



Molecular Identification of *Candida* Species Isolated from Onychomycosis with *In Vitro* Antifungal Susceptibility Profiles

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Abstract

Background: Onychomycosis is one of the most common fungal infections. The most common etiological agents of onychomycosis in Iran are *Candida* species, especially fingernails. It is more common in women than men, particularly workers in occupations requiring them to submerge their hands or feet in water for prolonged periods.

Objectives: The current study's main aim was to determine the abundance of candidal onychomycosis, identify the *Candida* species using molecular methods, and evaluate the *in vitro* antifungal susceptibility profiles.

Methods: One hundred forty samples were obtained from patients suspected of onychomycosis, and 51 (36.4%) *Candida* strains were identified by PCR-RFLP. The *in vitro* susceptibility of four triazole (fluconazole, itraconazole, voriconazole and posaconazole) antifungal drug testing of 51 *Candida* species was performed using broth microdilution.

Results: Direct microscopic examination by KOH 20% of 140 nail samples showed that 51 (36.4%) samples were positive in terms of fungal elements, with *Candida parapsilosis* complex being the most frequently isolated of patients, followed by *C. albicans* complex, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. guilliermondii*, *C. famata*, *C. kefyr*, and *Candida* species. All *Candida* species showed they were susceptible to four triazoles, except that five *C. krusei* were resistant to fluconazole. Only one *C. glabrata* isolates and one *C. parapsilosis* isolate were resistant to fluconazole.

Conclusions: The growing trend towards the frequency of fingernail onychomycosis in housewives has been noticeable in the last decades in Iran. Therefore, accurate identification of *Candida* species and perform *in vitro* antifungal susceptibility testing can aid physicians in choosing an effective potential drug for treating onychomycosis patients.

Keywords: Onychomycosis, PCR-RFLP, *In Vitro* Antifungal Susceptibility

1. Background

Onychomycosis is the most common fungal infection (1). Onychomycosis affects both fingernails and toenails; this complication is caused by both yeasts and molds (2). The etiological agents of onychomycosis differ in different countries, but according to the brief information in this context in Iran, onychomycosis by *Candida* species is more common, especially in fingernail infections (2-7). Up to 50% of all nail disorders are related to onychomycosis (1). The prevalence of onychomycosis is related to various factors such as gender, age, occupation, weather, living and traveling environment, and underlying diseases such as diabetes (1, 8).

Paronychia is a common clinical manifestation in

onychomycosis by yeasts at the proximal and lateral toenail and fingernail folds and is more common in women than in men, particularly workers in occupations that require them to have their hands or feet submerged in water for prolonged periods (e.g., dishwashers); middle-aged females are at the highest risk of onychomycosis (9). Onychomycosis is not life-threatening, but it can cause complications such as secondary bacterial infection, chronicity, changes in the shape and color of the nail, as well as difficulties related to treatment, and it can often cause social and occupational concerns (10, 11). Therefore, rapid diagnosis of onychomycosis and accurate identification of *Candida* species is very important because, in these conditions, management and treatment can be successful.

2. Objectives

In this study, we aimed to determine the abundance of candidal onychomycosis of patients referred to the Fasa medical mycology lab, identifying the *Candida* species using molecular methods and evaluating the *in vitro* antifungal susceptibility profiles via microdilution broth.

3. Methods

3.1. Clinical Sampling

The nails were sampled, including the matrix, bed, or plate that were discolored, thick, fragile, or cracked. In total, 140 clinical specimens were collected from the toenails or fingernails of patients suspected of onychomycosis (age range 3 - 74 years) referring to the medical mycology laboratory of Fasa University of Medical Sciences. The clinical specimens were initially examined by direct microscopic examination (KOH 20%) and then cultured on Sabouraud dextrose agar (SDA, Condalab, Madrid, Spain) supplemented with chloramphenicol and incubated at 35°C for two days. The grown *Candida* species were introductory identified by CHROMagar *Candida* medium (CHROMagar Company, Paris, France) (12).

3.2. Molecular Identification

Afterward, isolated *Candida* species were subjected to molecular identification (PCR-RFLP). Initially, gDNA extraction was done using the glass bead disruption method, then PCR amplification of ITS1-5.8S-ITS2 rDNA regions was performed in a final volume of 25 μ L by using the primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Metabion, Germany) (13, 14). Each reaction consisted of 1 μ L template DNA, 1 μ L of each primer at 10 pmol, 12.5 μ L MasterMix containing 5 mM dNTP, 0.5 U Taq DNA polymerase, and 10X PCR buffer (Ampliqon, Denmark) and 9.5 μ L double-distilled water. PCR conditions were adjusted with cycles of five minutes at 94°C for primary denaturation, followed by 35 cycles at 94°C (30 seconds), 55°C (45 seconds), and 72°C (60 seconds), and final extension at 72°C for seven minutes. Subsequently, PCR products were digested in a final reaction volume of 30 μ L containing 17 μ L water, 2 μ L of 10X FastDigest buffer, and 1 U of FastDigest restriction enzyme MspI (Fermentas, Thermo Fisher Scientific Inc., USA) and 10 μ L PCR product at 37°C for 10 minutes (12, 14).

3.3. In Vitro Susceptibility Testing

The *in vitro* susceptibility of four triazole antifungal drugs testing of 51 *Candida* species was based on broth microdilution method using minimum inhibitory concentrations (MICs) following the M27-A3 and M27-S4

guidelines of the Clinical and Laboratory Standards Institute (CLSI) (15, 16). Voriconazole (VRC), itraconazole (ITC) and posaconazole (POS) (All, Sigma-Aldrich, USA) were dissolved in dimethyl sulfoxide (Sigma, Germany) and fluconazole (FLC) (Sigma-Aldrich, USA) in deionized-distilled water. The antifungal agents were diluted in the standard RPMI-1640 medium (Sigma Chemical Co. Germany) buffered to pH 7.0 with 0.165 M-morpholine propane sulfonic acid (MOPS) (Sigma, Germany) with L-glutamine without bicarbonate to yield two times their concentrations. The buffer was dispensed into 96-well microdilution trays at a final concentration of 0.016 - 16 μ g/mL for ITC, VRC, and POS and 0.063 - 64 μ g/mL for FLC. Briefly, homogeneous yeast suspensions were spectrophotometrically measured at the 530 nm wavelength and a percent transmission within the 75 - 77% range. Therefore, the final densities of the stock inoculum suspensions of the tested isolates ranged between 2.5×10^3 and 5×10^3 colony forming units/mL. The 96-well microplates were incubated at 35°C and examined visually after 24 and 48 h to determine MIC values. The *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were quality control strains (15, 16).

4. Results

Generally, out of 140 nail samples, including 71 fingernails (46 female and 25 male) samples, 31 toenail samples (21 female and 10 male), and 38 samples of both fingernail and toenail (15 female and 4 male) were taken from 82 females (F) and 39 males (M). Direct microscopic examination by KOH 20% of 140 nail samples showed that 51 (36.4%) samples (42 female and 7 male) were positive in terms of fungal elements (yeast & budding and pseudohyphae) (Figure 1); also, the cultures of all 51 (36.4%) samples were positive. Out of 51 positive samples (49 patients), 41 (80.4%) samples (41 patients) were fingernail, 6 (11.8%) toenail samples (6 patients), and 4 (7.8%) finger & toe nail samples (2 patients) (Figure 2).

In this study by molecular methods (Figure 3), *C. parapsilosis* complex (n=14; 27.5%) was the most frequently isolated of patients (F = 12, M = 2), followed by *C. albicans* complex (n=12; 23.5%) (F=11, M=1), *C. glabrata* (n=6; 11.8%), (F=5, M=1), *C. krusei* (n=5; 9.8%) (F=4, M=1), *C. tropicalis* (n=5; 9.8%) (F=4, M=1), *C. guilliermondii* (n=3; 5.9%), (F=3), *C. famata* (n=3; 5.9%) (F=2, M=1), *C. kefyr* (n=2; 3.9%) (F=2) and *Candida* species (probably, *C. rugose*, *C. intermedia* or *C. lusitaniae*) (n=1; 1.9%) (F=1) (Table 1). The results of *in vitro* antifungal susceptibility testing of 51 *Candida* species revealed that all isolates of *C. albicans* complex, *C. tropicalis*, *C. famata*, *C. guilliermondii*, *C. kefyr*, and one *Candida* species were susceptible to four triazoles. Still, out of fourteen *C.*

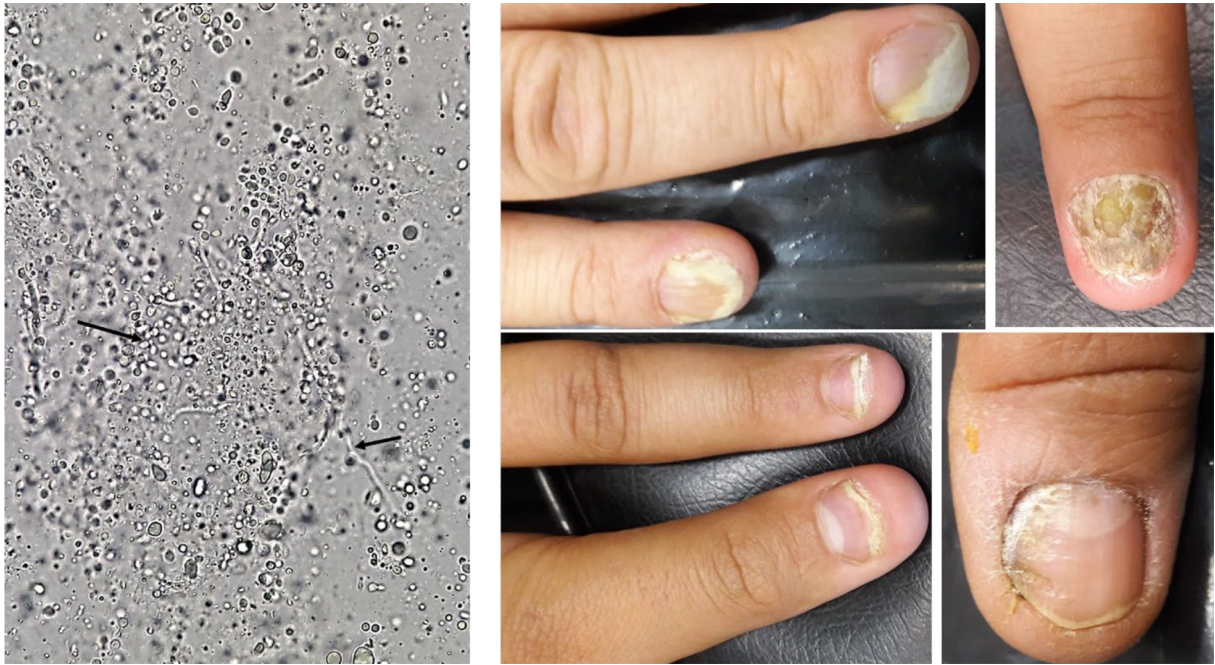


Figure 1. KOH smear ($\times 400$) shows yeast and budding and pseudohyphae in a fingernail sample of a patient with *Candida* onychomycosis (left) and some pictures of *Candida* paronychia and onychomycosis patients (right).

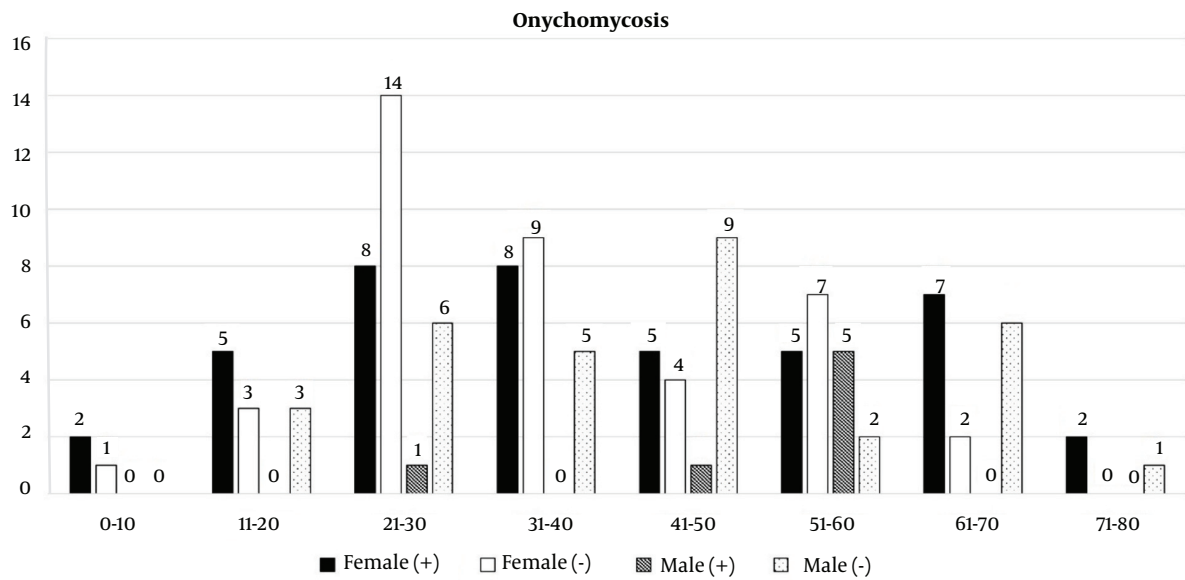


Figure 2. Frequency distribution chart of onychomycosis in patients, based on sex and age range.

parapsilosis complexes, a single isolate was resistant to FLC and susceptible dose-dependent (SDD) to ITC and POS. The six *C. glabrata* isolates were susceptible to VRC and POS, and five of them were susceptible dose-dependent (SDD) to FLC. Still, a *C. glabrata* was resistant to FLC, and two isolates showed dose-dependent (SDD) to ITC. Also, the five *C. krusei* isolates were resistant to FLC and susceptible to VRC, but out of five *C. krusei*, a single isolate was SDD to ITC and POS, and one isolate was resistant to itraconazole (Table 2).

5. Discussion

Onychomycosis occurs worldwide and accounts for up to 50% of nail diseases. Its prevalence varies across geographical areas and is influenced by the weather of the region, age, gender, social and occupational factors, the population of immune-deficient patients, and changes in lifestyle and everyday habits (17-19). The present study evaluated the abundance of candidal onychomycosis in different strata of Fasa, Iran, where 51 cases were recorded with candidal onychomycosis from 121 patients over 6 years (2017 - 2023). In this study, 82 (67.8%) of the patients were female, 22 (26.8%) of them were in the range 21 - 30 years, and 39 (32.2%) were male; of this number, 42 (51%) of females had candidal onychomycosis, while only 7 (18%) of males were positive. The abundance of candidal onychomycosis in different age groups showed that patients aged 51 to 60 (19.6%) and 21 to 30 (17.6%) were the most commonly affected, respectively.

Most epidemiological studies in recent years in Iran have reported onychomycosis "between" 35% to 56% of patients (2-7, 20, 21), which is consistent with the present study (36.4%). In all these studies, candidal onychomycosis was more frequent than onychomycosis by molds. In contrast, in the study by Haghani et al. (2019) (22), onychomycosis was diagnosed in 70% of cases, among which 51.1% were caused by non-dermatophyte moulds (NDMs), 35% by yeast and 10.6% by dermatophytes. Also, more positive cases were reported in females than males, more onychomycosis of fingernail than toenail, and most cases of onychomycosis were reported in the age range of 40 - 60 years (2-7, 20, 21), which is consistent with the present study. Also, in most of these studies, *C. albicans* was the most common cause of onychomycosis, followed by *C. parapsilosis* (2-7, 20, 21). In contrast, in the present study and the study of Pakshir et al. (2015) in Shiraz (3), the most common species was *C. parapsilosis*, followed by *C. albicans*. It is noteworthy that the cities of Fasa and Shiraz are located in the same province (Fars). Therefore, it seems that cases of onychomycosis should be investigated by the mycology laboratory in other cities of Fars province.

In a systematic review study in Iran, Rafat et al. (11) (2019) showed that yeasts were the cause of onychomycosis more than moulds, and females were more affected than males, and, in both genders, the highest infection rate occurred after age 50. In a worldwide review study, Piraccini and Alessandrini reported that onychomycosis was mainly caused by anthropophilic dermatophytes, in particular by *Trichophyton rubrum* and *T. interdigitale*, and yeasts such as *C. albicans* and *C. parapsilosis*; molds such as *Aspergillus* spp., represented the second cause of onychomycosis (1). In the study in Greece by Gregoriou et al. (17), in 27.99% of diagnosed onychomycosis cases, men were infected more often than women, and toenails were infected more than fingernails in both sexes. Also, Bodman and Krishnamurthy (2022) reported that onychomycosis of the toenail was much more prevalent (23).

In a retrospective study in Brazil, Arrua et al. showed that onychomycosis most affected women, which occurred mainly in adults (24). The toenails were the favorite yeast targets, and the prevalent yeasts were *C. tropicalis* and *C. krusei*. In another study in Egypt, Bedaiwy et al. showed that candidal onychomycosis was the 5th most common clinical type, *C. tropicalis* was the most prevalent causative species, fingernails were affected more frequently than toenails, and the infection was more common among females aged 41 to 50 years (mostly housewives) (18).

Today, with the emergence of resistant strains due to using azoles to prevent fungal infections, the *in vitro* antifungal susceptibility testing of *Candida* species for the proper treatment of patients is very important. However, in our study, the results of *in vitro* antifungal susceptibility testing of 51 *Candida* species revealed that all isolates (100%) of *C. albicans* complex, *C. tropicalis*, *C. famata*, *C. guilliermondii*, *C. kefyr*, and one *Candida* species were susceptible to four triazoles. Only one *C. parapsilosis* complex was resistant to FLC and SDD to ITC and POS. However, in some studies, *C. albicans* is demonstrated to be resistant to some antifungal drugs, especially fluconazole, which is widely used to prevent fungal infections (25-28). Different studies showed *in vitro* antifungal activity against various species of *Candida* in Iran. For instance, Aslani et al. in 2019 and 2021, reported that some of *C. albicans* isolates were resistant to FLC (4.3%, 3.5%), ITC (6.5%, 4.7%), VRC (2.1%, 1.1%) and POS (6.5%, 4.7%), and *C. non-albicans* were resistant to FLC (43.4%, 21.7%), ITC (12.9%, 25%) and POS (1.5%, 12.5%) (25, 26).

A study by Shokohi et al. in 2016 showed that 9.1%, 11.3%, and 9.1% of *Candida albicans* isolates were resistant to FLC, ITC, and VRC, respectively (28). Pakshir et al. showed only one isolate (2%) of *C. parapsilosis* was resistant to FLC, and two isolates (4%) were resistant to VRC. In contrast,

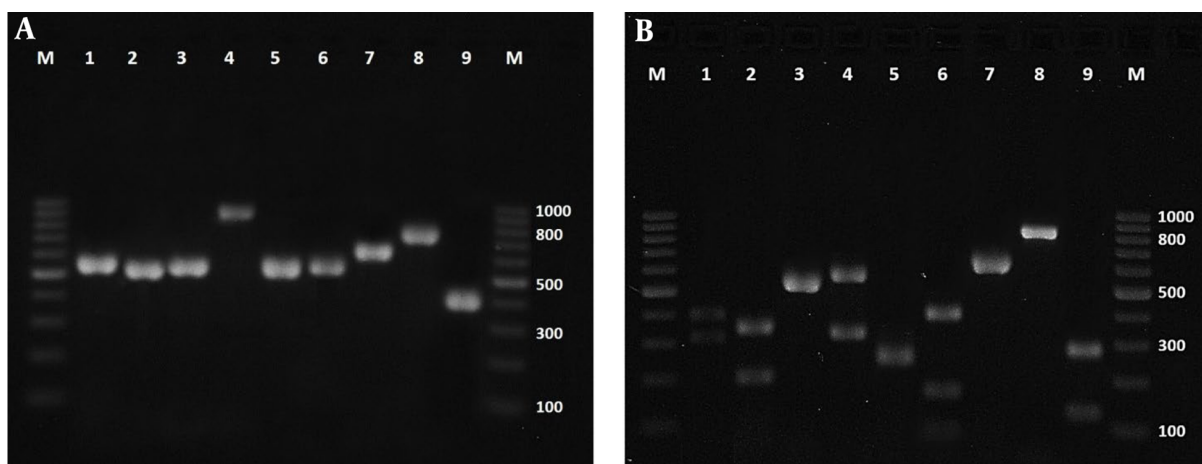


Figure 3. Agarose gel electrophoresis of ITS-PCR products of *Candida* species (left) and PCR-RFLP after digestion with *MspI* (right); lanes 1 - 9: *Candida albicans* complex, *C. tropicalis*, *C. parapsilosis* complex, *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. famata*, *C. kefyr* and *Candida* species (Probably, *C. rugose* or *C. intermedia* or *C. lusitanae*) respectively; Lane M: A 100-bp DNA size marker.

Table 1. Distribution of *Candida* Species in Patients and Size of ITS Region for Isolates in PCR and *MspI* PCR-RFLP

Isolate	Total	Fingernail	Toenail	Female	Male	Size of ITS PCR (bp)	Size of <i>MspI</i> PCR-RFLP (bp)
<i>Candida parapsilosis</i> complex	14	10	4	12	2	510 or 530	510 or 530
<i>Candida albicans</i> complex	12	12	-	11	1	537	239, 298
<i>Candida glabrata</i>	6	6	-	5	1	881	320, 561
<i>Candida krusei</i>	5	5	-	4	1	510	250, 260
<i>Candida tropicalis</i>	5	5	-	4	1	526	186, 340
<i>Candida guilliermondii</i>	3	2	1	3	-	607	82, 155, 370
<i>Candida famata</i>	3	2	1	2	1	639	639
<i>Candida kefyr</i>	2	1	1	2	-	720	720
<i>Candida</i> species	1	-	1	1	-	~390	~120, ~270
Total	51	43	8	44	7		

43% of *C. albicans* isolates and (54%) of *C. tropicalis* were resistant to VRC(3). Fortunately, in this study, most *Candida* isolates were susceptible to antifungal drugs and had low resistance against triazoles. It is noteworthy that in the present study, most referred women were housewives. In fact, it can be argued that women place more importance on beauty than men. At the same time, at the community level, there may be men suffering from onychomycosis but indifferent to it. Undoubtedly, onychomycosis is often regarded as a trivial cosmetic issue. However, its effect on one's quality of life may be underestimated as it can cause significant pain, affecting full mobility and activities. Patients working in moist environments such as dishwashing areas should be encouraged to wear gloves because humidity and maceration can cause onychomycosis by *Candida* species.

5.1. Conclusions

The prevalence of fingernail onychomycosis in Iranian housewives has increased in recent decades. In summary, this study showed that identifying *Candida* species by molecular methods such as PCR-RFLP and *in vitro* antifungal susceptibility tests can aid physicians in choosing effective drugs for treating onychomycosis. Therefore, it is necessary to have specialized medical mycology laboratories in every city.

Footnotes

Authors' Contribution: M.H A. conceived the study; M.H A. and Z. Sh. prepared the strains; M.H. A. and Z. Sh. performed the experiments; M.H A., and Z. Sh. analyzed the data; M.H A. prepared the manuscript and edited the

Table 2. In Vitro Susceptibility Testing of 51 Clinical Isolates of *Candida* Species to Four Antifungal Agents

Strains (No.) and Antifungal Drugs	S	SDD	R	MIC Range ($\mu\text{g/mL}$)
<i>Candida parapsilosis</i> complex (14)				
FLC	13	-	1	0.5 - 8
ITC	13	1	-	0.063 - 0.5
VRC	14	-	-	0.016 - 0.125
POS	13	1	-	0.063 - 0.25
<i>Candida albicans</i> complex (12)				
FLC	12	-	-	0.125 - 2
ITC	12	-	-	0.031 - 0.125
VRC	12	-	-	0.016 - 0.063
POS	12	-	-	0.031 - 0.125
<i>Candida glabrata</i> (6)				
FLC	-	5	1	4 - > 64
ITC	4	2	-	0.5 - 1
VRC	6	-	-	0.125 - 0.25
POS	6	-	-	0.25 - 0.5
<i>Candida krusei</i> (5)				
FLC	-	-	5	16 - 32
ITC	3	1	1	0.25 - 2
VRC	5	-	-	0.016 - 0.25
POS	4	1	-	0.25 - 1
<i>Candida tropicalis</i> (5)				
FLC	5	-	-	0.125 - 0.5
ITC	5	-	-	0.063 - 0.125
VRC	5	-	-	0.016 - 0.031
POS	5	-	-	0.063
<i>Candida guilliermondii</i> (3)				
FLC	3	-	-	0.5 - 1
ITC	3	-	-	0.063 - 0.125
VRC	3	-	-	0.016 - 0.031
POS	3	-	-	0.031 - 0.063
<i>Candida famata</i> (3)				
FLC	3	-	-	0.5 - 1
ITC	3	-	-	0.125 - 0.25
VRC	3	-	-	0.016
POS	3	-	-	0.125
<i>Candida kefyr</i> (2)				
FLC	2	-	-	0.25
ITC	2	-	-	0.125
VRC	2	-	-	0.016 - 0.031
POS	2	-	-	0.063 - 0.125
<i>Candida</i> species (1)				
FLC	1	-	-	0.5
ITC	1	-	-	0.125
VRC	1	-	-	0.016
POS	1	-	-	0.125

Abbreviations: MIC, minimum inhibitory concentration; S, susceptible; SDD, susceptible dose-dependent; R, resistant.

final article. All authors read and approved the final manuscript.

Conflict of Interests: The authors declare there is no conflict of interest.

Data Reproducibility: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This research was approved by the Ethics Committee of the Fasa University of Medical Sciences (ethical code: [IR.FUMS.REC.1397.067](#)), Fasa, Iran. Written informed consent was obtained from all patients.

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Informed Consent: Written informed consent was obtained from all patients.

References

- Piraccini BM, Alessandrini A. Onychomycosis: A review. *J Fungi (Basel)*. 2015;1(1):30–43. [PubMed ID: 29376897]. [PubMed Central ID: PMC5770011]. <https://doi.org/10.3390/jof1010030>.
- Afshar P, Khodavaisy S, Kalhori S, Ghasemi M, Razavyoon T. Onychomycosis in north-East of Iran. *Iran J Microbiol*. 2014;6(2):98–103. [PubMed ID: 25705360]. [PubMed Central ID: PMC4281668].
- Pakshir K, Zomorodian K, Zakaei A, Motamedi M, Rahimi Ghiasi M, Karamitalab M. Molecular identification and in-vitro antifungal susceptibility testing of Candida species isolated from patients with onychomycosis. *Curr Med Mycol*. 2015;1(4):26–32. <https://doi.org/10.18869/acadpub.cmm.1.4.26>.
- Fatahnia M, Jafarpour S, Rafiei A, Taghipour S, Makimura K, Rezaei-Matehkolaei A. Mycological aspects of onychomycosis in khuzestan province, Iran, a new scenario as shift from dermatophytes towards yeasts. *Curr Med Mycol*. 2017;3(4):26–31. <https://doi.org/10.29252/cmm.3.4.26>.
- 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. [PubMed ID: 25952331]. <https://doi.org/10.1016/j.mycmed.2015.03.007>.
- Mohammadi R, Badiee P, Badali H, Abastabar M, Safa AH, Hadipour M, et al. Use of restriction fragment length polymorphism to identify Candida species, related to onychomycosis. *Adv Biomed Res*. 2015;4:95. [PubMed ID: 26015921]. [PubMed Central ID: PMC4434489]. <https://doi.org/10.4103/2277-9175.156659>.
- Chadeganipour M, Nilipour S, Ahmadi G. Study of onychomycosis in Isfahan, Iran. *Mycoses*. 2010;53(2):153–7. [PubMed ID: 19298358]. <https://doi.org/10.1111/j.1439-0507.2008.01679.x>.
- Al-Mutairi N, Eassa BI, Al-Rqobah DA. Clinical and mycologic characteristics of onychomycosis in diabetic patients. *Acta Dermatovenerol Croat*. 2010;18(2):84–91. [PubMed ID: 20624357].
- Rerucha CM, Ewing JT, Oppenlander KE, Cowan WC. Acute hand infections. *Am Fam Physician*. 2019;99(4):228–36. [PubMed ID: 30763047].
- Malhotra S, Sharma S, Kaur N, Jangid K, Hans C. Prevalence of onychomycosis and its etiological agents in a tertiary care hospital in India: A five years study. *Adv Clin Med Microbiol*. 2016;2(6).
- Rafat Z, Hashemi SJ, Saboor-Yaraghi AA, Pouragha B, Taheriniya A, Moosavi A, et al. A systematic review and meta-analysis on the epidemiology, casual agents and demographic characteristics of onychomycosis in Iran. *J Mycol Med*. 2019;29(3):265–72. [PubMed ID: 31285126]. <https://doi.org/10.1016/j.mycmed.2019.05.004>.
- Shokohi T, Moradi N, Badram L, Badali H, Ataollahi MR, Afsarian SMH. Molecular identification of clinically common and uncommon yeast species. *Jundishapur J Microbiol*. 2018;11(10). <https://doi.org/10.5812/jjm.66240>.
- Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. *Nihon Ishinkin Gakkai Zasshi*. 2006;47(3):225–9. [PubMed ID: 16940958]. <https://doi.org/10.3314/jjmm.47.225>.
- Mohammadi R, Mirhendi H, Rezaei-Matehkolaei A, Ghahri M, Shidfar MR, Jalalizand N, et al. Molecular identification and distribution profile of Candida species isolated from Iranian patients. *Med Mycol*. 2013;51(6):657–63. [PubMed ID: 23470036]. <https://doi.org/10.3109/13693786.2013.770603>.
- Rex JH, Alexander BD, Andes D, Arthington-Skaggs B, Brown SD, Chaturvedi V, et al. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard; M27-A3. *Clin Lab Standard Inst*. 2008;28.
- Patrick J, Anderson M, Colard D, DeHoyos DR, Geller J, Kroll MH, et al. Implementation guide of POCTo1 for health care providers; approved guideline. *Clin Lab Standards Inst*. 2006;28(18):1–37.
- Gregoriou S, Mpali N, Vrioni G, Hatzidimitriou E, Chryssou SE, Rigopoulos D. Epidemiology of onychomycosis in an academic nail unit in South Greece during a three-year period. *Skin Appendage Disord*. 2020;6(2):102–7. [PubMed ID: 32258053]. [PubMed Central ID: PMC7109406]. <https://doi.org/10.1159/000504812>.
- Bedaiwy M, Metwally M, Elzawayy N, Saba HA. Epidemiology, causative agents and clinical features of onychomycosis in el-gharbia governorate. *Egypt J Bot*. 2017;0(0):187–96. <https://doi.org/10.21608/ejbo.2017.965.1080>.
- Sigurgeirsson B, Baran R. The prevalence of onychomycosis in the global population: a literature study. *J Eur Acad Dermatol Venereol*. 2014;28(11):1480–91. [PubMed ID: 24283696]. <https://doi.org/10.1111/jdv.12323>.
- Mikaeili A, Karimi I. The incidence of onychomycosis infection among patients referred to hospitals in Kermanshah province, western Iran. *Iran J Public Health*. 2013;42(3):320–5. [PubMed ID: 23641410]. [PubMed Central ID: PMC3633803].
- Hashemi SJ, Gerami M, Zibafar E, Daei M, Moazeni M, Nasrollahi A. Onychomycosis in Tehran: Mycological study of 504 patients. *Mycoses*. 2010;53(3):251–5. [PubMed ID: 19389065]. <https://doi.org/10.1111/j.1439-0507.2009.01703.x>.
- Haghani I, Shams-Ghahfarokhi M, Dalimi Asl A, Shokohi T, Hedayati MT. Molecular identification and antifungal susceptibility of clinical fungal isolates from onychomycosis (uncommon and emerging species). *Mycoses*. 2019;62(2):128–43. [PubMed ID: 30255665]. <https://doi.org/10.1111/myc.12854>.
- Bodman MA, Krishnamurthy K. Onychomycosis. *StatPearls*. Treasure Island (FL); 2022.
- Arrua JM, Rodrigues LA, Pereira FO, Lima EO. Prevalence of Candida tropicalis and Candida krusei in onychomycosis in Joao Pessoa, Paraiba, Brazil from 1999 to 2010. *An Acad Bras Cienc*. 2015;87(3):1819–22. [PubMed ID: 26375021]. <https://doi.org/10.1590/0001-3765201520130418>.
- Aslani N, Shokohi T, Ataollahi MR, Ansari S, Gholampour Y, Khani Jeihooni A, et al. In vitro activity of four triazole antifungal drugs against clinically common and uncommon yeast species. *Curr Med Mycol*. 2019. <https://doi.org/10.18502/cmm.5.4.1949>.
- Aslani N, Kokabi R, Moradi F, Abbasi K, Vaseghi N, Afsarian MH. Characterization of Candida species isolated from vulvovaginal candidiasis by MALDI-TOF with in vitro antifungal susceptibility profiles. *Curr Med Mycol*. 2022. <https://doi.org/10.18502/cmm.7.4.8405>.
- Amirrajab N, Badali H, Didehdar M, Afsarian MH, Mohammadi R, Lotfi N, et al. In vitro activities of six antifungal drugs against candida glabrata isolates: An emerging pathogen. *Jundishapur J Microbiol*. 2016;9(5). e36638. [PubMed ID: 27540459]. [PubMed Central ID: PMC4977912]. <https://doi.org/10.5812/jjm.36638>.
- Shokohi T, Badali H, Amirrajab N, Ataollahi MR, Kouhpayeh SA, Afsarian MH. In vitro activity of five antifungal agents against Candida albicans isolates, Sari, Iran. *Curr Med Mycol*. 2016;2(2):34–9. [PubMed ID: 28681018]. [PubMed Central ID: PMC5490303]. <https://doi.org/10.18869/acadpub.cmm.2.2.8>.