Isolation and Molecular Characterization of *Trichophyton rubrum* from Siddi Tribal Community Residing in North Karnataka Region, India

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ABSTRACT

BACKGROUND

Siddi community is the tribal community of African origin living in north Karnataka region since many generations. Few studies have shown the presence of rare dermatophytes of African origin in the Siddi tribal community. This study was performed to detect the presence of such dermatophytes of African origin in the Siddi community.

METHODS

A total of 1004 samples were collected from 937 patients with superficial infections out of which 102 samples have shown the dermatophytic growth on culture media. All the dermatophytes were identified by phenotypic and physiological characters.

RESULTS

Tinea unguium was the prevalent clinical condition and *Trichophyton mentagrophytes* was the commonest etiological agent to cause dermatophytosis. Two isolates have shown similar macroscopic and microscopic features of as dermatophyte of African origin species (*Trichophyton soudanense*) and subjected to sequencing of internal transcribed spacer region (ITS) and identified as *Trichophyton rubrum*.

CONCLUSIONS

Therefore, presence of rare African species was not seen in the migrated tribal community may be due to the existence of community since more than 5 generations and have adapted to local social and religious practices.

KEY WORDS

Siddi, Dermatophytes, *Trichophyton*, *Microsporon*, *Epidermophyton* and Tribal

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BACKGROUND

Dermatophytosis is the superficial fungal infection which mainly affects hair, nail and skin. Dermatophytes produce similar clinical symptoms which are difficult to be distinguished by clinical examination, and these infections are commonly known as tinea infections which are classified based on the site of involvement.⁽¹⁾ There are three ecological group of dermatophytes are known they are anthropophilic, zoophilic and geophilic. Anthropophilic species spreads from human to human and are confined to host. Animals serve as host for zoophilic species and spreads from animals to human. Geophilic species spreads from soil to human.⁽²⁾ Distribution of the dermatophytes are depends on the geographic location, migration, climate condition, Immune status of the host, socioeconomic condition and age.^(3,4) This superficial fungal infection is caused by three genera namely *Trichophyton*, *Microsporon* and *Epidermophyton*; of these *Trichophyton* is most common, which has 15 species and many varieties found within the species.⁽¹⁾ *Trichophyton* has considerable variation in their morphology as well as in geographical distribution. *T. rubrum* can be seen in many part of the world whereas *Trichophyton violaceum*, and *T. soudanense* found in trans-Sudan belt and central African regions.^(1,5,6)

Siddis are the tribal group having African ancestry brought to India during the 16th – 19th century by Portuguese and residing in Indian states like Gujarat, Karnataka, Andhra Pradesh, and Telangana.^(7,8) In Karnataka Siddis are distributed in Uttar Kannada District, although Siddi are residing in India since many generations and adapted to local and social lifestyle they form separate physical and genetic makeup compared to native Indians.^(9,10) Several previous study suggested that Siddi genomes have closest relation to the Africans.^(7,8) Few literature have shown the existence of rare geographically restricted African origin dermatophytes in the community.^(9,10) In the present study we screened Siddi tribal population to see the presence of rare geographically restricted dermatophytes.

METHODS

The study was conducted during 2015 to 2017 in north Karnataka region with the approval from institutional ethical committee (KLE/Ethic/2015-16/D-51). A total of 1004 samples were collected from 937 Siddi tribal population with suspected superficial fungal infection with required clinical details. Further all samples were subjected to direct microscopy by using 10% potassium hydroxide (KOH) and inoculated on to Sabouraud dextrose Agarose (SDA) (Hi-media) with cycloheximide (0.5% 0.5 mg/ml), chlortetracycline (0.1% 0.1 mg/ml) and Gentamicin (0.1% 0.1 mg/ml). Inoculated plates were incubated for 4 weeks after inoculation before designating the sample as no growth at 28°C. Identification of dermatophytes were done on the basis of Macroscopic and Microscopic features and urease hydrolysis.

DNA Extraction, PCR and Sequencing

Genomic DNA was extracted by phenol-chloroform isoamyl alcohol method as previously described (15) and eluted with 50 µl Tris-EDTA buffer. Amplification was carried out by polymerase chain reaction (PCR) using internal transcribe spacer (ITS); ITS 1 and ITS 4 primer (ITS1, 5' TCCGTAGGTGAACCTTGCGG 3', and ITS 4, 5' TCCTCCGCTTATTGATATGC 3'), amplification was done with final volume of 50 µl containing 0.5 µg of template DNA, 20 µl of Emerald Amp GT PCR Master Mix (2X premix composed of a DNA polymerase, optimized reaction buffer, dNTPs, and a density reagent) 15 pmol of each primer. PCR was performed in a thermo cycler (Eppendorf) with initial denaturation of 94°C for 6 minutes followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute 30 second and final extension at 72°C for 10 minutes. Separation of PCR products were carried out on 1.5% agarose gel stained with ethidium bromide followed by visualized in UV transilluminator and imaged. PCR product were subjected to sequencing both strands using ITS 1 and ITS 4 primer and Big Dye Terminator Cycle sequencing kit version 3.1 (Applied Biosystems), ABI 3130 genetic analyzer (Applied Biosystems) and analysis of all sequencing reaction. Sequences were compared with the GenBank DNA database using the NCBI BLAST tool (<https://blast.ncbi.nlm.nih.gov>), the ISHAM ITS database (<http://its.mycologylab.org/BioloMICSSequences.aspx>), and the CBS database (<http://www.westerdijknstitute.nl/Collections/BioloMICSSequences.aspx>).

Phylogenetic Analysis

Phylogenetic analysis of standard sequences retrieved from NCBI and study isolates were carried out by aligning sequences using multiple sequence alignment mode in ClustalX2 software. The aligned sequences were exported to Molecular Evolutionary Genetics Analysis software version 7 (MEGA7).⁽¹¹⁾ Neighbor joining tree was constructed with 1000 bootstrapping replicates by using Kimura 2 parameter model

RESULTS

A total of 937 patients were screened for superficial fungal infection, a total of 102 samples showed positive by both microscopy and culture from 90 patients for dermatophytes. Dermatophytes isolated are *T. mentagrophytes* [69 (67.64%)], *T. rubrum* [24 (25.49%)], *Trichophyton tonsurans* [3 (2.94%)], *Microsporum gypseum* [3 (2.94%)] and *Trichophyton terrestre* [1 (0.98%)]. In the present study two isolates have shown the macroscopic and macroscopic feature of dermatophyte of African origin, isolated from nine year old child who was suffering from tinea capitis since one month had history of soil and animal contact, lesions were spreading without any erythema and a fourteen year old girl with tinea unguium since one month with history of soil and animal contact. Both patients did not have history of antifungal treatment as well as self-medication.

Mycological Characters

One isolate shown slow growing growth after 9 days the colonies were waxy glabrous white without any macroconidia and microconidia the colony characters and microscopy were overlapping with the *T. rubrum*. Another strain was also slow growing, colony was flat and whitish suede-like texture later colony turned to yellow-orange in colour and microscopically no conidia, reflective branching was seen rarely.

PCR and Its Sequencing

Two phenotypically suspected dermatophytes of African species were sequenced and identified as *T. rubrum* (accession no: MT280227 and MT280226).

Phylogenetic analysis was done with the study isolates and standard CBS stains retrieved from the NCBI database [*T. violaceum* CBS 374.92 (NR_144901), *T. soudanense* IHEM 19751 (NR_155948) and *T. rubrum* CBS 392.58 (MH857821)] *T. Mentagrophytes* (KY761968) was taken as out-group. Both the isolate clustered with CBS reference strain *T. rubrum*.

DISCUSSION

Siddi community is the tribal community residing in many parts of India who has African origin and residing in India since more than five generations.^(9,12) Investigators in India have shown the presence of rare dermatophytes of African species in the Siddi tribal community. Hemashettar and Nadigir isolated *T. soudanense* in 1980 and in 1993 Hemashettar isolated *Trichophyton yaoundei* in the community.^(9,10) Few literature around the world have shown the presence of such infection in the native and African origin population.^(5,6,13-16) To see the presence of such rare dermatophytes of African species we have screened more than 2000 Siddi community persons residing in the north Karnataka region of India. Out of 1004 samples 102 samples have grown dermatophytes on culture media with prevalence of 9.6%. Tinea unguium (32%) was the most prevalent infection seen in the community followed by tinea corporis (27.45%), tinea capitis (25.49%), tinea cruris (13.72%) and tinea pedis (0.98%).

T. mentagrophytes was the commonest etiological agent seen in 67.64% followed by *T. rubrum* (25.49%) to cause dermatophytosis, whereas two dermatophytes which have shown phenotypic characters of dermatophytes of African origin like *T. violaceum* and *T. soudanense* from two different

patients with tinea capitis and tinea unguium respectively were identified as *T. rubrum*. both isolates were slow growing without any macro or micro conidia. Studies have shown the emergence of *T. violaceum* in the population of native local origin in India as well as in different parts of world.⁽¹⁷⁻²⁰⁾ even though it is considered as dermatophyte of African Species,^(5,6,13,14) whereas many studies have shown *T. soudanense* is isolated in migrated African community in many parts of the world and rarely infecting the people of native origin.^(6,13,14) In an attempt to isolate *T. violaceum*, and *T. soudanense* in Baltimore, Maryland, Shelley S. Magill et al have reported 88% of *T. violaceum*, and *T. soudanense* infection in migrated population of eastern Africa and west Africa living in America since three months to five years and 12% of infection U S origin population.⁽¹⁴⁾ In a retrospective study conducted by Konstantin V. Grigoryan et al and Elizabeth Gaviria Morales et al in Rochester, Minnesota, United States and Southern Switzerland respectively to observe the presence of *T. violaceum*, and *T. soudanense* have reported the infection in 82.7% of Africans migrated to America, 4.93% in African Americans and 3.7% in White population in Rochester, Minnesota, United States, whereas in southern Switzerland 77.27% *T. violaceum* infection was seen in African patients and 15.90% infection in originally from Switzerland.^(5,6) In the present study we have not found any such rare dermatophyte of African species. The possible reason for the decreased

prevalence or absence of rare exotic dermatophytes of African origin in Siddi community, a migrated African population might be due to their existence in India since more than five-generation,⁽⁷⁾ whereas others researchers have reported infection with dermatophyte of African species in the African origin population who are residing since one or two-generation.^(5,6,13)

A study conducted by Danielle Marcoux et al in 2018 have showed there was six fold rise in dermatophytes of African species in children of African origin residing in Canada since one or two generation.⁽¹³⁾ A detailed study on this African origin population residing in other parts of India may reveal the presence geographically restricted dermatophytes. Antifungal susceptibility testing of isolates may also reveal the sensitivity pattern against antifungal drugs, and helps the clinician to treat dermatophytosis infection in such particular area.

In dermatophytes, ITS region of rDNA is used for species identification as well as for understanding phylogenetic relationship between the species.⁽²¹⁾ This study confirms phenotypically identified dermatophyte of African species as *T. rubrum*, based on ITS region sequencing. Presence of these African anthropophilic dermatophyte species was observed in migrated African population in many other previous studies around the world.^(5,13,15) Whereas our study revealed absence of such rare exotic dermatophyte and *T. mentagrophytes* complex as prevalent etiological agent to cause dermatophytosis in Siddi tribal community as they stay in India since five generation and adapted to local and religious lifestyle.

CONCLUSIONS

Presence of rare African species was not seen in the migrated Siddi tribal community may be due to the existence of community in India for more than 5 generations and have adapted to local social and religious practices.

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