Medical Mycology Case Reports 43 (2024) 100640

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Three cases of vulvovaginal candidiasis due to *Candida nivariensis*

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ARTICLE INFO

Handling Editor: Dr Adilia Warris

Keywords:
Vulvovaginal candidiasis
Candida nivariensis
Fluconazole

ABSTRACT

Candida nivariensis is emerging as a highly resistant species of the *Candida glabrata* complex causing invasive and mucocutaneous infections. In this study, three cases of vulvovaginal candidiasis caused by *C. nivariensis* are described and identified by Internal Transcribed Spacer 1–2 sequencing. All isolates were susceptible in vitro to anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, itraconazole, amphotericin B, and showed dose-dependent susceptibility to fluconazole. In two patients, three doses of oral fluconazole were effective, while one patient developed clinical fluconazole resistance with a new relapse after 6 months. Increasing the weekly dose of fluconazole showed to be effective in this patient.

1. Introduction

Vulvovaginal candidiasis is a common disease caused by *Candida* species, with *Candida albicans* and *Candida glabrata* being the most important pathogens [1]. *C. glabrata* is generally less susceptible to fluconazole, a drug of choice for the treatment of vaginal candidiasis. *Candida nivariensis* is a fungal species that belongs to the *C. glabrata* complex and was first described in 2005 [2]. This complex consists of three species, including *C. glabrata*, *C. nivariensis*, and *C. bracarensis*. *C. nivariensis* is considered an emerging pathogen and causes a variety of clinical diseases [3–6]. Vulvovaginitis caused by this fungal species complex has been reported worldwide [7].

The morphological and biochemical characteristics of all three species of the *C. glabrata* complex are similar. Therefore, *C. nivariensis* can only be identified by molecular methods such as amplified fragment length polymorphism (AFLP), matrix-assisted laser desorption-time of flight (MALDI-TOF), and sequencing of Internal Transcribed Spacer (ITS) rDNA [8]. In addition, knowledge of the susceptibility of *C. nivariensis* to antifungal drugs is considered limited, as there are only a few isolated reports compared to the large number of reports on *C. glabrata* [7,9,10].

In this study, we report three cases of vulvovaginal candidiasis caused by *C. nivariensis*, in which fungal species was identified by ITS sequences, and assessed the antifungal susceptibility profile of these isolates.

2. Cases

Case 1. A 25-year-old woman presented to the Gynaecology Outpatient Department of Hue University of Medicine and Pharmacy (HUMP) Hospital with vulvar pruritus and profuse discharge (day 0). The patient had no previous medical history. Her vaginal symptoms included white cottage cheese-like discharge, redness and itching. Microscopic examination and culture of the vaginal swab revealed a *Candida* infection. The patient was then treated with 150 mg fluconazole orally every 72 h for 3 doses. A follow-up examination could not be performed as the patient did not return for treatment evaluation. However, after five months, the patient returned with vulvar pruritus and profuse discharge. Microscopic examination of vaginal swab and culture showed a relapse of vulvovaginal candidiasis. The patient was prescribed 150 mg fluconazole orally in 3 doses every 72 h, followed by 150 mg orally once a week for six months. The patient was monitored regularly. The *Candida* infection was negative by culture after two weeks of treatment. Even six months after treatment, there was no re-infection. The vaginal swabs from two hospital visits were cultured after admission. *C. nivariensis* was identified by molecular method described below, namely KS13, KS65.

Case 2. A 21-year-old woman with a history of bacterial vulvovaginitis visited the Hue Center for Reproductive Endocrinology and Infertility, HUMP Hospital to undergo in vitro fertilization. The vaginal sample was examined because she had white cottage cheese-like discharge and

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https://doi.org/10.1016/j.mmcr.2024.100640
Received 21 December 2023; Received in revised form 16 February 2024; Accepted 22 February 2024
Available online 27 February 2024
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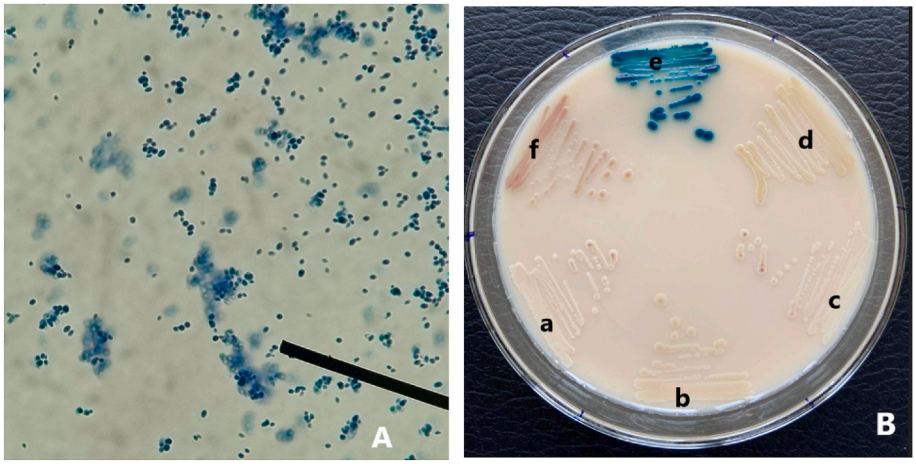

Fig. 1. A. Microscopic features of isolate VS01 from primary culture on Sabouraud-chloramphenicol medium, B. Colonies characteristics after three day culture of *Candida* spp. on Brilliance Candida medium: a. isolate VS01, b. isolate KS13, c. isolate VS12, d. isolate KS65, e. *Candida albicans* ATCC 90028, f. *Candida glabrata* ATCC 66032.

itching. Microscopic examination and culture suggested a *Candida* infection. The patient was treated with fluconazole 150 mg in 3 doses 72 h apart. There were no residual symptoms after one week of treament

Case 3. A 33-year-old female farmer who underwent in vitro fertilization at the Hue Center for Reproductive Endocrinology and Infertility, HUMP Hospital presented with cheesy vaginal discharge and itching. Routine examination with microscopy and culture was perfomed, and the patient was diagnosed with *Candida* infection. Fluconazole 150 mg was prescribed in 3 doses at 72 h intervals.

The *Candida* infection proved to be negative with culture in both patients after two weeks and no relapse after three months. The vaginal swabs from case 2 and case 3 were cultured and identified as *C. nivariensis*. The isolates were labelled VS01 and VS12 respectively.

Biochemical and antifungal susceptibility testing was performed on 4 isolates from 3 patients as described below.

2.1. Identification

Vaginal swabs were collected and sent to the Department of Parasitology for direct examination and culture. The pH vaginal secrections were around 4.5. A wet mount preparation with NaCl 0.9 % showed yeasts in round to oval and budding cell shape and negative detection for clue cells. The samples were initially cultured on Saubouraud-chloramphenicol medium (HiMedia, India) and microscopically showed round to oval yeast and budding cells with a size of 2–4 μm (Fig. 1A). All isolates were identified as non-*albicans Candida* by subculture in Brilliance Candida medium (Oxoide, England), with isolates VS01 and VS12 growing as white colonies and KS13 and KS65 as creamy white colonies, while *C. glabrata* ATCC 66032 showed beige-coloured colonies (Fig. 1B). Subsequent identification with API *Candida* (BioMerieux) showed only glucose assimilation, giving a probability of 47.9% for all isolates to be *C. glabrata*. Fungal DNA was extracted using the thermolysis method according to the protocol of Zang et al. [11]. Polymerase chain reaction (PCR) and sequencing were performed using universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) for the entire ITS1-2 region. PCRs were run in a volume of 25 μL with 200 μM of dNTPs, 1.5 mM of MgCl2, 0.2 μM primers, and 1.0 U Taq polymerase (Invitrogen, Waltham USA). PCR reactions were performed on a SureCycler 8800 using previously published cycling conditions. The PCR products were visualized in a 1.5% agarose gel in TAE 1X buffer with GelRed™ (Biotium, Fremont, USA) using a UV transilluminator with the band around 800 bp, while the bands of *C. albicans* ATCC 90028 and *C. glabrata* ATCC 66032 were approximately 600bp and 880bp, respectively (Fig. 2).

Fig. 2. Electrophoresis of the PCR products of four *C. nivariensis* isolates (L: 100bp ladder, C.al: *Candida albicans* ATCC 90028, C.gla: *Candida glabrata* ATCC 66032, NC: negative control, VS01: isolate VS01, VS12: isolate VS12, KS13: isolate KS13, KS65: isolate KS65).

The amplicons were sent to Malaysia's 1st Base DNA Sequencing Service (https://www.base-asia.com/dna-sequencing-services) for purification and Sanger sequencing. The newly generated sequences were analyzed using BLAST in GenBank. All isolates in these case reports were identified as *C. nivariensis*. The GenBank accession number of isolates KS13, KS65, VS01, and VS12 were OR690735, OR690736, OR690737, and OR690738 respectively.

2.2. Antifungal susceptibility testing

Antifungal susceptibility testing of each isolate was performed using

Table 1
Antifungal susceptibility testing results.

Patient	Isolate code	MIC values (μg/mL)								
		AND	MFG	CAS	FC	PZ	VRC	IZ	FLC	AMB
1	KS13	0.03	0.015	0.03	0.06	0.06	0.015	0.08	0.5	0.5
	KS65	0.03	0.015	0.03	0.25	0.5	0.06	0.25	4	1
2	VS01	0.015	0.008	0.03	0.5	0.25	0.03	0.12	2	1
3	VS12	0.06	0.015	0.06	0.12	0.5	0.12	0.5	8	1
C.albicans ATCC90028		0.03	0.015	0.06	0.12	0.03	0.008	0.06	0.5	0.5
C.glabrata ATCC 66032		0.06	0.015	0.25	<0.06	2	0.5	1	32	0.5

(AND: anidulafungin, MFG: micafungin, CAS: caspofungin, VRC: voriconazole, PZ: posaconazole, IZ: itraconazole, FLC: fluconazole, FC: 5-flucytosine, AMB: amphotericin B).

the Thermo Scientific™ Sensititre™ YeastOne™ YO10 AST (SYO) Plate. Results were interpreted according to the manufacturer's instructions. The dried SYO plates were rehydrated with the yeast suspension, distributing 100 μL into each well using a multichannel pipette. The plates were covered with adhesive seals and incubated at 35 °C for 24 h in a CO2-free incubator. MIC endpoints were read after 24 h of incubation. Evidence of yeast growth was determined by a color change from blue (negative, no growth) to red (positive, growth). MICs were interpreted according to M60-Ed2 [12], while itraconazole and amphotericin B were classified based on the epidemiological cut-off values (ECV) according to CLSI M59-Ed3 [13]. *C. albicans* ATCC 90028 and *C. glabrata* ATCC 66032 were used as standard reference strains.

The results showed that all isolates were susceptible to all drug tests. The details are listed in Table 1.

3. Discussion

Although non-*albicans Candida* infections are considered to have milder symptoms than *C. albicans* or asymptomatic infections, all cases of *C. nivariensis* in the present study were symptomatic, including the white cottage cheese-like discharge and itching. This result was similar to a study by Shi Y. et al. who showed that *Candida* vaginitis due to *C. nivariensis* was symptomatic [7].

C. glabrata is the most common of non-*albicans Candida* cause of vulvovaginitis [14], but so far only a few papers have been published on *C. nivariensis* [5,7,9]. This may be because the incidence of *C. nivariensis* is lower than that of *C. glabrata*, or the phenotypic characteristics of *C. nivariensis* are similar to those of *C. glabrata*, leading to misidentification and underestimation [5,15]. *C. nivariensis* has been previously reported from Vietnamese vaginal samples at a low rate (0.42%, n = 238) [16]. Regarding the identification methods of *C. nivariensis* in this study, the phenotypic characteristics of this species were found to be similar to those of *C. glabrata*. However, API 20C AUX demonstrated the ability to assimilate trehalose in *C. glabrata*, which was not observed in *C. nivariensis*. All our isolates were confirmed by ITS1-2 sequencing, but the difference between the PCR bands of *C. nivariensis* (760bp) and *C. glabrata* ATCC (880bp) could distinguish these species from each other. This finding was consistent with a previous study [17].

C. nivariensis was reported as a new pathogenic and multidrug-resistant pathogen in the first study by Borman and co-workers in 2008 [18]. It was reported to be less susceptible to itraconazole, fluconazole, voriconazole, and flucytosine than isolates of *C. glabrata* [18]. However, several studies in Southeast Asian countries have reported that *C. nivarinensis* is still susceptible to fluconazole [15,19]. Nowadays, the susceptibility of this fungal species to antifungal agents varies in different studies. While Shi Y. and co-workers showed a low mycological cure rate of *C. nivariensis* infection in patients with vaginitis [7], the isolates in their study were susceptible to common antifungal agents in vitro. According to the 2nd edition of M60 - Performance Standards from Antifungal Susceptibility Testing of Yeasts, all isolates in our study were considered sensitive to most of antifungal agents tested and dose-dependent susceptible to fluconazole. This result was consistent with the studies of Hou X. and co-authors, who tested fungal isolates collected from blood, ascitic fluid, and pus [20]. Additionally, all *C. nivariensis* isolated from blood were susceptible to amphotericin B, fluconazole, itraconazole, posaconazole, voriconazole, anidulafungin and micafungin, as shown in the study by Arastehefar et al. [4]. In contrast, isolates of *C. nivariensis* from invasive candidiasis have also been reported to be resistant to antifungal drugs or to have a high MIC of fluconazole [3,6]. Fluconazole remains the recommended oral drug for the treatment of vaginal candidiasis [21], and all patients in this study were successfully treated by fluconazole, with a follow-up period of six months. Although the antifungal susceptibility test result of isolate KS65 was still susceptible - dose dependent to fluconazole (MIC 0.25 μg/mL), the increase in the MIC of fluconazole and all other drugs tested indicates a possible trend towards secondary resistance (Table 1). In addition, the *C. glabrata* species complex is increasingly becoming one of the most important pathogens of invasive candidiasis with a high mortality rate and a low susceptibility to azoles, polyenes, and echinocandins, which are commonly used to treat invasive mycoses [22]. Therefore, topical antifungal drugs such as nystatin, clotrimazole, and miconazole might be preferred for the treatment of vulvovaginitis caused by *C. nivariensis* to reduce the risk resistance development. According to Fan et al., nystatin was also a good choice for the treatment of vulvovaginitis caused by non-*albicans Candida* [23].

From this report and the results from other studies mentioned above, it can be concluded that mycological examination and follow-up should be performed in vulvovaginal candidiasis caused by non-*albicans Candida* to enable directed treatment.

CRediT author contribution

Cases collection: Phuong Anh Ton Nu, Phuoc Vinh Nguyen, Minh Tam Le; Fungal culture and identification, antifungal susceptibility testing, molecular techniques: Phuong Anh Ton Nu, Thi Minh Chau Ngo, Thi Bich Thao Do, Thi Ngọc Thuy Ha, Giang Tran Thi; Patients follow-up: Phuong Anh Ton Nu, Minh Tam Le; Data Formal analysis: Thi Minh Chau Ngo, Phuong Anh Ton Nu, Cao Le Chi; Writing the Writing – original draft: Thi Minh Chau Ngo, Phuong Anh Ton Nu, Cao Le Chi; Writing – review & editing: Thi Minh Chau Ngo

Funding source

This work was supported by Hue University (Grand code: DHH2023-04-188).

Declaration of competing interest

The authors have no conflict of interest.

Acknowledgements

We would like to thank the patients and our colleagues in the Gynaecology Outpatient Department of Hue University of Medicine and

Pharmacy Hospital for their contributions to this study.

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