Isolation and Molecular Analysis of *Cladosporium Species* from Siddi Tribal Community Residing in North Karnataka Region, India

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Abstract

Dermatophytes are the common etiological agent to cause superficial mycosis, however non-dermatophytes like yeast and non dermatophyte mould (NDM) are emerging as potential agent to cause superficial mycosis. In this study, we evaluated epidemiological, clinical and mycological characters of non-dermatophytes causing superficial infection in the Siddi tribal community. A total of 1004 samples were collected from 937 Siddi community patients with superficial infection. In that 158 samples shown culture positive, *Candida species* (32, 20.25%) was the predominant agent to cause superficial infection other than dermatophytes followed by *Aspergillus species* (12, 7.59%), *Fusarium species* (10, 6.32%) and *cladosporium species* (2, 1.26%). Further, *Cladosporium species* isolated from tineacapitis infection was subjected to PCR, sequencing of ITS region and identified as *Cladosporium halotolerans.* Therefore, this study also revealed non dermatophytic fungi are emerging as important cause of superficial infection.

Key words: *Dermatophytes, Mould, Yeast, Trichophyton, Cladosporium, Onychomycosis and Tinea, Polymerase chain Reaction (PCR)*

Introduction

Superficial mycosis is the fungal infection of skin and its appendages like nail and hair, it is apublic health problem and of worldwide importance in developing countries. Potential reason for proliferation of the infection may be low economic status, poor hygiene, inadequate health facility and exchanging of footwear and cloth[(1)].Even though it is not a life threatening infection but may affect the social life and day to day activities. Dermatophytes are common cause of superficial mycosis worldwide, there is an increase in the infection by non-dermatophytemould (NDM) and yeast has been observed[(1,2)]. Change the incidence of infection by pathogen may affect the clinician capability to diagnose and can change the approach to treat[(3)].

Siddis are tribal community residing in India who brought from eastern African countries before many generations. They leave in different parts of Indian states like Andhrapradesh Maharashtra Gujarat and Karnataka. In Karnataka they reside in North Karnataka region and adapted to local social and religious lifestyle. We under took this study to see the common non dermatophytes causing the superficial fungal infection in Siddi tribal community[(4,5)].

Materials and Method

Study was conducted during 2015 to 2017in north Karnataka region. A total of 1004 clinical samples like hair nail and skin scrapping were collected from 937 Siddi tribal patients with suspected superficial fungal infection. Clinical details were collected with patient consent. Samples were subjected to direct microscopy

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by 10 % pottacium Hydroxide (KOH) and inoculated in pairs in plain SabourdoseDextrose Agar (SDA) and SDA with cycloheximide (0.5% 0.5 mg/ml), chlotetracycline (0.1% 0.1 mg/ml) and Gentamicine (0.1% 0.1 mg/ml) and incubated at room temperature. Cultures were noted for colony character, surface color and color on the reverse. Culture was identified by macroscopic and microscopic examination.

PCR and sequencing

Genomic DNA was extracted by phenol-chloroform isoamyl alcohol method and the extracted genomic material was eluted with 50 µl Tris-EDTA buffer. Polymerase chain reaction was carried out using internal transcribe spacer (ITS); ITS 1 and ITS 4 primer (ITS1, 5' TCCGTAGGTGAACCTTGCGG 3', and ITS 4, 5' TCCTCCGCTTATTGATATGC 3'), 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 30seconds, 58°C for 30 seconds and 72°C for 1 minute 30 second and final extension at 72°C for 10 minutes. PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized in UV transilluminator and imaged. PCR sequencing was done form PCR product using ITS 1 and ITS 4 primer and BigDye Terminator Cycle sequencing kit version 3.1 (Applied Biosystems). ABI 3130 genetic analyzer (Applied Biosystems) was used for purification 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.westerdijkinstitute.nl/Collections/BioloMICSSequences.aspx).

Phylogenetic analysis

Phylogenetic analysis of study isolates and standard sequences retrieved from NCBI were done 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)[6] and neighbor joining tree was constructed using Kimura 2 parameter model with 1000 bootstrapping replicates.

Results

A total of 1004 samples were collected from 937 patients with suspected superficial mycosis. Tineaunguim was the most commonly seen clinical condition followed by tineacorporis and tineacapitis.A total of 158 samples have shown the culture positive. Dermatophytes were the most common etiological agent isolated from 102 (64.55%) cases. Whereas non dermatophytes were isolated from 56 (35.44%) cases. Most of the patients were agriculturist (32, 57.14%) and field workers (9, 16.07%).Commonest clinical condition seen in superficial infection with nondermatophytes was tinea unguium (48, 85.71%) in which 52.08% cases were Proximal subungual onychomycosis (PSO), 22.91% of cases were white subungual onychomycosis, 16.66% of cases were Distal lateral subungual onychomycosis and 6.25% of cases were endonyxonychomycosis whereas, Tineacorporis (5, 8.92%) and Tineacapitis (3, 5.35%) was seen in 5 (8.92%) and 3 (5.35%) cases respectively.

In this study, Candida species (32, 20.25%) was the predominant agent to cause superficial infection other than dermatophytes followed by *Aspergillus species* (12, 7.59%), *Fusarium species* (10, 6.32%) and *cladosporium species* (2, 1.26%). Further repeat sample was collect to confirm the infection with *Cladosporium species*.

Cladosporium species was isolated from twin's patients of age 7years old with tineacapitus infection since from one year. Morphological feature included unbranched cylindrical conidiophores bearing ovoidal to ellipsoidal intercalary and terminal conidia.

Both *Cladosporium specis* were used to sequence ITS region and were identified as *Cladosporium halotolerans*(accession number MT588811 and MT588810) Phylogenetic analysis of both strains were done with standerd *Cladosporium species* stains retrieved from the NCBI database [*Cladosporium halotolerans* (LN834374, LN834375 and DQ780364), *Cladosporium angustisporum* (LN834356), *Cladosporium asperulatum* (LN834357), *Cladosporium allicinum* (LN834353*), Cladosporium cladosporioides* (LN834358), *Cladosporium flabelliforme* (LN834361), *Cladosporium funiculosum* (LN834364), *Cladosporium*

herbarum (LN834378), *Cladosporium subinflatum* (LN834391), *Cladosporium tenuissimum* (LN834398)]. Study isolates have clustered with *Cladosporium halotolerans*.

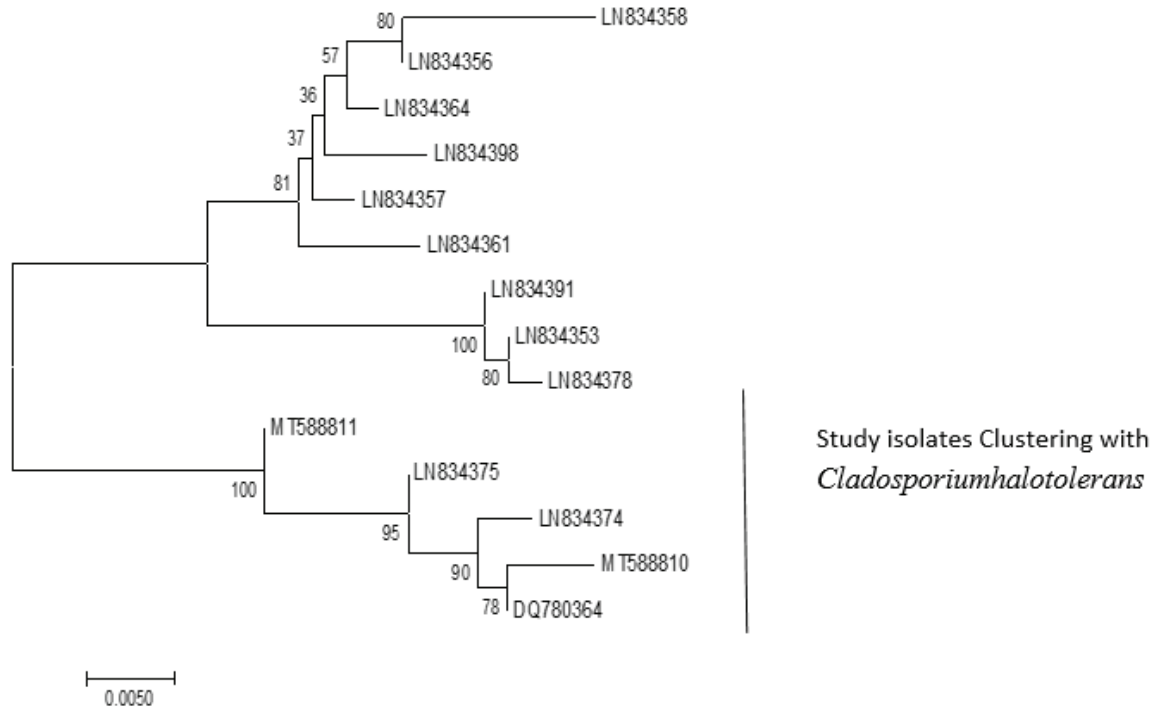

Figure.1 Neighbor–joining (NJ) derived dendrogram of internal transcribe spacer (ITS) gene based sequences. Phylogenetic analysis was done with the study isolates and standerd CBS stains retrived from the NCBI database [*Cladosporiumhalotolerans* **(LN834374, LN834375 and DQ780364),** *Cladosporiumangustisporum* **(LN834356),** *Cladosporiumasperulatum* **(LN834357),** *Cladosporiumallicinum* **(LN834353***), Cladosporiumcladosporioides* **(LN834358),** *Cladosporiumflabelliforme* **(LN834361),** *Cladosporiumfuniculosum* **(LN834364),** *Cladosporiumherbarum* **(LN834378),** *Cladosporiumsubinflatum* **(LN834391),** *Cladosporiumtenuissimum* **(LN834398)] study isolates (MT588811 and MT588810) have clustered with** *Cladosporiumhalotolerans***.**

Discussion

The present study showed 5.97% prevalence of superficial infection with non dermatophytes including yeast and moulds other than dermatophytes. It appears tineaunguium as the common clinical condition accounted for 85.71%, the risk factor for increase prevalence of tineaunguium in superficial mycosis by non dermatophytes might be due to frequent contact with soil during their day today life, Age, history of similar infection or frequent sharing of foot ware. Tineacorporis and tineacapitiswas seen in 8.92% and 5.35% of the cases respectively. Similarly Kaur et.al. in 2015 found nail infection the most common site to cause superficial infection(7),whereas Lakshmanan et al. in 2015 have found skin is the common site of superficial infection followed by nail and hair[8]. Infection rate was highest in the outdoor workers like farmers (57.14%) and daily wage workers (16.07%) which in accordance with studies done in other parts of India[9–11].Candida species (20.25%) was the predominant agent followed by *Aspergillus species*(12, 7.59%), *Fusarium species*(10, 6.32%) and *Cladosporiumhalotolerans*(2, 1.26%). Hazarika et.al in 2020 reported NDM were more prevalent than yeast to cause superficial infection[12]. Kaur et.al. in 2015 have found NDM was the most common agent to cause superficial mycosis followed by dermatophytes and yeasts[7]. *Cladosporium halotolerans*has been isolated from twin's patients with tineacapitis to confirm the infection with *Cladosporium halotolerans* repeat

samples were collected from both the patient shown the growth *Cladosporium halotolerans*.*Cladosporium* usually considered as indoor fungus being isolated in the environmental sources and geographic location, however many species of *Cladosporium species* are important pathogens to cause infection in plants animal and even in humans[13,14]. In most of the cases *Cladosporium species* isolated lack molecular confirmation, however in the present study *Cladosporium species* isolated were sequenced using ITS region and identified as *Cladosporiumhalotolerans*. Possible reason for the isolation of fungus like *Cladosporiumhalotolerans*in Siddi tribal community might be due to their low socioeconomic condition and poor hygiene, as majority of the Siddi tribal community live their life in poverty.

In conclusion, this study showed other than dermatophyte, non-dermatophytes like yeast and non dermatophyticmoulds are also responsible for the superficial infection in the Siddi tribal community with prevalence rate of 5.97%. Tineaungum was the most commonly seen clinical condition to cause superficial infection by non dermatophytes in 85.71% of cases. *Cladosporium halotolerans* which is considered as the indoor fungus can also cause superficial infection like tineacapitis in the Siddi tribal community, a detailed study on Siddi tribal community and native Indians to find out such fungal superficial infection may reveal interesting findings, which may further help clinician diagnosis and may change the approach to treat.

Acknowledgments: Nil

Source of Funding: Self

Conflict of Interest: Nil

Ethical Clearance: Taken

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