ISSN NO. 2320-5407

Journal hmepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Epidemiology and In-Vitro Antifungal Susceptibility Testing of Dermatophytes in Hyderabad, India

Humera Q. F. Ansari[1], Mizbah B. Patel[2], Juwairriyyah A. W. Siddiqui[2]

1.M.D., (Associate Professor, Deccan College of Medical Sciences)

2.Final Year, M.B.B.S (Deccan College of Medical Sciences)

Manuscript Info *Abstract*

Manuscript History:

Received: 12 December 2013
Final Accepted: 25 January 2014
Published Online: February 2014

Key words:
Dermatophytosis, Anthropophilic, Trichophytonrubrum, Antifungal Susceptibility, Disk diffusion..

Corresponding Author

Humera Q. F. Ansari

The objectives of the study were to find the epidemiological patterns of dermatophytic infections in Hyderabad, India;to estimate their prevalence in the study population and to explore the in-vitro antifungal susceptibility patterns of the isolates. Fifty(50) clinical samples from patients of age between 18-55 years, with lesions suggestive of Tinea infection were collected and processed. Samples included skin, hair, nail from adult patients. KOH mount was done for direct exam. Culture was done on Sabouraud's dextrose agar with antibiotics and cycloheximide and on dermatophyte test medium. Lactophenol cotton blue mounts and urease test were performed for species identification. Antifungal sensitivity testing was done using a disk-diffusion method on RPMI-1640 medium with L-glutamine and without bicarbonate buffered with MOPS and supplemented with 1.5% Bactoagar. Females constituted 54% of the study group and males constituted 46%. Tineacapitis was common in the younger males (18-30 years), with Tineapedis in (31-42 years) and Tineacruris in the older age group of (43-55 years), Tineaunguium was the commonest across most age groups of females. Among detectionmethods for dermatophyticfungi, culture proved the better method with a detection of 74% as compared to 68% for fungalhyphae by KOH mount.Trichophytonrubrum was the commonest isolate in cases with Tineacruris, Tineacorporis and Tineamanuum.The study showed an isolation rate of 75%, which is on the higher side as patients were mostly from lower socio-economic strata, living in overcrowded dwellings, aggravating the infectiousness of these dermatophytic fungi..

INTRODUCTION

Dermatophytosis is one of the commonest of skin infections. The dermatophytes are a group of closely related fungi that have the capacity to invade the keratinized tissues of skin and its appendages, the skin, hair, and nails, and cause an infection, dermatophytosis,commonly referred to as ringworm (Weitzman and Summerbell, 1995). Infection with these agents is limited to the non-living cornified layers of the skin, as these fungi are unable to penetrate deeper tissues or organs of immunocompetent hosts (Dei-Cas and Vernes, 1986; King et al., 1975).The etiological agents of dermatophytosis are classified into three genera,Trichophyton, Microsporum and Epidermophyton, based on a scheme devised by Emmons (Emmons, 1934). Dermatophytes cause communicable disease, which is seldom seen with other fungi.Infections occur from contact with infected humans, animals, or soil. Consequently,dermatophyte species are divided into anthropophilic, zoophilic and geophilic species, respectively, on the basis of their primary habitat association (Agello, 1968, 1977; Matsumota and Ajello, 1987; Georg, 1960).

Most estimates show that anthropophiles constitute two-third of the primary mammalian pathogens (Ajello and Cheng, 1967; Ajello and Georg, 1957, Chmel, 1980). Although most anthropophilicspecies have welldefined areas ofendemicity,T.rubrumandT.tonsuransare cosmopolitan in distribution (Mayr, 1989; Tanaka et al., 1992;

Rippon, 1988). Infections caused by dermatophytes are classified clinically based on the anatomic locations. They are Tineabarbae (ringworm of the beard and moustache), Tineacapitis(Scalp, eyebrows and eyelashes), Tineacorporis (glabrous skin), Tineacruris(groin), Tinea imbricate (ring worm caused by T.concentricum), Tineamanuum (hand),Tineapedis (feet) and Tineaunguium (nails).

These infections are bothendemic and epidemic, and spread easily and rapidly, especially in the lower socio-economic strata thatrepresent most of the patient population seeking medical attention in India. The condition requires an early and safe therapy to prevent further spread among contacts.

The objectives of the current study were to find the epidemiological patterns of dermatophytic infections in the city of Hyderabad, India; to estimate their prevalence in the study population and to explore the in-vitro antifungal susceptibility patterns of the isolates.

MATERIALS AND METHODS:

Fifty, (50) clinical samples from patients with lesions suggestive of Tinea infection were collected and processed. Necessary clinical data were obtained from the patients using a pre-designed proforma.

CLINICAL SPECIMENS: Skin, hair, nail from adult patients in the age group of 18 to 55 years.

COLLECTION AND TRANSPORT OF SPECIMENS:

1. Skin was disinfected with alcohol and scraped from centre to edge, crossing the margin of the lesion, using a sterile scalpel blade or edge of a microscopic glass slide.
2. Hair fluorescing under the Wood's Lamp was plucked and processed.
3. Infected part of the nail was clipped or cut with scissors.
4. Vesicles were also clipped and examined thoroughly.

All these material were collected in a black paper for better visualization.

KOH MOUNT OR DIRECT EXAM:

The microscopic examination of KOH wet mounts of keratinous material is simple and reliable. Skin scales, and hair stubs were examined within 15 to 20 minutes after preparing the KOH mount but nails were kept in KOH overnight and were examined the next morning.

In the KOH wet mount, the fungus was seen as branching hyaline mycelia, which frequently show arthrospores production. Ectothrix and Endothrix types of infections were also differentiated in the wet mount prepared from hair.

CULTURE:

The clinical specimens were inoculated on fungal culture media irrespective of the direct exam findings.

SDA – Sabouraud's Dextrose Agar: Dermatophytes grow easily on Sabouraud's dextrose agar with antibiotics and cycloheximide.The growth was slow and it took anywhere between 10 days and three weeks in time to occur. The colonies were identified based on their macroscopic appearance such as color of the surface and reverse, topography, texture and rate of growth. Teased mounts were prepared, stained with Lactophenol Cotton Blue and examined for identifying microscopic morphology, especially for the presence, appearance and arrangement of Macroconidia and Microconidia.

Dermatophyte Test Medium: At 25°C, it was used to isolate and distinguish dermatophytes from the fungal or bacterial contaminants found in cutaneous lesions. The dermatophytesturn the medium red by raising the pH through metabolic activity while most fungi and bacteria do not.

BIOCHEMICAL TEST:

The trichophyton species were subjected to the urease test.

Urease test:This was done on Christensen's medium. Trichophytonmentagrophyteshydrolysedurea giving positive results, while Trichophytonrubrum showed negative results.

ANTIFUNGAL SENSITIVITY TESTING:

This was performed using a disk diffusion method that mainly followed the CLSI document M44-A under optimal conditions. The medium used was RPMI-1640 medium with L-glutamine and without bicarbonate buffered with MOPS and supplemented with 1.5% Bacto agar. (Sigma – Aldrich, MO, USA)

Inoculum Preparation: Inoculum suspensions were adjusted to a concentration of 10^6 CFU/ml and diluted 1:100 in RPMI to achieve a final concentration of 10^4 CFU/ml.

Antifungal Agents: Itraconazole(Janssen), Terbinafine(Novartis),Voriconazole(Pfizer) ,Ravuconazole(Bristol-myerssquibb), and Micafungin(AstellasPharma) were obtained from manufacturers as standard powder. All drugs were dissolved in dimethylsulfoxide to obtain stock solutions of 50,100,200,400, 800 µg/ml, except for terbinafine stock solutions, which were 6.25, 12.5, 25, 50 and 100 µg/ml. Blank paper disks were impregnated with 20 µL of each of the concentrations of the stock solutions.

Method: The surface of the agar medium was inoculated by streaking several times a sterile swab that was dipped into each adjusted inoculum. After 5 to 10 minutes, disks were dispensed onto the surface of the inoculated agar plates. The plates were incubated at 28°C and the inhibition zone diameters were measured in millimeters.

OBSERVATIONS AND RESULTS:

The present research included 50 cases in the age-group of 18-55 years via a multicentric study involving peripheral health centers attached to a tertiary care hospital in Hyderabad, India.

Of the 50 patients, 23 were males and 27 were females. The common infections among maleswere Tineacruris, Tineapedis and Tinea capitis (6 each), whereas,Tineaunguium (8/27) was the most common among females, followed by Tinea corporis (7/27) and Tinea manuum (6/27).

Looking at the clinical manifestation with respect to age, Tineacruris was the predominant clinical condition in the 18-30 years and 43-55 years age-groups, whereas, Tineaunguium wasmost common in the 31-42 years age-group.

Among males, Tinea capitis was common in the 18-30 years age group, Tineapedis among 31-42 years age-group and Tineacruris among 43-55 years age-group. Among females, Tinea unguium was common in 18-30 years age-group. Both Tineaunguium and Tineacorporis were common among 31-42 years age-group, whereas, Tineacruris, Tineacorporis and Tineamanuum were evenly distributed in the 43-55 years age-group. (Table 1)

Comparing methods of detection, KOH mount was positive in 34 (68%) and negative in 16 (32%) of the samples tested. Culture was positive in 37 (74%) of the 50 samples tested, whereas, 13(26%) tested negative(Figure2

The prevalence pattern of dermatophytic fungi revealed T. rubrum (4/11) to be the most common isolate in cases of Tineacruris, followed by (4/6) in Tineamanuum, Tineacorporis (3/11) and Tinea pedis (4/7). T.violaceum was the most isolated fungus (4/6) in cases of Tinea capitis. In Tineaunguium, the fungi, T.mentagrophytes and 'E.floccosum were equally isolated (2 each), see(Table 2, Figure 1)

Inhibition zone diameters (IZD)were obtained for each drug and species combination. The ranges and arithmetic means of the IZDwere calculated for each combination of species and drug (Table 3). The IZD were read at 5 days of incubation. All strains showed measurable inhibition zones, without microcolonies inside them. Voriconazole showed the widest IZD,whileMicafungin did not show any inhibition zone.(Figure 3)

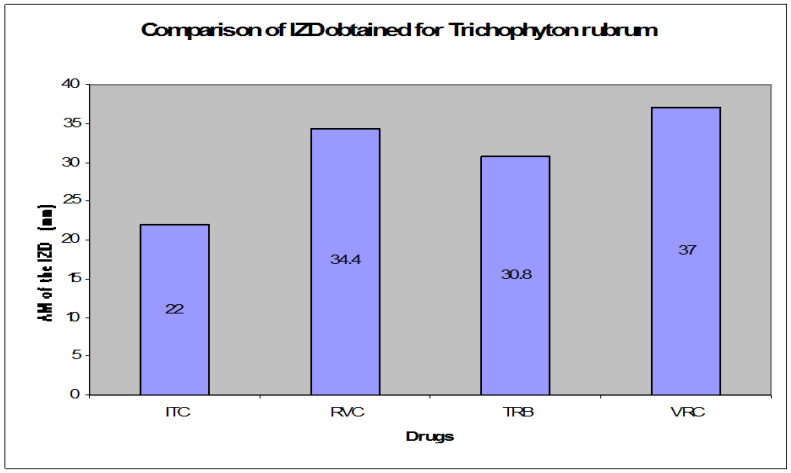
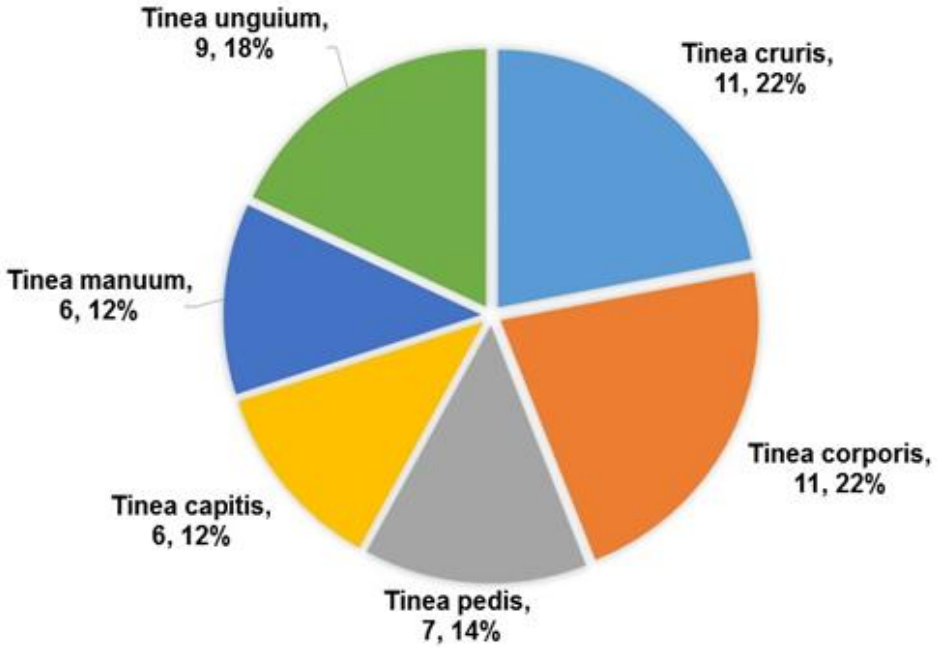


FIGURE 3-

Figure 1: Distribution of Clinical Manifestations



i

Figure 2: Positivity Pattern of KOH Mount and Fungal Culture

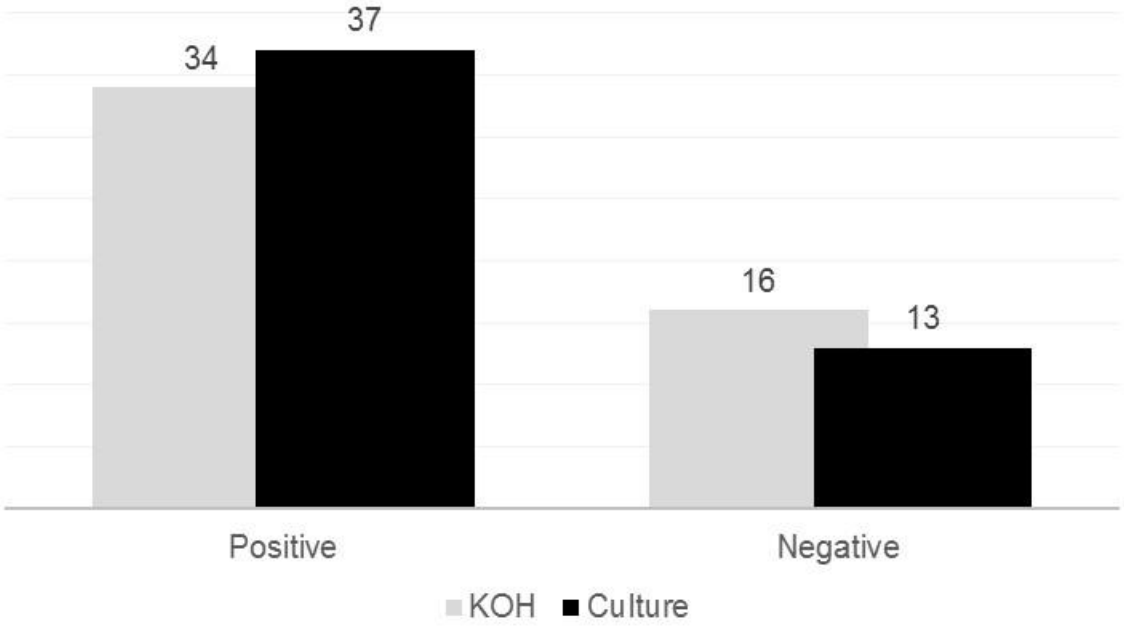


Table 1: Details of samples with reference to clinical manifestation, sex and different age group (n, %).

Clinical Manifestation	Males (n, %)				Females (n, %)			
	Total	18-30 yrs	31-42 yrs	43-55 Yrs	Total	18-30 yrs	31- 42 Yrs	43-55 Yrs
Tineacruris	6 (26.09)	4 (66.67)	0 (0)	2 (33.33)	5 (18.52)	4 (80)	0 (0)	1 (20)
Tineacorporis	4 (17.39)	2 (50)	1 (25)	1 (25)	7 (25.93)	3 (42.86)	3 (42.86)	1(14.29)
Tineapedis	6 (26.09)	2 (33.33)	3 (50)	1 (16.67)	1 (3.70)	1 (100)	0 (0)	0 (0)
Tineacapitis	6 (26.09)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tineamanuum	0 (0)	0 (0)	0 (0)	0 (0)	6 (22.22)	4 (66.67)	1 (16.67)	1(16.67)
Tineaunguium	1 (4.35)	0 (0)	1 (100)	0 (0)	8 (29.63)	5 (62.5)	3 (37.5)	0 (0)
Total	23 (100)	14 (60.87)	5 (21.74)	4 (17.39)	27 (100)	17 62.96)	7 (25.93)	3 (11.11)

Table 2: Prevalence pattern of dermatophytic fungi (n,%).

Clinical Manifestation	Total number of samples (n, %)	T. rubrum (n,%)	T.mentagr-ophytes (n,%)	T.verrucos --um (n,%)	T.violace-um (n,%)	M. canis (n,%)	M.gypseu m (n,%)	E.floccosu--m (n,%)
Tineacruris	11 (22%)	4(36.36%)	2 (18.18%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (27.27%)
Tineacorporis	11 (22%)	3 (27.27%)	0 (0%)	2 (18.18%)	2(18.18%)	1 (9.09%)	1(9.09%)	0 (0%)
Tineapedis	7 (14%)	4 (57.14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tineacapitis	6 (12%)	2(33.33%)	0 (0%)	0 (0%)	4(66.67%)	0 (0%)	0 (0%)	0 (0%)
Tineamanuum	6 (12%)	4(66.67%)	0 (0%)	0 (0%)	1(16.67%)	0 (0%)	0 (0%)	0 (0%)
Tineaunguium	9 (18%)	0 (0%)	2 (22.22%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (22.22%)
Total	50 (100%)	17 (34%)	4 (8%)	2 (4%)	7 (14%)	1 (2%)	1 (2%)	5 (10%)

Table 3: Antifungal sensitivity testing.

Species (no. of Strains)	Drug	Inhibition Zone Diameters (IZD) with RPMI	
		Range	A.M.
Trychophyton rubrum (17)	ITC RVC TRB VRC	15 – 30 26 – 40 23 – 36 30 – 42	22.0 34.4 30.8 37.0
Trichophyton violaceum (7)	ITC RVC TRB VRC	19 – 26 27 – 34 35 – 44 23 – 30	23.1 30.7 39.5 27.4
Trichophyton mentagrophytes (4)	ITC RVC TRB VRC	19 – 24 23 – 30 25 – 29 30 – 36	21.7 25.7 26.5 32.8
Microsporum gypseum(1)	ITC RVC TRB VRC	23 – 33 28 – 36 31 – 37 33 – 39	27.2 31.2 34.1 35.0
Epidermophyton flocossum(5)	ITC RVC TRB VRC	20 – 26 21 – 32 21 – 27 25 – 33	22.8 26.6 24.2 28.7

DISCUSSION:

Dermatophytoses are superficial infections of keratinised tissue, the skin, hair and nails, caused by dermatophytes. The prevalence of dermatophytosis is determined by environmental conditions, personal hygiene and individuals' susceptibility. The variation in clinical presentation is related to the species of the fungus, size of the inoculum, the involved sites, and the immune status of the host.

The zoophilic and geophilicdermatophytes cause inflammatory lesions, which respond well to therapy and heal spontaneously, on occasion. Chronic dermatophytosis refers to persistent dermatophytosis that runs a chronic course with episodes of remission and exacerbation for more than a year.

In the present study, among males, Tineacapitis was common in the younger age group of 18-30 years, consistentwith other studies from the Indian sub-continent.[27-28]Tineacapitis is generally seen as a disease of adolescent and young adultsand is influenced by the male sex hormones (Aly et al., 2000; Asawanoda and Taylor, 1999; Aste et al., 1996; Attapattu, 1989; Higgins et al., 2000).

TineaPedis is the infection of the plantar aspect of the foot, toes and interdigital web spaces. The warmth and moisture produced by the shoes help establish and maintain the infection. It is frequently seen among individuals wearing shoes for long hours, and hence the condition is named the "Athlete's foot".Use of socks and shoes create a damp environment with sweating, facilitating fungal growth. Tineapedis was common among the 31-42 years old males of the higher income group,which constituted most the cases in this age group.

Tineacruris occurs worldwide, but is relatively more prevalent in tropical countries. It is the dermatophytic infection of the groin and is mostly present in men with an underlying predisposing factor such as long-term use of tight-fitting garments (Chakrabarti et al., 1992; Ekanem and Gugnani, 1987). Tineacruris, which affects the region of the thighs, buttocks and groin, is commonly seen in people wearing tight underwear. It was a common predilection in the 43-55 years age group of male patients (Elewski, 1998, Evans, 1998).

TineaUnguium is the dermatophyte infection of the nail plates and is largely a disease of adults. It begins under the free edge of the nail plate or along the lateral nail fold and may continue until the entire nail plate and nail bed are infected (Banerjee et al., 1990).Tineaunguium was commonly observed both in the 18-30 years and 31-42

years age-groups, among females. Infection of the nail was most common, as this group of patients consisted ofeither house-wives or home maids and engaged more often in activities that involved soaking of hands in water (Bharadwaj et al., 1987; Blecher and Korting, 1993).

Comparing methods of detection, 34 (68%) samples showed a positivity pattern of fungal hyphae by KOH mount, whereas,fungal culture was positive in 37 (74%) of the 50 samples tested, showing culture as the better method of detection of dermatophytes from clinical material, which is consistent with an Indian study from Tiruchirapalli,Tamilnadu (Srinivasan et al., 2012).

Trichophytonrubrum was the commonest isolate in cases of Tinea cruris, Tinea corporis, Tinea manuum and Tinea pedis, with T. violaceum emerging as the common etiological agent in Tinea capitis. Tinea unguium was caused by the anthropophilic species,T.mentagrophytes and E.floccosum. Because of their adaptability to a wide range of environmental conditions and their intimate association with humans, these fungi occur wherever humans dwell.

The results of previous studies of the in-vitro susceptibility of dermatophytes to antifungals have shown variations due to type of medium used, inoculum sizeand incubation period. More extensive or severe fungal infections require treatment with oral drug formulations of Terbinafine or Itraconazole (Gupta et al., 1998; Roberts 1997, Saul et al., 1987). This study concentrated on the most commonly used drugs for the treatment of dermatophytoses alongwith newer agents such asRavuconazole and Voriconazole (Ghannoum et al., 2004; Gupta et al., 2005; Perea et al., 2001). With an increasing number of available drugs for the treatment of dermatophytoses, there is the need for a reference in-vitro susceptibility testing method and also for evaluation of more clinical specimens using different antifungal susceptibility testing methods. In the present study, an in-vitro antifungal susceptibility testing method for testing dermatophytes was identified and standardized.

The study showed an isolation rate of 75%, which is higher than normally seen, because most of the patients were from a lower socio-economic strata and came from over-crowded livingconditions, which could have aggravated the infectiousness of these dermatophytic fungi.

CONFLICT OF INTEREST STATEMENT:

We declare that we have no conflict of interest.

ACKNOWLEDGEMENT:

The authors thank the Indian Council of Medical Research (ICMR) for funding the research.

REFERENCES:

1. Ajello, L. (1968): A taxonomic review of the dermatophytes and related species. Sabourau-dia. 6:147-159.
2. Ajello, L. (1977): Taxonomy of the dermatophytes a review of their imperfect and perfect states P.289-297. In .K. Iwata (ed.). Recent advances in medical and veterinary mycology. University of Tokyo Press, Tokyo.
3. Ajello, L, and Cheng, S. L. (1967): The perfect state of Trichophytonmentagrophytes. Sabouraudia. 5:230-234.
4. Ajello, L., and Georg, L.K. (1957): In vitro hair cultures for differentiating between atypical isolates of Trichophytonmentagrophytes and Trichopythonrubrum. Mycopathol Mycol. Appl. 8:3-17.
5. Aly R, Hay RJ, and Del Placio A, et al., (2000): Epidemiology of tineacapitis. Med Mycol, 38:183-188.
6. Asawanonda P and Taylor CR, (1999): Wood's light in dermatology. Int J Dermatol, 38:801-807.
7. Aste, N, Pau, M, and Biggio, P, (1996): Tineacapitis in adults. Mycoses, 39:299-301.
8. Attapattu M.C., (1989): A study of tineacapitis in Sri Lanka. J Med Vet Mycol, 27:27-32.
9. Banerjee, U., Sethi, M, and Pasricha, J.S., (1990): Study of onychomycosis in India, Mycoses, 33:411-415.
10. Bharadwaj, G., Hajini, G.H., Khan J.A., et al., (1987):Dermatophytes in Kashmir, India. Mykosen, 30:135-138.
11. Blecher, P., and Korting, H.C., (1993): A new combined diagnostic approach to clinically and microscopically suspected onychomycosis unproven by culture; Mycoses, 36:321-324.
12. Chakrabarti, A., Sharma, S.C., and Talwar, P. (1992). Isolation of dermatophytes from clinically normal sites in patients with tineacruris. Mycopathologia. 120:139-141.
13. Chmel, L., (1980): Zoophilic dermatophytes and infections in man Medical Mycology, (Suppl.) 8: 61-66.
14. Dei-Cas, E. and Vernes, A.,(1986): Parasitic adaptation of pathogenic fungi to mammalian hosts. Critical Review in Microbiology 13: 173-218.

15. Ekanem, L.S., and Gugnani, H.C. (1987): Fungal species isolated from skin and nail lesions of hand and feet of patients suspected of mycotic lesions. Mykosen. 30:493-498.
16. Elewski, B.E. (1998):Onychomycosis pathogenesis, diagonis and management. Clin. Microbiol. Rev. 11:415-429.
17. Emmons, C. W. (1934):Dermatophytes: natural groupings based on the form of the spores and accessory organs. Arch. Dermatol. Syhilol. 30: 337-362.
18. Evans, E.G. (1998): Causative pathogens in onychomycosis and possibility of treatment resistance: a review. J Am AcadDermatol. 38: S32-36.
19. Georg, L.K. (1960): Epidemiology of the dermatophytoses: sources of infection, modes of transmission and epidemicity. Ann. N.Y. Acad. Sci. 89: 69-77.
20. Ghannoum, M.A., Chaturvedi, V., and Espinel, A., et al., (2004): Intra and Inter laboratory study of a method for testing the antifungal susceptibilities of dermatophytes. J ClinMicrobiol, 42:2977-2979.
21. Gupta, A.K., Einarson, T.R., Summerbell, R.C., and Shear, N.H. (1998): An overview of topical antifungal therapy in dermatomycoses: A North American perspective. Drugs, 55:645-674.
22. Gupta, A.K., Kohli, Y., and Batra, R., (2005). In vitro activities of posaconazole, ravuconazole, terbinafine, itraconazole and fluconazole againstdermatophyte, yeast and non-dermatophyte species. Med Mycol. 43:179-185.
23. Higgins, E.M., Fuller, H.C., Smith, C.H. et al. (2000): Guidelines for the management of tineacapitis. Br H. Dermatol. 143:53-58.
24. King, R.D., Khan, H. A., Foye, J. C., Greenberg, J. H, and Jones, H.E.,(1975): Transferrin, iron and dermatophytes I. serum dermatophytes inhibitory component definitely identified as unsaturated transferrin J. Lab. Clin. Med. 86: 204-212.
25. Kannan, P.,Janaki, C., and Selvi, G. S., (2006): Prevalence of dermatophytes and other fungal agents isolated from clinical samples. Indian J Med Microbiol, 24: 212 – 215.
26. Matsumoto, T., and Ajello, L., (1987): Current taxonomic concepts pertaining to the dermatophytes and related fungi Int. J. Dermatol. 26: 491-499.
27. Mayr, A.(1989): Infections which humans in the householdtransmit to dogs and cats. Zentralbl, Bakteriol. Mikrobiol. Hyg. Ser., B 187: 508-526.
28. Pere , S., Fothergill, A.W., Sutton, D. A., and Rinaldi, M.,G., (2001): Comparison of in vitro activities of Voriconazole and five established antifungal agents against different species of dermatophytes using broth macrodilution method. J ClinMicrobiol, 39:385-388.
29. Rippon, J. W.,(1988): Medical mycology. The Pathogenic Fungi and the Pathogenic Actinomycetes; 3rd ed. W.B. Saunders, Philadelphia.
30. Roberts, D.T., (1997): Oral terbinafine(Lamisil) in the treatment of fungal infection of the skin and the nails. Dermatology, 194(Suppl.1):37-39.
31. Saul, A., Bonifaz, A., and Arias, J., (1987):Intraconazole in the treatment of superficial mycoses: an open trial of 40 cases. Rev Infect Dis, 9(Suppl.1) S1000-S103.
32. Srinivasan, Balakumar, and Solomon, Jeeva,(2012): Epidemiology of dermatophytosis in and around Tiruchirapalli, Tamilnadu, India. Asian Pacific Journal of Tropical Disease, 286-289.
33. Tanaka, S.,.Summerbell, R.C., Tsuboi, R., Kaaman, T., Sohnle, P.G, Matsumoto, T., and Ray, T.L.(1992): Advances in dermatophytes and dermatophytosis. J. Med. Vet. Mycol. 30 (Suppl.1): 29-39.Weitzman, I., and Summerbell, R. C. (1995). The dermatophytes, Clinical Microbiology Review. 8:240-259