International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 469-476
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

INCIDENCE AND PREDOMINANCE OF DERMATOPHYTES AMONG THE PATIENTS WITH SUPERFICIAL SKIN INFECTION IN A TERTIARY CARE CENTRE IN BIRGUNJ

Dipak Bhargava[1*], Suman Kumar Halder[2], Keshab Chandra Mondal[2], Sanjay Kar[3]

[1]Department of Microbiology, National Medical College and Teaching Hospital, Parsa, Birgunj-18, Nepal.
[2]Department of Microbiology, Vidyasagar University, Midnapore-721102, West Bengal, India.
[3]Department of Botany, Midnapore College, Midnapore -721101, West Bengal, India.

*Corresponding Author Email: dipak.bhargava80@gmail.com

ABSTRACT

Dermatophytes are keratinophilic fungi, which causes the superficial mycotic manifestations known as dermatophytosis. The present work was intended to determine the predominance and antimycotic sensitivity pattern of dermatophytes among the patients. Clinical samples were collected from August, 2017 to May 2018, among the 230 patients presenting characteristic lesions of dermatophytosis. Clinical specimens collected were processed by means of potassium hydroxide wet mount, culture and the biochemical tests to identify the dermatophytes. In this present work antimycotic sensitivity testing by the disc diffusion method was also conducted. Of 230 clinical samples, the maximum number of dermatophytosis was in the age group of 31-40 years. Out of the 230 clinical samples, 47.3% were positive in the potassium hydroxide wet mount and a sum of 53.9% was positive for the dermatophytic growth in culture. Frequency of Tinea corporis association is significantly higher than Tinea cruris ($p < 0.05$) and Trichophyton rubrum was the predominant etiological agent with 53.2% of the isolates. Antimycotic susceptibility test of the isolated Dermatophytes shows maximum susceptiblity against itraconazole (10mcg) and miconazole (30mcg), whereas least susceptible towards fluconazole (10mcg) and ketoconazole (50mcg). Systemic surveillance of antifungal susceptibility test should be carried out to resolve the optimal treatment regimen.

KEY WORDS

Antifungal susceptibility test, Dermatophytes, Dermatophytosis, KOH wet mount

1. INTRODUCTION

Dermatophytes are a group of morphologically and physiologically allied moulds which causes the common form of superficial mycotic infection known as Dermatophytosis. The universe has been depicted as a natural terrain for the dermatophytes. Dermatophytes possess two important features: they are keratinophilic and keratinolytic agents. They most commonly infect the keratinized non- living tissues of skin, hair and nails. According to Emmon's morphological classification, the dermatophytes are classified into three anamorphic genera -*Trichophyton*, *Microsporum* and *Epidermophyton* based on conidial morphology. The clinical manifestations caused by the dermatophytes is commonly referred to as ringworm. The World Health Organization estimates universal occurrence of dermatomycoses to be related to 20% [1].

The dermatophytes are considerably changing in different parts of the world. The growth of dermatophytes was found to be increased at the exterior temperature of 25-28° C and membrane mycosis is continued by warm and humid conditions [2, 3]. The infection spreads easily by direct contact from infected humans and animals or through fomites [4]. In

addition, the episode of dermatomycoses is greater in population with little socioeconomic status and also in close proximity of animals [5, 6]. However, floor covering, garments, linens, furnishings and barber shop instruments, are the vital foundation of dermatophytes. Though the infection named dermatomycoses is not invasive and uncomplicated its widespread nature and the treatment cost is one of the key public health concern and causes enormous dent to the financial status of the countries like Nepal & India. Although many International studies on epidemiology of dermatophytosis are available but there is a very less number of works carried out on the incidence and the predominance of dermatophytosis in Birgunj, Nepal. Therefore, in view of this an effort has been made to frame this work on the incidence and the predominance of Dermatophytes among the patients with superficial skin infection attending the skin and venereal disease department of a tertiary care hospital in Birgunj, Nepal.

2. MATERIAL AND METHODS

2.1 Collection of specimens

A total of 230 patients presenting lesions characteristic of dermatophytic infection based on the doctors' provisional diagnosis from the outpatient department of skin and venereal disease department from August, 2017 to May 2018 were included in this study. The samples were collected and sent to Department of Microbiology of National Medical College and Teaching Hospital, Birgunj, Nepal. The patients included in this study are of all age groups and from both the sexes.

Diverse dermatophytic clinical conditions such as tinea cruris, tinea corporis, tinea pedis, tinea unguium, and tinea manuum were observed in patients. To collect the specimen for the diagnosis the suspected ringworm lesions were cleaned with 70% ethyl alcohol using sterile cotton. Clinical skin lesions were scraped from the centre to the edge of the infected area. In case of suspected dermatophytic infection of the hair, the hair follicle with the basal root was plucked using sterile tweezers. In case of nail infection, the brittle part of nail or the scrapings from near the nail bed were collected. All the clinical samples were collected in a thick black art paper to retain the moisture, folded and transported to the laboratory [7, 8].

2.2 Direct Microscopy

Skin scrapings and hair follicles with basal root were treated with a drop of an aqueous solution of 10% (w/v) potassium hydroxide (KOH) with parker ink on a clean grease free microscopic slide. The brittle part of nail clippings was mounted with fresh 20% or 40% (w/v) KOH with parker ink was used, as the material is hard to digest. After 5 minutes of mounting, the prepared slides were observed under low (×10) and high (×40) power magnification for the existence of fungal elements.

2.3 Culture

The isolation of dermatophytes from the clinical samples was performed in Sabouraud's dextrose agar containing chloramphenicol (0.05%) and cycloheximide (0.5%). Cycloheximide in agars was used to isolate the dermatophyte by inhibiting several fungi, including *Aspergillus* and the mucoraceous moulds *Rhizopus*, *Absidia,* and *Mucor* [9]. It acts as a semi-selective medium, since it reduces the growth of other fungi. Dermatophyte Test Medium (DTM) was also used to isolate the dermatophytes. Any type of dermatophytic growth in DTM was indicated by red colour, which indicates alkalinity generated by dermatophytic growth. The culture plates inoculated were incubated at overturned position for 4-6 weeks at 25-30˚ C aerobically. The culture plates inoculated with the clinical specimens were observed twice a week for any type of dermatophytic growth.

Growths of fungal colonies suspected of dermatophytic growth were subcultured on Potato Dextrose Agar plate to stimulate sporulation. To rule out the growth of non dermatophytic growth or any contamination the following sets of criteria was followed. (i) abnormality consistent with superficial mycoses, (ii) positive KOH preparation, the presence of filamentous fungi in biological fluid material, (iii) failure to isolate a dermatophyte culture, and (iv) the growth of non-dermatophyte moulds in three successive occasions at least, with a minimum of 2-week interval [10].

Dermatophytic growth on the culture plate was examined by evaluating the macroscopic and microscopic features of the colonies. For macroscopic identification; rate of the growth of the colonies, surface growth characteristics of the colonies and the pigmentation produced by the colonies on the front and the reverse side of the growth.

Microscopically the isolates were identified by introducing a portion of a colony from SDA/PDA to a clean grease free microscopic slide and stained with lactophenol cotton blue (LPCB). The LPCB mount was covered with clean cover slip and observed

microscopically under 10x and 40x magnification. Beside the above mentioned macroscopic and microscopic evaluations, the biochemical test like christensens urea agar test, hair perforation test was also performed to differentiate the different dermatophytes. Fungal morphology was compared with the color atlas of reference text books and identified [11, 12]. The cultured agar plates were incubated maximum for a period of 4 weeks before declaring the culture plates as negative.

2.4 Antimycotic sensitivity testing

Antimycotic sensitivity testing by disc diffusion method using antimycotic sensitivity disc was carried out in Mueller Hinton Agar containing 2% glucose & 0.5 mcg/ml methylene blue dye medium.

2.4.1 Preparation of Inoculum

The inoculum was prepared by taking 4 to 5 singly isolated colonies from the 24 hours grown culture in Sabouraud Dextrose Agar and incubated at 37°C. and the colonies are emulsified in 5 ml of sterile saline. The emulsified suspension is vortexed and the turbidity is adjusted to yield 1×10^6- 5×10^6 cells/ml (i.e. 0.5 McFarland standard).

2.4.2 Test Procedure

Sterile cotton swab stick was dipped into the prepared inoculums and the soaked swab stick was rotated strongly against the upper inside wall of the tube to utter the extra fluid. The whole agar surface of the Mueller Hinton Agar containing 2% glucose & 0.5 mcg/ml methylene blue dye medium was streaked with the dipped swab stick three times. In between each streaking the entire plate was moved at 60° angle. The inoculated inoculum in the plate was allowed to dry with the lid on the plate for 10 minutes. The antifungal discs were placed in the plate aseptically with centers at least 24 mm. apart. After the antifungal discs were applied the plates were inverted and placed in an incubator set at 37°C within 10 minutes. The plates were interpreted after every alternate day for the zone of inhibition. The following antifungal discs Fluconazole (10 mcg), Ketoconazole (50 mcg), Itraconazole (10 mcg), Miconazole (30 mcg) and Nystatin (50 mcg) were used for the antifungal sensitivity testing (Himedia, Mumbai).

2.5 Statistical analysis

To ensure consistency of the findings of the antifungal susceptibility testing the antifungal susceptibility test was performed in triplicate under aseptic condition. The data was analyzed and expressed as mean ± standard deviation. Statistical analysis was performed using Sigma Plot 11.0 (USA).

3. RESULT

In the current work, among the 230 collected clinical samples of the suspected cases of dermatophytosis 135 (57.4%) were from males and 95 (41.3%) from females. From this study it was visible that the maximum number of dermatophytosis was in the age group of 31-40 years (Table 1). Whereas, the sex wise breakdown of the clinical samples also shows the preponderance of the males over the females (Table 1).

Table 1. Age and Sex wise Distribution of patients

Age of the patients (in years)	Number of cases		Total (Percentage)
	Males	Females	
≤ 10	3	2	5 (2.1%)
11-20	14	17	31 (13.4%)
21-30	42	28	70 (30.4%)
31-40	53	34	87 (37.8%)
41-60	23	14	37 (16.0%)

Out of the total number of 230 clinical samples investigated for the dermatophytes 182 were skin scrapings, 42 were nail clippings and 6 were hair plucking with roots. In order to detect the dermatophytic elements in the above-mentioned clinical samples, the procedure of direct microscopy by means of KOH wet mount is done. Among the 230 clinical samples, 109 were positive in the direct microscopy of KOH wet mount and a sum of 124 was positive for the dermatophytic growth in culture. In totality 83 was positive for KOH wet mount and culture, whereas, 26 cases were positive for KOH wet mount and downbeat for the culture. Similarly, 41 cases were negative for KOH wet mount and positive for dermatophytic growth culture. Simultaneously, on the other hand 80 cases were negative for both KOH wet mount and the culture for the dermatophytic growth (Table 2).

Table 2. Comparison between the KOH wet mount and dermatophytic cultures (N = 230)

	Dermatophytic growth in culture	No growth of Dermatophyte in culture	Total
KOH wet mount Positive	83 (36.0%)	26 (11.3%)	109 (47.3%)
KOH wet mount Negative	41(17.8%)	80 (34.7%)	121 (52.6%)
Total	124 (53.9%)	106 (46.0%)	230 (100%)

According to the clinical presentation with reference to gender the clinical samples were analyzed. The data clearly demonstrates that *Tinea corporis* was the most common standing at 66.5% of all the cases followed by *Tinea cruris* (15.2%) and *Tinea unguium* (7.3%), whereas *Tinea barbae* (0.43%) and the *Tinea faciei* (2.6%) are at the lowest. The gender wise analysis of the clinical manifestation also shows that *Tinea corporis* and *Tinea cruris* was the commonest lesion and *Tinea barbae* and *Tinea faciei* was at its lowest. The particulars of the clinical presentation with reference to the gender were presented in Fig. 1.

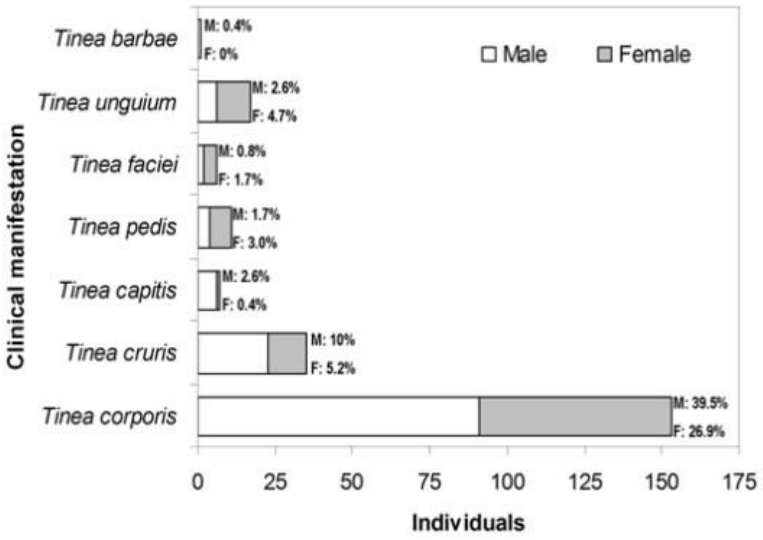

Fig 1. Frequency of clinical presentation with reference to Gender (M: male, F: female)

In this study, among the 230 clinical samples, 124 were positive for the dermatophytes in culture. Out of these 124 culture positive samples 66 (53.2%) were of *T. rubrum* isolates followed by *T. mentagrophytes* 35 (28.2%) and *T. violaceum* 10 (8.6%). Whereas, *M. gypseum* and *E. floccosum* were the least number of isolates grown in culture (Table 3). Frequency of *Tinea corporis* association is significantly higher than *Tinea cruris* ($p < 0.05$), *Tinea pedis* ($p < 0.01$), *Tinea unguium* ($p < 0.01$) associated cases with *T. rubrum*. Apart from the predominant association, frequency of *T. mentagrophytes* association with different *Tinea* is also significantly higher than *T. violaceum* ($p < 0.001$), *M. gypseum* ($p < 0.001$) and *E. floccosum* ($p < 0.001$) associated cases.

In this recent work it has been found that the highest percentage of the isolate i.e. *T. rubrum* was found to be maximum susceptible against miconazole (30 mcg), whereas, the same sps. was found to be less susceptible against fluconazole (10 mcg). The other species of *Trichophyton* i.e. *T. violaceum* was found to be maximum susceptible against itraconazole (10 mcg) and miconazole (30 mcg) and least susceptible against ketoconazole (50 mcg). Among the isolates the second highest percentage of the isolate i.e. *T. mentagrophytes* was found to be maximum susceptible against miconazole (30 mcg) followed by itraconazole (10 mcg) and less susceptible towards fluconazole (10 mcg) and ketoconazole (50 mcg). Likewise, the growth of *E. floccosum* was inhibited maximally by nystatin (50 mcg) whereas ketoconazole (50 mcg) was found as least effective. The isolates which were least in number were also found to be maximum susceptible against miconazole (30 mcg), itraconazole (10 mcg) and nystatin (50 mcg), whereas they were also less susceptible against fluconazole (10 mcg) and ketoconazole (50 mcg) (Table 4).

Table 3. Association of various Dermatophytes isolates with different clinical presentations

Dermatophytes isolates	*Tinea corporis*	*Tinea cruris*	*Tinea capitis*	*Tinea pedis*	*Tinea faciei*	*Tinea unguium*	*Tinea barbae*	Total
T.rubrum	48	9	0	2	0	7	0	66 (53.22%)
T. violaceum	0	4	3	1	0	2	0	10 (8.06%)
T.mentagrophytes	23	7	1	3	1	0	0	35 (28.22%)
M. gypseum	3	0	0	2	0	2	1	8 (6.45%)
E. floccosum	2	1	1	0	0	0	1	5 (4.03%)
Total	76 (61.29%)	21 (16.93%)	5 (4.03%)	8 (6.45%)	1 (0.81%)	11 (8.87%)	2 (1.61%)	

Table 4. Antifungal susceptibility pattern of the isolated Dermatophytes

Antifungal agents	Dermatophytic growth (Zone of Inhibition in mm)				
	T. rubrum	***T. violaceum***	***T. mentagrophytes***	***M. gypseum***	***E. floccosum***
Fluconazole (10 mcg)	12.3 ± 0.5d	15.3 ± 0.4d	10.1 ± 0.5e	12.5 ± 0.5e	13.3 ± 0.5d
Ketoconazole (50 mcg)	15.4 ± 0.5c	14.2 ± 0.5e	12.3 ± 0.5d	15.7 ± 0.4d	10.5 ± 0.5e
Itraconazole (10 mcg)	19.2 ± 0.3b	21.5 ± 0.5a	22.3 ± 0.4b	24.4 ± 0.5b	17.3 ± 0.5c
Miconazole (30 mcg)	21.3 ± 0.5a	19.4 ± 0.3b	23.5 ± 0.5a	26.6 ± 0.3a	18.5 ± 0.5b
Nystatin (50 mcg)	20.5 ± 0.5a	16.4 ± 0.5c	19.6 ± 0.4c	20.5 ± 0.5c	19.5 ± 0.5a

Values within a column followed by different upper-case letters are significantly different (P<0.05) in Duncan's multiple range test

4. DISCUSSION

Dermatophytes are group of fungi (*Trichophyton, Microsporum and Epidermophyton*) which invades the keratinized tissues by means of the enzyme keratinase and cause dermatophytosis [7]. The developing countries the dermatophytic infection is more prevalent and it is increasing in this part of the world. In spite of this fact studies on the dermatophytic infections in Nepal, more specifically in Birgunj is scarce. Therefore, through this recent work an attempt has been made to determine the incidence and predominance of dermatophytic infection among the patients attending a tertiary care teaching hospital in Bigunj, Nepal.

In the present research work it has been evident from the Table 1 that the dermatophytic infection was more ubiquitous in the age group of 31 – 40 (37.8%) followed by 21-30 (30.4%). This study was in consensus with the study conducted by Prasad P.V.S who showed that the common age group involved in Dermatophytosis is 21-40 yrs [13]. The dominance of the dermatophytic infection in the above-mentioned age group may be due to increased physical exertion resulting in increased perspiration which produces a moist environment in the body, favoring the growth of dermatophytes. Moreover, compared to other age group, socialization with different people in the age group of 21-40 years is high which also contributes to the wide spread of the infection.

In this work it has been also observed from the Table 1 that the infection of dermatophytes is more predominant among the males (57.4%) than in females (41.3%) with the male to female ratio 4:3. The result was in complete accord with the work of Vijayakumar et al. [14]. The predominance of males over the females may be due to increased outdoor exposure and more physical work that results in increased sweating and less cosmetic consciousness compared to females [15].

The precise identification of the dermatophytosis is based on direct microscopy (i.e. KOH wet mount), fungal culture and sensitivity tests. These procedures are of immense help for the purpose of treatment. In our study out of the total 230 cases 109 (47.3%) were

positive in KOH wet mount and a whole of 124 (53.9%) were positive in culture. This finding was in total harmony with Grover and Sen et al. [16, 17]. In this present work 121 (52.6%) were negative for any fungal elements in KOH wet mount, whereas 41(17.8%) were positive for dermatophytic growth in culture. Therefore from Table 2 it is evident that the detection rate of dermatophytes through culture was greater than that of the direct microscopy. This type of results might be due to the drying procedure, which has been sponsored also by the work of Milne [18]. The result of our study was also in accord with the results of Madhavi et al. [19]. The result from the Table 2 clearly emphasizes the significance of the culture and the direct microscopy by KOH wet mount for the pinpoint diagnosis of superficial mycoses.

In the present work, among the 230 cases analyzed *Tinea corporis* was the most clinical type (66.5%), followed by *Tinea cruris* (15.2%) and *Tinea unguium* (7.3%). The finding was comparable to the work of Venkatesan et al., who have also reported that *T. corporis* was the most common clinical type [20]. This result was in total concurrence with Sumana et al. and Sumana et al. [21, 22].

Several researchers have reported that *Trichophyton* species have been the most important causative agent of dermatophytosis compared to other two genuses *Microsporum* and *Epidermophyton* [14]. In our present work among the 230 suspected dermatophytosis cases the preponderance of *Trichophyton* was found to be the maximum, with 111 isolates out of 124 dermatophytic isolates. Whereas, the isolates of *Microsporum* and *Epidermophyton* were very negligible in number. Among all the *Trichophyton* isolates *Trichophyton rubrum* was the most common isolate (53.2%), followed by *Trichophyton mentagrophytes* (28.2%) and *Trichophyton violaceum* (8.6%). The differentiation of *T. rubrum* and *T. mentagrophytes* was done by urea hydrolysis test, pigment production on potato dextrose agar, and macroscopic observation. In the present times it has been found that the number of *T. mentagrophytes* was increased gradually, but in our work it is the second most common isolate. This was in concurrence with the work of Kumar et al., Venkatesan et al., and Srinivasan et al. [15, 20, 23]. In comparison to *Trichophyton* isolates *Microsporum* and *Epidermophyton* count up for very small percentage, which was very much comparable to the work of Aggarwal et al. Patel et al. and Nawal et al. [24, 25, 26]

The essential management for all superficial mycotic manifestations, excluding the hairy regions, is right use of antifungal agents [27]. In this part of the world, it has been observed that over the counter availability of the steroids is very common and people with superficial mycotic manifestations preferred to take the steroids directly instead of consulting the dermatologists. Research conducted in different parts of the world shows that steroids only decreases irritation and that is observed clinically as decreased erythema and pruritus and patients will sense symptomatically better, and they take this as a cure. As soon as patient stop application, the erythema and pruritus reappear. After this patient keep on reuse it without completing the dose, which will promote the resistance towards the antifungal agents. In our work we have also carried out the antifungal susceptibility tests for the isolated dermatophytes where it has been clearly evident that the maximum isolates of the dermatophytes were less susceptible towards Fluconazole (10mcg) and ketoconazole (50mcg), whereas, majority of them were found to be susceptible against miconazole (30mcg), itraconazole (10mcg) and nystatin (50mcg) (Table 2). This work has been in complete consensus with the work of Gupta et al. and Mukherjee et al. [28, 29].

5. CONCLUSION:

Dermatophytosis is the most prevalent fungal manifestations in this part of the world and has an effect on individuals of diverse age groups. These fungal manifestations of the individuals will lead to their worse quality of life and financial burden due to the cost of the treatment. In this current work an attempt has been made to confirm the dermatophytic infections through microscopic and culture characteristics. It has been evident from this work that the pervasiveness of both the lab procedures among the patients was found to be on the higher side. From the present study it has been observed that the incidence of the dermatophytosis was found to be maximum in the age group of 31-40 and predominance of the males for the clinical manifestation of the dermatophytosis was greater than the females. The present study clearly showed that *Tinea corporis* was the prevalent clinical appearance involving 66.5% of the total cases of the dermatophytosis. In this work culture for the isolation

of the dermatophytes from the suspected clinical cases was conducted, which reveals that among the 124 positive isolates of the dermatophytes 90% represented *T. rubrum, T. mentagrophytes* and *T. violaceum* respectively, whereas, *M. gypsum* and *E. floccosum* were isolated in limited numbers in the suspected clinical cases of dermatophytosis. This identification of the fungal isolates by the microscope and the culture was instrumental in the treatment of the dermatophytosis. As a matter of fact in this study an effort has been made to observe the antifungal susceptibility pattern of the isolated dermatophytes. The isolated dermatophytes were found to be maximum susceptible towards miconazole (30 mcg) and itraconazole (10mcg) and shows least susceptibility towards fluconazole (10 mcg) and ketoconazole (50 mcg). Dermatophytosis remains a significant superficial mycotic manifestation throughout the universe and the symptoms of it remain unacceptably high in this part of the world. This is the high time that the dermatophytes must be diagnosed by means of direct microscopy and the culture methods. The widespread use of nonculture methods in the recent times is a major challenge for the diagnosis of the dermatophytosis and its resistance patterns. Moreover, routine antifungal susceptibility testing is not performed in most settings because of cost and reproducibility problems. Systemic surveillance of antifungal susceptibility test should be carried out for continuous monitoring of the antifungal susceptibility in dermatophytes to resolve the optimal treatment regimen.

ACKNOWLEDGEMENTS

The authors would like to thank the management of National Medical College and Teaching Hospital for their kind support for this work. Likewise, thanks are due to laboratory technician of Microbiology laboratory Mrs. Reecha Das for her technical assistance during this work.

REFERENCES

[1] Marques S.A., Robles A.M., Tortorano A.M., Tuculet M.A., Negroni R., Mendes R.P., Mycoses associated with AIDS in the third world. Med Mycol, 38(S1): 269–279, (2000).

[2] Male, O., in: Hawksworth, D.l. (Ed.), Frontiers in Mycology, Cab International, Wallingford 1990, pp.131–156.

[3] Havlickova B., Viktor A.C., Markus F., Epidemiological trends in skin mycoses worldwide. Mycoses, 4 (S51): 2-15, (2008).

[4] Nweze E.I., Dermatophytosis in Western Africa: a review. Pak J Biol Sci, 13: 649-656, (2010).

[5] Farzana A.N., Prevalence and Etiology of Dermatomycoses in Rajshahi, Bangladesh. J Life Earth Sci, 2(2): 75-78, (2007).

[6] Mikaili A., Chalabi M., Ghashghaie A., Mostafaie A., Immunization against bovine dermatophytosis with live *Trichophyton verrucosum*. Afr J Microbiol Res, 6(23): 4950-4953, (2012).

[7] Weitzman I., Summerbell R.C., The dermatophytes. Clin Microbiol Rev, 8: 240-259, (1995).

[8] Kane, J., Summerbell, R.C., in: Kane, J., Summerbell, R.C., Sigler, L., Krajden, S., Land, G. (Eds.), Laboratory handbook of dermatophytes: A clinical guide and laboratory manual of dermatophytes and other filamentous fungi from skin, hair and nails, Star Publishing Company, Belmont 1997, pp. 33-44.

[9] Isenberg, H.D., in: Gracia, L.S., Isenberg, H.D. (Eds.), Clinical Microbiology Procedure Handbook, 2nd ed. ASM Press, Washington DC 2004, pp. 8.0.1–8.10.7.

[10] Shemer A., Davidovici B., Grunwald M.H., Trau H., Amichai B., New criteria for the laboratory diagnosis of non dermatophyte moulds in onychomycosis. Br J Dermatol, 160: 37- 39, (2009).

[11] Ellis, D., Davis, S., Alexiou, H., Handke, R., Bartely, R., in: Ellis, D., Davis, S., Alexiou, H., Handke, R., Bartely, R. (Eds.), Descriptions of Medical Fungi, Nexus Print Solutions, Adelaide 2007, pp.4–169.

[12] Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Winn, W.C. Color Atlas and Text Book of Diagnostic Microbiology, 5th Edn, Lippincott Williams and Wilkins publisher: 983–1069, (1997).

[13] Prasad P.V.S., A study of chronic dermatophyte infection in a rural hospital. Indian J Dermatol Venereol Leprol, 71: 21-29, (2005).

[14] Vijayakumar R., Vijayaraman R.S., Rangarajan S., Kindo A.J., Incidence and prevalence of dermatophytosis in and around Chennai, Tamilnadu, India. Int J Res Med Sci, 4(3): 695-700, (2016).

[15] Kumar K., Kindo A.J., Kalyani J., Anandan S., Clinico–Mycological Profile of Dermatophytic Skin Infections in A Tertiary Care Center–A Cross Sectional Study. Sri Ramachandra J Med, 1 (2): 12-15, (2007).

[16] Grover S., Roy P., Clinico-mycological profile of superficial mycosis in a hospital in North-East India. Med J Armed Forces India, 59(2): 114–116, (2003).

[17] Sen SS., Rasul E.S., Dermatophytosis in Assam. Indian J Med Microbiol, 24(1): 77–78, (2006).

[18] Milne, L.J.R., in: Collee, J.C., Duguid, J.P., Fraser, A.G., Marmion, B.P. (Eds.), Practical Medical Microbiology, 13th ed, Churchill Livingstone, Edinburgh 1989, pp. 676–677.

[19] Madhavi S., Rao M.V., Jyothsna K., Mycological study of Dermatophytosis in rural population. Ann Biol Res, 2(3): 88–93, (2011).

[20] Venkatesan G., Singh A.J.A.R., Murugesan A.G., Janaki C., Shankar SG., *Trichophyton rubrum*-the predominant etiological agent in human dermatophytoses in Chennai India. Afr Journal Microbiol Res, 1(1): 9–12, (2007).

[21] Sumana MN., Rajagopal V., A study of dermatophytes and their in-vitro anti-fungal sensitivity. Indian J Pathol Microbiol, 45(2): 169–172, (2002).

[22] Sumana V., Singaracharya M.A., Dermatophytosis in Khammam. Indian J Microbiol, 47(2): 287–289, (2004).

[23] Balakumar S., Rajan S., Thiyagarajan T., Jeeva S., Epidemiology of dermatophytosis in and around Tiruchirapalli, Tamilnadu, India. Asian Pac J Trop Dis, 2 (4): 286-289, (2012).

[24] Aggarwal A., Arora U., Khanna S., Clinical and mycological study of superficial mycoses in Amritsar. Indian J Dermatol, 47(4): 218–220, (2002).

Received:06.05.18, Accepted: 09.06.18, Published:01.07.2018

[25] Patel P., Mulla S., Patel D., Shrimali G.A., Study of superficial mycosis in south gujarat region. Natl J Community Med, 1(2): 85–88, (2010).

[26] Nawal P., Patel S., Patel M., Soni S., Khandelwal N., A study of superficial mycosis in tertiary care hospital. Natl J of Integr Res Med, 3(1): 95–99, (2012).

[27] Hiroshi, S., Shimizu's Textbook of Dermatology, 2nd Edn, Hokkaido University Press, Sapporo 2007.

[28] Gupta A.K., Cooper E.A., Update in antifungal therapy of dermatophytosis. Mycopathologia, 166: 353-367, (2008).

[29] Mukherjee P.K., Leidich S.D., Isham N., Leitner I., Ryder N.S., Ghannoum M.A., Clinical *Trichophyton rubrum* strain exhibiting primary resistance to terbinafine. Antimicrob Agents Chemother, 47: 82-86, (2003).

***Corresponding Author:**
Dipak Bhargava*
Email: **dipak.bhargava80@gmail.com**