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Documentation of yeast-like pathogens causing onychomycosis from Doda Region of Jammu and Kashmir (India)

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ABSTRACT

Onychomycosis is a fungal infection of the nail bed and nail plate predominantly caused by anthropophilic dermatophytes. However, these days, non dermatophytes, yeasts and yeast-like pathogens are continuously emerging as important etiological agents of onychodystrophy. It affects particularly the nails of elders, diabetics, immune compromised individuals, smokers and patients with psoriasis, reduced peripheral circulation or tinea pedis, as well as those with history of nail trauma or with family history of onychomycosis. The treatment of onychomycosis is dependent on several factors, including the type of onychomycosis and causative organism. A number of techniques have been used in the past to accurately diagnose onychomycosis and among these, microscopy and culturing are being used most frequently. However, these techniques are not completely reliable for confirming the identity of yeast and yeast-like pathogens. Therefore, during the present investigation, both mycological and molecular techniques were attempted to identify the yeast-like species causing onychomycosis. On this basis, three yeast-like species viz., *Candida parapsilosis* (Ashford) Langeron & Talice, *C. tropicalis* (Castellani) Berkhout and *Aureobasidium pullulans* (de Bary & Lowenthal) Arnaud were identified to cause finger and toe nail infections among the residents of hilly Doda region (J.&K.), India.

KEYWORDS: Onychomycosis, *Candida, Aureobasidium,* NCBI, MEGA6 software, GenBank

INTRODUCTION

Onychomycosis is a chronic mycotic nail infection caused by different species of dermatophytes, saprophytic moulds, yeasts and yeast-like fungi and is known to be associated with significant physical and psychological morbidity (Elewski, 1998; Lungran *et al*., 2014). Earlier it was known to be caused by dermatophytes quite frequently, however the latest reports confirm a gradual increase of such cases where yeasts and other non-dermatophytic fungi (NDF) are the causal agents (Kaur *et al*., 2007; Kotwal and Sumbali, 2016; Kotwal *et al*., 2018). Species of *Candida* are the most common causes of fungal infections worldwide and are even known to cause candidal onychomycosis, an infection of the nail plate that generally occurs in patients with mucocutaneous candidiasis. Among the *Candida* species, *C. albicans*, *C. tropicalis* and *C. parapsilosis* are the main yeasts responsible for onychomycosis in India (Kaur *et al*., 2007; Veer *et al*., 2007; Rakshit *et al*., 2014) and elsewhere (Velez *et al*., 1997; Gupta *et al*., 2003; Alvarez *et al*., 2004). Generally, *Candida* species are harmless commensals or endosymbionts of hosts including humans where they inhabit skin and mucosa as innocuous commensals (Jayatilake *et al*., 2009). However, when the host immunity is compromised or the mucosal barriers are disrupted, *Candida* species have the ability to invade and cause diseases, ranging from moderate superficial lesions to chronic systemic infections (Jayatilake *et al*., 2009). In superficial candidiasis, one or more mucocutaneous tissues including skin, mucous membrane and hair are involved, but when nails and finger webs are involved it is known as candidal onychomycosis (Jesudanam *et al*., 2002). Generally, the ability of *Candida* species to invade the nail plate and cause onychodystrophy is indistinguishable from those caused by dermatophytes.

On the other hand, *Aureobasidium pullullans,* a dematiaceous black, saprophytic, yeast-like fungus can also cause nail infections and has been isolated from soil, decaying plant debris, human hair and skin (Hawkes *et al*., 2005). Since last few years, yeast species have emerged as important pathogens of onychomycosis representing up to 49.1% of all nail infection cases in Brazil (de Araujo *et al*., 2003; Figueiredo *et al*., 2007; Godoy- Martinez *et al*., 2009), 59.1% cases in Rome (Mercantini *et al*., 1996) and 75% cases in Libya (Ellabib *et al*., 2002).

In view of the variety of yeasts being implicated as etiological agents of onychomycosis, it has become difficult for any clinician to decide for management protocol. This has become more important because some of the yeast species have already become resistant to antifungals (Warnock, 1992; Kwok *et al*., 1998; Rao *et al*., 2004; Whaley *et al*., 2016). Therefore, the aim of the present study was to identify the predominant yeasts and yeast-like pathogens of onychomycosis in our geographic location (Doda region*,* J & K, India) by using mycological and molecular techniques.

MATERIALS AND METHODS

Sampling was done from clinically suspected cases of onychomycosis from Doda region of Jammu & Kashmir (India). A detailed history of occupation, duration of infection, predisposing factors and personal habits were obtained from the patients. Different clinical patterns of onychomycosis were recorded and written consent from each patient was obtained. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Ethical Committee.

Nail scrapings and clippings were obtained according to standard procedures (Elewski, 1998; Gupta *et al*., 2004). The affected area of the nail was first cleaned with 70% ethanol and the nail or subungual scrapings were collected with a surgical blade in sterilized polythene bags. Direct microscopic observations were conducted by placing the infected nail sample in 2-3 drops of 20% potassium hydroxide (KOH) prepared in 36% dimethyl sulfoxide (DMSO) to detect any fungal hypha or spore. The samples irrespective of

the negative or positive direct microscopic examination were then cultured on Sabouraud Dextrose Agar (SDA) medium supplemented with Chloramphenicol (0.040 g/L) and Cycloheximide (0.500 g/L) and incubated at 28±2°C for 8-14 days. Isolation of the same fungus in culture on more than two consecutive occasions was taken as the criterion to consider the fungus as a probable pathogen. On the basis of growth rate, colony morphology and lactophenol cotton blue mounts, the yeast-like pathogens were identified mycologically by following the descriptions given by Barron (1972) and Domsch *et al.* (1980). Later, they were grown on Hicrome *Candida* Differential Agar medium (HiMedia) for identification of *Candida* species on the basis of colony characters.

DNA Extraction, Amplification and Sequencing of yeasts ITS1-5.8S-ITS2 Region: The genomic DNA of the isolated yeast strains were extracted by using ZR Fungal MiniPrepTM kit (Zymo Research). The quality and quantity of genomic DNA was checked spectrophotometrically by using NanoDrop 2000. The ITS1-5.8S rDNA-ITS2 region was amplified by using universal primers ITS5: 5´-GGAAGTAAAAGTCGTAACAAGG-3´ and ITS4: 5´-TCCTCCGCTTATTGATATGC-3´. The amplification was done by following the protocol of Arora *et al.* (2016) with slight modifications. PCR was performed in a 50 µL reaction volume with 25 µL of ReadyMix Taq PCR Reaction Mix (SIGMA-ALDRICH, India), 2.50 µL of 10 µM each primer, 2 µL of yeast genomic DNA (100 ng) and 18 µL of molecular biology grade water. The thermal programme was followed as: initial denaturation 95 °C for 5 min; 35 cycles of 30 s at 94 °C, 45 s at 56 °C and 60 s at 72 °C; and a final extension of 10 min at 72°C. The PCR product was cleaned with HiPurA™ PCR product purification kit (HiMedia Laboratories, India).

Phylogenetic Analysis of Yeasts strains: The amplified ITS region was sequenced in both directions by ABI 377 DNA sequencer using the Big Dye Terminator v3.1 cycle sequencing kit (Thermo Fischer Scientific). To identify the yeasts strains, the sequences obtained were used as query sequence for similarity search by using BLAST algorithm against the database maintained at National Center for Biotechnology Information, Maryland (USA) (http://www.ncbi.nlm.nih.gov). The ITS1-5.8S rDNA-ITS2 sequences of the strains G1, G2, G3, C1 and C2 were aligned with the most similar reference sequences of the taxa by using the clustal W module of the MEGA6 software (Tamura *et al.*, 2013). A phylogenetic tree was constructed subsequently and analyzed for evolutionary distances by the neighbor-joining method (Saitou and Nei, 1987). The robustness of clades was estimated by bootstrap analysis with 1000 replications.

The pure cultures of isolated pathogenic yeasts causing onychomycosis were maintained on PDA slants under specific codes (G1, G2, G3, C1 and C2) and deposited in the Col Sir R.N. Chopra Microbial Resource Center, Indian Institute of Integrative Medicine (IIIM-CSIR), Jammu (J&K, State) under the accession numbers MRCJ-692, MRCJ-693, MRCJ-694, MRCJ-695 and MRCJ-696, respectively.

RESULTS

From February 2017 to January 2019, 50 patients with presumptive diagnosis of onychomycosis were detected from the hilly region of Doda (J.&K.), India. Of these, 8 patients were detected with nails infected with 3 species of yeast belonging to 2 genera (**Table 1**). The clinical and mycological features of the eight case reports are described below:

Table 1: Clinical, mycological and ITS region sequence based taxonomic designation of yeast strains involved in onychomycosis.

Case number & Occupation of the patient	Site of Infection	Code number of yeast strain	Identification based on cultural and morphological features	Identification with ITS sequence analysis (NCBI Acc. No.)	%age Homology with Reference Sequence
Case 1 Student and part-time farmer	Finger nails	C1/ MRCJ-695	*Candida parapsilosis*	*Candida parapsilosis* (MF380666)	99%; *Candida parapsilosis* KY102304
Case 2 Student	Finger nails	C1/ MRCJ-695	*Candida parapsilosis*	*Candida parapsilosis* (MF380666)	99%; *Candida parapsilosis* KY102304
Case 3 Retired Postman	Toe thumb nail	C2/ MRCJ-696	*Candida parapsilosis*	*Candida parapsilosis* (MF380667)	99%; *Candida parapsilosis* KY102304
Case 4 Student and part-time farmer	Finger nails	G2/ MRCJ-693	*Candida tropicalis*	*Candida tropicalis* (MF380669)	96%; *Candida tropicalis* KY102470
Case 5 Retired Government employee	Finger nails	G3/ MRCJ-694	*Candida tropicalis*	*Candida tropicalis* (MF380670)	99%;*Candida tropicalis* KY102459
Case 6 Vegetable vendor	Finger nails	G2/ MRCJ-693	*Candida tropicalis*	*Candida tropicalis* (MF380669)	96%;*Candida tropicalis* KY102470
Case 7 Juice Vendor	Finger nails	G1/ MRCJ-692	*Aureobasidium* sp.	*Aureobasidium pullulans* (MF380668)	98 %; *Aureobasidium pullulans* KF996495
Case 8 Farmer	Toe nails	G1/ MRCJ-692	*Aureobasidium* sp.	*Aureobasidium pullulans* (MF380668)	98 %; *Aureobasidium pullulans* KF996495

A. Clinical signs of onychomycotic case reports infected by *Candida parapsilosis* (Ashford) Langeron & Talice:

Case 1: Onychomycosis was detected in a 21 years old male college student who was also engaged in part-time paddy cultivation. All the finger nails of his left hand excepting the little finger were involved. On examination, the nails of his thumb, index and ring finger showed onycholysis from the distal region, whereas the nail of middle finger showed onychodystrophy, wherein the nail plate was totally destroyed and crumble of nail pieces came out. Swelling was also noticed in the middle finger that gave pain to the patient. The infection evolved 5 years ago from the distal end of the middle nail and progressed gradually to other nails and skin (**Fig. 1a**). On mycological examination, it was found to be caused by *C. parapsilosis* (Code C1).

Case 2: Onychomycosis was detected in a 24 years old boy, whose nails of the index, middle and ring fingers of the right hand were associated with fungal invasion. The ring finger was highly infected and onycholysis was clearly visible (**Fig. 1b**). On mycological examination, it was found to be caused by *C. parapsilosis* (Code C1).

Case 3: Onychomycosis was detected in a 70 years old retired

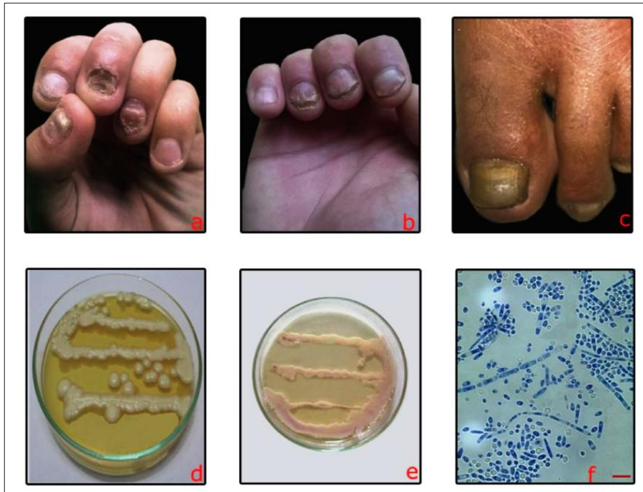

Fig. (1 a-b): Onychomycotic finger nails of case 1 and 2.
c: Onychomycotic toe nails of case 3.
d: Colonies of *Candida parapsilosis* growing on SDA medium.
e: Light pink colonies of *C. parapsilosis* on HiCrome *Candida* Differential Agar medium.
f: Photomicrograph showing curved pseudohyphae, blastoconidia and budding cells of *C. Parapsilosis*.

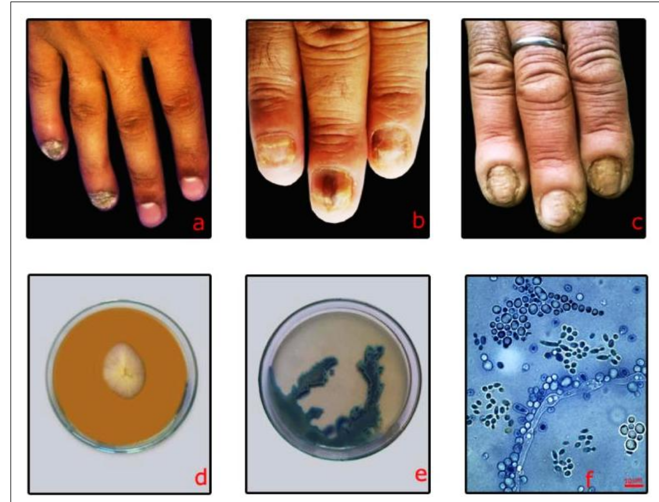

Fig. (2 a-c): Onychomycotic finger nails of case 4, 5 and 6.
d: Colony of *Candida tropicalis* growing from the nail sample on SDA medium.
e: Metallic blue colonies of *C. tropicalis* on HiCrome *Candida* Differential Agar medium.
f: Photomicrograph showing pseudohyphae with local swelling, blasto-conidia and budding cells of *C. Tropicalis*.

postman, whose thumb nail of the right foot was affected. The colour of the infected nail was light brown, texture was slimy (**Fig. 1c**) and the patient felt irritation on the surrounding areas of the thumb. Infection occurred due to the wearing of rubber shoes and excessive sweating. The patient was also suffering from Type 2 diabetes mellitus. On mycological examination, onychomycosis was found to be caused by *C. parapsilosis* (Code C2).

Colony Characteristics of *C. parapsilosis*: Colonies on SDA after 10 days of incubation attained a diameter of 7-12 mm. To look at colonies were smooth, slimy, slightly raised at the centre, having fringed margins; white to off-white (**Fig 1d**). On Hicrome *Candida* Differential Agar medium, colonies attained a diameter of 4-10 mm after 5 days of incubation. These were smooth, slimy and light pink in color (**Fig. 1e**). The pseudomycelium present was curved, 1.3-2.5 µm in width; budding cells moderate, blastoconidia abundant, smooth-walled, ovate to oval, measuring 2-8 x 1.8-3.0 µm in size (**Fig. 1f**).

B. Clinical signs of onychomycotic case reports infected by *Candida tropicalis* (Castellani) Berkhout:

Case 4: A male student of 24 years old was detected with nail dystrophy. The nails of his right hand ring finger and little finger were associated with onychomycosis. Total nail dystrophy was clearly visible. The infection had begun 3 years back due to continuous contact with marshy soil of the paddy field where he worked as a part timer. The infection spread to the entire nail plates showing onycholysis of the nail (**Fig. 2a**). On mycological examination, it was found to be caused by *C. tropicalis* (Code G2).

Case 5: Onychomycosis was detected in a 62 years old retired government employee. His nails of the index, middle and ring finger of left hand were involved. Onycholysis started from the distal region of the nails and spread to the posterior region. The colour of the nails was pale brownish and the nail plate was deformed (**Fig. 2b**). The nail infection evolved 5 years back after experiencing nail trauma due to the jerk of falling window pane of the bus. The patient was also suffering from Type 2 diabetes mellitus. On mycological examination, it was found to be caused by *C. tropicalis* (Code G3).

Case 6: Nail infection was detected in a 54 years old vegetable vendor on the index, middle and ring finger of the right hand. The eponychium region of the infected nails showed swelling, whereas the texture of the nails was rough and crumbly. The surrounding skin was dry with developing cracks. Infection occurred some 3 years back (**Fig. 2c**). On mycological examination, it was found to be caused by *C. tropicalis* (Code G2).

Colony characteristics of *C. tropicalis*: Colonies on SDA medium attained a diameter of 23-28 mm after 10 days of incubation. The colonies were smooth, slimy, having umbonate centre and slightly radially furrowed margin, off white in colour (**Fig. 2d**). On Hicrome *Candida* Differential Agar medium, colonies attained a diameter of 5-12 mm after 5 days of incubation.These were smooth, slimy having undulating margins and metallic blue colour (**Fig. 2e**). Pseudomycelium with local swelling present, width 2.5- 6.0 µm. Budding cells and blastoconidia abundant; conidia large, smooth-walled, oval to ellipsoidal, present in small groups along the pseudhyphae (**Fig. 2f**), measuring 3-11 x 2.3-5.8 µm.

C. Clinical signs of onychomycotic case reports infected by *Aureobasidium pullulans* (de Bary & Lowenthal) Arnaud:

Case 7: Onychomycosis was detected in a 45 years old juice

vendor. The nail of his middle finger of the right hand was black, slimy and soft textured. The nail started dystrophy from the distal region. On the same hand, the nail of his ring finger was also completely deformed with pieces of nail shedding out. The infection started 5 years back probably due to regular contact of his nails with the acidic constituents of the fruit juice (**Fig. 3a**). The patient was also suffering from Type 2 diabetes mellitus. On mycological examination, it was found to be caused by *A. pullulans* (Code G1).

Case 8: Onychomycosis was detected in the toe nails of a 35 years old person. His thumb nail and two finger nails of the toe showed nail dystrophy (**Fig. 3b**). The nails showed discolouration with brownish pigmentation. The infection evolved 2 years back due to the wearing of rubber shoes. On mycological examination, it was found to be caused by *A. pullulans* (Code G1).

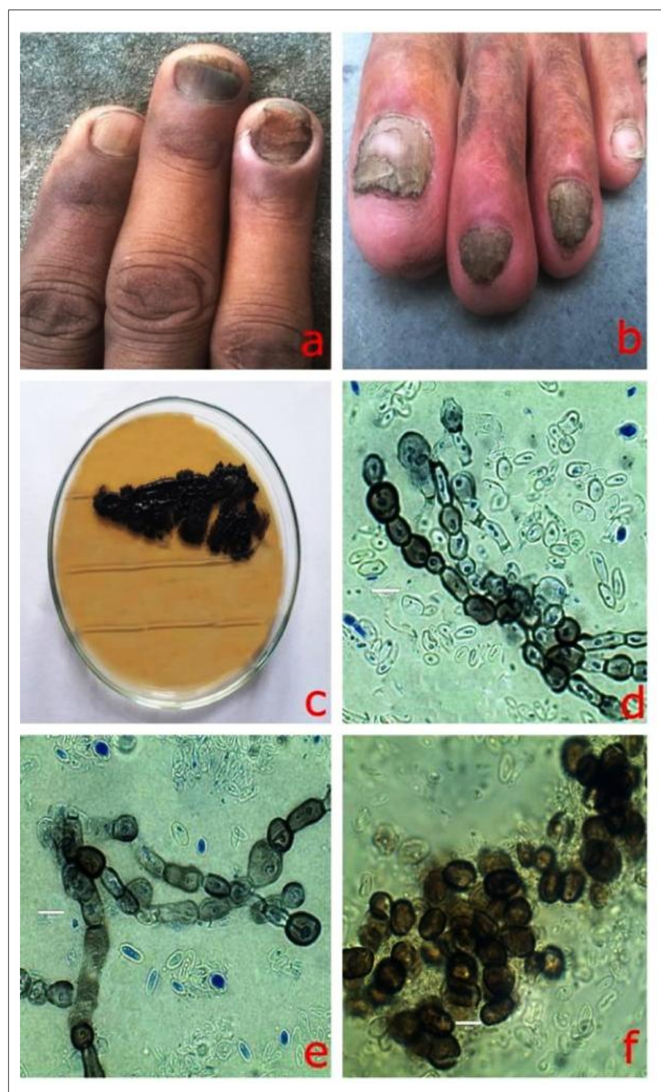

Fig. 3 a: Onychomycotic finger nails of case 7.
b: Onychomycotic toe nails of case 8.
c: Black coloured colonies of *Aureobasidium pullulans* growing on SDA medium.
d-e: Photomicrographs showing hyphae with arthrospore-like appearance and small, non-septate, ovate to ellipsoidal conidia of *A. pullulans*.
f: Photomicrograph showing thick walled chlamydospores of *A. Pullulans*.

Colony characteristics of *Aureobasidium pullulans:*

Colonies on SDA medium attained a diameter of 20-25 mm after 15 days of incubation at 28 ± 2ºC.These were slimy, shining, yeast-like, with spreading margins. The colony color is initially light brown, which turn to black with age and blackish brown on the reverse side (**Fig. 3c**). Hyphae irregularly branched, smooth- walled, appeared as arthrospore-like due to the presence of thick double-walled septa (**Fig. 3d,e**), measuring 8.0-15.0 x 4.0-6.0 µm in size. Many areas possesses elongated chains of spherical to broadly ovate 10-15 µm wide cells. Conidia hyaline, smooth, produced in slime, non- septate, ovate to ellipsoidal (**Fig. 3d,e**), measuring 3.8-10.2 x 3.0-4.5 µm in size. Also produce secondary smaller conidia by budding in yeast-like manner. Chlamydospore-like cells usually formed, which are thick-walled, spherical to ovate, measuring 9-14 µm in diameter (**Fig. 3f**).

Molecular characterization: To confirm the yeast strains up to species level, molecular identification was employed. The ITS1-5.8S rDNA-ITS2 region was amplified and sequenced. The GenBank search for DNA sequence similarity revealed that ITS1-5.8S-ITS2 sequence of yeast strains G1, G2 and C1 showed more than 96% homology to those of *Aureobasidium pullulans* (KF996495), *Candida tropicalis* (KY102470) and *Candida parapsilosis* (KY102304), respectively (**Table1**) and forms clades with the retrieved sequences. The ITS sequences of G3 and C2 form clades with *C. tropicalis* and *C. parapsilosis*, respectively. Sequences of the maximum identity greater than 90% were retrieved, aligned with the sequences of yeast strains, using clustal W module of MEGA6 software further subjected to neighbor-joining (NJ) analysis to obtain the phenogram (**Fig. 4**). The authentication of the phenogram was designated in terms of bootstrap values given at branch nodes. The contiguous rDNA sequences of the yeasts strains G1, G2, G3, C1 and C2 have been submitted to GenBank under the accession number MF380668, MF380669, MF380670, MF380666, MF380667.

Clinical, mycological and ITS region sequence based taxonomic designation of yeast strains is discussed in **Table 1**. The neighbor-joining tree of yeast strains based on ITS1-5.8S-ITS2 rDNA sequences is shown in **Fig. 4**.

DISCUSSION

Yeasts and yeast-like species were earlier regarded as contaminants but are now increasingly being recognised as pathogens of onychomycosis (Karmakar *et al*., 1995; Manzano-Gayosso *et al*., 2008). Though dermatophytes have been considered as the major pathogens, yet infections due to non-dermatophytic moulds and yeasts have been reported worldwide (Ertam *et al*., 2008). After the dermatophytes as the most important onychomycotic causal agents, *Candida* species have emerged as second-line pathogens (Jayatilake *et al*., 2009; Manzano- Gayosso *et al*., 2008), generally occurring in the patients having chronic mucocutaneous candidiasis (Kaur *et al*., 2008).

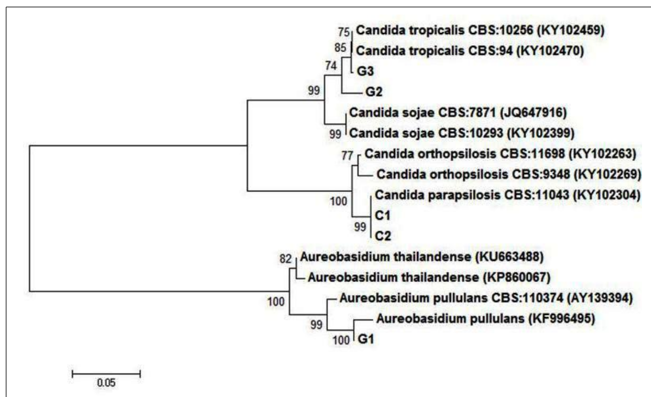

Fig. 4: Neighbor-joining tree of yeast strains based on ITS1-5.8S-ITS2 rDNA sequences. Confidence values above 50% obtained from a 1,000-replicate bootstrap analysis are shown at the branch nodes.

During the present study, yeast species were recovered from 16% of the onychomycotic cases (8/50). Among the yeast species isolated, *Candida parapsilosis* and *C. tropicalis* were the most prevalent species. Our results are very much similar to other studies conducted from Australia (McAleer, 1981), Belgium (Achten and Wanet-Rouard, 1978), Brazil (Godoy-Martinez *et al*., 2009; Arrua *et al*., 2015), Canada (Daniel *et al*., 1998), Chile (Fich *et al*., 2014), Colombia (Alvarez *et al*., 2004), Mexico (Manzano-Gayosso *et al*., 2008), Saudi Arabia (Al-Sogair *et al*.,1991) and Spain (Velez *et al*., 1997), where candidal onychomycosis was detected to be more prevalent then primary dermatophytic onychomycosis. Prevalence percentage of candidal onychomycosis varies from place to place and these differences are usually attributed to geographical location and changing environmental conditions (Rakshit *et al*., 2014). Among the *Candida* species, *C. albicans* is the main yeast species responsible for onychomycosis (Daniel, 1991) and has been reported as the major pathogen of candidal onychomycosis from Barcelona and Roma (Torres-Rodriguez and Lopez-Jodra, 2000), Malaysia (Ng *et al*., 1999), Italy (Mercantini *et al*., 1996) and India (Veer *et al*., 2007). However, role of *C. parapsilosis* and *C. tropicalis* as etiological agents of onychomycosis has also been reported from Spain (Velez *et al*., 1997), Belgium (Pierard, 2001), Brazil (Figueiredo *et al*., 2007), Iran (Asadi *et al*., 2009) and Chile (Fich *et al*., 2014). Similarly, while studying onychomycosis among children, *C. albicans, C. parapsilosis* and *C. krusei* were isolated as the etiological agents from Italy (Romano *et al*., 2005), whereas from infected nails of some premature infants, *C. tropicalis* and *C. parapsilosis* have been isolated as main onychomycotic agents (Chun *et al*., 2004; Koklu *et al*., 2007).

In the present study, candidal onychomycosis of fingernails was detected to be more prevalent than that of the toenails. Similar findings have been recorded by many other researchers also (Velez *et al*., 1997; Veer *et al*., 2007; Figueiredo *et al*., 2007; Godoy- Martinez *et al*., 2009; Rakshit *et al*., 2014; Fich *et al*., 2014). *Candida* species usually invade the nail plate directly and show severe dystrophic changes like nail folding, marked thickening, distoration, fragmentation and pigmentation of nail tissue (Jayatilake *et al*., 2009). More association of *Candida* species with fingernails than the toenails is often attributed to the predictable occupational trauma in farmers, vendors, housewives, sweepers, etc (Chi *et al*., 2005). In the onychomycotic cases detected by us from the hilly Doda region of Jammu & Kashmir, prolonged contact of fingernails with moisture and working in marshy areas probably led to easy invasion of nails by the yeast species.

Aureobasidium pullulans a saprophytic dematiaceous black, yeast-like fungus is reported rarely to cause nail infections (de Oliveira *et al*., 2013; Ding *et al*., 2017). It is widely distributed in the environment and has been isolated from soil, decaying plant debris, human hair and skin (Bolignano and Criseo, 2003; Chan *et al*., 2011). During the present study, *Aureobasidium pullulans* was isolated from infected finger and toe nails, which proves that it is capable of causing onychomycosis. *A. pullulans* is most commonly recognised as an etiologic agent of a number of human diseases (de Morais *et al*., 2011). It can cause infection in any part of the body depending on the nature of the exposure (inhalation, wound, ingestion, etc) and as such the type and severity of symptoms can vary considerably (de Morais *et al*., 2011). Usually, it causes different types of cutaneous and subcutaneous infections, peritonitis in patients undergoing peritoneal dialysis, pulmonary mycosis, fungemia, etc. (Hawkes *et al*., 2005). In addition, splenic and mandibular abscess, meningitis, eye infections, tonsil infections and disseminated infections have been described in the literature (Pikazis *et al*., 2009; Joshi *et al*., 2010). Its pathogenic ability is more in humans suffering of immune-suppression (de Oliveira *et al*., 2013).

Identification of yeast species has generally been achieved by different researchers by simple mycological observations like microscopy and culturing (Alvarez *et al*., 2004; Rakshit *et al*., 2014; Fich *et al*., 2014). However, these techniques are not completely reliable for confirming the identity of yeast pathogens, which is very essential for management of the disease. Therefore, proper diagnosis of yeast and yeast-like pathogens by using both mycological and molecular techniques is required (Kotwal *et al*., 2018; Kotwal and Sumbali; 2018). Our findings show that *Candida* species can still be identified by using HiCrome *Candida* Differential Agar medium, as the results obtained were similar to those obtained by using molecular techniques (**Table 1**). However, for other yeast-like species, taxonomic identification is entirely dependent on molecular analysis.

CONCLUSION

Candida has emerged as an important pathogen of onychomycosis necessitating accurate diagnosis for appropriate therapy. In addition, *Aureobasidium pullulans*, which is capable of causing a variety of human diseases, was also detected to be capable of causing onychomycosis. In both the cases, clinical diagnosis is often confusing because of the varied clinical presentations. Therefore, mycological and molecular identification is required to ascertain the exact etiological agent, which is extremely important for choosing therapeutants of choice. Farmers were found to be more affected than any other occupational group, suggesting that

exposure to water and soil leads to invasion of soft tissues around the nails by yeast and yeast-like species, which may eventually spread to the nail plate and results in its destruction. Survey of literature shows that *A. pullulans* has not been reported so far as an etiological agent of onychomycosis from North-India, hence it constitutes a new report.

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