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Isolation and identification of dermatophytes by arbitrarily primed PCR from clinical samples.

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Abstract

Background: Dermatophytes are a group of fungi which are responsible for most superficial infections of the skin, hair, or nails. It is divided into three genera of molds: *Epidermophyton, Trichophyton,* and *Microsporum.* Arbitrarily primed PCR (AP-PCR) is a species-specific, rapid, and sensitive method for the identification of dermatophytes.

Objective: To identify different dermatophytic species with KOH, culture methods and AP-PCR.

Methods: 320 clinically diagnosed cases of dermatophytic infections were selected, of which 272 were skin and 48 were nails which was identified by10% KOH by direct microscopy, SDA culture method and AP-PCR methods. The isolated culture further confirmed by ITS primers.

Result: In the present study, identification of seven dermatophytes species (*Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum, Trichophyton tonsurans, Microsporum gypseum, Trichophyton verrucosum, and Microsporum canis)* was done using random AP-PCR primers OPAA11, OPU15, OPAA11, and OPD 18.

Conclusion: AP-PCR is a more sensitive and rapid method to identify dermatophyte species in comparison with conventional morphological culture methods.

Keywords: AP-PCR; Dermatophytes; Superficial infections; *Trichophyton* species; Phylogenetic analysis

Introduction

During the last 100 years, the dermatophytes pattern has changed markedly over the globe with differences based on the modern life, geographic area, socioeconomic factors, intensification of travel, as well as migration of population [1,2]. Dermatophytes are a group of fungi that have a high affinity for the keratinized tissues and are responsible for most superficial infections that affect human skin, hair, or nails. It is divided into three genera of molds: *Epidermophyton, Trichophoryton,* and *Microsporum.* On the basis of mode of transmission, dermatophytes have been again classified as anthropophilic, zoophilic, and geophilic. Anthropophilic dermatophytes are associated with humans' beings [3]. Zoophilic dermatophytes usually infect animals but easily get transferred from animals to human beings. Geophilic dermatophytes associated with soil may cause human and animal infections [4]. Lastly, the dermatophytes also categorized on the basis of anatomical sites-*Tinea capitis* (head), *Tinea faciei* (face), *Tinea barbae* (beard), *Tinea corporis* (body), *Tinea manuum* (hand), *Tinea cruris* ("Jocks itch"; groin), *Tinea pedis* (foot), and *Tinea unguium* (nail) [5].

According to the World Health Organization, the worldwide prevalence of dermatophytes is 25% (4). The prevalence of dermatophyte infection is mostly found in tropical and subtropical

countries because the hot and humid climate is favourable for the growth of dermatophytes [6]. Other causes of dermatophytes infection are poor personal hygiene, humid weather, living in an overcrowded area, sharing clothes, sharing fomites, close contact with infected people, pets, domestic animals, and farming occupations that involve direct contact with soil [7].

Despite the high prevalence of dermatophyte infection, this infection has been investigated poorly at molecular levels [8]. Most laboratories rely on culture-based diagnosis for dermatophytes, which is a time-consuming process. But over the past two decades, many molecular methods have been developed, including species-specific, rapid, and sensitive methods for the identification of dermatophytes [9].

The present research focused on the isolation and identification of the dermatophytes from clinical samples at the genus and species level with four arbitrarily primed primers, along with the culture characteristics.

Material and methods

In the present, a total of 320 clinically diagnosed cases of dermatophyte infections were collected, in which 272 skin scrapings and 48 nail clippings were sampled in the Outpatient Department of Dermatology.

Inclusion criteria: All new cases of dermatophytes infection attending the Outpatient Department of Dermatology, Adesh Institute of Medical Science and Research, Adesh University, Bathinda, were included in the study.

Exclusion criteria: Patients on antifungal drugs and with non-dermatophytic fungal infections were excluded from the study.

Ethical Approval

The cross-sectional study was carried out after the approval of the Institutional Research Committee and Ethical Committee of the Adesh University, Bathinda (AU/ECFM/38/2018). Written informed consent was obtained from all the participants, and confidentiality of the data was maintained throughout the study. A complete medical history, including age, gender, family history of fungal infection, occupation, any allergies, duration of infection, and other demographic profiles, was assessed. The types and numbers of lesions were observed, and the presence of inflammation at the infection site and the edges of the lesions were recorded.

Collection of nail samples

The nails of the hands and feet of the participants were washed using soap and water, and after drying, the nails were cleaned with 70% alcohol to remove contaminants such as bacteria and some opportunistic fungi that may be present at the surface of the nail. The samples were taken from the deeper part of the discoloured or dystrophic parts of the nail with a sterile surgical blade or pre-sterilized nail clippers. The nail scraping was collected in sterilized eppendrof tubes, which were labelled to provide details on name, gender, age, OPD number, and infection site. All samples were kept at room temperature because dermatophytes grow better at room temperature.

Collection of skin samples

Skin was decontaminated with 70% alcohol to remove surface dirt, bacterial contamination, and some opportunistic fungi that may be present at the surface of the lesions. A 5–10 mg skin sample was collected from the erythematous, peripheral, actively growing margins of the lesions. For sample collection, a sterile 2 ml eppendrof vial was held immediately below the lesion to be sampled, and scales were flaked into it by using the blunt edge of a sterile surgical blade. While lesions on the glabrous skin were difficult to scratch, very few samples were obtained from such participants. For collecting samples from delicate areas such as the area around the eyes and face,

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the cello-tape method was adopted. The cello tape strip was pressed against the lesion, peeled off, and processed further for direct microscopy and growth of fungus on media plates.

Methods for investigation

Each of the collected samples was divided into three parts: the first part was used for direct microscopic examination with 10% KOH for skin samples and 20% KOH for nail samples. The second part was inoculated on SDA media for pure culture growth. The third part was used for DNA extraction from the samples.

Direct microscopic examination

The samples were used for direct microscopic examination with 10% KOH for skin samples and 20% KOH for nail samples. The slides were examined under a 10X objective lens followed by a 40X lens to study the detail of fungal hyphae, spores, and budding cells. Further confirmation was done under the oil immersion lens (100X) of the Olympus CX31RTSF microscope for branching and septate hyphae, and images were captured and saved.

Identification of Isolates

After direct microscopic examination, the specimens were inoculated on Sabourad dextrose agar medium (SDA) with gentamycin (20 mg/ml). The inoculated plates were incubated for a week at room temperature. The growth of fungi on SDA media was examined to study the colony morphology based on colony characters on the obverse, which were recorded as white, pearl, ivory, consistent cottony, velvety, fluffy, and suede. Colony characters on the reverse were recorded as the presence or absence of pigment, appearance, and diffusion.

In microscopic examination, the cultures were observed under a microscope using low (10X) and high power (40X, 100X) objectives lens. The culture slides were prepared by taking a loopful of mycelium on a glass slide, which was mixed with lactophenol blue. The species was identified on the basis of hyphae, fruiting bodies, and the microconidia (drop-like, tear-shaped, in bunches, spherical, etc.) and macroconidia (spiral, pencil-shaped, pyriform, septations, etc.).

Molecular detection of dermatophytes

DNA was extracted from skin, nails, and pure cultures of dermatophytes by crushing the samples with a mortar and pestle. The crushed samples were transferred into 15-ml falcon's tubes with the addition of 500 µl of digestion buffer, Proteinase K, and 10% SDS (sodium dodecyl sulphate) for 2-3 hours in a water bath at 65 °C with intermittent shaking. After incubation, the samples were vortex vigorously, followed by the addition of CTAB and NaCl, and incubated at 56 °C in a water bath for 2 hours. Then add an equal amount of chloroform and isoamyl alcohol in 24:1. Centrifuge the samples at 7000 rpm for 10 min. at 4°C and transfer the supernatant into fresh tubes. Phenol-chloroform-isoamyl alcohol (PCI-25:24:1) was added after slow mixing of the content by movement of tubes horizontally and vertically. The sample tubes were centrifuged again and transferred the supernatant into fresh tubes, followed by the addition of chilled isopropanol, and incubated for 30 min. at -20°C. The tubes were centrifuged again at 7000 rpm for 10 min at 4 °C, the supernatant was discarded, and the pellets were washed twice with 70% ethanol. The DNA pellets were air dried for an hour and suspended in 50 µl of sterilized MilliQ water.

Arbitrarily primed polymerase chain reaction amplification

Arbitrarily primed PCR was conducted for all isolates of skin, nails, and cultures with each primer separately. Amplification reaction was performed with a total reaction mixture of 20 µl in which DNA was 3 µl, master mix 10 µl, and 2 µl random primers, i.e., OPAA11 (5'-ACCCGACCTG-3'), OPU15 (5'-ACGGGCCAGT-3'), OPAA17 (5'-GAGCCCG ACT-3'), and OPD18 (5'-GAGAGCCAAC-3'), and 5 µl MilliQ water. The PCR cycling was 32 cycles of 94°C for 30s, 36°C for 45s, and 72°C for 90s. After PCR amplification, the samples were electrophoresed

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on a 1.2% agarose gel in 1X TAE buffer, stained with ethidium bromide, and visualized in the Biorad gel EZ imager. The different molecular weight bands that appeared on the gel were interpreted with known-size DNA ladder molecular weight markers and previous reported data by Zarrin *et al.*[10].

Segments of DNA comprising the internal transcribed region (ITS) were amplified with primers ITS1 and ITS4.

The present study involved the identification of dermatophytes by using primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5TCCCTCCGCTTATTGATATGC 3') to amplify ITS region, and ITS sequencing was done at Central University, Bathinda. ITS sequences were subjected to BLAST (Basic Local Alignment Search Tool) searches at GenBank. Species identification was completed through sequence relationships among reference sequences in the constructed ITS database from the NCBI (National Center for Biotechnology Information). Multiple ITS sequence alignments and calculations of the similarity score between the query sequence and reference sequence were performed using the algorithms of 'Align' by searching databases using the BLASTN algorithm of the BLAST sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/) from the NCBI. These sequences were identified by NCBI Blast and submitted to the gene bank.

Phylogenetic Analysis

Phylogenetic analysis was performed using the software package MEGA-6. The alignment tool MEGA-6 was used to align the sequences from the database. The evolutionary distances were computed using the DNA Maximum Composite Likelihood method [11].

Results

In the present study, 320 samples were collected in the Out Patient Department of Dermatology at a tertiary care hospital. Out of 320 samples, 272 were from skin and 48 were from nails. The age of 320 participants varied between 7 and 70 years, of which 165 (51.5%) were males and 155 (48.4%) were females (Table 1). According to the classification on the basis of the habitat, the most common type of dermatophyte was *anthropophilic,* which was isolated from 284 (88.75%) cases, followed by *zoophilic* type 11 (3.4%) and *geophilic* type 8 (2.5%) cases. In the 303 cases of dermatophytes infection, *Tinea corporis* was the most common type infection present in 153 (50.5%) cases followed by *Tinea cruris* in 89 (29.37%) cases, *Tinea unguium* in 46 (15.2%) cases, *Tinea faciei* in 10 (3.3%) cases, and 5 (1.65%) cases *of Tinea pedis* (Table 2).

All 320 samples were subjected to dermatophyte identification by three methods: with 20% KOH direct microscopy, the culturing of samples on SDA media, and AP-PCR. Out of 320 cases, 270 were positive for culturing on SDA media, whereas with AP-PCR, 303 cases were positive for dermatophytes and 10 cases were negative. With all the methods, 310 cases were positive for dermatophytes and non-dermatophytic fungi (Table 3). *Trichophyton mentagrophytes* was the most commonly isolated dermatophyte, which was isolated from 129 (41.61%) participants, followed by *Trichophyton rubrum* with 98 (31.61%) isolations. *Epidermophyton floccosum* was isolated from 42 (13.54%) cases, followed by *Trichophy tontonsurans,* which was isolated from 15 (4.83%) cases. Of the 7 (2.25%) isolates of *Trichophyton verrucosum, Microsporum gypseum* was isolated from 8 (2.58%) cases, and *Microsporum canis* was isolated from 4 (1.3%) cases (Table 2). Non-dermatophyte *Aspergillus flavus* was isolated from 7 (2.25%) cases, while 10 cases were negative in the KOH examination, SDA media, and AP-PCR method (Table 3).

Only two species of dermatophytes were isolated from the nail specimen: *Trichophyton rubrum,* which infected 32 (66%) cases, followed by *Trichophyton mentagrophytes,* which infected 14 (29%) cases. *Aspergillus flavus,* a non-dermatophyte, was isolated from seven cases.

Arbitrarily primed polymerase chain reaction-based identification

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In the present study, characterization of seven dermatophyte species (*Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum, Trichophytonton surans, Microsporum gypseum, Trichophyton verrucosum, and Microsporum canis)* was done using random AP-PCR primers OPAA11, OPU15, OPAA11, and OPD 18 (Table 4) after amplification different fragment length bands appeared on the gel (Figure 1a & b).

Identification of dermatophyte with ITS primers

The ITS1 and ITS4 region of dermatophyte species isolated from cases with *Tinea cruris* and *Tinea corporis* infections were sequenced (Table 5). The sequences obtained were subjected to the basic local alignment search tool (BLAST) and aligned with other similar sequences downloaded from GenBank using Molecular Evolutionary Genetics Analysis (MEGA6) program. Alignments were manually edited wherever necessary. The sequences obtained for ITS1 and ITS4 regions were split into different data sets in order to determine phylogenetic relationship of the seven species at the familial and species level. On the basis of ITS sequencing results, from the dermatophyte species only *Trichophyton interdigitale* and *Trichophyton mentagrophytes* were identified. All the *Trichophyton mentagrophyte* isolates belonged to *Tinea corporis.* The species *Trichophyton inderdigitale* and *Trichophyton mentagrophyte* were isolated from *Tinea cruris.*

Discussion

Around the world, dermatophytosis is the most common cutaneous infection and has become a public health problem. In tropical and subtropical countries, dermatophytes are one of the predominant fungi. The epidemiology of dermatophytes has changed drastically in the last few decades due to lifestyle modifications, globalization, drug therapy, and the socioeconomic conditions of people [12]. Dermatophytosis is prevalent in tropical and subtropical countries like India due to a hot and humid climate, overcrowded living conditions, low socio-economic conditions, an unhygienic working environment, a lack of health care facilities, a lack of understanding about health education, and more outdoor activities related to agriculture and farming, etc. Therefore, there is an absolute need to develop economic, rapid, and precise identification of the dermatophytes causing dermatophytosis with good laboratory methods for appropriate treatment and prevention measures [13].

In the present study efforts have been made to identify dermatophytes species by AP-PCR from skin and nail samples collected from 320 cases after obtaining the written informed consent. ITS (Internal Transcribed Spacer) region 1 and 4 was for the species *Trichophyton mentagrophyte* and *Trichophyton interdigitale* to examine their phylogenetic relationships.

The most common age group among the 320 cases of severe dermatophytes infection was 21–30 years (25%), of which 45 were males and 36 were females. The second most affected age group was 31- 40 years (25%) in which 33 were males and 48 were females (Table 1). Both of these age groups are in the most productive stage of their lives, and the cases in this age group indulge more in day-to-day outdoor activities that involve physical work and agricultural activities. Most of the female cases in the group are housewives belonging to rural areas, and these were involved in farming activities and had frequent contact with domestic animals. Kumar, [14] and Khade *et al.* [15] also reported that the occurrence of *tinea* infection was common in the age group of 21–30 years, followed by the age group of 31–40 years. Similarly, in the present study, Verma and Madhu (16) also reported that the age group of 31–40 years in females is more prone to infection due to *Tinea cruris* and *Tinea corporis*. In the present study *Tinea corporis* was the major clinical type of infection, with 159 (49.68%) cases.

In Asia, different dermatophytes are prevalent in different geographical regions. The commonest clinical pattern in India is *Tinea corporis,* and in Saudi Arabia it is *Tinea capitis.* In

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China, Japan, and Turkey, *Tinea pedis* is the most frequently reported dermatophyte [17]. Naseri *et al*. [18] reported that *Tinea corporis* is the main clinical form of dermatophyte infection in Iran.

Worldwide, the prevalence of dermatophytes infection is 20–25%, and *Tinea corporis* was the most common fungal infection, followed by *Tinea cruris* [19, 20], which is similar to the results of the present study (Table 2), in which *Tinea corporis* (49.68%) was more prevalent than *Tinea cruris* (30.31%).

Similar to the present study, Pathania *et al*. [21] also reported that 37% of patients had dermatophytes infection for less than 6 months, 25% had it for 6–12 months, and 38% had infection for more than one year. While Mitruka *et al*. [22] reported that the majority of cases had infections lasting less than 3 months.

In the present study, phenotypic and genotypic identification of dermatophytes species was done, and the results obtained from the culture method were further confirmed using AP-PCR.

The most commonly isolated fungi in the present study was *Trichophyton mentagrophytes* followed by *Trichophyton rubrum*, *Epidermophyton floccosum*, *Trichophyton tonsurans*, *Microsporum gypseum*, *Trichophyton verrucosum* and *Microsporum canis* (Table 2). In the present study dermatophyte *Microsporum gypseum* (geophilic) and *Microsporum canis* (zoophilic) have been identified for the first time in the present region.

Amplification of genomic DNA from seven dermatophyte species with four random primers generated distinct banding patterns according to the separation of fragments on gel electrophoresis. The banding patterns generated by the primers OPAA11, OPU15, OPAA17, and OPD18 on each of the seven species were distinct and accurately identified the dermatophytes species (Table 4; Figure 1a &b).

In the present study, AP-PCR has been used as a molecular diagnostic technique for the differentiation of the dermatophyte species. This is cost-effective as compared to real-time PCR and nested PCR. Previously, Zarrin *et al.* [10] also reported the four random primers, such as OPAA11, OPU15, OPAA17, and OPD18, which was used in the present study for the identification of dermatophyte species. This is in agreement with the present study, while Girgis *et al.* [23] successfully used only one primer, OPAA17.

Spiliopoulou *et al*. [24] also reported that *Trichophyton rubrum is* the major pathogen causing nail infections, followed by *Trichophyton mentagrophytes*. On the contrary, in some studies, *Trichophyton mentagrophytes* has been reported as the most prevalent dermatophytes in nail [25,26].

Bhatia and Sharma [13] conducted a study in three different areas of Himachal Pradesh, and in the current study also, *Trichophyto mentagrophyte* was the most predominant species (63.11% isolates), followed by *Trichophyton rubrum* (35.1%), and 1.35% of the isolates belonged to *Microsporum gypseum.*

A similar observation was reported by Gürcan *et al*. [27] and Nagaral *et al*. [28]: *Trichophyton rubrum* was the most common isolated fungus, followed by *Trichthe mostyton mentagrophytes.* These works also reported a study on the basis that in southern India, *Trichophyton rubrum* was the main species, followed by *Trichophyton mentagrophytes.* The high prevalence of the *Trichophyton mentagrophytes* in India may be due to their better adhesion to the *stratum corneum* surface [27,28]. Four random primers, OPAA11, OPU15, OPAA17, and OPD18, developed by Zarrin *et al*. [10], differentiated all the seven dermatophyte species identified on the basis of the culture method. Different researchers have used these four primers singly or in combination [29,30,31]. Liu *et al*. [30] were successful in differentiating 20–25 species using these four primers. Other than the use of nested PCR for differentiation of dermatophyte species.

Elavarashi *et al*. [32] used the ITS region and PCR-RFLP for the direct identification of dermatophyte species from skin and nail samples. The authors concluded that amplification of the dermatophyte-specific primer was appropriate for the identification of dermatophyte directly from samples. *Trichophyton rubrum* was reported to be the most detected fungus, followed by *Trichophyton mentgrophytes,* by using PCR targeting the ITS region and RFLP analysis [32].

In the present study, *Trichophyton rubrum* was the most frequently isolated fungus from the nails, and *Trichophyton mentagrophytes* was mostly isolated from the skin specimens. A similar observation was reported by Spiliopoulou *et al*. [24], in which *Trichophyton rubrum* was the most frequently isolated dermatophyte from nail samples, followed by *Trichophyton mentagrophytes* [24].

In the present study *Trichophyton mentagrophytes* was the most predominant etiological agent causing dermatophytosis, identified in 129 samples by conventional culture techniques, followed by *Epidermophyton floccosum,* identified from 42 cases. *Microsporum gypseum was* identified in 08 cases. A total of seven species belonging to the three genera *Trichophyton, Epidermophyton,* and *Microsporum* were identified on the basis of conventional techniques. *Trichophyton mentagrophytes* being the most prevalent species in the present study, an effort has also been made to examine the relationship between *Trichophyton mentagrophytes* isolated from clinical-type *Tinea corporis* and *Tinea cruris*.

The genomic DNA of *Trichophyton mentagrophytes isolated* from these clinical types was used for the amplification of ITS1 and ITS4 primers. The amplified region thus obtained was eluted and sequenced. The result of DNA sequencing was analyzed using the BLASTn program. The DNA sequences were aligned with other similar sequences downloaded from GenBank using the Molecular Evolutionary Genetics Analysis (MEGA X) program.

The sequences of *Trichophyton mentagrophytes* isolated from clinical types *Tinea corporis* and *Tinea cruris* were submitted to the GenBank database, which has been assigned an accession number accordingly. Accession MN999935, MN999936, MN999937, MN999940, and MN999941 have been identified as *Trichophyton mentagrophytes,* and these showed query coverage of 52%, 54%, 76%, 40% and 47%, respectively. Whereas, accessions MN999938 and MN999939 identified as *having Trichophyton interdigitale showed* query coverage of 85% and 99%, respectively.

Although according to Makimura *et al*. [33] and Malinovschie *et al*. [34], the nucleotide sequence of the ITS1 region of pathogenic dermatophytes was useful for identification of dermatophytes at the species level and also to understand the phylogenetic relationship within dermatophytes, the present results have clearly demonstrated that ITS1 and ITS4 have not been able to do so accurately, affecting the cure rate.

Author contributions.

PK contributed substantially to the concept and design of the research, reviewed the literature, and its analysis and interpretation. SK contributed substantially to acquiring the data. PK contributed to drafting the manuscript and edited the manuscript critically for important intellectual content. Both authors approved the final version submitted for publication and take responsibility for statements made in the published article.

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Conflict of interest statement.

None of the authors has any potential or actual conflict of interest to disclose in relation to the present article.

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Data sharing statement.

Statistical summaries of the data generated and analyzed for the present study are included in this published article. Further details of the data are available from the corresponding author.

Reference

1. Hayette MP, Sacheli R. Dermatophytosis trends in epidemiology and diagnostic approach. Current Fungal Infection Reports. 2015; 9(3):164-179.
2. Gupta A, Kaur S, and Gill, P.K. (2018). Combination Drug Treatment In Tinea Infections Of Cutaneous Soft Tissues. Journal of Medicine and Health Research. 3(1): 16-23.
3. Oguntade TO. Studies on Dermatophytes Isolated from Patients in Two Tertiary Health Institutions in Lagos State, Nigeria (Doctoral dissertation); 2012.
4. English M. P. The epidemiology of animal ringworm in man. British Journal of Dermatology, 1972; 86 (8):78-87.
5. Datt S, Datt T. Pathogenesis and Clinical Significance of Dermatophytes. International Journal of Curent Microbiology and Applied Science. 2019; 8 (11), 1877-1886.
6. Sharma V, Kumawat TK, Sharma A, Seth R. Chandra S. Dermatophytes: Diagnosis of dermatophytosis and its treatment. African Journal of Microbiology. 2015; 9 (19): 1286-93.
7. Lakshmipathy DT, Kannabiran K. Review on dermatomycosis: pathogenesis and treatment. Natural Science. 2010; 2 (07):726.
8. Kaur S. Predisposing Factors and Seasonal Distribution of Tenia Infections in Tertiary Care Hospital. International Journal of Microbiology Research. 2019; 11 (7):1641-1644.
9. Poyyamozhi JS, Lakshmanan A. Profile of dermatophyte infections among rural population: A facility based prospective observational study. International Journal of Community Medicine and Public Health. 2018; 5: 1354- 1359.
10. Zarrin M, Salehi Z, Mahmoudabadi AZ. Identification of dermatophytes by arbitrarily primed PCR. Asian Biomedicine. 2015; 9(3): 291-298.
11. Tamura K, Nei M, Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences. 2004; 101(30): 11030-11035.
12. Asticcioli S, Di Silverio A, Sacco L, Fusi I, Vincenti L, Romero E. New Microbiologica. 2008; 31:543-548.
13. Bhatia VK, Sharma PC. Epidemiological studies on dermatophytosis in human patients in Himachal Pradesh, India. Springer Plus. 2014; 3 (1):134.
14. Kumar S. Clinico Microbial Identification and Characterization of Dermatophytes and Mrsa in Jaipur. Ph.D. Thesis JECRC University; 2017.
15. Khade AS, Burute SR, Deogude SS, Jadhav P, Ramanand SJ. A Study of Clinical Profile of Dermatophytosis with a Changing Clinical Pattern at a Tertiary Care Centre. Journal of Medical Science and Clinical Research. 2018; 6 (05):622-670
16. Verma S, Madhu R. The great Indian epidemic of superficial dermatophytosis: An appraisal. Indian Journal of Dermatology. 2017; 62(3):227.
17. Rudramurthy SM, Shaw D. Epidemiology of Superficial Fungal Infections in Asia. In Clinical Practice of Medical Mycology in Asia: 2020; 9-37.
18. Naseri A, Fata A, Najafzadeh MJ, Shokri H. Surveillance of dermatophytosis in northeast of Iran (Mashhad) and review of published studies. Mycopathologia. 2013; 176(3-4): 247-253.

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IFS 2020 4.085

19. Kanaan ICS, Santos TBPD, Kac BK, Souza AMVD, Cerqueira AMMD. Majocchi's granuloma-Case report. Anais Brasileiros de Dermatologia. 2015; 90 (2): 251-253.

20. Konda C, Surekha JK, Jahnavi I, Madhuri DS, Nagamani K. Isolation and Identification of Dermatophytes in a Tertiary Care Hospital. International Journal of Current Microbiology and Applied Sciences. 2017; 6(2): 4088-4101.

21. Pathania S, Rudramurthy SM, Narang T, Saikia UN, Dogra S. A prospective study of the epidemiological and clinical patterns of recurrent dermatophytosis at a tertiary care hospital in India. Indian Journal of Dermatology, Venereology, and Leprology. 2018; 84 (6):678.

22. Mitruka B, Gill AK, Kaur N, Mittal RK, Mahajan A, Kaur A. Mycological analysis of 150 cases of dermatophytosis of skin, hair and nail attending the outpatient department of skin and venereology. Annals of Applied of Bio Sciences. 2016; 3: A170-A182.

23. Girgis SA, El-Fakkar NMZ, Bard H, Shaker OA, Metwally FE, Bassim, HH. Genotypic identification and antifungal susceptibility pattern of dermatophytes isolated from clinical specimens of dermatophytosis in Egyptian patients. Egyptian Dermatology Online Journal. 2006; 2 (2): 1-23.

24. Spiliopoulou A, Bartzavali C, Jelastopulu E, Anastassiou ED, Christofidou M. Evaluation of a commercial PCR test for the diagnosis of dermatophyte nail infections. Journal of Medical Microbiology. 2015; 64 (1): 25-31.

25. Adekhandi S, Pal S, Sharma N, Juyal D, Sharma M, Dimri D. Incidence and epidemiology of onychomycosis in patients visiting a tertiary care hospital in India. Cutis. 2015; 95 (1): E20-E25.

26. Sanjiv A, Shalini M, Charoo H. Etiological agents of onychomycosis from a tertiary care hospital in Central Delhi, India. Indian Journal of Fundamental Applied Life Science. 2011; 1: 11-4.

27. Guercan S, Tikveşli M, Eskiocak M, Kilic H, Otkun M. Investigation of the agents and risk factors of dermatophytosis: a hospital-based study. Mikrobiyoloji Bulteni. 2008; 42 (1): 95-102.

28. Nagaral GV, Veerabhadra GG, Sudha P, Jagadevi. Prevalence of *tinea corporis* and *tinea cruris* in Chitradurga rural population. Indian Journal of Clinical Experimental Dermatology. 2018; 4(3): 221-225.

29. Liu D, Coloe S, Baird R, Pedersen J. Molecular determination of dermatophyte fungi using the arbitrarily primed polymerase chain reaction. British Journal of Dermatology. 1997; 137 (3): 351-355.

30. Liu D, Coloe S, Baird R, Pedersen J. Application of PCR to the identification of dermatophyte fungi. Journal of Medical Microbiology. 2000; 49 (6):493-497.

31. Khaled JM, Golah HA, Khalel AS, Alharbi NS, Mothana RA. Dermatophyte and non dermatophyte fungi in Riyadh City, Saudi Arabia. Saudi Journal of Biological Sciences. 2015; 22 (5): 604-609.

32. Elavarashi E, Kindo AJ, Kalyani J. Optimization of PCR—RFLP directly from the skin and nails in cases of dermatophytosis, targeting the ITS and the 18S ribosomal DNA regions. Journal of Clinical and Diagnostic Research. 2013; 7(4): 646.

33. Makimura K, Tamura Y, Mochizuki T, Hasegawa A, Tajiri Y, Hanazawa R, Yamaguchi H. Phylogenetic classification and species identification of dermatophyte strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 region. Journal of Clinical Microbiology, 1999; 37 (4): 920-924.

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www.journalofresearch.org
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SJIF 2020: 6.224
IFS 2020 4.085

34. Malinovschi G. Rapid PCR based identification of two medically important dermatophyte fungi, Microsporum canis and Trichophyton tonsurans. Acta Biologica Szegediensis. 2009; 53(1): 51-54.

Journal of research in health science
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Figure legends:

Figure 1a. Amplified products of genomic DNA from *Trichophyton rubrum*, Lanes 1- 4 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 obtained from *Trichophyton rubrum*, respectively. Lanes 5-8 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Trichophyton verrucosum*. Lanes 9-12 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Trichophyton mentagrophytes* respectively. Lane M is molecular weight maker on both side of the gel**.**

Figure 1b. Amplified products of genomic DNA from *Microsporum gypseum,* Lanes 1- 4 show the products of primers OPAA11, OPU15, OPAA17 and OPD18. Lanes 5-8 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Epidermophyton floccosum*. Lanes 9-12 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Microsporum canis*, respectively**.**

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Table 1. Demographics of dermatophytes infected cases.

Age group	**Male**	**Female**	**Total**	**Percentage**
00-10	02	01	03	0.93%
11 -20	28	04	32	10.0%
21-30	45	36	81	25.3%
31-40	33	48	81	25.3%
41-50	24	37	61	19.0%
51-60	22	19	41	12.8%
61-70	11	10	21	06.5%
Total	**165**	**155**	**320**	**100%**
Occupation				
House wife	00	143	143	44.60%
Agriculture	57	0	57	17.80%
Student	34	7	41	12.80%
Armed force	30	0	30	9.30%
Professionals	15	5	20	6.20%
Labour	15	0	15	4.60%
Business	14	0	14	4.30%
Total	165	155	320	100%
Family transmission				
Transmitted	036	052	088	27.50
Not transmitted	129	103	232	72.50
Total	**165**	**155**	**320**	**100%**

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Area					
Rural	1	906	197	16	61.5
Urban	4	79	423	13	38.4
Total	**65**	**155**	**120**	**3%**	**100**

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Table 2. Isolated dermatophytes species and their relation to the clinical type of cases

Species of dermatophytes						
	Tinea corporis	***Tin eacruris***	***T inea u nguium***	***inea aciei***	***inea edis***	**otal**
T.mentagrophytes	66	41	15	5	2	29
T. rubrum	29	37	31		1	8
E.floccosum	34	08	-			2
T.tonsurans	12	03	-			5
M.gypseum	07	-	-		1	8
T.verrucosum	05	-	-	2		7
M.canis	-	-	-	3	1	4
Aspergillusflavus	-	-	-			7
Total	153	89	46	0	5	10

Table 3. Comparison of culture and AP-PCR for identification of dermatophytes

Total no of patient	Culture	AP-PCR
263	+	+
7	+	-
40	**-**	+
10	-	-

Table 4. Details of amplified fragment size (in base pairs) by four AP-PCR primers obtained from genomic DNA of dermatophyte species.

Dermatophyte Species	**Primer**			
	OPAA11	**OPU15**	**OPAA17**	**OPD18**
T.mentagrophytes	350-750	250-400-450-600	300-450-500-750-900	350-400-750-1000
T.rubrum	350-450-650	250-400-550-750-	300-500-700-800-	350-400-650-800-900-

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		800	1200	1300
E.floccosum	350-450-650-750	250-400-600-650-750-900	300-500-750-850	250-350-400-600-800-900
T.tonsurans	350-450-600-750-850	250-300-400-500-650-800-900	300-400-500-700-900-1200	350-600-800-1000-1400
M.gypseum	300-450-600-700-800-1000-1200	300-450-550-650-750-850-1000-1200	350-450-500-650-800-900	350-450-600-700-800-900
T.verrucosum	350-400-600	250-350-750	300-500-800	350-450-600-700-800-900-1000-1100
M. canis	350-450-650-750	250-350-450-600	200-300-450-700-900	250-400-500-700-800-1000

.

Table 5. GenBank accession numbers and identified species identity

r. no.	Gene Bank accession number	Clinical type	Sequence isolated from culture	Identified species	Maximum Identity %
.	MN999935	*T.corporis*	*T.mentagrophytes*	*T.mentagrophytes*	52 %
.	MN999936	*T.corporis*	*T.mentagrophytes*	*T.mentagrophytes*	54 %
.	MN999940	*T.corporis*	*T.mentagrophytes*	*T.mentagrophytes*	40 %
.	MN999937	*T.corporis*	*T.mentagrophytes*	*T.mentagrophytes*	76 %
.	MN999938	*T.cruris*	*T.mentagrophytes*	*T. interdigitale*	85 %
.	MN999939	*T.cruris*	*T.mentagrophytes*	*T. interdigitale*	99 %
.	MN999941	*T.cruris*	*T.mentagrophytes*	*T.mentagrophytes*	47 %

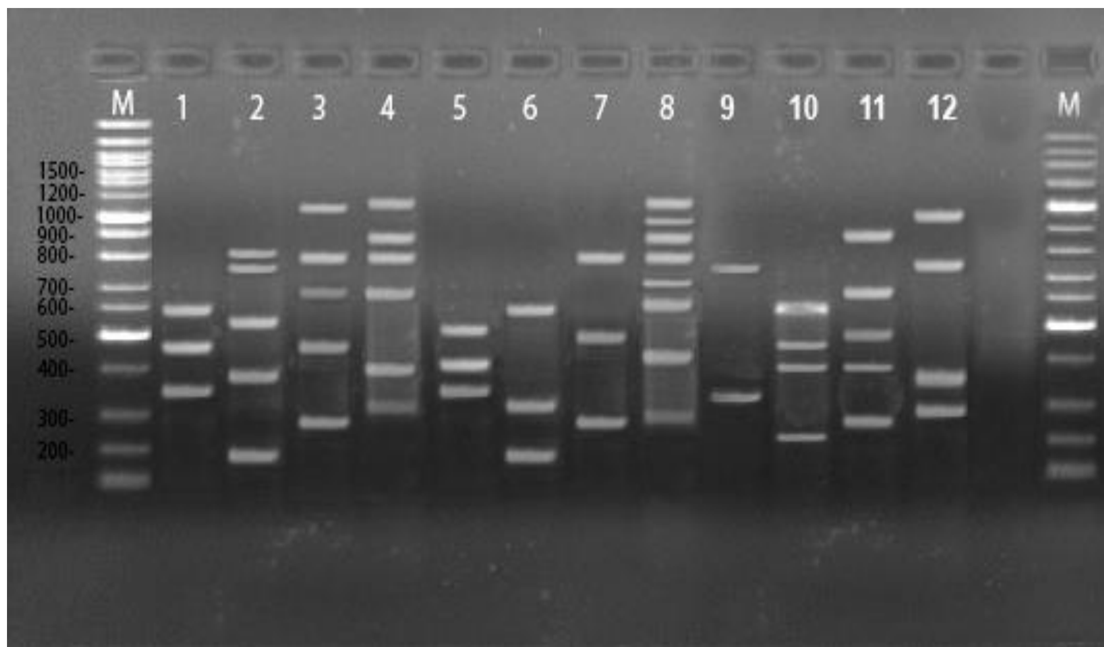

Figure 1a

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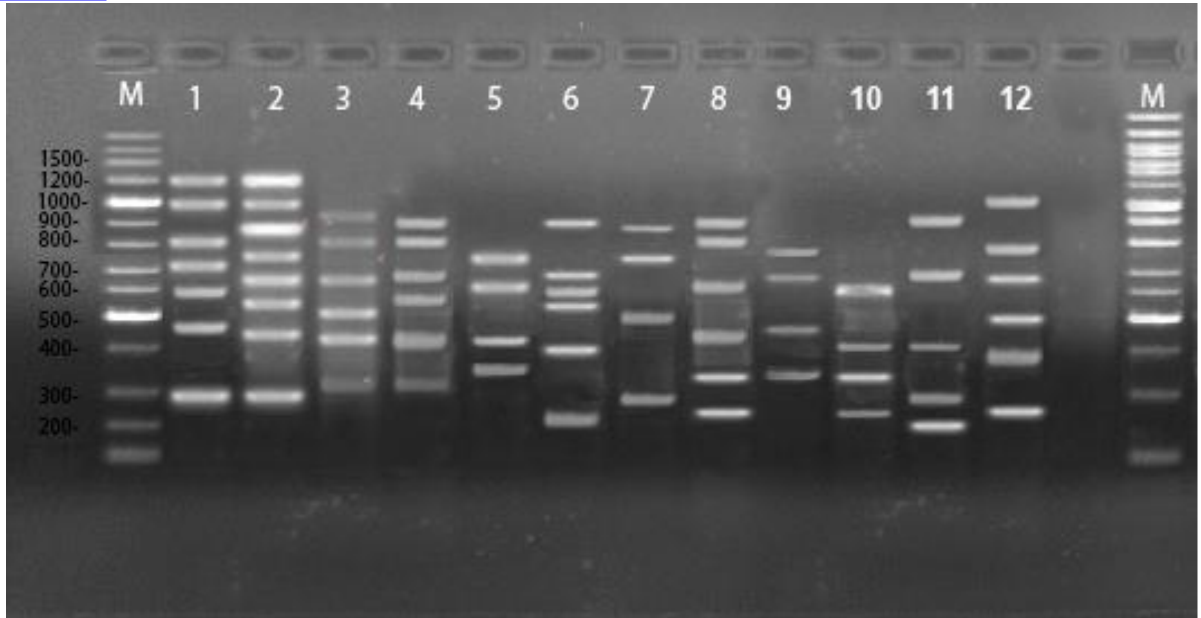

Figure 1b