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# Opportunistic yeasts causing onychomycosis among some elderly residents of Rajouri district, J&K (India)

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**ABSTRACT**

**Onychomycosis is the fungal infection of toenails and fingernails, which can be caused by dermatophytes, non-dermatophytic filamentous fungi and yeasts. Among these, yeasts are a group of fungi widely distributed in nature and can be found in the soil, air, water, food, etc. In human beings, they are occasionally a part of the normal microbiota of gastrointestinal tract, respiratory tract, reproductive tract and skin. However, these can cause superficial as well as systemic infections when a person is immunocompetent or his/her normal flora gets altered. In the last few decades, yeasts are emerging as the common etiological agents of onychomycosis. During a survey conducted for the first time in Rajouri district (Union Territory of Jammu and Kashmir), toenails and fingernails of the elderly residents were visually scanned for dystrophies and sampled for knowing the etiologic agents. Investigations revealed the presence of three yeast species *viz., Candida albicans, Trichosporon asahii* and *Rhodotorula mucilaginosa* associated with the dystrophied nails of some of the residents.**

**Keywords:** Onychomycosis, *Candida albicans, Trichosporon asahii, Rhodotorula mucilaginosa*

## INTRODUCTION

Onychomycosis is a common nail infection caused by fungal organisms. Earlier, onychomycosis was referred to as dermatophytic nail infection but these days it is used as a general term to denote any fungal nail infection (Weitzman and Summerbell, 1995). It is often regarded as a cosmetic problem of minor importance, despite clearly diseased appearance associated with this condition (Elewski, 1998). It is usually caused by three groups of fungal pathogens, dermatophytes, non-dermatophytic moulds and yeasts (Welsh *et al.,* 2010*;* Shimoyama *et al.,* 2018). According to Moreno and Arenas (2010), it affects 10% of the total population and represents about 50% of all onychopathies.

Approximately 5-10 % of the nail infections are known to be caused by yeasts (Manzano-Gayosso *et al.,* 2010; Capoor *et al.,* 2013). *Candida* species are the most common pathogenic yeasts causing onychomycosis with *Candida albicans* as the predominant species in most of the yeast-caused onychomycotic cases (Gupta *et al.,* 2000; Ellabib *et al.,* 2002; Pontes *et al.,* 2002; Vender *et al.,* 2006; Seebacher *et al.,* 2007; Veer *et al.,* 2007; Chadeganipour and Mohammadi, 2016). *Candida* species are also known to cause other nail syndromes, such as, onycholysis and paronychia (Andre and Achten, 1987). Generally, three patterns of nail diseases have been reported due to *Candida* (i) total dystrophic onychomycosis in chronic mucocutaneous candidiasis (ii) distal and lateral nail dystrophy associated with onycholysis (iii) proximal and lateral nail dystrophy (Hay *et al.,* 1988). Among the *Candida* species, *C. albicans* is a member of healthy microbiota, which asymptomatically colonizes the gastrointestinal (GI) tract, reproductive tract, oral cavity and skin of humans (Kennedy and Volz, 1985; Kumamoto, 2002; Achkar and Fries, 2010; Ganguly and Mitchell, 2011; Kumamoto, 2011). It is generally classified as an opportunistic fungal pathogen because it usually causes diseases in those individuals who are immunocompromised or whose natural flora has been altered. It can cause two major types of infections in human *viz.,* superficial infections and life threatening systemic infections (Calderone and Clancy, 2012).

In the last few decades, species of *Trichosporon* have also emerged as important opportunistic pathogens in the immuno-compromised individuals. Especially *T. asahii* is the most important yeast after *Candida* species, which causes systemic infections (Rodrigues *et al.,* 2006). Infections by *Trichosporon* species are frequently associated with high mortality up to 80% (Chagas-Neto *et al.,* 2008). *Trichosporon* species are basidiomyceteous yeast-like anamorphic organisms that are widely distributed in nature and found predominantly in the tropical and temperate areas (Colombo *et al.,* 2011). These can be found in the soil, water, air, food, decomposing wood, bird droppings, etc. These are known to colonize gastrointestinal, respiratory, skin and urinary tract of humans (Walsh *et al.,* 2004; Sugita *et al.,* 2005; Fuentefria *et al.,* 2008; Cafarchia *et al.,* 2008; Davies and Thornton, 2014). This genus was first designated by Beigel (1865), who reported this microorganism causing a benign hair infection (Colombo *et al.,* 2011). It includes about 51 species and atleast 16 of them have clinical relevance (Gueho *et al.,* 1992; Colombo *et al.,* 2011).

According to Pfaller *et al.* (2009), *Rhodotorula* species are found to be the fourth most frequently isolated species from clinical specimens. In human beings, *Rhodotorula mucilaginosa* is commonly reported as an etiological agent of opportunistic infections in immuno-compromised individuals (Wirth and Goldani, 2012). It is a basidiomyceteous pigmented yeast, which is a normal inhabitant but can cause opportunistic infections associated with endocarditis, blood stream, peritonitis, meningitis and endophthalamitis (Duggal *et al.,* 2011; Garcia-Suarez *et al.,* 2011; Kim *et al.,* 2013; Mohd Nor *et al.,* 2015; Kitazawa *et al.,* 2018).

During the present study, three yeasts species *viz., Candida albicans, Trichosporon asahii* and *Rhodotorula mucilaginosa* were isolated from the dystrophied nails of elderly individuals of district Rajouri (J&K).

## MATERIALS AND METHODS

**Collection of dystrophied nails:** During the study period (August 2016-September 2019), dystrophied fingernail and

toenail samples were collected from the elders (above 40 years age) residing in district Rajouri. A detailed data sheet of each patient specifying history of occupation, duration of infection, predisposing factors, clinical patterns of onychomycosis, his/her consent to give infected nail samples, etc., were recorded.

Nail scrapings and clippings were obtained according to standard procedures given by Elewski (1998). The affected area surrounding the nail was first cleaned with 70% ethanol to remove any superficial organism. Then the nail or subungual scrapings were collected with a sterilized surgical blade. These were kept in sterilized polythene bags and brought to the laboratory for further investigations.

**Isolations:** The dystrophied nail samples were cultured on Sabouraud dextrose agar (SDA) medium supplemented with chloramphenicol (0.05 mg/ml) and incubated at 28°C for 9-14 days. Repeated isolation of the same fungus in culture on more than two consecutive occasions was taken as the criterion to consider it as a probable pathogen and then it was sub-cultured on appropriate medium for identification up to species level.

**Ethical clearance:** Ethical clearance granted to this study was in accordance with the ethical standards of the Animal and Human Experimentation Ethics Committee (AHEEC), University of Jammu, Jammu (JU/SBT/17/1360 Dated: 22/12/17), which follows the guidelines laid by Indian Council of Medical Research (ICMR), New Delhi, India.

**Mycological identification and photography:** Identification of the recovered organisms was done by studying their growth rate, colony morphology and other microscopic characteristics by preparing lactophenol cotton blue mounts. Relevant literature and various keys were used for morphological identification. The current names of the recovered pathogens were updated by following Index Fungorum (www.indexfungorum.org). Their mycological characters were observed on potato dextrose agar (PDA) medium, Hichrome *Candida* differential agar (CDA) medium and Sabouraud dextrose agar (SDA) medium. The mounts were viewed at 1000X on an Olympus compound microscope where measurements and other diagnostic features were recorded. Photographs of colonies grown on different media were taken by using Canon camera (model IXUS 125 HS 16.1 mega pixels) and the diagnostic features were recorded micro photographically on a Nikon microscope (model Eclipse E400) fitted with Samsung SDC-312 digital camera.

**Molecular identification**: To confirm the identity of the recovered pathogens, molecular identification was carried out at the sequencing facility of National Centre for Microbial Resource (NCMR), Pune (India). For this, the genomic DNA was isolated by following the standard phenol/chloroform extraction method of Sambrook *et al.* (1989). It was followed by PCR amplification of the ITS regions using universal primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS 4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). The amplified products were purified by PEG-NaCl precipitation and sequenced directly on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster city, CA) as per manufacturer's instructions. The sequencing was chiefly carried out using Lasergene package followed by NCBI-BLAST against sequences from type material for tentative identification. Finally, the confirmed sequences were submitted to Genbank, National Centre for Biotechnology Information (NCBI), Maryland, USA to obtain GenBank accession number.

## RESULTS

The dystrophied fingernail and toenail samples of some residents of Rajouri district of J&K (India) yielded three yeast species *viz., Candida albicans, Trichosporon asahii* and *Rhodotorula mucilaginosa* when cultured on Sabouraud's dextrose agar (SDA) medium. The onychomycotic case details, cultural and microscopic details of the etiological agents of nail dystrophies are described below:

**Case 1:** Onychomycosis was detected in a 50 years old housewife whose thumb nail of right hand was involved. The infection had evolved 2 years back from the proximal end of the nail plate and slowly progressed towards the lateral sides. The colour of the nail was yellowish at the middle and purplish at the lateral side. Onycholysis was also observed. These symptoms suggest a case of proximal lateral subungual onychomycosis (PLSO) (**Fig.1a**).

In this case, repeated isolations yielded *Candida albicans* (Robin) Berkhout as the etiological agent.

On SDA medium, the colonies were creamish in colour. But on Hichrome *Candida* differential agar (CDA) medium,

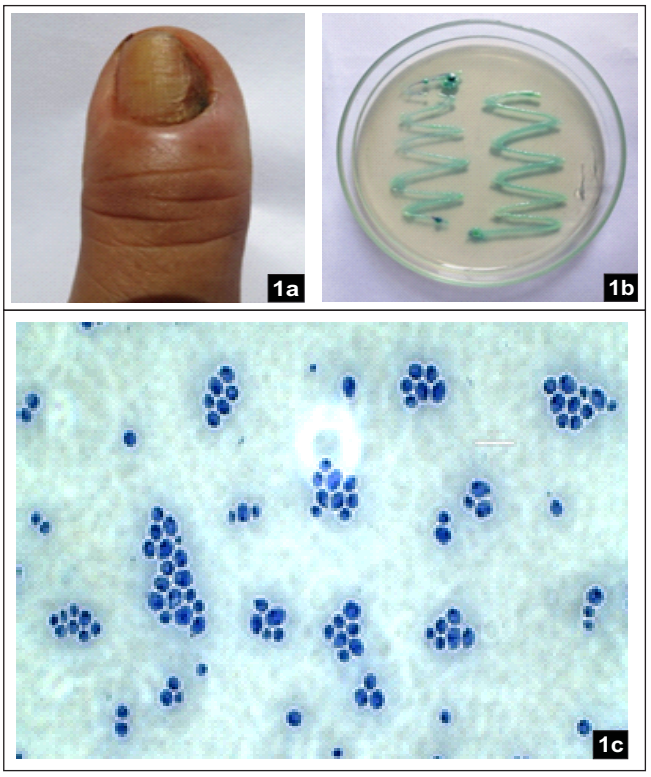


**Fig. 1:** a) Dystrophied thumb nail (right hand) of case 1. b) Colonies of *Candida albicans* on CDA medium. c) Subglobose to oval shaped blastoconidia (Bar =10 µm)

4.0-9.0 x 2.6-4.0 µm **(Fig. 1 b-c).**

**Case 2:** Onychomycosis was detected in a 43 year old farmer whose big toenail of right foot was involved with infection. The infection had evolved 3 years back from the distal edge of the nail. The colour of the nail was normal. But at one side of the nail plate onycholysis and hyperkeratosis was observed. The patient had pain in the lateral nail fold (perionychium) and the symptoms suggest a case of distal lateral subungual onychomycosis (DLSO) **(Fig. 2 a).** In this case, *Trichosporon asahii* Akagi ex Sugita, A. Nishikawa & Shinoda was recovered as the etiological agent.

Colonies on SDA medium were fuzzy, dry, white with raised centres and smooth margins, attaining a diameter of 12-15 mm after 12 days of incubation at 28°C. On microscopic examination, pseudo-mycelium was observed; arthroconidia cylindrical to ellipsoidal, measuring 5.0-14.0 x 1.9-3.1 µm. Blastoconidia abundant, smooth and sub-globose, measuring 3.5-5.1 x 3.0-3.7 µm **(Fig. 2 b-c).**

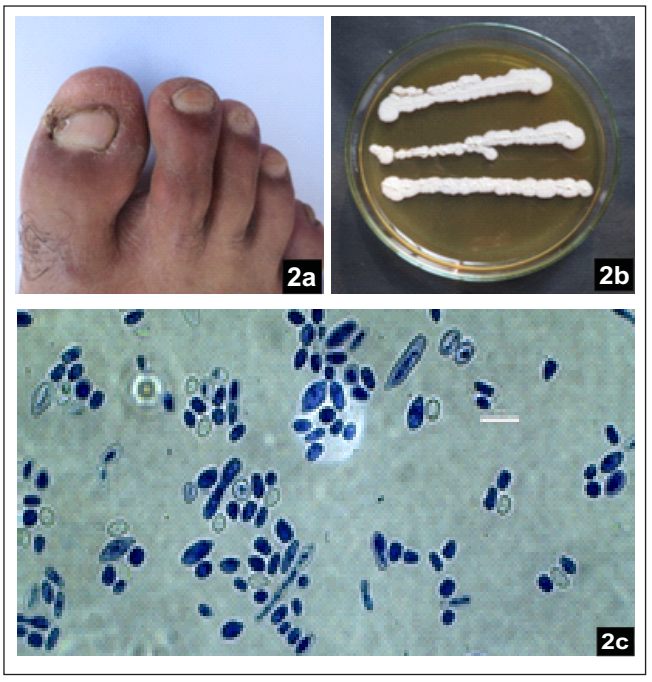


**Fig. 2:** a) Dystrophied big toenail (right foot) of case 2. b) Colonies of *Trichosporon asahii* on SDA medium. c) Arthroconidia and blastoconidia (Bar = 10 µm)

**Case 3:** Onychomycosis was detected in a 57 year old housewife, whose little fingernail of left hand was involved. The infection had evolved 1 year back from the distal edge of the nail plate and slowly covered whole of the nail plate. The whole nail plate became dystrophied and the colour of the nail turned brownish with white patches. The symptoms suggest a case of total dystrophic onychomycosis (TDO) **(Fig. 3a)**. In this case, *Rhodotorula mucilaginosa* (A. Jorg.) F.C. Harrison was recovered as the etiological agent.

Colonies on SDA medium were mucilaginous, smooth, glistening orange in colour, attaining a diameter of 12 to 14 mm in 5 days of incubation at 28°C. On microscopic examination, smooth, spheroidal to oval budding cells were observed without pseudohyphae **(Fig. 3b-c)**.

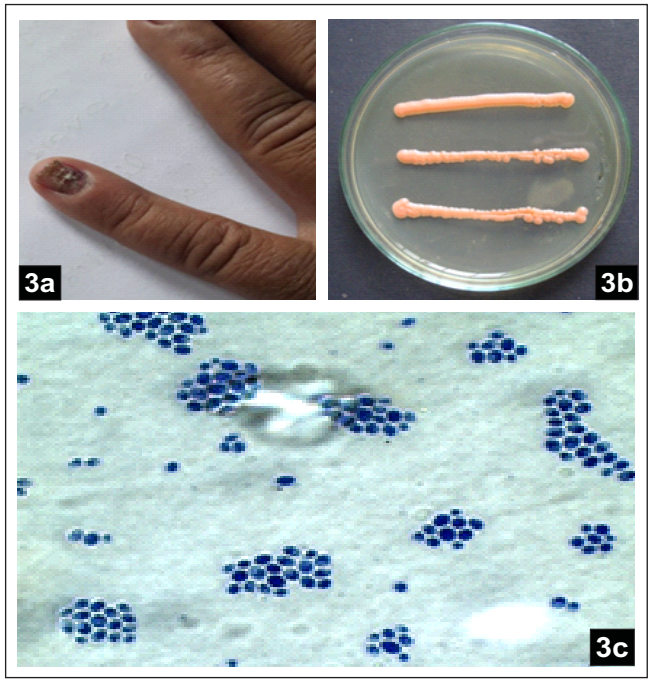


**Fig. 3:** a) Dystrophied little fingernail (left hand) of case 3. b) Colonies of *Rhodotorula mucilaginosa* on SDA medium. c) Spheroidal to oval budding cells (Bar = 10 µm)

**Molecular characterization of the recovered yeasts**

Molecular identification of the recovered yeasts was done by using rRNA gene sequencing. The obtained internal transcribed spacer (ITS) sequences were aligned using BLAST tool against the NCBI nucleotide database (http://blast.ncbi.nlm.nih.gov/) for most homologous sequences. The percentage similarity of the identified fungal pathogens using NCBI- BLASTn tool was above 99%. The fungal pathogens were identified as *Candida albicans* (Acc. No. MT337592.1), *Trichosporon asahii* (Acc. No. MT337741.1) and *Rhodotorula mucilaginosa* (Acc. No. MT328138.1). The strain numbers given to these pathogens are Y-II, B-II and Y-I respectively.

Identical sequences greater than 90% were retrieved and aligned with the sequence of YI, YII and BII, using Clustal W module of Mega-X software. The evolutionary history was inferred by using the Neighbor-Joining method to obtain the phylogenetic tree. The tree authentication was designated in terms of bootstrap values (above 50%) given at branch nodes. The fungus *Fusarium solani* from the class *Sordariomycetes* was used as outgroup **(Fig. 4)**.

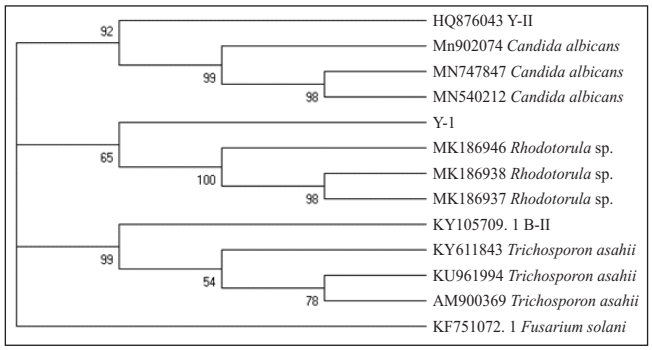


**Fig. 4**: Neighbour-joining tree of Y-I, Y-II and B-II strain based on ITS sequences. Confidence values (above 50%) obtained from a 500 replicate bootstrap analysis are shown at branch nodes. *Fusarium solani* was used as an outgroup.

## DISCUSSION

Onychomycosis is the infection of the toenails and the fingernails caused by fungal organisms, which may be filamentous or yeasts. Nail infections caused by yeasts are increasing day by day and the most commonly isolated yeasts are *Candida* species like *C. albicans, C. parapsilosis, C. tropicalis, C. guilliermondi, C. glabrata, C. krusei* and *C. famata* (Escobar *et al.,* 1999; Gautret *et al.,* 2000; Han *et al.,* 2000; Ellabib *et al.,* 2002; Pontes *et al.,* 2002; Foster *et al.,* 2004; Brilhante *et al.,* 2005; Seebacher *et al.,* 2007). According to Hay *et al.,* (1988), onychomycosis caused by *Candida* species are generally restricted to patients having chronic mucocutaneous candidosis (CMC) or as secondary infection in chronic paronychia. In addition, as observed in the present investigation, *Candida* onychomycosis occurs more commonly on the fingernails than on the toenails. Similar observations have been recorded by Andre and Achten (1987), Lwanga and Lemeshow (1991), Grover (2003), Veer *et al.* (2007), Kaur *et al.* (2008) and Gianni *et al.* (2001). So far, among the *Candida* species, *C. albicans* is the only yeast capable of invading the nail plate and causing total dystrophy of the nail, thus producing a clinical picture that is similar to dermatophytic onychomycosis (Upma and Bajaj, 2016). *C. albicans* has been reported as an onychomycotic agent from different parts of the world *viz.,* Italy (Romano *et al.,* 2005), New York (Scher *et al.,* 2007), Iran (Asadi *et al.,* 2009; Aghamirian and Ghiasian, 2010), Nigeria (Efuntoye *et al.,* 2011), Pakistan (Farwa *et al.,* 2011), Saudi Arabia (Venugopal and Venugopal, 1992), Brazil (Godoy-Martinez *et al.,* 2009) and India (Sen *et al.,* 2018).

Few species of *Trichosporon* have also emerged as important opportunistic pathogens in the immuno-compromised individuals. They have been isolated from the skin of healthy individuals, human faecal samples and patients with atopic dermatitis (Middelhoven *et al.,* 2004; Zhang *et al.,* 2011; Hamad *et al.,* 2012; Gouba *et al.,* 2014). During the present study, *T. asahii* (earlier known as *T. beigelii*) was recovered from the dystrophied big toenail of a farmer. Earlier, *T. asahii* has been recovered as an etiologic agent of onychomycosis from Korea (Han *et al.,* 2000), Columbia (Alvarez *et al.,* 2004), Brazil (Souza *et al.,* 2007) and Qatar (Taj-Aldeen *et al.,* 2009). Few other species of *Trichosporon* like *T. cutaneum, T. capitatum, T. mucoides, T. inkin, T. ovoides* and *T. dohaense* have also been recovered as etiological agents of onychomycosis from different parts of the globe (Restrepo and Uribe, 1976; Oyeka and Ugwu, 2002; Archer-Dubon *et al.,* 2003; Svejgaard and Nilsson, 2004; Mugge *et al.,* 2006; Taj-Aldeen *et al.,* 2009; Sageerabanoo *et al.,* 2011; Capoor *et al.,* 2013; Ortega-Springall *et al.,* 2015; Magalhaes *et al.,* 2016). Recently, Kotwal *et al.* (2018) recovered three species of *Trichosporon viz., T. asahii, T. asteroides* and *T. faecale* from the dystrophied and infected nails of three members of the same family from district Doda of Jammu and Kashmir (UT).

Another onychomycotic genus, *Rhodotorula* is a normal inhabitant of the human microbiota but can also cause opportunistic infections in humans. In the present investigation, *R. mucilaginosa* was recovered from the dystrophied nail of a housewife. It has been suggested by several workers that the tropical conditions may favour the establishment of some yeasts including *Rhodotorula* species in the interdigital areas of hand and feet (Mc Ginnis *et al.,* 1975; Mok and Barreto de Silva, 1984). Some species of *Rhodotorula* causing onychomycosis have been reported by Zhou *et al.* (2014), Uludag Altun *et al.* (2014) and Mot *et al.* (2017). From Brazil, *Rhodotorula mucilaginosa* has been reported to be the primary causative agent of onychomycosis in a 57 year old immuno-competent patient whose nail of the hallux was affected only (da Cunha *et al.,* 2009). Onychomycosis caused by *R. glutinis* has been reported by Maurya *et al.* (2015). Another study reported that the nails subject to psoriasis were secondarily infected by *R. mucilaginosa* (Martini *et al.,* 2013). In both the cases, infection appeared in toenail and fingernails of immuno-competent individuals. Similarly, a recent study reported *R. mucilaginosa* causing toenail mycotic dystrophy in an immuno-competent young adult (Ge *et al.,* 2019).

## CONCLUSION

In the present investigation, *Candida albicans, Trichosporon asahii* and *Rhodotorula mucilaginosa* are being reported for the first time as the etiological agents of onychomycosis among elderly residents of Rajouri district of Jammu and Kashmir (UT). Patients under study belonged to the rural areas and were exposed to some predisposing factors of onychomycosis like poor hygiene, illiteracy, poverty, no access to specialist doctors, frequent exposure to water and mud, climatic conditions, and frequent work trauma.

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