Case Report

Coinfection of Toenail Onychomycosis Caused by *Rhodotorula mucilaginosa* and *Candida glabrata* in an Immunocompromised Adult: A Case Report and Literature Review

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Abstract

Background: *Rhodotorula mucilaginosa* and *Candida glabrata* have emerged as potential pathogens, particularly in immunosuppressed hosts. This study aimed to present a case of coinfection of *Candida glabrata* and *Rhodotorula mucilaginosa* in a 35-year-old immunosuppressed female with onychomycosis on the first and second left toenails.

Cases Report: Causative agents were identified according to morphology, microscopic studies, culture, and DNA molecular analysis. *Candida glabrata* demonstrated high minimum inhibitory concentrations against the tested antifungals except itraconazole. Moreover, *Rhodotorula mucilaginosa* had shown low minimum inhibitory concentrations against clotrimazole and ketoconazole at a dilution of 0.25 µg/ml. Itraconazole is administered at 200 mg twice daily for one week for toenails and as pulse treatment (for one week a month) at 5 mg/kg daily with topical clotrimazole.

Conclusion: Clinical improvement was noted in the patient's clinical examination after ten months. Information about the increasing resistance to antifungal agents helps decide antifungal prophylaxis and select the empirical therapy for cancer patients.

Keywords: Onychomycosis, *Rhodotorula mucilaginosa*, *Candida glabrata*, Coinfection, Drug resistance, Antifungal agents

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Introduction

Onychomycosis is a fungal nail infection with crumbly thickening, onycholysis, splitting, subungual hyperkeratosis, roughening, white patches, and discoloration[1,2]. This fungal infection is a significant health problem and described by extreme chronicity and difficulty in treatment[3]. It is the most frequent nail disease among adults, affecting nearly 8% of individuals referred to general medical and dermatology clinics[4].

Natural barriers in the nails usually prevent fungal

infection development. However, these protective mechanisms can be affected by several factors. Aging, humidity, nail deformities, occlusive footwear, repeated nail trauma, onychodystrophy, vascular disease, diabetes mellitus, HIV infection, genetic predisposition, immunosuppressive drugs, broad-spectrum antibiotics, and immunosuppression should be considered as predisposing factors for onychomycosis[2,5-7].

Onychomycosis tends to be caused by dermatophytes, non-dermatophyte molds, and yeasts [7]. *Candida spp*. are the most prevalent etiological yeasts[8]. *Rhodotorula*, another yeast pathogen, is recently reported as the causative agent of this infection[3]. Onychomycosis caused by environmental yeasts is an uncommon infection. It is hard to eradicate due to its resistance to common antifungals[8]. This study aimed to report a case of coinfection of *Candida glabrata* and *Rhodotorula mucilaginosa* in a 35-year-old immunosuppressive female with onychomycosis.

Case Report

A 35-year-old immunocompromised female patient was admitted to the Medical Mycology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, in October 2019. She complained of deformity and yellow-brown discoloration of the first and second left toenails detached from the nail bed which started ten months earlier. She received fluconazole (FCZ) as prophylaxis before and during chemotherapy. During the physical examination, thickening, onycholysis, and subungual hyperkeratosis were observed in various degrees.

In the first toe, the infection had destroyed the right upper side of the nail plate. The patient had been diagnosed with breast cancer two years earlier and underwent eight cycles of chemotherapy. At admission, she was using corticosteroids and Tamoxifen (Nolvamon) 20 mg daily and FCZ as antifungal prophylaxis.

Laboratory examinations were as follows: white blood cells 7.8×10^3/mm3 (polymorphonuclear leukocytes 59%, lymphocytes 41%), hemoglobin 14.2 g/dl, hematocrit 43.5%, platelets 248×10^3/mm3 (normal 150-450), erythrocyte sedimentation rate 32 mm/h (normal: <15 mm/h), C-reactive protein 2 mg/dl (normal: <0.5 mg/dl), and normal renal and liver function tests. It should also be noted that results of abdominal and renal ultra-sound studies were normal.

Nail sampling was performed by clipping and scrapping the nail bed with a sterilized scalpel. Afterward, the samples were placed on a sterile petri dish and divided into four groups. The first group of samples was treated with 10% potassium hydroxide and dimethyl sulfoxide 40% (KOH/DMSO) (Merck, Germany) and observed under light microscopy to detect fungal elements in the samples directly.

The second group of scrapped nail pieces was inoculated in Sabouraud Dextrose Agar (SDA; Merck, Germany). The cream-colored yeast-like colonies were developed after 24 h at 37 °C. After four days, pinkish-orange pigmented colonies were suspiciously revealed *Rhodotorula* (Figure 1).

Accurate identification was accomplished via sequencing analysis. Briefly, the genomic DNA of the strains was isolated from fresh colonies using a method described previously [9]. Afterward, the internal transcribed spacer (ITS) regions of the rDNA gene of isolates were amplified by the universal fungal primers, ITS1 (5´-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5´-TCCTCCGCTTATTGATATGC-3') [10]. The obtained sequences were compared with similar sequences in Genbank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The DNA sequences of the ITS1 and ITS4 regions matched and showed 100% similarity with those of *R. mucilaginosa* 1.P2F (Genbank MT476908) and *C. glabrata* CNRMA10.308 (Genbank KP131701), respectively.

Antifungal susceptibility tests were carried out by broth microdilution method based on Clinical and Laboratory Standards Institute guidelines, document M27-S3 [11]. The minimal inhibitory concentrations (MICs) were described as the lowest concentration at which no growth occurred, which led to the results summarized in Table 1. Antifungal agent powders were bought from Sigma, USA. *Candida parapsilosis* ATCC 22019 was checked for quality control. It should be mentioned that each test was carried out twice.

A B C

Figure 1. The yeast colonies on SDA at the first cultivation of specimens (A). The cream colored colonies of *Candida glabrata* (B) and orange-colored mucoid colonies of *Rhodotorula mucilaginosa* (C) on SDA after sub cultivation.

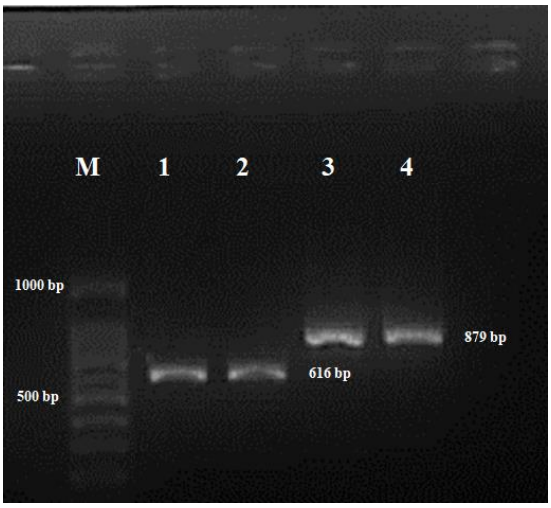
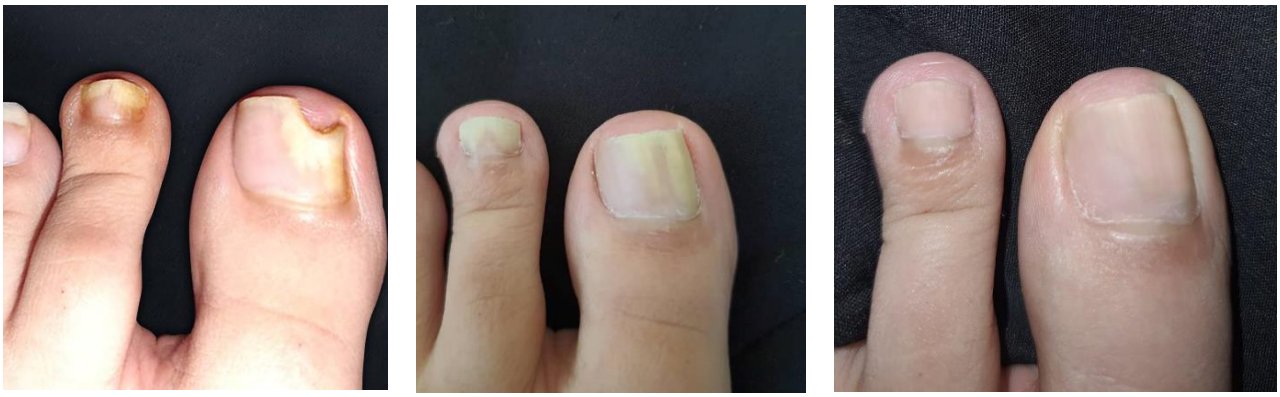


Figure 2. Molecular identification of yeast species using the ITS-PCR: Lanes 1, 2 *R. mucilaginosa* (616 bp), lanes 3, 4 *C. glabrata* (879 bp), lane M: molecular size marker 100 bp.



A B C

Figure 3. Left thickened toenails with yellow discoloration (A). Improved nail after five months (B) and healed nail after ten months (C).

Based on Table 1, *C. glabrata* demonstrated high MICs against tested antifungals except for itraconazole (ICZ). *R. mucilaginosa* had a low MIC against clotrimazole (CTZ) and ketoconazole (KCZ) at

Table 1: *In vitro* antifungal susceptibility pattern of isolates.

Drug	***Rhodotorula mucilaginosa***	***Candida glabrata***
	MIC (µg/ml)	
Fluconazole	>64	32
Itraconazole	4	**0.125**
Voriconazole	4	2
Posaconazole	4	2
Clotrimazole	**0.25**	4
Ketoconazole	**0.25**	>2
Amphotericin B	>8	>16

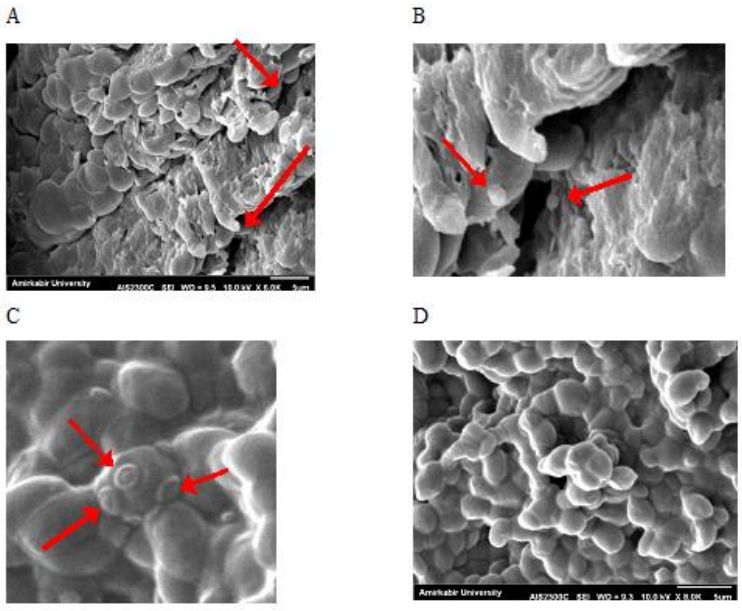


Figure 4. SEM results illustrate the organization of keratin layers (a major component of the nail) and grooves (arrow) on these layer (A). Yeasts adhered to keratin layers inside the grooves of the nail plate (B). The budding of a blastoconidia (arrow) on yeast attached to the nail plate (C). Yeast growth with spiral pattern on the nail (D).

a dilution of 0.25 µg/ml. Hence, a depigmentation test was used to select a drug with higher efficacy, as described previously[12]. There was a significant association between prophylaxis with fluconazole and resistance to fluconazole ($p<0.0001$).

ICZ is administered in a dose of 200 mg twice a day for one week for toenails and as pulse treatment (for one week a month) at 5 mg/kg daily with topical CTZ. The patient was healed for ten months without recurrence (Figure 3).

The fourth group was prepared according to a previously published study[13]. The affected nail plate (the width >4mm) was eliminated and fixed. They were washed, post-fixated, dehydrated, displaced, dried, sprayed, and finally chemically dried in the following steps. Scanning Electron Microscopy (SEM;

Table 2: Overview of four reported articles of onychomycosis due to antifungal resistant *Candida glabrata* or *Rhodotorula mucilaginosa* isolates.

No.	Study/Country	Microorganism	Antifungal Agent(s)	Total No. isolates/ resistant isolates	MICs rates of resistant isolates (µg/mL)	Susceptibility testing method
1	Khosravi *et al*. 2008/Iran [14]	*Candida glabrata*	FLU	4/4	≤ 11	CLSI
2	da Cunha *et al*. 2009/Brazil [23]	*Rhodotorula mucilaginosa*	FCZ	1/1	≥ 128	ATBF3, bioM´erieux
			ICZ		≥ 4	
			vcz		4	EUCAST
3	Manzano-Gayosso *et al*. 2011/Mexico [24]	*Candida glabrata*	ICZ	4/4	≥ 1	CLSI
			KCZ		≥ 1	
			FCZ		≥ 64	
4	Gai Ge *et al*. 2019/China [8]	*Rhodotorula mucilaginosa*	FCZ	1/1	8	CLSI
			ICZ		0.125	

FLU: Flucytosin; FCZ: Fluconazole; ICZ: Itraconazole; vcz: Voriconazole; KCZ: Ketoconazole

AIS2100, Seron Technology, South Korea) was applied to investigate the samples (Figure 4).

The SEM images showed the keratin layers that compose the nail plate and the presence of several grooves on the keratin layer (Figure 4. A). It was observed that the yeast adhered to keratin layers, preferentially inside the grooves of the nail plate (Figure 4. B). The budding of the yeast was attached to the nail plate (Figure 4. C). Vast amounts of yeasts presenting a spiral pattern of growth were also commonly observed inside the nail grooves (Figure 4. D). The SEM image allowed us to see with more detail and real depth the budding yeast's spiral pattern and the conidia's fit in the nail groove region.

Discussion

As far as we know, it is the first report of coinfection of *C. glabrata* and *R. mucilaginous* causing onychomycosis in a 35-year-old immunosuppressed female. It should be mentioned that the incidence rate of onychomycosis increases with age[14]. According to an Iranian study, 80% of patients with onychomycosis were female with a mean age of 38.6 years[14]. The increased incidence rate in older patients can be attributed to frequent trauma, nail abnormalities, long-term contact with water, and immune system disorders[14-16]. Repeated direct smear and culture of specimens after many rounds of pure isolation revealed the yeast structures. These isolates were accurately detected as *C. glabrata* and *R. mucilaginosa* by using a molecular test[17].

An increasing rate of candidiasis has been observed among patients with immune deficiency. The *C. glabrata* is a nondimorphic yeast pathogen presented as a small blastoconidium. On SDA, the creamy, smooth, and glistening colonies of *C. glabrata* are relatively distinguishable from other *Candida* colonies

due to their small size[18-20]. *Rhodotorula spp.*-the fourth most prevalent non-*candida* yeasts- can produce coral or red pigmented colonies [7,21].

Therapeutic options against *Rhodotorula* infections remain controversial. Various antifungals have been prescribed to eradicate these infections, such as amphotericin B (AMB), 5-flucytosine (5-FLU), ICZ, and FCZ[7]. According to the results of antifungal susceptibility tests, isolates exhibited resistance against terbinafine, FCZ, voriconazole (VOR), posaconazole (POS), and AMB. This patient received ICZ therapy based on the susceptible results of this agent, which affected onychomycosis due to *C. glabrata*. The ICZ is a good choice that penetrates the nail matrix and remains active for months[22]. Given the findings of present and previous studies, it seems that FCZ, ICZ, and VOR are ineffective against most *Rhodotorula* strains in vitro[23]. However, in contrast to our findings, AMB is considered a reasonable choice for empirical therapy[23].

In an investigation by Khosravi et al., four *C. glabrata* strains were isolated from patients with onychomycosis. In the study above, all *C. glabrata* isolates were resistant to FCZ and susceptible to AMB, ICZ, KCZ, and FCZ[14]. Results of another study highlighted that an isolate of *R. mucilaginosa* was resistant to FCZ, ICZ, and VOR[17]. Another study found that *R. mucilaginosa* was susceptible to 5-FLU, AMB, and ICZ[23]. Antifungal resistant mechanisms of *Rhodotorula* isolates against FCZ are not discussed clearly; however, the frequent patterns of elevated MICs imply the possible presence of intrinsic resistance[7].

Conclusion

In this study, the isolates were resistant to terbinafine, FCZ, VOR, posaconazole, and AMB. Susceptibility test results and the clinical evidence showed that treatment with ICZ and CTZ were available and reasonable for this onychomycosis case. In the present study, FCZ was used as a prophylactic agent to protect the patient against invasive fungal infections. It seems that exposure to FCZ may be an independent risk factor for resistance, increasing the colonization of drug-resistant fungal spp. in cancer patients.

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