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Research Article

Incidence and prevalence of dermatophytosis in and around Chennai, Tamilnadu, India

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ABSTRACT

Background: Dermatophytes are group of fungi that infect keratinized tissues of human and animals. The group consist of three different genera namely, *Trichophyton*, *Microsporum*, *Epidermophyton* and several species within each genera. Among *Trichophyton*, *Trichophyton rubrum* is predominant, followed by various strains of *Trichophyton mentagrophytes*, which include both anthropophiles and zoophiles. Prevalence of dermatophytes varies with location and environmental condition. The infection is common worldwide with higher prevalence in tropical countries like India. Molecular diagnosis renders accurate identification of clinical dermatophyte isolates to species level. The main objective of this study was to determine the prevalence of dermatophytoses, isolate and identify the dermatophyte from samples of clinically suspected cases attending tertiary care centre using conventional and molecular methods.
Methods: A total of 210 patients showing lesions typical of dermatophytes infection from outpatient Department of dermatology were sent to mycology unit, Department of Microbiology for the period of April 2011-March 2014 were studied. Diagnosis was confirmed by conventional and polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique.
Results: Out of 210 samples received, tinea corporis was the predominant clinical site which was followed by tinea cruris. A total of 143 dermatophytes were isolated from the clinical samples. *T. rubrum* was the predominant etiological agent with 70/143 isolates and *T. mentagrophytes* was the second most common with 64/143 isolates. Amplification of internal transcribed spacers (ITS) was successful in all the clinical isolates by PCR and produced species specific banding pattern in RFLP using restriction enzyme Mva I.
Conclusions: Among dermatophytoses, *T. rubrum* was the predominant etiological agent present in the whole of Chennai District and *T. mentagrophytes* takes the second place.

Keywords: Molecular speciation, Internal transcribed spacers, Mva I, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, PCR-RFLP

INTRODUCTION

Dermatophytoses is a superficial infection caused by a group of fungi, dermatophytes. The infection is common world-wide with higher prevalence in tropical countries.[1] The dermatophytoses infection is commonly referred as ringworm due to the appearance of the lesion. Dermatophytes comprise of three major genera, *Trichophyton*, *Microsporum* and *Epidermophyton*, of the class hyphomycetes and division deuteromycota. They

are keratinophilic in nature and have the ability to colonise keratinized non-living tissues such as skin, hair and nail in human and animals.[2] The infection spreads easily by direct contact from infected humans and animals or through fomites.[3]

Although the infection is not invasive and easy to cure, its widespread nature and cost of the treatment is a major public health problem and causes colossal damage to the economic status of the tropical countries like India.[4] There are not many cases reported recently on the prevalence of the dermatophytosis in Chennai, India, therefore, this work was framed to study the epidemiology and prevalence of dermatophyte strains from patients attending the dermatology outpatient unit from a tertiary care centre in Chennai, Tamilnadu, India.

METHODS

Collection of specimens

Total of 210 patients showing lesions typical of dermatophytes infection based on the clinicians' preliminary diagnosis from outpatient Department of Dermatology from April 2011-March 2014, were sent to Mycology Unit, Department of Microbiology, Sri Ramachandra Medical Center, Chennai, India. Patients of all age groups and both sexes were included in the study.

Different tinea conditions such as tinea corporis, tinea capitis, tinea cruris, tinea pedis, tinea unguium, tinea faciei, and tinea manuum were observed in patients. The lesions were scraped from centre to edge of the infected area. Other dermatophytoses, such as tinea pedis and tinea manuum were scraped in such a way that the whole infected area is represented. In tinea capitis and tinea barbae, the hair with basal root portion was plucked using sterile tweezers and small portions of hair roots were epilated. In tinea unguium infection, the debris from beneath the distal end of the nails, scrapings from near the nail bed were collected. Close clipping of the infected nail end was performed wherever scrapings were not possible. Samples were collected in a thick black chart paper, folded and transported.

Direct microscopy

Scrapings and hair were mounted in fresh 10% KOH with parker ink and observed under 400x magnifications for septate hyphae. For nail clippings, fresh 20% or 40% KOH with parker ink was used, as the material is hard to digest.

Culture

For primary isolation of dermatopytes from clinical samples, Sabouraud's dextrose agar with cycloheximide was used as semi-selective medium, since cycloheximide reduces the growth of non-dermatophytic fungi. Dermatophyte test medium was also used for all the samples as a colour change to red indicates alkalinity generated by dermatophyte growth. The samples were inoculated in both Sabouraud's dextrose agar with cycloheximide and dermatophyte test medium in duplicates and incubated at 25°C and 37°C respectively.

Lactophenol cotton blue (LPCB) mount

The LPCB mount was covered with clean glass coverslip, heated gently and observed under 100 and 400 magnifications.

Slide culture technique

All the isolates for which the morphology was not clear in LPCB were subjected to slide culture technique. The slide culture technique permits the microscopic observation of the undisturbed relationship of spores to hyphae.

All the clinical isolates which were identified based on phenotypic method were subjected to genotypic method using PCR-RFLP.

DNA isolation

DNA was extracted from all the clinical isolates by phenol:chloroform method with certain modifications.[5] Briefly, the culture was suspended in 400µl lysis buffer (10mM TRIS, (pH - 8), 1mM EDTA (pH - 8), 3% SDS and 100 mM NaCl) in a 1.5ml microfuge tube. About 20 µl of proteinase K (1mg/ml) (merck genei) was added and incubated at 56ºC for 30 minutes. It was boiled for 1 minute. About 400µl of phenol:chloroform (sigma) (1:1) mixture was added, vortexed and centrifuged at 10,000 rpm for 10 minutes. The aqueous layer was transferred to a new microfuge tube and equal volume of chloroform was added, vortexed and centrifuged at 10,000 rpm for 10 minutes. The aqueous layer was transferred to a new microfuge tube. DNA was precipitated using equal volume of ice cold isopropyl alcohol and washed twice with 70% ethanol. The pellet was dissolved in 40 µl sterile nuclease free water and stored at -20ºC until use.

Amplification of ITS region

PCR amplification of ITS1 and ITS 2 region was carried out using universal fungal primers ITS 1 (5' – TCC GTA GGT GAA CCT GCG G – 3') and ITS4 (5' – TCC TCC GCT TAT TGA TAT GC – 3'). The reaction mix contained 25 µl PCR master mix (merck genei), 50 pmol universal fungal primers, ITS-1 (sigma) and ITS-4 (sigma) each, 1 µl of template DNA and the volume made up to 50 µl with nuclease free water. Amplification was carried out for 35 cycles under following conditions: initial denaturation at 95ºC for 5 min, denaturation at 95ºC for 30 sec, annealing at 56ºC for 30 sec, extension at 72ºC for 30 sec and final extension at 72ºC for 5 min.

Restriction fragment length polymorphism

The PCR products were subjected to restriction analysis using Mva I restriction enzyme (thermo fishers). The reaction mix had 2 µl of enzyme buffer, 5 Units of Mva I enzyme and 10µl of PCR product, the volume was made up to 20µl with nuclease free water. The reaction mix was incubated at 37ºC for 1 hour.

Agarose gel electrophoresis

Agarose gel was prepared in 1X TAE and 1µl of EtBr (10mg/ml) was added to it. The PCR products and RFLP products were electrophoresed in 1.5% and 2% agarose respectively for 45-60 minutes, at 50 V. The products were visualized under UV illumination.

RESULTS

Sex distribution

From the study, it was found that, out of the 210 patients suspected with dermatophytosis, male were infected more (120) than female (90) in the ratio of 4:3. The sex distributions among various clinical types are tabulated (Table 1).

Table 1: Details of samples with reference to sex and clinical manifestation.

Clinical Manifestation	Number of Samples n (%)	Sex	
		Male	Female
		n (%)	n (%)
Tinea corporis	133 (63.27)	70 (33.33)	63 (30.00)
Tinea cruris	29 (13.86)	27 (12.88)	2 (0.95)
Tinea unguium	19 (9.04)	10 (4.76)	9 (4.28)
Tinea manuum	9 (4.3)	4 (1.90)	5 (2.38)
Tinea pedis	8 (3.81)	2 (0.95)	6 (2.86)
Tinea capitis	6 (2.86)	4 (1.90)	2 (0.95)
Tinea faceii	5 (2.38)	2 (0.95)	3 (1.43)
Tinea barbae	1 (0.48)	1 (0.48)	-
	210 (100)	120 (57.15)	90 (42.85)

Age distribution

Dermatopyhytic infection was found more in the age group of 21-40 years with 103/210 patients, followed by age group of 41-60 years with 61/210 patients, age group of 11-20 years with 28/210 patients, old age group, 61-80 years with 17/210 patients, and very old age group (>81 years) with only one patient.

Area distribution

Patients were from in and around Chennai district. Most of the patients were from West Chennai (74/210) and West suburbs of Chennai (67/210). Sixteen patients were from South Chennai. Eleven patients were from both Central Chennai and South-Western suburbs of Chennai. Two patients from both North Chennai and North suburbs of Chennai. 27 Patients were from outside Chennai District.

Clinical manifestation

Samples were collected from patient's various anatomical sites such as epidermal layers of skin, hair and nail. Among them tinea corporis was predominant in 133/210 (63.27%) patients followed by tinea cruris in 29/210 (13.86%) patients. Tinea unguium was found in 19/210 (9.04%) patients, tinea manuum was observed in 9 (4.30%) patients, tinea pedis was seen in 8 (3.81%) patients and tinea capitis, tinea facei and tinea barbae were seen in six (2.86%), five (2.38%) and one (0.48%) patient respectively.

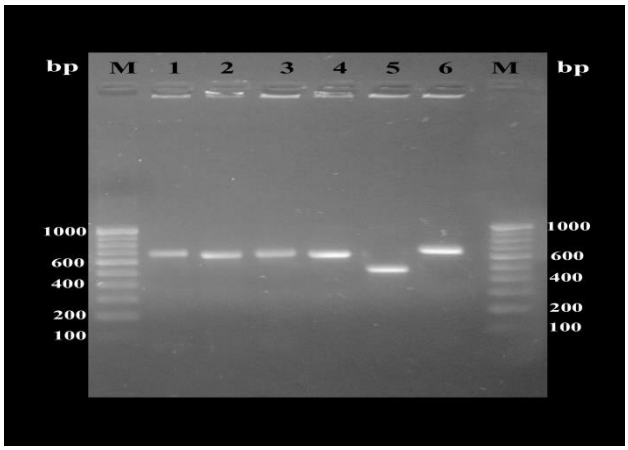

Figure 1: PCR products of ITS1-ITS2 regions from representative clinical isolates of dermatophytes species. Lanes 1-5: *T. rubrum, T. mentagrophytes, T. tonsurans, M. canis, M.gypseum and E. floccosum*, respectively. Lanes M: 100 bp DNA ladder.

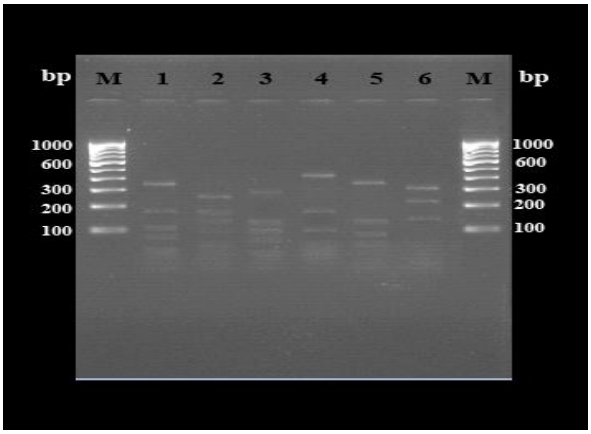

Figure 2: Mva I digested products from representative clinicial isolates of dermatophytes species. Lanes 1-5: *T. rubrum, T. mentagrophytes, T. tonsurans, M. canis, M.gypseum and E. floccosum*, respectively. Lanes M: 100 bp DNA ladder.

Molecular identification

PCR amplified ITS-1 and ITS-2 region of all 143 dermatophytes isolates using universal fungal primers ITS-1 and ITS-4. Amplicon size of 650-800bp was obtained from all 143 clinical isolates of dermatophytes (Figure 1). The restriction digestion of PCR amplicon using restriction enzyme *Mva* I was performed for all the clinical isolates, which yielded four to five bands in each isolates with different banding pattern which is unique to each species (Figure 2) making it easy to distinguish one species from other.

Culture

Based on the conventional and molecular methods, out of 210 clinical samples, 143 (68%) were positive for dermatophyte growth. *T. rubrum* was predominant with 70 isolates (48.95%) followed by *T. mentagrophytes* with 64 isolates (44.75%). Other isolates were, *T. tonsurans* 5 (3.50%) isolates, *M.gypseum* 2 (1.40%), one *M. canis* (0.70%) and *E. floccosum* (0.70%) (Table 2).

Table 2: Correlation of Clinical Manifestation with dermatophytes isolates.

Dermatophyte	Clinical manifestation						Total
	Tinea corporis	Tinea cruris	Tinea unguium	Tinea pedis	Tinea manuun	Tinea capitis	
T. rubrum	47	10	8	3	2	-	70
T. mentagrophytes	40	9	6	4	4	1	64
T. tonsurans	3	-	-	-	-	2	5
M. gypseum	1	-	-	-	1	-	2
M. canis	1	-	-	-	-	-	1
E. floccosum	1						1
	93	19	14	7	7	3	143

DISCUSSION

In the present study, 210 clinically suspected dermatophytoses cases were studied. Earlier studies have confirmed that infection with dermatophytes was more frequent in males compared to females.[6-10] In our study, among 210 patients who were diagnosed with dermatophytoses infection, the males were 57% which is marginally higher than the percentage of females (43%) with the male to female ratio 4:3. The reason for increased percentage of males may be due to the fact of increased outdoor exposure and more physical work that results in increased sweating and less cosmetic consciousness compared to females.[8]

The study shows that the dermatophyte infection is predominant in the adult age group (21 - 40 years). The reason for this may be due to increased level of physical activity in the particular age group and this leads to excessive sweating which favours the growth of dermatophytes. Socialization with different people is also high compared to other age groups which eventually help in spreading of infection. This finding correlates with the earlier studies.[8,11,12]

Among all the clinical types, tinea corporis was the predominant one with 133 (63.27%) out of 210 samples. The finding is comparable with the earlier studies from Tamil Nadu, Madhya Pradesh, Manipal and Kashmir.[8-10,13-15] Apart from India, tinea corporis had been reported as most predominant clinical type in Brazil and Spain.[16,17] Tinea cruris was the next dominant clinical type with 29 (13.86%) samples, followed by tinea unguinum 19 (9.04%). Tinea cruris is more prevalant in men compared to women. The findings were backed by earlier studies.[9,18,19] This may be due to exhausting physical activity in open environment leading to excess sweating and the use of tightly worn synthetic clothes resulting in increased humidity and temperature of the body which makes skin as a suitable growth environment for dermatophytes.[9] These conditions are shown to be associated with the incidence of tinea corporis and tinea cruris.[1,18] Other clinical types such as tinea manuum, tinea pedis, tinea capitis, tinea faceii and tinea barbae were found less frequent. The details of sample with reference to the sex and the clinical manifestation have been shown in Table I.

Trichophyton species have been a major causative agent of dermatophytosis than the other two genuses, *Microsporum* and E*pidermophyton*. In our study, among 210 dermatophytosis cases studied, *Trichophyton* was found to be the predominant etiological agent with 139 isolates out of 143 dermatophyte isolates, as only negligible number of isolates of M*icrosporum* and *Epidermophyton* were grown. *T. rubrum* was the most predominant isolate (48.95% growth) like demonstrated by other studies earlier in India.[8,9,10,20] In recent years, prevalence of *T.mentagrophytes*is increasing gradually but in our study we have obtained 44.75% isolates and is second most common isolate next to *T. rubrum*.[8,9,10] This finding is in slight variation to the previous study,

although *T. mentagrophytes* was again the second most common in all the previous studies, the number of isolates were very less compared to *T. rubrum*.[8,9,10] Apart from *T. rubrum* and *T. mentagrophytes*, *T. tonsurans* was also isolated from 5 samples. *Microsporum* was represented by two *M. gyseum* and one *M. canis* isolates. *E. floccosum* was represented by only one isolate. Compared to *Trichophyton*, the other two genuses were very few to represent. Generally, *Microsporum* and *Epidermophyton* are accounted for very low percentage compared to *Trichophyton* species.[18,20] Correlation between the etiological agents with clinical manifestation of infection is indicated in Table II.

The increased incidence of dermatophytoses could be due to environmental conditions such as humid weather and hot temperature of the geographical location in and around Chennai district. Apart from the environmental condition, poor personal hygiene along with poor illiteracy plays a major role in influencing the higher incidence of dermatophytosis.[1] The present study also shows that male are more prone than females. This can be correlated with the occupation of the person. On the other hand, social stigma present in the rural population of Tamilnadu which influences non-reporting of female patients to the hospital may also be the factor for showing less frequency in females.[18,19]

CONCLUSION

To conclude, dermatophytoses are worldwide distributed with increased incidence especially in tropical countries like India. Several factors such as age, sex, illiteracy, poor hygiene and social economy influence the infection with dermatophytes. In the present study we have attempted to understand the epidemiological status of the dermatophytes in and around Chennai, Tamilnadu, India. Tinea corporis was the predominant clinical site from which dermatophytes were isolated. *T. rubrum* and *T. mentagrophytes* have been the major etiological agents and that has been evinced by our study.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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