

Clinico - mycological study on superficial fungal infections in tertiary care hospital and a profile of their antifungal susceptibility pattern

Kumaran Ganesan^{1,*}, AS Shameem Banu², Riswana Jasmine³

¹Assistant Professor, ²Professor & HOD, Dept. of Microbiology, Chettinad Hospital & Research Institute, Chennai, Tamil Nadu,

³Senior Registrar, Dept. of Dermatology, Sundaram Medical Foundation, Chennai, Tamil Nadu

***Corresponding Author:**

Email: kumaranganesan15@gmail.com

Abstract

Background: Superficial fungal infections are one among the most common fungal diseases in the world. Dermatophytosis is common in tropical countries like India and may reach epidemic proportions in areas with high rate of humidity and over population and poor hygienic conditions.

Material & Method: The present study was undertaken to isolate, identify and determine the antifungal susceptibility of various fungal agents causing mycoses among the patients attending the Dermatology outpatient (OP) Chettinad hospital and research institute. Clinical samples from 200 patients were subjected to KOH examination and culture isolation. Causative agents were identified by colony morphology, pigment production and by microscopic examination. Antifungal susceptibility testing was done by broth dilution detecting the minimum inhibitory concentration.

Result: In total of 200 specimens cultured 98 dermatophytes were isolated and 12 non dermatophytic fungus were isolated which includes 5 isolates of *Candida albicans* and 7 isolates of *Candida non albicans*. *Trichophyton mentagrophyte* was the predominant dermatophyte isolate found in 62 cases of dermatophytosis followed by *T.rubrum* 22. This study showed among 98 isolates of dermatophytes, lowest value of MIC sensitivity was recorded in terbinafine as 0.03µg/ml and highest value was recorded with fluconazole 32µg/ml.

Conclusion: It is concluded that causative fungal agent for cutaneous mycoses in addition to dermatophytes and non-dermatophytes fungi *Candida* species also play an important role. Terbinafine was found to be the most potent drug for treating dermatophytic infections.

Keywords: Dermatophytes, *T.mentagrophytes*, *T.tonsurans*, *M.canis*

Introduction

Cutaneous fungal infections have been reported worldwide as being one of the most common human infectious diseases in clinical practice. A skin infection due to dermatophytes has become a significant health problem affecting children, adolescents and adults. In spite of therapeutic advances in the last decades, the prevalence of cutaneous mycoses is still increasing. Cutaneous fungal infections can be caused by dermatophytes, yeasts and non-dermatophyte moulds, although dermatophytes cause most of the cutaneous fungal infections. A single species might be involved in several clinical types each with its distinct pathology.⁽¹⁾ The severity of these reactions is related to the immune status of the host as well as to the strain and species of the organism causing the infection. The prevalence of dermatophytoses varies in different geographical locations.⁽²⁾ Surveillance for fungal infections is important to define their burden and trends to provide the infrastructure needed to perform various epidemiological and laboratory studies and to evaluate interventions. The prognosis among these immunocompromised patients is very poor and therefore early diagnosis and initiation of treatment is essential.⁽³⁾ There are many antifungal agents that are used to treat dermatophytosis. With the increase in drugs available for treatment, it is required to know their resistance profile as well.⁽⁴⁾ The present study was

conducted to isolate and identify the fungal strains by phenotypic method from patients suffering with superficial fungal infections and also to study their profile of antifungal susceptibility pattern.

Methodology

The study population included 200 patients with clinically suspected superficial fungal infections, who attended the outpatient department of dermatology at Chettinad hospital & research institute, Chennai. A detailed clinical and family history was taken. Skin scales, nail and hair fragments were collected from patients with cutaneous infection according to the site of infection.⁽⁵⁾

All the specimens were collected in dark paper sachets so that the small amount of specimens were seen easily for processing. These papers were sterilized in autoclave for 15 min at 121°C. Direct microscopic examination was undertaken in 20% potassium hydroxide (KOH) wet mount for hair and nail, 10% potassium hydroxide wet mount for skin scraping. All the collected specimens were inoculated into two sets of fungal culture media such as sabourauds dextrose agar with gentamicin and cyclohexamide for detection of dermatophytes and sabourauds dextrose agar with gentamicin to detect the growth of other non-dermatophyte species in the clinical samples.

The culture tubes were incubated at two different temperature 28°C and 37°C. After incubation culture growth was observed every two days and the tubes were discarded only after six weeks in the absence of growth. Culture identification done based on growth rate, temperature, colony characteristics, colour, texture and pigment production. The culture tubes contain mould group of fungal growth were inoculated into dermatophyte test medium and potato dextrose agar for better growth of sporulation and conidating fungus.

The microscopic examinations of fungal growth were identified by lactophenol cotton blue stain. Dermatophytes were identified depends on nature of mycelium and formation of macro and micro conidia of the isolates. The slide culture was performed for doubtful morphological isolates to identify the accurate structure of fungi or undisturbed morphological structure. Urease test and hair perforation test were performed to differentiate Trichophyton species. Non dermatophytic fungi were isolated such as Candida species in all culture media contain yeast fungi. Further identification of Candida species were done by inoculating the yeast colonies into corn meal agar and chrom agar medium.

All identified isolates were undertaken to perform antifungal susceptibility testing by minimum inhibitory concentration method for dermatophyte isolates to determine their in vitro activity of anti fungal drugs. Drugs selected for antifungal susceptibility testing were griseofulvin, fluconazole, itraconazole and terbinafine (CLSI guideline). These drugs were used in powder form as supplied by manufacturer (Jansen pharmaceutical & Pfizer international). Fluconazole was dissolved in sterile distilled water. Water insoluble drugs like griseofulvin & itraconazole were dissolved in dimethyl sulfoxide and terbinafine was dissolved in dimethyl sulfoxide with 5% tween 80. Stock solutions of each drug were prepared at an initial concentration of 1000 µg/ml. Further dilutions were used to get the required dilutions for each drug was made in distilled water.

Dermatophyte isolates were grown on potato dextrose agar at 28°C for four to five days. Mature colonies were covered with 1 ml of sterile 0.85% saline, and colony suspension were prepared and kept to settle down for 10 mins. The conidia were counted in the suspension by using hemocytometer then the concentration was adjusted.

The tests were done in 96 well microtitre plates and incubated at 28°C for 4 days. Inoculated each well on the day of the test with 0.1 ml of the 2 x conidial inoculums suspension and 0.1 ml of dilution of antibiotics. Test quality control (QC) and reference organisms in the same manner and include each time an isolate was tested. After incubation MIC was taken as the lowest concentration of antifungal agent that substantially inhibit growth of the organism as detected visually.

Antifungal drug susceptibility testing for non-dermatophyte isolates of candida species were done by disk diffusion method by using following drugs miconazole, fluconazole, itraconazole and ketoconazole.

Results

The study population which included 200 patients with clinically suspected superficial fungal infections was subjected to mycological examination. Out of 200 patients 80 (40%) were females and 120 (60%) were males. Out of which maximum cases with infection were between 21-30 years of age (31%). Among these age group 44 were males and 18 were females.

Out of 200 patients from whom the specimens were collected, 80 (40%) cases were farmers, 64 (32%) cases were construction workers, 36 (18%) cases were students and 20 (10%) were housewives.

Out of total 200 specimens collected 108 (54%) were skin scrapings, 78 (39%) were hair samples and 14 (7%) were nail clippings. The maximum numbers of specimens were skin scrapings, among these 65 specimens were collected from male and 43 from female.

Out of 200 specimens the KOH wet mount was positive for fungal elements in 130 (65%) samples and culture positivity was 110 (55%). Among these culture positive isolates 98 (49%) were dermatophytes and 12 (6%) were non dermatophytes, 70 (35%) did not show evidence of the fungi on direct microscopy and 90 (45%) not grown in culture.

Among the culture positive 98 dermatophytes and 12 were non dermatophytic fungus which includes 5 isolates of Candida albicans and 7 isolates of Candida non albicans. Other than Candida species no other non dermatophytic fungi were grown in this study. There was no growth in 90 (45%) of the total specimens (Table I).

Table 1: Distribution of culture positive isolates among clinical specimens

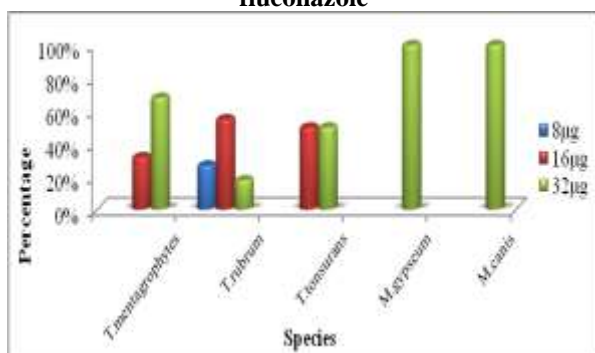
Name of the species	Total number	Skin	Hair	Nail	Total percentage
Trichophyton mentagrophytes	62	36	26	-	31%
Trichophyton rubrum	22	16	4	2	11%
Trichophyton tonsurans	4	4	-	-	2%
Microsporum gypseum	4	2	2	-	2%
Microsporum canis	6	4	2	-	3%
Candida species	12	6	2	4	6%
No growth	90	40	42	8	45%

In the total 98 dermatophytes, 88 isolates belonged to the Trichophyton species of which 62 isolates were Trichophyton mentagrophytes followed by 22 isolates of Trichophyton rubrum, 4 isolates of Trichophyton

tensurans, 10 isolates belonged to *Microsporum* species of which 4 isolates were *Microsporum gypseum* and 6 isolates were *Microsporum canis* (Table I).

This study showed among 98 isolates of dermatophytes, lowest value of MIC sensitivity was recorded in terbinafine as 0.03µg/ml and highest value was recorded with fluconazole 32µg/ml. (Graph 1, 2)

Graph 1: Percentage of sensitivity to drug fluconazole



Graph 2: Percentage of sensitivity to drug terbinafine

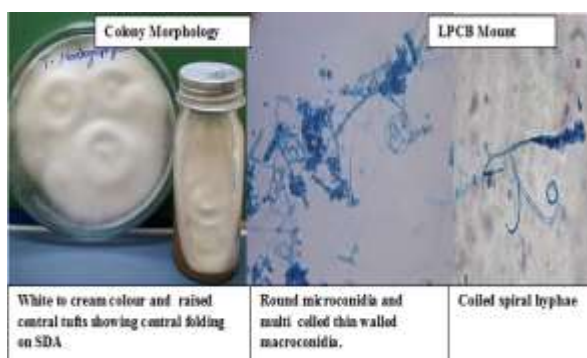
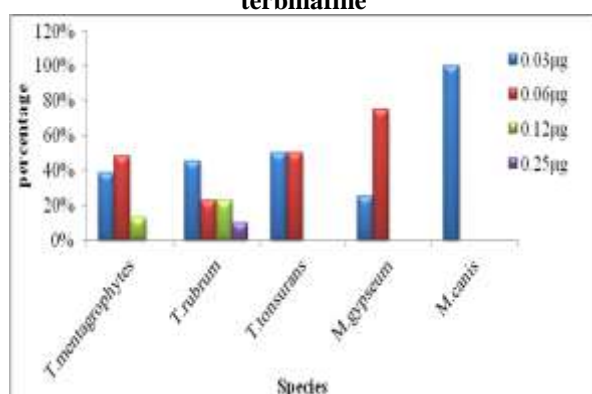


Fig. 1: Trichophyton mentagrophytes

Most of the dermatophyte species showed similar pattern of susceptibility to each antifungal agent tested. The determinations of susceptible pattern of isolates were identified by high MIC value.

Antifungal drug susceptibility testing for non-dermatophyte isolates of candida species were done by

disk diffusion method which showed sensitive to all candida species.

Discussion

In the present study out of 200 samples processed 60% were from males and 40% were from females. Male predominant occurs due to their occupation, frequent interaction with overcrowded people, poor personal hygiene and most of them were working as exhaustive physical worker like farmer. My study showed that maximum of 31% had tinea infections in age group between 21 to 30 years. Roy et al⁽⁶⁾ observed 40% which showed high prevalence of tinea infections compare to my study observation.

My study included 200 patients from whom the specimens were collected, 96 (48%) cases did not suffer from the same infection before, 50 (25%) cases had previous history of disease, 44 (22%) patients had contact history with infected person in their house. Similar findings were seen in study by Suganthi et al⁽⁷⁾ who also had shown 49% cases did not suffer from the same infection before which was correlated with my study.

Tinea corporis was the commonest lesion accounting for 23% of the cases in my study followed by Tinea capitis 21% and Tinea cruris 18%. This observation correlated with Nawal P, Patel et al⁽⁸⁾ which showed Tinea corporis 40.8%, followed by Tinea cruris 27.8% cases. Among the tinea corporis infections most of the clinical condition include lesion in exposed part of the human skin. Similar findings have been shown by Venkatesan et al.⁽⁹⁾

Agrawalla et al⁽¹⁰⁾ and Grando et al⁽¹¹⁾ conducted a study on clinico-mycological study of dermatophytosis observed that more than 20% of patients had two or more clinical types but my study did not show patients with dual clinical presentation which indicates no one had any risk factors for extensive tinea infection.

My study observed KOH mount was positive for fungal elements in 65% cases which correlated with Singh S and Beena PM⁽¹²⁾ who had shown KOH mount positivity as 61%.

Trichophyton mentagrophyte was the predominant dermatophyte isolate found in 62 cases of dermatophytosis followed by T.rubrum 22. We did not observe any Epidermophyton species in my present study. My study was comparable with Kansra S et al⁽¹³⁾ who observed that 65 cases were Trichophyton mentagrophytes and this was correlated with my study.

The pattern and isolation rate of non dermatophytic fungi obtained in my study was comparable with N Dako JA et al⁽¹⁴⁾ showed that out of 100 specimens 16 non-dermatophyte fungi were isolated which includes 6 isolates of Candida species which had correlated with my study and 10 isolates of Aspergillus species which did not correlate.

Low MIC values for terbinafine drug have been observed in my study which also correlated with Kansra

S et. al⁽¹³⁾ and Fernández-Torres et. al.⁽¹⁵⁾ They had also reported terbinafine was the most effective drug in their study. Haroon et. al⁽¹⁶⁾ observed terbinafine is most active drug and also it has perfect in vitro potency and wide spectrum activity against all dermatophyte species. Monitoring the resistance pattern also is useful because detection of resistance for different fungi also gives evidence to emerging threats of fungal infections.

Matnani G et. al⁽¹⁷⁾ who had isolated total of 27 *Candida albicans* and anti-fungal susceptibility testing was done by disc-diffusion method by using anti-fungal drug fluconazole. All the isolates were sensitive in this study which correlated with my study.

Conclusion

Dermatophytic infections are one of the most common infectious diseases. Isolation rate of fungus depends on the good culture techniques with aseptic precautions which prevents the contaminants to over grow in the culture. *Trichophyton* species was the predominant causative agent of dermatophytic infections.

Minimum inhibitory concentration need to be correlated with clinical outcome to develop interpretive breakpoint, which may specify the cause for lack of clinical response and detection of resistance. Terbinafine was found to be the most potent drug for treating dermatophytic infections.

References

- Hanumanthappa H, Sarojini K, Shilpashree P, Muddapur SB. Clinicomycological study of 150 cases of dermatophytosis in a tertiary care hospital in South India. *Indian journal of dermatology*. 2012 Jul;57(4):322.
- Tsuboi R, Ogawa H, Bramono K, Richardson MD, Shankland GS, Crozier WJ, Sei Y, Ninomiya J, Nakabayashi A, Takaiuchi I, Payne CD. Pathogenesis of superficial mycoses. *Journal of medical and veterinary mycology*. 1994 Jan; 32(sup 1):91.
- Mishra M, Mishra S, Singh PC, Mishra BC. Clinicomycological profile of superficial mycoses. *Indian journal of dermatology, venereology, and leprology*. 1998 Nov;64(6):283.
- Hussein MR. Mucocutaneous Splendore-Hoeppli phenomenon. *Journal of cutaneous pathology*. 2008 Nov;35(11):979.
- Jagdish Chander. *Textbook of Medical Mycology*. 3rd Ed. Mehta Publishers, 2009;580.
- Grover S, Roy P. Clinico-mycological profile of superficial mycosis in a hospital in North-East India. *Medical Journal Armed Forces India*. 2003 Apr;59(2):114.
- Suganthi et al. A study of prevalence of dermatophytes in north chennai and a profile of their antifungal susceptibility pattern March 2006.
- Nawal P, Patel S, Patel M, Soni S, Khandelwal N. A study of superficial mycoses in tertiary care hospital. *National Journal of Integrated Research in Medicine*. 2012 Mar;3(1):90.
- Venkatesan G, Singh AR, Murugesan AG, Janaki C, Shankar SG. *Trichophyton rubrum* the predominant etiological agent in human dermatophytoses in Chennai,

- India. *African Journal of Microbiology Research*. 2007 May;1(1):9.
- Agarwalla A, Jacob M, Sethi M, Parija SC, Singh NP. A Clinico-Mycological Study of Dermatophytoses in Nepal. *The Journal of dermatology*. 2001 Jan;28(1):16.
- Grando SA, Hostager BS, Herron MJ, Dahl MV, Nelson RD. Binding of *Trichophyton rubrum* Mannan to Human Monocytes In Vitro. *Journal of investigative dermatology*. 1992 Jun;98(6):876.
- Singh S, Beena PM. Profile of dermatophyte infections in Baroda. *Indian journal of dermatology, venereology, and leprology*. 2003 Jul;69(4):281.
- Kansra S, Devi P, Sidhu S, Malhotra A. Prevalence of Dermatophytoses and Their Antifungal Susceptibility in a Tertiary Care Hospital of North India. *International Journal of Scientific Research*. 2016 Mar;5(3):450.
- Ndako J, Osemwegie OO, Spencer TH, Olopade BK, Yunusa GA, Banda J. Prevalence of dermatophytes and other associated fungi among school children. *Global Advanced Research Journal of Medicine and Medical Sciences*. 2012;1(3):49.
- Fernández-Torres B, Carrillo-Muñoz A, Ortoneda M, Pujolz I, Pastor FJ, Guarro J. Inter laboratory evaluation of the Etest® for antifungal susceptibility testing of dermatophytes. *Medical Mycology*. 2003 Jan;41(2):125.
- Haroon TS, Hussain I, Aman S, Jahangir M, Kazmi AH, Sami AR, Nagi AH, Alvi KH, Iqbal N, Khan KA, Aziz R. A randomized double-blind comparative study of terbinafine for 1, 2 and 4 weeks in tinea capitis. *British Journal of Dermatology*. 1996 Jul;135(1):86.
- Matnani G, Roy I, Gandham N, Mandal A, Ujagare M, Jadhav SV. Identification and antifungal susceptibility testing of fungal infections in clinical samples of suspected superficial fungal infections. *International Journal of Medical and Clinical Research*. 2012 Jan;3(7):215.