

# Chapter 8

## *Candida* Onychomycosis

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### Key Features

- *Candida* species have emerged as second-line pathogens related to onychomycosis.
- *Candida* onychomycosis is increasingly found in individuals with defective immunity and that can be related with occupational aspects.
- *Candida* species is considered as one of the most important causes of fingernail onychomycosis, especially in women.
- The organism invades the nail plate and may cause paronychia, onycholysis, and melanonychia.
- The diagnostic route includes a complete interrogatory based on the patients' history: a physical examination and microscopy and culture of nail specimens.

### Introduction

Onychomycosis is a fungal infection of nails that represents about 30 % of superficial mycotic infections. It is caused by dermatophytes, or non-dermatophytic molds, while *Candida* species have emerged as second-line pathogens [1]. This term is derived from the Greek words *onychos* (meaning nail) and *mycosis* (meaning fungal infection) [2]. But, it is traditionally referred to as non-dermatophytic infection of the nail, while *tinea unguium* specifically describes a dermatophytic invasion of the nail plate, but is now used as a general term to denote any fungal nail infection [3].

The importance of onychomycosis is often underestimated. In fact, onychomycosis can have significant negative effects on patients' emotional, social, and

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occupational functioning and can, in addition, consume a sizable proportion of healthcare dollars. Affected patients may experience embarrassment in social and work situations, where they feel blighted or unclean, unwilling to allow their hands or feet to be seen [3]. Far more than being a simple cosmetic problem, infected nails serve as a chronic reservoir of infection which can give rise to repeated mycotic infections of the skin [4]. In spite of improved personal hygiene and living environment, onychomycosis continues to spread and persist [5].

## Epidemiology

Onychomycosis encloses all fungal infections of the nail due to filamentous and yeast fungi, accounting for up to 50 % of all nail disorders which range from distal and lateral subungual, superficial, proximal subungual, and dystrophic onychomycosis; however, immunocompromised hosts can present a more significant health problem [6].

Over time, as the etiology of onychomycosis, yeasts from the genus *Candida* have emerged as important etiologic agents [7], so *Candida* onychomycosis is increasingly found in individuals having defective immunity consequential to aging, diabetes mellitus, vascular diseases, HIV infection, and drug therapies such as immunosuppressives and broad-spectrum antibiotics. Breached local immunity at the nail complex due to trauma, chronic exposure to moisture, and chemicals including smoke, detergents, soap, etc., also contributes to *Candida* onychomycosis [1]. Moreover, numerous factors exist for increasing the prevalence of onychomycosis in modern life such as wearing of shoes especially high-heeled shoes, high-moisture areas (gymnasium and wrestling mattresses by great numbers of people), use of broad-spectrum antibiotics, and corticosteroid therapy. *Candida* species is considered as one of the most important causes of fingernail onychomycosis, especially in women [6].

*Candida* onychomycosis constitutes an important health problem that can be related with occupational aspects, and this genus of yeasts may be the principal causative agent of fungal nail infections in some regions. Many authors around the world have documented that this condition affects mainly adult patients of female gender and toenails are more frequently involved than fingernails. The causative species varies around the world, and in some regions, non-*albicans* species have shown an increase of its frequency.

In a multicenter study conducted by Álvarez and colleagues in Colombia, from a total of 299 patients with nail lesions, onychomycosis was found in 183 cases (141 in toenails and 38 in fingernails), with a predominance in females (53 in males and 126 in females), and 4 cases in toenails and fingernails simultaneously (all females). Yeasts were identified in 40.7 % and *Candida albicans* was the most commonly isolated yeast species [8].

In a retrospective, observational, and descriptive study of fungal cultures conducted by Fich in Chile, specimens obtained from patients between December 2007

and December 2010 were analyzed. 29.1 % of cases with positive cultures were men and 70.9 % were women. *Candida* was retrieved from 467 of 8443 specimens (52 % fingernails and 48 % toenails), with a prevalence of 43.3 % for *C. parapsilosis*, while isolates of *Candida guilliermondii* were seen in 24.2 %, those of *Candida albicans* were present in 23.6 % of cases, those of *Candida* spp. were 4.3 %, and there were 4.71 % of cases of other isolates [9]. According to these data, Torres and colleagues in a retrospective study conducted in Mexico reported a frequency of *Candida* onychomycosis in 57.54 % of *Candida* mucocutaneous infections in a period of 6 years, with predominance in female gender of 65.49 % and a predilection of toenails' involvement in comparison with fingernails (86.01 % vs 13.99 %), and they reported a frequency of *C. albicans* in 42.80 %, *Candida krusei* in 28.41 %, *Candida tropicalis* in 15.86 %, and *Candida glabrata* in 12.91 % [10].

In a study with 140 patients conducted in Tehran, results show that females are more infected than males. The most common age group infected was 41–60 years (40.7 %). Toenails were affected more frequently than fingernails, and dystrophic onychomycosis was the most common clinical type (seen in 39.2 % of patients). Yeasts were the most frequent etiologic agents isolated (71.4 %), followed by non-dermatophytic molds in 17.1 % and dermatophytes in 11.5 % of patients [11]. According to these demographic data, in a study conducted in Paraiba, Brazil, from 1999 to 2010, women were the most affected by onychomycosis, which occur preferentially in adults, toenails are the favorite yeast targets, and the prevalent yeasts were *Candida tropicalis* and *C. krusei* [7].

Jesudanam et al., in India, studied 448 patients with nail abnormalities; and they reported *Candida* onychomycosis in 58.82 % of a total of 204 cases with positive direct microscopy, culture, or both, with a major prevalence in housewives (33.33 % of the total) [4]. Meanwhile, in Lebanon, Ellabib conducted a study with 500 patients. Yeasts of the genus *Candida* (*C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. guilliermondii*, and *C. tropicalis*) were identified as the dominant agents in women (96 %); in contrast, dermatophytes were predominant in men (80 %) [12].

During a period of 10 years (2003–2012), Afshar et al. conducted a study in Iran with 1100 patients suspected with onychomycosis, and from 464 cultures, *Candida* spp. was isolated in 61.9 % of the cases, as the most common agents of onychomycosis [13].

On the other hand, Segal and colleagues analyzed epidemiologic parameters of onychomycosis in Israel. Data of a cohort of 27,093 patients, which were collected from six centers during a 10-year period, revealed that dermatophytes were the main causative agents of toenail onychomycosis, while *Candida parapsilosis* was the most frequent agent in women fingernails; and its frequency is increased with age [14]. A similar frequency of genera of causative agents was obtained by Seck et al. in Senegal, where dermatophytes predominate, but *Candida albicans* occupied the second position, with 90.86 % of isolated yeasts, and molds were isolated in nine cases (3.02 %) (all cases predominated in toenails) [15]. On the other hand, in a study conducted in Taiwan with 375 patients with onychomycosis, Chi et al. reported the isolation of dermatophytes in 227 patients (60.5 %), *Candida* in 118 (31.5 %), and molds in 30 (8 %) [16].

In Argentina, Nazar and colleagues studied 414 patients with onychopathies, and they reported a prevalence of the toenail and fingernail mycoses of 78 % and 58 %, respectively. The major etiologic agents were *Trichophyton rubrum*, *Candida* spp., and *Trichophyton mentagrophytes* [17].

Diabetic patients have a special predisposition to be affected with fungal infections and *Candida* onychomycosis as Imbert reports in Mexico. In an observational, descriptive, and transversal study made with 261 diabetic patients from public institutions of health of the state of Hidalgo, Mexico, he revealed onychomycosis was caused by *Candida guilliermondii*, *C. parapsilosis*, *C. glabrata*, *Candida* spp., and unidentified molds and yeasts [18].

Papini, in Italy, reported that diabetic patients showed onychomycosis in 53.3 % and foot skin mycosis in 46.7 % of the cases, with a prevalence of both fungal infections significantly higher than that observed in nondiabetic individuals. *Candida* spp., *Fusarium* spp., *Aspergillus* spp., and other molds were found in about 1/3 onychomycosis [19].

With respect to onychomycosis in childhood, in a study conducted in Korea with 59 children, 2.3 % of onychomycosis in general population is seen in children. In toenails, *Trichophyton rubrum* was reported in 51.3 %, followed by *Candida albicans* (10.2 %), *C. parapsilosis* (5.1 %), *C. tropicalis* (2.6 %), and *C. guilliermondii* (2.6 %); but in fingernails, *C. albicans* was the most common isolated pathogen (50.0 %) followed by *T. rubrum* (10.0 %) [20]. In another study, conducted by Gulgum in Turkey with 8122 schoolchildren (aged 5–16 years), culture-positive onychomycosis was detected in 27 cases, and yeasts were isolated in 10/27 cases (37.1 %), with a predominance of *Candida glabrata* (14.8 %) [21].

In a retrospective study conducted in Serbia, in a period from 2011 to 2015, out of 761 patients who underwent clinical and mycological examinations, 137 had *Candida* species isolated from nails. The dominant species was *Candida albicans* (36.59 %), *C. parapsilosis* (23.78 %), *C. krusei* (9.76 %), and *C. guilliermondii* (6.71 %) [22].

## Clinical Features

The organism invades the entire nail plate and may cause other clinical syndromes, including onycholysis and paronychia. These forms occur more commonly in women and often affect the middle finger. It may be related with professional aspects where the repeated contact with water or humidity predisposes to this condition [3, 23]. *Candida* onychomycosis can be divided into three general categories:

1. Infection beginning as an infection of the structures surrounding the nail (paronychia). This is the most common type of *Candida* onychomycosis (Fig. 8.1). It starts as an edematous, reddened pad surrounding the nail plate; later, it can develop a periungual abscess. *Candida* spp. only penetrate the nail plate only secondarily after it has attacked the soft tissue around the nail

**Fig. 8.1** Paronychia

(unlike dermatophytic invasion). After infection of the nail matrix occurs, transverse depressions (Beau's lines) may appear in the nail plate, which becomes convex, rough, and, ultimately, dystrophic [3, 23].

2. *Candida* granuloma, which is more frequent in individuals with chronic mucocutaneous *Candida* infections, accounts for less than 1 % of onychomycosis cases. This condition is seen in immunocompromised patients and involves direct invasion of the nail plate, resulting, in advanced cases, in swelling of the proximal and lateral nail folds until the digit develops a pseudo-clubbing appearance.
3. Finally, *Candida* onycholysis. This form is more frequent in fingernails than toenails, and it occurs when the nail plate has separated from the nail bed. Distal subungual hyperkeratosis can be seen as a yellowish-gray mass that lifts off the nail plate. The lesion resembles that seen in patients with distal subungual onychomycosis [3].

Other clinical findings comprise fungal melanonychia (Fig. 8.2), with a greenish, brownish, or even black nail discoloration [23].

## Diagnostic Clues

The diagnostic route of onychomycosis (caused by dermatophytes, *Candida* species, or other molds or yeasts) includes a complete interrogatory based on the patients' history: a physical examination and microscopy and culture of nail specimens [5].

The clinical presentation of dystrophic nails (with or without discoloration of the nail plate) should alert the clinician to the possibility of onychomycosis; however, it's necessary to use appropriate diagnostic techniques including direct microscopy and fungal culture to ensure correct diagnosis and treatment. The clinical appearance

**Fig. 8.2** Fungal melanonychia due to *Candida*



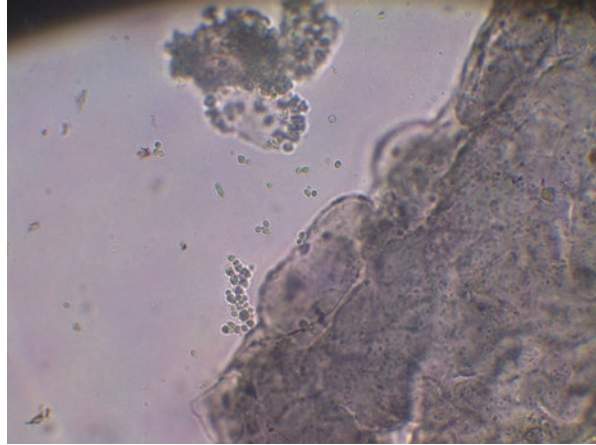
of the nail will help to recognize fungal from nonfungal etiologies of nail dystrophies. A detailed interrogation of patient's history can help to obtain information about predisposing factors for onychomycosis such as diabetes mellitus, older age, hyperhidrosis, professional occupations, hobbies, onychogryphosis, nail trauma, poor peripheral circulation, and immunosuppression [3].

Direct examination of a nail sample with potassium/sodium hydroxide (KOH or NaOH) or Chlorazol Black is the first step to support a clinical diagnosis of onychomycosis. The specimen is obtained by scraping of nail plate in its ventral portion (hyponychium), and it should be divided into two portions for direct microscopy and culture. To identify the causative agent, the laboratory culture of sampled material is necessary to identify the specific etiologic agent.

The specimen can be mounted in a solution of 20–25 % KOH or NaOH mixed with dimethyl sulfoxide and examined, first under  $\times 10$  and after under  $\times 40$  magnification (if a suggestive structure is present) (Fig. 8.3). When the sample is counterstained with chitin-specific Chlorazol Black, fungal structures are accentuated (it takes a dark green or blackish color); this is of particular value if the number of fungal elements is sparse, and it can help to discriminate contaminants such as cotton or elastic fibers, reducing the number of false-positive identifications [3, 23].

It is important to understand the limitations of direct microscopy in diagnosing the cause of onychomycosis, because this technique only serves as a screening test for the presence or absence of fungi, but it could be negative in approximately 15 % of cases, and this technique cannot identify the specific causative agents [3, 23]. Moreover, the sensitivity and specificity of this test are strongly influenced by the clinician's training. When examining a KOH preparation, the presence of round or ovoid spores suggests yeasts, but pseudohyphae or true hyphae could be present too. Almost half of all specimens taken from onychomycotic nails fail to yield a pathogen in culture. Other diagnostic techniques for a direct examination include Gram, Papanicolaou, Wright, or PAS (periodic acid-Schiff) stains or, in case of its availability, fluorescence microscopy with calcofluor white stain.

**Fig. 8.3** Budding yeasts in direct microscopy (Chlorazol Black, 40X)



In onychomycosis, direct microscopy is the most efficient screening technique; however, careful matching of microscopic and culture results is necessary for the clinician to be confident of the diagnosis to avoid fungal resistance and therapeutic fails [3, 23].

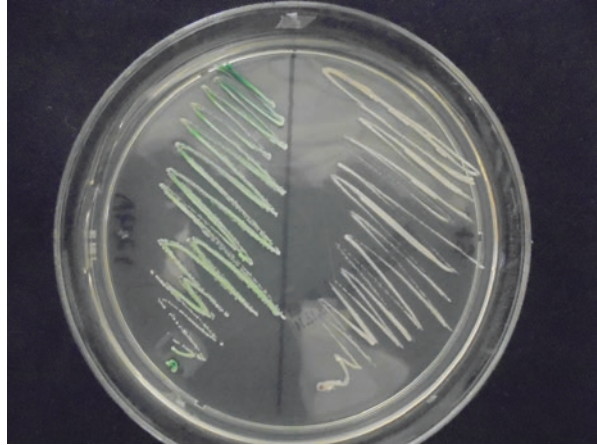
Culture at room temperature (or at 37 ° C) is the only method by which the causative agents can be recognized. Routine culture media such as Sabouraud (with or without chloramphenicol), malt extract, or mycobiotic agar are adequate to obtain the fungal isolates, but cycloheximide inhibits the growth of *Candida parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. zeylanoides*. The inoculation must be done rapidly because fungal and bacterial contaminants may obscure the real nail pathogen. After 28–72 h at 37 ° C, colonies grow. These organisms produce white, smooth, and brilliant colonies with a creamy consistence. The identification of *Candida* species is very important to establish the therapeutic regimen. Some chromogenic culture media have been developed to identify the most frequent *Candida* species. These include CHROMagar®, Biggy® (Fig. 8.4), or Candida-ID® media, and they can identify some species such as *Candida albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* [23].

Other no routine laboratory tests to identify different *Candida* species include serum-induced filamentation (to recognize *C. albicans*), tetrazolium salt assays, and sensitivity to cycloheximide test.

Also, some systematic biochemical tests are available to identify some species, such as API 20C, API 32C, Vitek®, Uni-Yeast Tek®, Minitek®, Yeast-Ident®, and MicroScan®, which are based on the metabolic and physiologic characteristics of this genus of yeasts [23]. Other identification tools such as ViteK MS®, MALDI TOF/TOF®, and mass spectrometers are used to a wide range of fungi including *Candia* spp.

Nowadays, molecular diagnostics are a useful tool to specific identification of particular *Candida* species, and it can be helpful at the moment of deciding the antifungal regime, because some species have an intrinsic drug resistance.

**Fig. 8.4** Greenish colony of *Candida albicans* (CHROMagar® culture medium)



DNA sequencing-based tests are very specific to identify and differentiate among several species of *Candida* and other microorganisms. In some research centers of China, PCR-based assays combined with internal transcribed spacers sequencing such as ITS1-5.8S-ITS2 rDNA regions are performed to reveal the prevalence of *Candida* species including emerging species in onychomycosis [24, 25]. Other assay procedures consisted of PCR amplification of the ITS using universal primers, followed by hybridization of the digoxigenin-labeled amplicons to probes on the array [26]. For example, differentiation of *C. parapsilosis* complex species (*Candida parapsilosis* sensu stricto, *C. metapsilosis*, and *C. orthopsilosis*) is necessary, because *C. parapsilosis* has shown an intrinsic resistance to fluconazole in vitro. One method to help is the amplification of the secondary alcohol dehydrogenase (SADH) gene and digestion by the restriction enzyme Ban I [27].

However, the majority of these laboratory tools have a restricted availability, so a good clinical examination with a correct sampling of nail plate for direct microscopy and culture is still the most useful diagnostic strategy.

### Summary for The Clinician

Onychomycosis encloses all fungal infections of the nail due to filamentous and yeast fungi, accounted for up to 50 % of all nail disorders. It is caused by dermatophytes or non-dermatophytic molds; however, *Candida* species have emerged as second-line pathogens, because *Candida* onychomycosis is increasingly found in individuals having defective immunity consequential to aging, diabetes mellitus, vascular diseases, HIV infection, and drug therapies such as immunosuppressives and broad-spectrum antibiotics. Moreover, numerous factors exist for increasing the prevalence of onychomycosis in modern life such as wearing of shoes especially high-heeled shoes,



high-moisture areas (gymnasium and wrestling mattresses by great numbers of people), use of broad-spectrum antibiotics, and corticosteroid therapy. *Candida* onychomycosis constitutes an important health problem that also can be related with occupational aspects, and this genus of yeasts may be the principal causative agent of fungal nail infections in some regions. The causative species varies around the world, and in some regions, non-*albicans* species have showed an increase of its frequency.

The importance of onychomycosis is often underestimated. In fact, onychomycosis can have significant negative effects on patients' emotional, social, and occupational functioning and can, in addition, consume a sizable proportion of healthcare dollars. Far more than being a simple cosmetic problem, infected nails serve as a chronic reservoir of infection which can give rise to repeated mycotic infections of the skin. In spite of improved personal hygiene and living environment, onychomycosis continues to spread and persist.

### **Clinical Pearls**

It may be related with immunosuppression or professional aspects where the repeated contact with water or humidity predisposes to this condition.

The organism causes three clinical syndromes: melanonychia, paronychia, and onycholysis.

Paronychia is the most common type of *Candida* onychomycosis.

Melanonychia adopts a greenish, brownish, or even black discoloration.

*Candida* granuloma is more frequent in individuals with chronic mucocutaneous *Candida* infections, and it appears as swelling of the proximal and lateral nail folds until the digit develops a pseudo-clubbing appearance.

The clinical presentation of dystrophic nails (with or without discoloration of the nail plate) should alert the clinician to the possibility of onychomycosis; however, it's necessary to use direct microscopy and fungal culture to ensure correct diagnosis and treatment.

## **References**

1. Jayatilake JA, Tilakaratne WM, Panagoda GJ. *Candida* onychomycosis: a mini-review. *Mycopathologia*. 2009;168(4):165–73.
2. Martínez-Herrera EO, Arroyo-Camarena S, Tejada-García DL, Porras-López CF, Arenas R. Onychomycosis due to opportunistic molds. *An Bras Dermatol*. 2015;90(3):334–7.
3. Elewski BE. Onychomycosis: pathogenesis, diagnosis, and management. *Clin Microbiol Rev*. 1998;11(3):415–29.

4. Jesudanam TM, Rao GR, Lakshmi DJ, Kumari GR. Onychomycosis: a significant medical problem. *Indian J Dermatol Venereol Leprol.* 2002;68(6):326–9.
5. Kaur R, Kashyap B, Bhalla P. Onychomycosis—epidemiology, diagnosis and management. *Indian J Med Microbiol.* 2008;26(2):108–16.
6. Mohammadi R, Badiee P, Badali H, Abastabar M, Safa AH, Hadipour M, Yazdani H, Heshmat F. Use of restriction fragment length polymorphism to identify *Candida* species, related to onychomycosis. *Adv Biomed Res.* 2015;4:95.
7. Arrua JM, Rodrigues LA, Pereira FO, Lima EO. Prevalence of *Candida tropicalis* and *Candida krusei* in onychomycosis in João Pessoa, Paraíba, Brazil from 1999 to 2010. *An Acad Bras Cienc.* 2015;87(3):1819–22.
8. Alvarez MI, González LA, Castro LA. Onychomycosis in Cali, Colombia. *Mycopathologia.* 2004;158(2):181–6.
9. Fich F, Abarzúa-Araya A, Pérez M, Nauh Y, León E. *Candida parapsilosis* and *Candida guilliermondii*: emerging pathogens in nail candidiasis. *Indian J Dermatol.* 2014;59(1):24–9.
10. Torres – Guerrero E, del Vasquez ME, Arenas R. Infecciones por *Candida* spp. Datos clínico-epidemiológicos y de tipificación de *Candida* en un hospital de segundo nivel. *Dermatología CMQ.* 2014;12(1):18–23.
11. Soltani M, Khosravi AR, Shokri H, Sharifzadeh A, Balal A. A study of onychomycosis in patients attending a dermatology center in Tehran, Iran. *J Mycol Med.* 2015;25(2):e81–7.
12. Ellabib MS, Agaj M, Khalifa Z, Kavanagh K. Yeasts of the genus *Candida* are the dominant cause of onychomycosis in Libyan women but not men: results of a 2-year surveillance study. *Br J Dermatol.* 2002;146(6):1038–41.
13. Afshar P, Khodavaisy S, Kalhori S, Ghasemi M, Razavyoon T. Onychomycosis in north-East of iran. *Iran J Microbiol.* 2014;6(2):98–103.
14. Segal R, Shemer A, Hochberg M, Keness Y, Shvarzman R, Mandelblat M, Frenkel M, Segal E. Onychomycosis in Israel: epidemiological aspects. *Mycoses.* 2015;58(3):133–9.
15. Seck MC, Ndiaye D, Diongue K, Ndiaye M, Badiane AS, Sow D, Sylla K, Tine R, Ndiaye JL, Faye B, Ndir O. Mycological profile of onychomycosis in Dakar (Senegal). *J Mycol Med.* 2014;24(2):124–8.
16. Chi CC, Wang SH, Chou MC. The causative pathogens of onychomycosis in southern Taiwan. *Mycoses.* 2005;48(6):413–20.
17. Nazar JR, Gerosa PE, Díaz OA. Onychomycoses: epidemiology, causative agents and assessment of diagnostic laboratory methods. *Rev Argent Microbiol.* 2012;44(1):21–5.
18. Imbert JL, G Gomez JV, Escudero RB, Blasco JL. Onychomycosis by yeast not common in diabetics of a health center. *Semergen.* 2015;42(7):449–57 pii: S1138-3593(15)00307-X. doi: [10.1016/j.semereg.2015.08.006](https://doi.org/10.1016/j.semereg.2015.08.006). [Epub ahead of print].
19. Papini M, Cicoletti M, Fabrizi V, Landucci P. Skin and nail mycoses in patients with diabetic foot. *G Ital Dermatol Venereol.* 2013;148(6):603–8.
20. Kim DM, Suh MK, Ha GY. Onychomycosis in children: an experience of 59 cases. *Ann Dermatol.* 2013;25(3):327–34.
21. Gulgun M, Balci E, Karaoglu A, Kesik V, Babacan O, Fidanci MK, Turker T, Tok D, Koc N. Prevalence and risk factors of onychomycosis in primary school children living in rural and urban areas in Central Anatolia of Turkey. *Indian J Dermatol Venereol Leprol.* 2013;79(6):777–82.
22. Otašević S, Barac A, Pekmezovic M, Tasic S, Ignjatović A, Momčilović S, Stojanović P, Arsic Arsenijević V, Hay R. The prevalence of *Candida* onychomycosis in Southeastern Serbia from 2011 to 2015. *Mycoses.* 2016;59(3):167–72.
23. Arenas R. *Micología médica Ilustrada*. 5th ed. México: McGraw Hill Companies; 2014. p. 239–60.
24. Feng X, Ling B, Yang X, Liao W, Pan W, Yao Z. Molecular Identification of *Candida* Species isolated from onychomycosis in Shanghai, China. *Mycopathologia.* 2015;180(5-6):365–71.

25. Galán-Sánchez F, García-Agudo L, García-Martos P, Rodríguez-Iglesias M. *Candida galli* as a cause of tinea unguium-molecular characterization of a rare clinical fungal entity. *Mycopathologia*. 2014;178(3-4):303–6.
26. Han HW, Hsu MM, Choi JS, Hsu CK, Hsieh HY, Li HC, Chang HC, Chang TC. Rapid detection of dermatophytes and *Candida albicans* in onychomycosis specimens by an oligonucleotide array. *BMC Infect Dis*. 2014;14:581.
27. Ataides FS, Costa CR, Souza LK, Fernandes O, Jesuino RS, Silva Mdo R. Molecular identification and antifungal susceptibility profiles of *Candida parapsilosis* complex species isolated from culture collection of clinical samples. *Rev Soc Bras Med Trop*. 2015;48(4):454–9.