

MYCOLOGICAL STUDY ON TRICHOPHYTON INTERDIGITALE ISOLATED FROM CLINICALLY DIAGNOSED CASES OF DERMATOPHYTE INFECTIONArchana Dukare¹, Dr. Ravindra Khadse*¹, Smita Boyar², Sonal Chavan¹, Sharmila Raut¹¹Indira Gandhi Government Medical College, Nagpur.²Government Veterinary College, Nagpur.

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ABSTRACT

Introduction: Dermatophytosis is one of the most commonly encountered cutaneous fungal infections worldwide. Recently, marked increase in the isolation of *Trichophyton interdigitale*. The changing epidemiology of fungal infection is not only of public health interest as a “new” pathogen to an area but also is of clinical and microbiological importance. **Material and Methods:** It is the clinico-mycological study of 250 dermatophytoses cases to find out trichophyton species as causative agent. The isolation and identification of fungus is performed by conventional methods like direct microscopy and culture on sabouraud's dextrose agar, dermatophyte test medium. The *T. interdigitale* is differentiated from *T. mentagrophytes* by MALDI-TOFMS technique. **Results:** The dermatophytes were isolated in 132(52.8%) cases of suspected dermatophytoses. *Trichophyton* 113(85.6%) is the commonest isolated dermatophytes in which *T. rubrum* 40(30.3%), *T. mentagrophytes* 37(28.02%) followed by *T. tonsurans* 23(17.42%), *T. schoenleii* 7(5.30%). Out of 37 isolates of *T. mentagrophytes*, 10(27.02%) were *T. interdigitale*. The history of contact with animals observed in 50% cases The commonest age group affected by *T. interdigitale* was 16-30 years with male preponderance. *T. interdigitale* isolated from cases of tinea unguium (4), tinea pedis (3), and each one with tinea corporis, tinea barbae, tinea capitis. **Conclusion:** It is imperative to be aware of the changing patterns of causative fungi for making adequate strategies for prevention and treatment. The conventional methods had failed to identify *T. interdigitale* strains, while MALDI-TOFMS analysis enabled the distinct classification as trichophyton species of *T. interdigitale* and helpful for the identification of dermatophyte species.

KEYWORDS: Dermatophytosis, *Trichophyton interdigitale*, MALDI-TOF.**INTRODUCTION**

Dermatophytosis is also called ringworm infections and it is the most common cause of fungal infections worldwide. Dermatophytes are grouped into three genera namely *Trichophyton*, *Microsporum* and *Epidermophyton*. Dermatophytes are also classified according to their habitat, being either anthropophilic associated with humans, zoophilic associated with animals or geophilic associated with soil. Anthropophilic species are responsible for the majority of human infections and tend to be chronic with little inflammation. Infection caused by zoophilic and geophilic species are associated with acute inflammation.^[1] The distribution of the dermatophytosis and their etiological agents varies with geographical location and depends on several factors, such as lifestyle, type of the population, migration of people and climatic conditions, therefore some species are widely distributed whereas others are geographically restricted.^[2]

Previously, *Trichophyton rubrum* was the predominant pathogen causing dermatophytosis,^[2,3,4,5] but now there is change in trend of disease causing agent. However, dermatophytosis caused by *T. interdigitale*, an emerging pathogen have been observed in the past five years. It is the second-most commonly isolated fungus causing tinea infections in humans and one of the most common fungi that cause zoonotic skin diseases.^[6] It belongs to *T. mentagrophyte* complex. *T. mentagrophyte* complex consists of several anamorphs and three teleomorphs (*Arthroderma vanbreuseghemii*, *A. benhamiae*, and *A. simii*) and are usually isolated from pets, such as guinea pigs and rabbits. This fungus can cause inflammatory tinea corporis, tinea facie and tinea capitis in humans.^[7]

According to recent molecular studies, the species *T. mentagrophyte* is synonymous with only the zoophilic subspecies *T. mentagrophyte* var. *quinckeanum*. The anthropophilic subspecies of *T. mentagrophytes*, as well as many of the zoophilic strains, formerly differentiated as var. *mentagrophytes* or var. *granulosum*, are

indistinguishable and are now designated *T. interdigitale*. The morphological differentiation between *T. interdigitale*, *T. mentagrophyte*, and its anamorph of *A. benhamiae* is impossible.^[8,9] In such cases, molecular identification is helpful for the confirmation of species. Rapid identification of dermatophyte species and knowledge of their host preference and ecology play an important role in epidemiology, public health issue and infection control. The availability of scanty data on *T. interdigitale* prompted us to present study which was conducted to know the prevalence, etiology and common clinical presentations of dermatophytosis and use of MALDI-TOF as diagnostic laboratory methods for the purpose of species identification.

MATERIALS AND METHODS

This is a cross-sectional and observational study over a period from January 2017 to September 2017 conducted at Indira Gandhi Medical College & Hospital, Nagpur in central India. The study population comprised of 250 clinically suspected cases of ringworm infection, attending Dermatology outpatients department. Detailed history of onset of disease, duration of symptoms, trauma, occupation, drugs, associated co-morbid conditions, family and personal history was taken. Enquiries were also made as to exposure to animals, cases or any other suspected sources.

These samples were collected and processed as per standard guidelines.^[10,11,12,13] The affected area of all types of lesions were swabbed with 70% alcohol. The active edge of skin lesion scraped with sterile blunt scalpel. The nails were scraped deeply enough to obtain recently affected nail tissue as well as nail clippings were also collected. The scrapings were taken from scalp lesions and few affected hairs were also epilated and collected with sterilized forceps. Usually basal portion of hair (hair stub) was collected as the fungus was found in this area. The scrapings were collected on a piece of sterile brown paper and transported to the laboratory within 2 hours for microscopic and cultural analysis. The samples were divided into two portions; one for direct KOH examination and remaining part was used to inoculate onto Sabouraud dextrose agar with chloramphenicol (SDCA), Sabouraud dextrose agar with chloramphenicol & cyclohexamide (SDCCA) and Dermatophyte test medium (DTM) with supplement to isolate the causative dermatophytes.

The identification and specification of dermatophytes were carried out by standard procedures.^[10,13] Skin and hair specimens were subjected to 10% KOH solution and kept at room temperature for 30 minutes while nail clippings and scrapings were kept in 40% KOH solution for overnight. Subsequently examination was done for branching and septate hyphae. The samples were inoculated on Sabourauds dextrose agar (SDA) with chloramphenicol (0.05mg/ml) & cyclohexamide (0.5mg/ml) and on DTM slopes for isolating. The tubes were incubated at 37°C and also at room temperature

25°C to achieve good growth. The tubes were examined at regular intervals for evidence of fungal growth for four weeks. Any visible growth on SDCA or SDCCA was examined for colony morphology, texture and pigmentation on obverse & reverse surface.

The slide culture technique (Riddel Slide culture Method), urease test, rice grain test and hair perforation test performed for fungus identification and lactophenol cotton blue mount was prepared for colonies to examine the hyphal structure, different vegetative structures formed like microconidia, macroconidia and chlamydoconidia. were performed.

The identification of *T. interdigitale* from *T. mentagrophytes* was performed by MALDI-TOF. The dermatophyte proteins were extracted using the procedure for molds.^[15] A piece of mycelium was gently scraped from the culture plate with a scalpel and suspended in 900 µl absolute ethanol and 300 µl HPLC water. The sample was centrifuged at 13,000 g for 10 min, with the resulting pellet re-suspended in 12.5 µl of 70% formic acid and incubated for 5 min at room temperature. Subsequently, 12.5 µl of 100% acetonitrile was added during 10 min at room temperature and then centrifuged at 13,000 g for 10 min. One µl of supernatant was spotted onto a MTP 384 target plate polished steel TF and allowed to air dry. Then, the spot was covered with 1 µl matrix solution [alpha-cyano-4-hydroxycinnamic acid] saturated in 50 acetonitrile: 25 HPLC water: 25 10% TFA and was allowed to air dry. The standard was used for instrument calibration.^[16] The MALDI-TOF MS assays were performed on an Ultra-Flex (BIOMERIEUX) mass spectrometer, according to the manufacturer's instructions.

RESULTS

A total of 250 samples were collected from patients with clinically suspected tinea infection. The dermatophytes were isolated in 132 (52.8%) cases. Among these dermatophytes, trichophyton 113(85.6%) is the most common genus, in which *T. rubrum*(30.3%) is most common species followed by *T. mentagrophyte* (28.02%), *T. tonsurans* (17.42%), *T. concentricum* (3.03%), *T. verrucosum* (1.51%), *T. schoenleinii* (5.30%).

Table 1: No. of Trichophyton strains isolated from samples (n=113).

Fungi	No. of strains (%)
<i>T. rubrum</i>	40 (30.30%)
<i>T. mentagrophytes</i>	37 (28.02%)
<i>T. tonsurans</i>	23 (17.42%)
<i>T. concentricum</i>	4 (3.03%)
<i>T. verrucosum</i>	2(1.51%)
<i>T. schoenleinii</i>	7 (5.30%)

Out of 37 isolates of *T. mentagrophyte*, *T. interdigitalis* identified in 10(27.02%) samples by MALDI-TOF. *T.*

interdigitale isolated from cases of tinea unguium (4), tinea pedis (3), and each one with tinea corporis, tinea barbae, tinea capitis. They were in the age group of 16-30 years with male to female ratio 1:0.43. The characteristic of zoophilic strains of *T. interdigitale* was

in 5(50%) cases with history of contact with animals. They presented with 5-8 weeks history of skin lesions showing inflammatory signs along with scaling and numerous yellow crusts attached to the lesions' surface.

Table 2: Details of cases of infection with *T. interdigitale* (n=10).

Duration of illness in weeks	Occupation Related History	H/O contact with animals	Diagnosis	KOH Mount
5	Student	Dog(pet)	<i>T. pedis</i>	Positive
7	Farmer	-	<i>T. unguium</i>	Negative
6	Farmer	Indirect contact	<i>T. corporis</i>	Positive
7	Student	Cat(pet)	<i>T. unguium</i>	Positive
5	Auto-Driver	-	<i>T. unguium</i>	Negative
5	House Maid	-	<i>T. capitis</i>	Positive
5	Farmer	Indirect contact	<i>T. unguium</i>	Positive
8	Student	Dog(pet)	<i>T. barbae</i>	Positive
6	Student	-	<i>T. pedis</i>	Negative
7	Student	-	<i>T. pedis</i>	Positive



Figure 1



Figure 2



Figure 3



Figure 4

Figure 1: Trichophyton interdigitale: white, flat, radiating thallus on Sabouraud's dextrose agar.

Figure 2: Reverse pigment shows yellow-brown pigment.

Figure 3: Trichophyton interdigitale; powdery, granular surface on the thallus.

Figure 4: Urease activity on a urea agar.

DISCUSSION

Dermatophytosis has a wide geographical distribution; the species of dermatophyte causing infection may vary from region to region and are geographically restricted except some species like *Trichophyton rubrum* which have a cosmopolitan distribution.^[3] In our study of 250 cases, *Trichophyton* is the most common genus, in which *T. rubrum* is most common species. This is comparable to the previous studies conducted by Sara Asticoli et al, A laksmanan et al, Doddamani PV et al, Lakshmi poiuri et al.^[2,4,5,6]

Recently, there was a marked increase in the isolation of *Trichophyton interdigitale*. The changing epidemiology of this fungal infection is not only of public health interest as a "new" pathogen to an area but also is of clinical and microbiological importance to suggesting an increasing incidence.^[6] *T. interdigitale* was previously known as *T. mentagrophyte*. But now on the basis of molecular biological analyses of dermatophyte DNA, *T. interdigitale* is classified under separate new species.

In order to confirm results obtained from cultures, we use MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry. Now a days VITEK MS Plus, bioMérieux, Nürtingen, Germany, a software and database, constitutes a quick and specific procedure for the identification of bacteria and fungi.^[14] Here, samples are prepared and analyzed without prior purification, eventually yielding an unequivocal fingerprint mass spectrum of the microorganism. This fingerprint is individual and therefore, used in the identification of species, subspecies, and ultimately even strains. Protein mass spectra allow for the detection of genus, species, type and strain specific signals. The sample material used is fungal colonies of dermatophyte embedded into a matrix and subsequently desorbed and ionized by means of a laser. Acceleration of ions generated in the gaseous phase by an electromagnetic field is then followed by time-dependent detection after a flight distance of 1.2 meters. Flight times may then be matched with molecular masses according to prior calibration. This method offers an easy and outstandingly accurate way to differentiate all clinically relevant

dermatophytes and even rare species, provided they have been entered into the database.

The anthropophilic strains of *T. interdigitale* tend to cause tinea unguium and tinea pedis, and less often, tinea corporis.^[7] The morphological differentiation between anthropophilic and zoophilic strains of *T. interdigitale* is often problematic.^[9] In present study, five strains were macroscopically characterized by a beige, powdery surface and presented microscopically with numerous, thin walled clavate macroconidia and round microconidia. These morphological features, combined with the strong inflammatory lesion, indicate the zoophilic characteristics of the isolate in this case, which may originate from the dog and cat or may be indirect contact with animals in farmers. The study from China observed significant increase in *T. interdigitale* infection associated with animal. In Germany as well *Trichophyton interdigitale* is the second most common dermatophyte infection.^[9] Remaining five strains macroscopically showed white, cottony, flat, radiating thallus like growth, indicating the anthropophilic strain.

This study showed predominance of younger patients which was observed previously in studies from India.^[17,20] The higher incidence of toe nail infection in younger population could be attributed to the higher occupational exposure and sports related trauma and use of occlusive footwear. In addition, younger population is usually cosmetically conscious and therefore seeks early and frequent dermatologic consultation. In our study, males were affected more than females. Our findings are in accordance with those reported by Vijaya et al.^[21] Higher incidence in male might be because of more pronounced outdoor activity in men resulting in higher incidence of trauma which thus increases susceptibility to infection caused by anthropophilic and zoophilic strain of *Trichophyton interdigitale*.

We reported *T. interdigitale* isolates maximum from 4 cases of tinea unguium followed by 3 cases of tinea pedis. A study from Delhi observed that *T. interdigitale* is most common etiological fungus for onychomycosis.^[17] Kai Wen Zhuang et al reported *T. interdigitale* to be the most isolated dermatophyte from *Tinea faciei* cases.^[7] The zoophilic strain of *Trichophyton interdigitale* causing of tinea barbae had been reported by R. Trotha et al.^[22]

CONCLUSIONS

There is a continuous change in the clinical pattern as well as the geographical region of infection. Therefore, it is imperative to be aware of these changing patterns and causative fungi for making adequate strategies for prevention and treatment of the infections. The conventional culture methods had failed to identify these strains, while MALDI-TOFMS analysis enabled the distinct classification as trichophyton species of *T. interdigitale* and helpful for the identification of dermatophyte species. Although MALDI-TOF is

extremely useful in unequivocally identifying trichophyton species of *T. interdigitale* colonies, the differentiation between anthropophilic and zoophilic strain of *T. interdigitale*, is not possible.

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