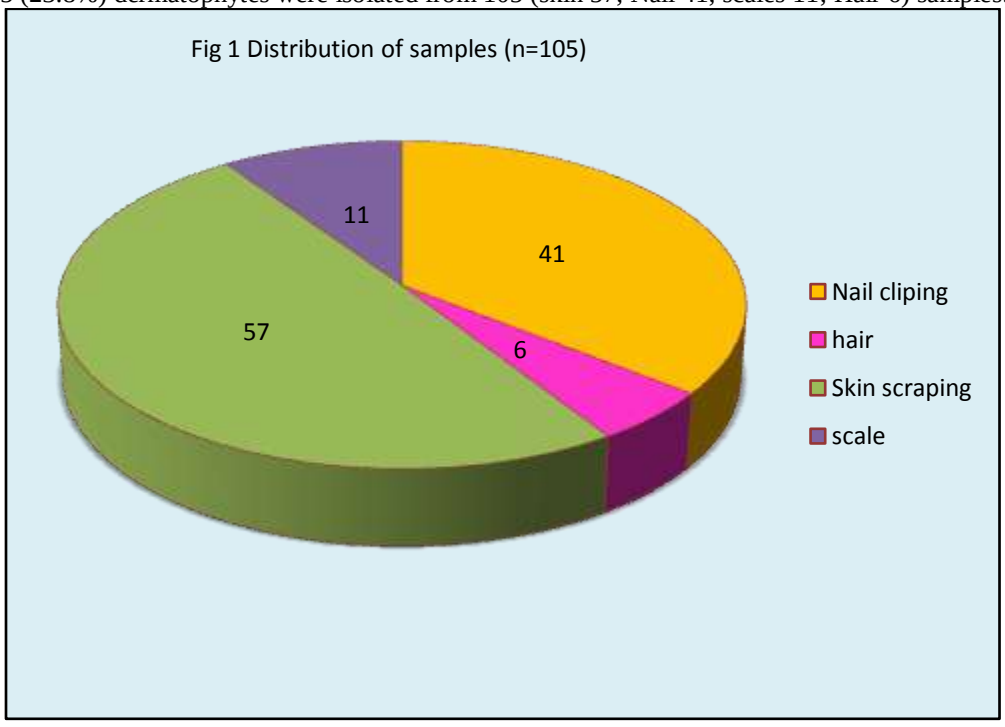
Material & Methods:-

A prospective study was conducted on patients with superficial fungal infections over a period of 11 months (October 2018 to August 2019). Various samples like skin scrapings, scales, hair and nail clippings were processed by standard fungal culture methods.[6] AFST was performed by using E-test strips (HiMedia) of fluconazole, itraconazole and terbinafine on Sabourauds dextrose agar plates and interpreted according to CLSI (M38A).[7]

Inoculum suspension of dermatophytes were prepared in sterile saline from 7-10 days old cultures on potato dextrose agar slants incubated at 30^{o}C.Hyphal fragments and conidia were harvested with sterile wet swabs in saline, vortexed for 20 seconds, and then kept at room temperature for 15-20 min to enable heavy, hyphal fragments and conidia to settle down. Homogenous suspensions of the supernatant were adjusted to $1.0X10^{6}$ cells/ml. The lawn cultures were made on SDA plates and E strip was placed.Plates incubated at 30^{o}C for 4-7 days. MICs were noted.[8]*Candida parapsilosis*ATCC22019 and *Candida krusei*ATCC6258 were used as quality control strains for AFST. The results of susceptibility were communicated to dermatologists and response to treatment recorded.

Results:-

A total of 25 (23.8%) dermatophytes were isolated from 105 (skin 57, Nail 41, scales 11, Hair 6) samples. (fig 1)



Out of 25 culture positive patients, 18 presented as tineacorporis (69%), 3 as tineacruris (11%), 3 onchomycosis (11%), 1 each (4%) as tineacapitis&tinea incognito. T. tonsurans was most common dermatophyte 40% (n=10), followed by T. rubrum 36% (n=9), T. mentagrophytes 12% (n=3) and M. canis 8% (n=2) and T. megninii 4% (n=1). (fig1,2,and fig 3)

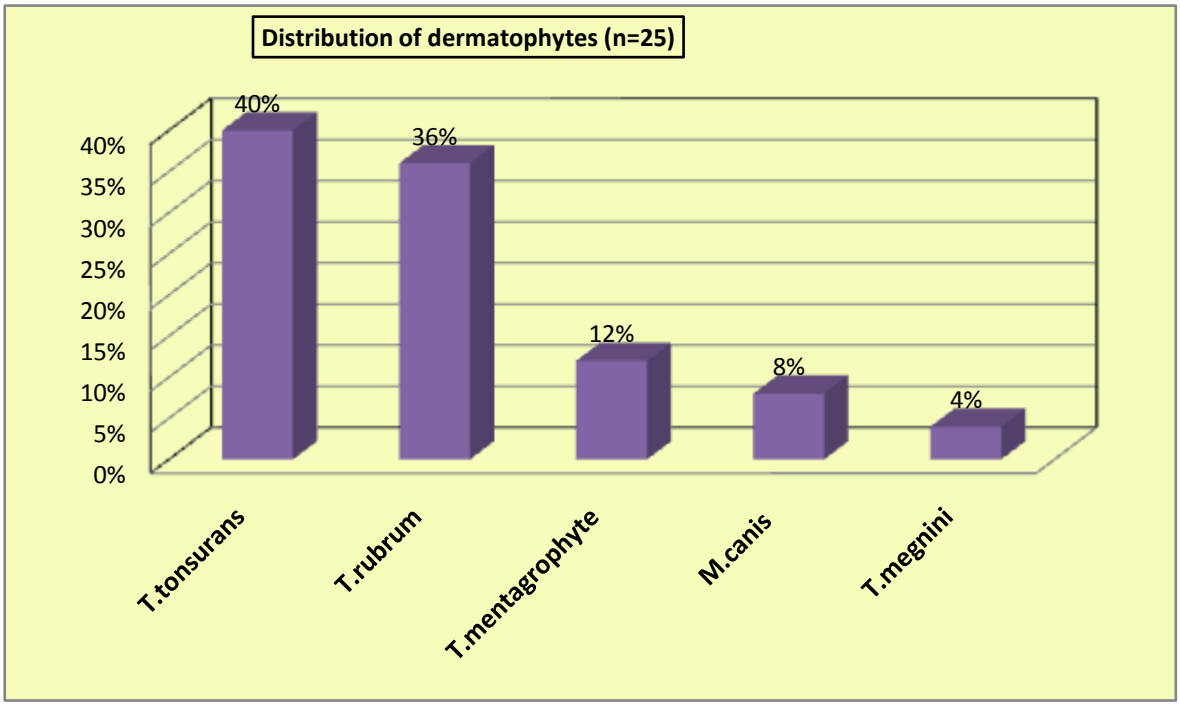


Table 1:- Clinico-mycological correlation.

Dermatophyte species (N=25)	Clinical pattern (N=25)				
	Tineacorporis	Tineacruris	Tinea incognito	Tineacapitis	Onycho-mycosis
T. tonsurans (n=11)	8	2	0	0	1
T. rubrum (n=9)	5	1	1	0	2
T.mentagrophytes (n=3)	3	0	0	0	0
M.canis (n=2)	1	0	0	1	0
T.megnini (n=1)	1	0	0	0	0

Table 1 shows most common site with the species of dermatophyte isolated from the site of fungal infection.

Antifungal Susceptibility testing (table 2, fig 5 and fig 6) of all 25 isolates revealed maximum resistance to fluconazole (88%) followed by terbinafine (56%) and least to itraconazole (8%).

Out of 25 fungi, (table 3) 21 isolates were sensitive to itraconazole (0.023 to 0.75 mcg/ml) wheras a single isolate of *T. rubrum and T. tonsurans* each were resistant to itraconazole. Two isolates of *T.tonsurans* showed lower MICs for itraconazole (0.023mcg/ml). For terbinafine (0.002-0.008mcg/ml), 14 isolates (56%) showed resistance with MICs >32 mcg/ml. For fluconazole (range 0.5-4 mcg/ml) only 3 isolates showed MIC in susceptible range while22 were resistant MICs >256mcg/ml. The results were communicated with dermatologists and appropriate changes were made in patient therapy.

Table 2:- Antifungal susceptibility testing (n=25).

Antifungal agents	Susceptible	Resistant
fluconazole	03 (0.5-4mcg/ml)	22 (> 256 mcg/ml)
Terbinafine	11 (0..002-0.008 mcg/ml)	14 (> 32 mcg/ml)
Itraconazole	23 (0.023-0.75 mcg/ml)	02 (2 mcg/ml)

Table 3:- AFST of different species (n =25).

Dermatophyte species (n=25)	Fluconazole		Itraconazole		Terbinafine	
	S	R	S	R	S	R
T. tonsurans (n=11)	0	11	10	1	8	4
T.rubrum (n=9)	2	7	8	1	7	4
T.mentagrophyte (n=3)	0	3	3	0	2	1
M.canis(n=2)	0	2	2	0	2	0
T. megnini (n=1)	1	0	1	0	0	1

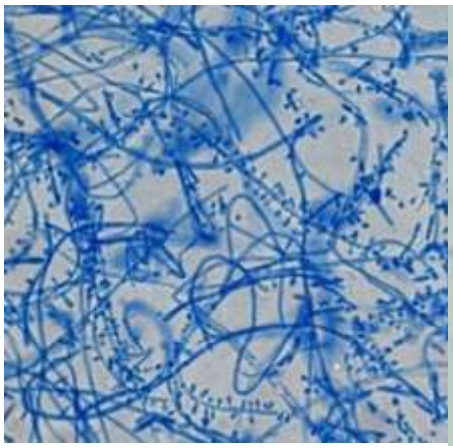


Fig 1 *T.rubrum*-bird on fence appearance

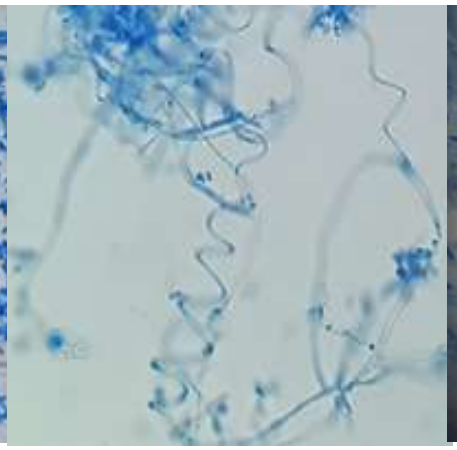


Fig 2- spiral hyphae of *T.tonsurans*

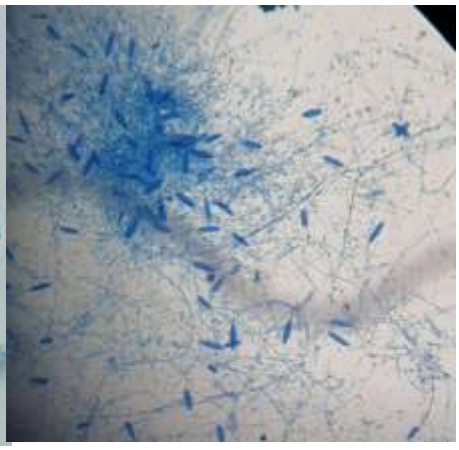


fig 3- Macroconidia of M.canis

Fig 5- Itraconazole MIC – 0.125 mcg/ml

fig 6- fluconazole MIC > 25 mcg/ml

Discussion:-

The overall prevalence of dermatophytes in the present study was 23.8% affecting mainly age group of 21-35 yrs and Tineacorporis was the most common (69%) presentation. An Indian study from south India[1] has reported a similar prevalence of 27.6% with common age group affected as 30-45 yrs and Tineacorporis (78.1%) as common presentation similar to findings of present study. However another Indian study has reported a strikingly high prevalence of 70% dermatophytoses affecting age group 21-35 yrs but Tineacapitis (29.2%) as common clinical presentation.[9]

In the present study mycological profile of fungi revealed *T.tonsurans* (n=11) as most common dermatophyte isolated followed by *T. rubrum* (n=9),*T.mentagrophytes* (n=3), *M.canis* (n=2) and *T.megnini* (n=1). Grover et al (Calcutta 2003)[9] and Weizman et al (USA 1998) [10]also reported *T. tonsurans* as most common dermatophyte isolated but Other studies have reported other dermatophytes as most common isolate. Patel et al (Gujrat 2010)[11] and Laxman et al (Chennai 2015)[1] has reported *T. rubrum*,Muhsin et al (Iraq 1999)[12] found *E.floccosum* as most common species. In another studies by Fortuno et al (Spain, 1997) [13]*Microsporumcanis* was the common fungus while Nowick et al (Polland, 1996)[14] reported *T.mentagrophyte* as most common fungus isolated.

In the present study Antifungal Susceptibility testingwas performed by E test. In the present study, all 25 isolates of dermatophyte revealed maximum resistance to fluconazole (88%) followed by terbinafine (56%) and least to itraconazole (8%). While in other studies by Singh et al (Canada, 2007)[5] AFST was performed by disk diffusion method and maximum resistance was found towards fluconazole followed byitraconazole and terbinafine. They found 64% fluconazole resistance in their study. Gupta et al (Toronto, 2005) [15] performed AFST by broth Microdilution method and demonstrated maximum resistance to fluconazole followed by itraconazole followed by terbinafine. The emergence of resistance to antifungal drugs in dermatophytes emphasizes need for judicious use of antifungal drugs. It warrants strict adherence to antifungal policies.

This study also highlights utility of E test for performing AFST. CLSI recommended Broth microdilution test is cumbersome to do in routine day to day practice. E test is a simple, rapid and convenient test for performing AFST of dermatophytes.

Conclusion:-

The emergence of resistant dermatophytesemphasises the need of antifungal drug susceptibility tests, antifungal stewardship and strong antifungal policy to enable the clinician to start suitable antifungals to avoid antifungal resistance and treatment failure.

Support of fund-
Nil.

Conflict of interest-
Nil.

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