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Molecular identification and antifungal susceptibility profile of rare and emerging yeast species causing onychomycosis

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Abstract

Objectives Onychomycosis is a nail infection caused by various fungal agents. In recent years, there has been an increase in cases of onychomycosis caused by rare fungi, which are often challenging to identify using conventional methods. Sometimes, these unusual species exhibit different antifungal susceptibility patterns compared to more common yeast species. Therefore, this study aimed to identify rare yeast species causing onychomycosis and assess their antifungal susceptibility profiles.

Results Nineteen rare and emerging yeast isolates, representing seven species, including *Candida orthopsilosis* (*n* = 9, 47.36%), *Clavispora lusitaniae* (formerly *Candida lusitaniae*) (*n* = 2, 10.52%), *Wickerhamiella pararugosa* (formerly *Candida pararugosa*) (*n* = 2, 10.52%), *Naganishia diffluens* (formerly *Cryptococcus diffluens*) (*n* = 2, 10.52%), *Wickerhamomyces anomalus* (*n* = 2, 10.52%), *Cyberlindnera fabianii* (*n* = 1, 5.26%), and *Meyerozyma caribbica* (formerly *Candida fermentati*) (*n* = 1, 5.26%), were identified. Most rare yeast agents exhibited high minimum inhibitory concentration (MIC) values for fluconazole.

Keywords Onychomycosis, Rare yeast, *Candida*, *Naganishia*, *Wickerhamomyces*, Antifungal susceptibility

Introduction

Fungal nail infections, commonly known as onychomycosis, are caused by various fungi, such as dermatophytes, yeasts, and non-dermatophyte molds. The prevalence of the disease worldwide is about 2–30% [1]. Onychomycosis accounts for 50% of all nail disorders and 30% of superficial fungal infections [2, 3]. Among yeasts, *Candida* species are the most common etiological agents of onychomycosis. However, recent studies have highlighted the role of uncommon yeast species in causing this condition [4]. A rare yeast is an infrequent species that rarely causes infections. While less frequently encountered in onychomycosis than common pathogens like *Candida albicans* or dermatophytes, these yeasts can still lead to nail infections with varying treatment responses [5].

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Recent reports indicate a rise in rare yeast infections, highlighting their emerging role as potential pathogens in onychomycosis [6–8].

Accurate identification of the etiologic agent of onychomycosis is essential for therapy management, as the choice of antifungal therapy for nail infection depends on the causative agent. Conventional methods, such as direct microscopic examination and culture, are commonly used in the diagnosis of onychomycosis; however, these methods are not precise in distinguishing between yeast genera/species [8, 9].

Polymerase chain reaction (PCR) assays are highly effective due to their specificity and sensitivity, significantly advancing our understanding of the fungal diversity associated with onychomycosis [10–12]. Several PCR-based methods have been developed to identify fungi, including nested PCR [13, 14], PCR-RFLP [15], probe-based and SYBR green-based quantitative PCR [16], and PCR sequencing [17–20]. While some in-house PCR methods are used for fungal identification, their ability to identify fungi is limited to a few species. Although methods like PCR-RFLP, PCR-FSP, and multiplex PCR have significant advantages over conventional mycological methods, they do not encompass all species. They are limited in identifying fungi according to their defined algorithms. In contrast, PCR sequencing has proven to be the most effective approach, offering superior capability in identifying yeast species. This method allows for precise identification and differentiation of yeast by comparing specific genomic regions, such as the internal transcribed spacer (ITS) region, to reference sequences [21, 22]. The ITS1 and ITS2 regions are considered as fungal barcodes due to their high degree of variation among fungi. Those regions are widely used for species and intra-species yeast identification too [11].

As mentioned before, effective treatment of onychomycosis relies on accurate fungal identification and the susceptibility of the causative species to antifungal agents. The most commonly prescribed antifungal treatments for yeast-induced onychomycosis are triazoles, such as fluconazole and itraconazole. However, treatment failure rates with azoles range from 25 to 40%, primarily due to the acquired or inherent resistance of yeasts to these drugs. The incorrect identification of the causative organism, the emergence of new fungal species resistant to conventional antifungal drugs, the poor drug penetration into the nail, and choosing an inappropriate antifungal against the yeast involved are the most important reasons for those failures. On the other hand, the side effects and impact of failed antifungal treatment should be considered [10, 23]. The aim of this study was to identify rare yeast species causing onychomycosis by PCR sequencing and to determine their antifungal susceptibility profiles.

Materials and methods

Yeast isolates

In this study, 19 yeast isolates out of a total of 105 isolates recovered from patients with onychomycosis referred to Dr. Kharazi's medical mycology laboratory in Shiraz, Iran, from June 2019 to March 2022 were analyzed. The nail specimens were cultured on Sabouraud dextrose agar (SDA; Merck, Germany) supplemented with chloramphenicol (0.05 mg/ml) and incubated at 35 °C for 24–72 h. Out of all the yeast isolates, 86 isolates were successfully identified using molecular methods, including the multiplex PCR and PCR-restriction fragment length polymorphism (RFLP), and the results were presented previously [24]. Therefore, this study builds upon previous research by including yeast isolates that were not definitively identified at the species level by applied molecular methods.

DNA extraction

At first, to obtain fresh colonies, the yeast isolates were cultured on SDA and incubated at 35 °C for 48 h, followed by DNA extraction using the precipitation method with saturated lithium acetate [25]. Briefly, one microbiological loop from colonies was transferred to a 1.5-ml tube containing 300 µl of DNA lysis buffer (100 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.5% w/v SDS, and 100 mM NaCl) and placed in boiling water for 15 min. Then 100 µl of lithium acetate (2.5 M) were added and kept at -20 °C for 60 min. After centrifugation for 5 min at 12,000 rpm, the supernatant was transferred to a new tube. An equal volume of cold isopropanol was added to the supernatant and kept at -20 °C for 30 min. After centrifuging at 10,000 rpm for 15 min, the supernatant was removed, and 300 µl of cold 70% ethanol was added to the sediment and centrifuged at 12,000 rpm for 5 min. In the final step, the tubes were air-dried upside down for 30 min at room temperature. Finally, 50 µl of double-distilled sterile water (DDW) was added to the pellet, and the solution was used as a DNA template. The quantity and quality of the extracted DNA were checked by determining the A260/A280 ratio using a NanoDrop 2000 spectrophotometer and electrophoresis of the PCR product on a 1% agarose gel, respectively.

Molecular identification of yeasts

Yeast identification at the species level was performed by the PCR sequencing method as described previously [25]. The ITS1–5.8S–ITS2 region of rDNA was amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [26]. The PCR reactions contained 20 µl of 2X premix (Ampliqon, Denmark), 1.5 µl of extracted DNA, and 25 µM of each primer, and the final volume was adjusted to 40 µl with DDW. The PCR thermal conditions

were as follows: an initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 45 s, and extension at 72 °C for 45 s, with a final extension step at 72 °C for 5 min. The PCR products were then sequenced using an automated DNA sequencer (ABI Prism 3730 Genetic Analyzer; Applied Biosystems). The reliability of the DNA sequences was checked using Chromas software version 2.6.6. To identify species, the sequences were subjected to nucleotide BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with relevant, dependable reference sequences deposited in the GenBank. Finally, the nucleotide sequences were submitted to GenBank.

Antifungal susceptibility testing

Minimum inhibitory concentration (MIC) values of five imidazole antifungals, including clotrimazole, sertaconazole, tioconazole, fenticonazole, and isoconazole, as well as two triazoles, fluconazole and itraconazole (Sigma, St. Louis, MO, USA), were determined using the broth microdilution method according to the M27-A3/S4 protocol documented by the Clinical and Laboratory Standards Institute (CLSI) [27]. In addition, the susceptibility of isolates to nystatin (Sigma, Germany) was determined. Briefly, RPMI 1640 (Sigma, St. Louis, Missouri, USA) buffered to pH 7.0 using 0.165 N-morpholinopropanesulfonic acid (MOPS) (Sigma, USA) was used to perform susceptibility tests. The concentration ranges of the tested antifungal agents were as follows: 0.03–16 µg/ml for itraconazole, sertaconazole, tioconazole, isoconazole, clotrimazole, and nystatin; 0.12–64 µg/ml for fluconazole; and 0.06–32 µg/ml for fenticonazole.

The lowest concentrations of antifungals that resulted in a 50% reduction in fungal growth after 24 to 48 h of incubation, compared to control wells (wells without antifungal agents), were considered the MIC values for azoles. Additionally, the lowest concentration that achieved no visible growth of isolates (100% inhibition) was regarded as the MIC for nystatin. Quality control included *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019). The breakpoints defined for *Candida parapsilosis* were applied to *Candida orthopsilosis* [27, 28]. Furthermore, the wild-type phenotypes of *Candida orthopsilosis* and *Clavispora lusitaniae* were determined as described by Pfaller et al. [29].

Table 1 Frequency of rare and emerging yeasts causing onychomycosis identified by PCR sequencing

Yeast species	Frequency No. (%)	GenBank accession number	E-value	Percent identity (%)	Rare and emerging classification (Ref.)
Candida orthopsilosis (*n* = 9)	9 (47.36)	OR734352	0.0	99.15	Emerging* species [7, 30]
		OR734353	0.0	100	
		OR734354	0.0	99.37	
		OR734355	0.0	100	
		OR734356	0.0	99.79	
		OR734357	0.0	100	
		OR734358	0.0	99.79	
		OR734359	0.0	99.36	
		OR734360	0.0	99.36	
Clavispora lusitaniae (*n* = 2)	2 (10.52)	OR734347	1e-175	99.14	Rare# species [26, 31]
		OR734348	1e-175	98.86	
Wickerhamiella pararugosa (*n* = 2)	2 (10.52)	OR734349	0.0	99.48	Emerging species [7]
		OR734350	0.0	98.19	
Naganishia diffluens (*n* = 2)	2 (10.52)	OR734345	0.0	100	Emerging species [10]
		OR734346	0.0	99.33	
Wickerhamomyces anomalus (*n* = 2)	2 (10.52)	OR734361	0.0	98.29	Emerging species [5, 10]
		OR734362	0.0	99.83	
Cyberlindnera fabianii (*n* = 1)	1 (5.26)	OR734344	0.0	99.16	Emerging species [32, 33]
Meyerozyma caribbica (*n* = 1)	1 (5.26)	OR734351	0.0	99.65	Rare species [34]

* Emerging means that these yeast species have been added to the fungal agents causing onychomycosis in recent years

\# Rare means that onychomycosis caused by these yeast species has a low incidence/prevalence

Results

Nineteen rare and emerging yeast isolates, representing seven species, including *Candida* (*C.*) *orthopsilosis* (*n* = 9, 47.36%), *Clavispora* (*Cl.*) *lusitaniae* (*n* = 2, 10.52%), *Wickerhamiella* (*Wi.*) *pararugosa* (*n* = 2, 10.52%), *Naganishia* (*N.*) *diffluens* (*n* = 2, 10.52%), *Wickerhamomyces* (*W.*) *anomalus* (*n* = 2, 10.52%), *Cyberlindnera* (*Cy.*) *fabianii* (*n* = 1, 5.26%), and *Meyerozyma* (*M.*) *caribbica* (*n* = 1, 5.26%), were identified. All yeast ITS sequences were assigned an accession number (OR734344–OR734362). The frequency of rare and emerging yeasts identified in this study is shown in Table 1. Clinical forms of onychomycosis caused by the rare and emerging yeast species are shown in Fig. 1.

The results of antifungal susceptibility testing are summarized in Table 2. Based on the MICs, all *C. orthopsilosis* isolates were classified as wild-type for fluconazole (MIC ≤ 2 µg/ml). Following the breakpoints defined for *C. parapsilosis*, all *C. orthopsilosis* strains were considered susceptible to both fluconazole and itraconazole. Furthermore, two *Cl. lusitaniae* strains in this study were identified as wild-type strains for itraconazole (MIC ≤ 0.5 µg/ml), while one of these strains was classified as a non-wild-type phenotype for fluconazole (MIC > 2 µg/ml). Among the tested species, *Wi. pararugosa* exhibited the lowest MIC for fluconazole compared to other species.

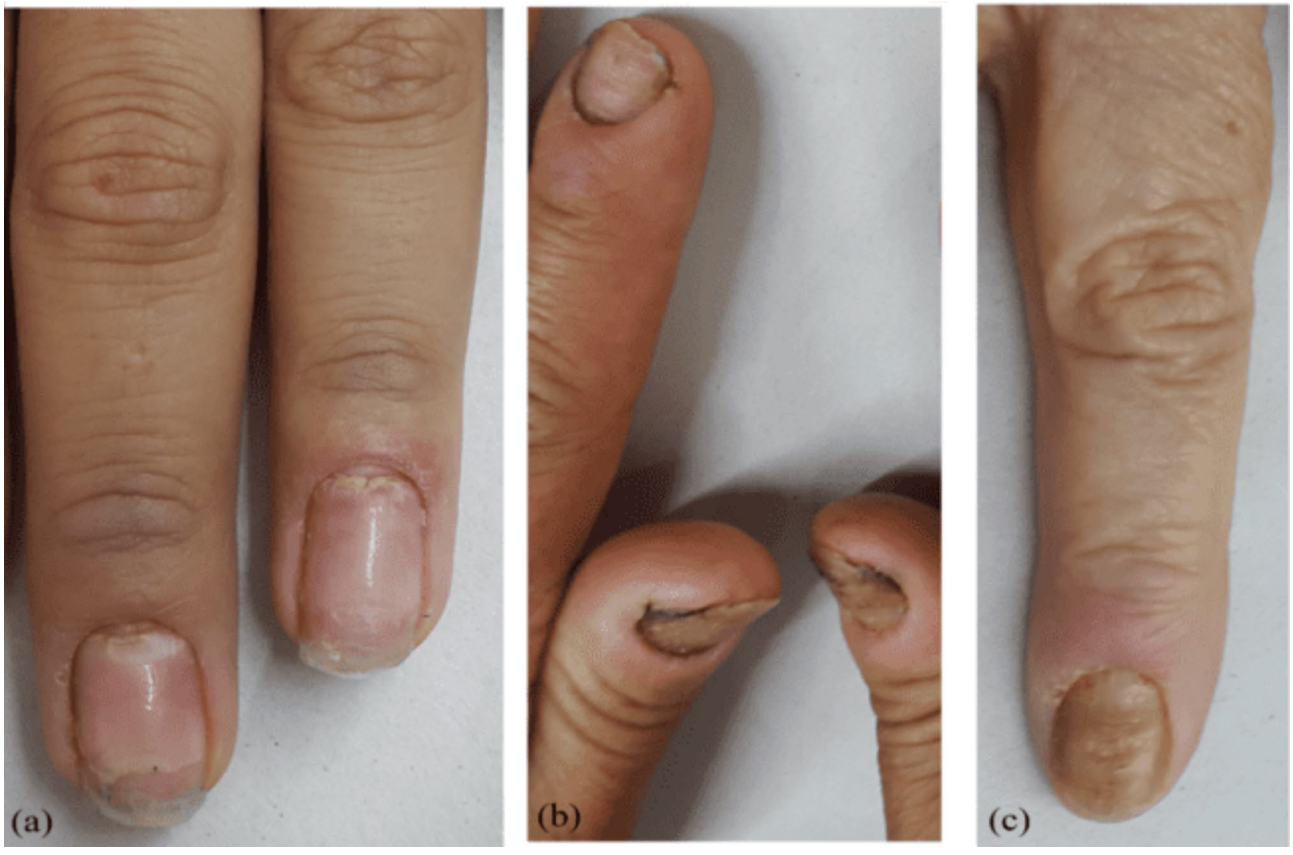

Fig. 1 Nails with onychomycosis caused by rare and emerging yeast species. (**a**) Proximal onychomycosis and onycholysis. (**b**) Longitudinal melanonychia with blackish discoloration. The pigmented line is visible in the lateral nail folds. (**c**) Paronychia and onychodystrophy

The MIC values for *N. diffluens* ranged from 4 to 8 µg/ml for fluconazole, while the MICs for other azole antifungals were ≤2 µg/ml. *W. anomalus* showed MIC values of 2–4 µg/ml for fluconazole and ≤0.5 µg/ml for other azoles. For nystatin, the MIC range across all isolates varied from 2 to 8 µg/ml. With the exception of *M. caribbica*, the remaining isolates displayed low MIC values for sertaconazole, tioconazole, fenticonazole, and isoconazole.

Discussion

The increasing incidence of infections caused by rare yeast species in onychomycosis highlights a significant clinical concern that demands attention. Recent studies indicate that *Candida* species, especially *C. albicans*, are the most common agents of onychomycosis. While less prevalent agents are emerging as important etiological agents of onychomycosis, they pose therapeutic challenges, making their study and identification essential [5, 6].

Our findings revealed that the incidence of onychomycosis caused by rare and emerging yeast strains is relatively high, with 19 (18.09%) isolates among the 105 yeast isolates. Although rare yeasts are important causative agents for onychomycosis, they are neglected because of the use of conventional methods in most mycology laboratories. Using those methods, identification of yeast agents, especially cryptic isolates, is not possible at the species level in most cases [35]. Rapid and accurate identification of the etiological agent of onychomycosis using reliable and specific methods not only allows for appropriate antifungal therapy management but also improves our epidemiologic knowledge of onychomycosis etiology. Furthermore, rare fungal agents might exhibit different antifungal susceptibility patterns compared to more common causative agents. Evaluating the susceptibility profiles of rare fungal species is crucial to guiding appropriate therapeutic interventions.

Molecular methods such as PCR sequencing provide accurate and correct identification of yeast isolates causing onychomycosis at the species level, which is essential for selecting an appropriate therapeutic strategy and preventing severe nail damage [36]. Some studies used the PCR sequencing method to identify the rare and emerging yeast species causing onychomycosis [10, 26, 34]. On the other hand, several studies used PCR-RFLP [35–37], PCR-FSP [38], and real-time PCR [39] to identify yeast species causing onychomycosis. Although these

Table 2 In-vitro susceptibility of rare and emerging yeasts isolated from onychomycosis to antifungal agents

Yeast species	Antifungal	Range	GM	MIC* (µg/mL)								
				0.03	0.06	0.12	0.25	0.5	1	2	4	8
Candida orthopsilosis (*n* = 9)	Nystatin	2–8	3.17							4	4	1
	Fluconazole	0.25–0.5	0.46				1	8				
	Clotrimazole	0.03–0.12	0.04	6	2	1						
	Itraconazole	0.03–0.12	0.04	5	2	2						
	Sertaconazole	0.03–0.25	0.16	1	1	1	5	1				
	Tioconazole	0.06–0.5	0.08		7	1		1				
	Fenticonazole	0.12-2	0.28			2	5	1		1		
	Isoconazole	0.03–0.25	0.07	3	2	2	2					
Clavispora lusitaniae (*n* = 2)	Nystatin	4	4								2	
	Fluconazole	0.5-8	2					1				1
	Clotrimazole	0.03–0.25	0.08	1			1					
	Itraconazole	0.06–0.25	0.12		1		1					
	Sertaconazole	0.12–0.25	0.17			1	1					
	Tioconazole	0.03–0.12	0.06	1		1						
	Fenticonazole	0.25	0.25				2					
	Isoconazole	0.03–0.25	0.08	1			1					
Wickerhamiella pararugosa (*n* = 2)	Nystatin	4	4								2	
	Fluconazole	0.25	0.25				2					
	Clotrimazole	0.03	0.03	2								
	Itraconazole	0.03–0.06	0.04	1	1							
	Sertaconazole	0.03–0.06	0.04	1	1							
	Tioconazole	0.03	0.03	2								
	Fenticonazole	0.12	0.12			2						
	Isoconazole	0.03–0.12	0.06	1		1						
Naganishia diffluens (*n* = 2)	Nystatin	4	4								2	
	Fluconazole	4–8	5.65								1	1
	Clotrimazole	0.5-2	1					1		1		
	Itraconazole	0.03–0.06	0.04	1	1							
	Sertaconazole	0.12–0.25	0.17			1	1					
	Tioconazole	0.25-1	0.5				1		1			
	Fenticonazole	0.06	0.06		2							
	Isoconazole	0.03–0.06	0.04	1	1							
Wickerhamomyces anomalus (*n* = 2)	Nystatin	1–4	2						1		1	
	Fluconazole	2–4	2.82							1	1	
	Clotrimazole	0.25–0.5	0.35				1	1				
	Itraconazole	0.06–0.25	0.12		1		1					
	Sertaconazole	0.12–0.5	0.24			1		1				
	Tioconazole	0.06	0.06		2							
	Fenticonazole	0.12–0.5	0.24			1		1				
	Isoconazole	0.06–0.25	0.12		1		1					
Cyberlindnera fabianii (*n* = 1)	Nystatin	4	-								1	
	Fluconazole	2	-							1		
	Clotrimazole	0.12	-			1						
	Itraconazole	0.25	-				1					
	Sertaconazole	0.12	-			1						
	Tioconazole	0.06	-		1							
	Fenticonazole	0.12	-			1						
	Isoconazole	0.25	-				1					

Table 2 (continued)

Yeast species	Antifungal	Range	GM	MIC* (µg/mL)								
				0.03	0.06	0.12	0.25	0.5	1	2	4	8
Meyerozyma caribbica (*n* = 1)	Nystatin	8	-									1
	Fluconazole	4	-								1	
	Clotrimazole	0.12	-			1						
	Itraconazole	1	-						1			
	Sertaconazole	1	-						1			
	Tioconazole	1	-						1			
	Fenticonazole	2	-						1			
	Isoconazole	1	-						1			

GM: Geometric Mean, MIC: Minimum Inhibitory Concentration

*MIC determination (visual): ≥ 50% growth inhibition (azoles) or 100% inhibition (nystatin) vs. drug-free control

methods have been used to identify common yeast species, they are unable to identify rare and emerging species [40]. In our previous study [24], multiplex PCR and PCR-RFLP methods were used, but these methods were unable to identify rare and uncommon yeast agents. In the current report, following the use of PCR sequencing method, unknown yeast species in our previous study were identified.

Although *C. albicans* [41–43], followed by *C. parapsilosis* [3, 44, 45], are the most prevalent *Candida* species isolated and identified from onychomycosis using PCR-based methods, non-sequencing molecular approaches cannot distinguish and name the *sensu stricto* species in each complex. As a result, certain species, such as *C. orthopsilosis*, have been overlooked in prior reports because of their proximity to the complexes dominating spices. But recently, rare and less-prevalent *Candida* species, including *C. orthopsilosis* and *Wi. pararugosa*, were isolated and reported from nail specimens and proposed as emerging yeast agents causing onychomycosis [7]. The findings of the present study indicate that *C. orthopsilosis* is an important species of nail infection. Although onychomycosis caused by *C. orthopsilosis* has been documented in some studies (12, 24, 25, 27).

Cl. lusitaniae is an opportunistic yeast that commonly affects immunocompromised patients and can cause systemic infections. Some strains of this species are resistant to many antifungal drugs, such as amphotericin B, fluconazole, and 5-fluorocytosine. So far, *Cl. lusitaniae* has been reported as the etiological cause of candidemia, vaginitis, and peritonitis in humans [46]. Similarly to our study, in various reports [3, 47, 48], *Cl. lusitaniae* has been one of the causative agents of onychomycosis. Its increasing presence should be considered alarming.

Wi. pararugosa is considered a rare pathogen of the bloodstream, oral cavity, and onychomycosis. This yeast has been isolated from dairy products, which is probably the route of transmission to humans [49]. *Wi. pararugosa* is mentioned as a potential probiotic that resists the toxic effects of bile and gastric secretions [50]. There have been reports of bloodstream infections caused by this yeast [50, 51]. In line with the results obtained by Feng et al. [7], in our study, *Wi. pararugosa* was one of the emerging yeast strains isolated from the nail specimens. Increased cases and reports of this yeast as a causal agent for onychomycosis show an increasing potential for this yeast to infect nail tissue, particularly in those who work with dairy products or come into contact with the environment.

Some *Naganishia* (*Cryptococcus*) species, such as *N. albida* and *N. diffluens*, have been reported as emerging pathogens of onychomycosis [8]. There is a report of a case of subcutaneous cryptococcosis caused by *N. diffluens*, and this yeast species was isolated from the skin of healthy individuals and atopic dermatitis patients too [52]. In the present study, two cases of onychomycosis caused by *N. diffluens* were observed. Similarly, Haghani et al. [10] have isolated this yeast from a patient with onychomycosis.

W. anomalus is widely found in the environment and rarely isolated from clinical specimens. This yeast agent is considered a biological control agent due to its antimicrobial properties [6]. However, in some literature, infections caused by *W. anomalus*, such as meningitis and nosocomial bloodstream infections, have been reported, especially in neonates and pediatrics. In addition, *W. anomalus* is one of the most important emerging yeast agents with high clinical implications [5]. In accordance with our findings, in some studies [10, 48], *W. anomalus* has been reported as the causative agent of onychomycosis.

Cy. fabianii is another rare yeast with low pathogenicity that has been reported in some fungemia cases [6, 53, 54].

M. caribbica has often been considered in the food industry due to its fermentability. Human infections caused by this yeast agent are rare. However, cases of fungemia, vaginitis, and keratitis due to *M. caribbica* have been reported [34]. Similar to the present study, Montoya et al. [34] reported a case of onychomycosis

caused by *M. caribbica*. To our knowledge, this is the first report of *M. caribbica* as an etiological agent of onychomycosis in Iran.

Importantly, rare fungal agents might exhibit different susceptibility profiles to antifungals compared to more common causative agents. Therefore, understanding the susceptibility patterns of rare species is essential for developing effective therapeutic strategies against these clinically significant fungal agents [36]. For instance, the MIC range for *C. orthopsilosis* has shown variability across different studies. Our findings indicate that the MIC range for *C. orthopsilosis* is 0.25–0.5 µg/mL for fluconazole, which aligns with other studies conducted in Iran [36, 55]. However, some studies have reported higher MIC values for this species [56]. Although *C. parapsilosis* and *C. orthopsilosis* belong to the same complex, the MIC ranges for antifungal agents can differ between the two species, as indicated in various studies. The study by Borman et al. reported MIC values ranging from 0.12 to 2 µg/mL for most *Cl. lusitaniae* isolates, with some exhibiting MIC values of ≥ 4 µg/mL [57]. Additionally, the MIC range for itraconazole against *Cl. lusitaniae* was found to be 0.016–0.25 µg/mL, which is consistent with our results. These findings further support the excellent activity of triazoles against *Cl. lusitaniae*, as all isolates demonstrated high susceptibility to both fluconazole and itraconazole. In another study, Borman et al. also noted that the highest frequency of *N. diffluens* isolates exhibited a fluconazole MIC range of 8–16 µg/mL, aligning with our findings. However, the MIC values for *Wi. pararugosa* in our study were lower than those reported by Borman et al. [58]. Overall, rare *Candida* species demonstrated higher MICs for azole drugs, including fluconazole and itraconazole, compared to more common species. These findings underscore the critical importance of accurately identifying yeast species and determining their MIC values to ensure the appropriate selection of antifungal therapy.

Limitations

A limitation of this study is that nail specimens were collected from only one medical mycology center. Therefore, the conclusions might not be generalizable to the increasing prevalence of onychomycosis caused by rare and emerging yeast species in different geographical regions.

Conclusion

In this study, a broader range of rare yeast strains was identified as etiologic agents of onychomycosis. To identify rare yeasts, reliable and standard reference methods, such as PCR sequencing, should be employed. Conventional methods and certain molecular methods, including PCR-RFLP and PCR-FSP, are insufficient for the precise identification of rare yeast species. Rare fungal species causing onychomycosis exhibit distinct antifungal susceptibility profiles, necessitating tailored treatment approaches. These findings underscore the importance of species-level identification and in-vitro susceptibility testing to guide therapy, particularly for infections caused by rare yeasts.

Abbreviations

C.	*Candida*
Cl.	*Clavispora*
Wi.	*Wickerhamiella*
N.	*Naganishia*
W.	*Wickerhamomyces*
Cy.	*Cyberlindnera*
M.	*Meyerozyma*
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
ITS	Internal transcribed spacer
SDA	Sabouraud dextrose agar
EDTA	Ethylenediaminetetraacetic acid
SDS	Sodium dodecyl sulfate
DDW	Double-distilled sterile water
MIC	Minimal inhibitory concentration
CLSI	Clinical and laboratory standards institute
MOPS	Morpholinopropanesulfonic acid
GM	Geometric mean

Author contributions

A.J: Investigation, Methodology, Writing-Original draft preparation, and Conceptualization. E.E, A.G, M.J, and M.K: Investigation. M.M: Investigation and Methodology. S.Y: Methodology. H.K: Project administration, Conceptualization, Reviewing, Editing, and Validation.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. The data that support the findings of this study, including accession numbers (OR734344-62) and nucleotide sequences, are openly available in the NCBI GenBank database at https://www.ncbi.nlm.nih.gov/genbank/.

Declarations

Ethics approval and consent to participate

This study was performed in line with the principles of the national norms and standards for conducting medical research in Iran and in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html). The study has been approved by the Research Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1402.069). Written informed consent forms were obtained from all patients for participation in all stages of the study, including the use of clinical nail specimens and the imaging of infected nails. In the case of minors, consent was obtained from parents or legal guardians after being informed of participation in all stages of the study, including the use of nail specimens and photography of nails.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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