Int J Clin Exp Pathol 2016;9(10):10774-10777
www.ijcep.com /ISSN:1936-2625/IJCEP0034994

Case Report

Candida nivariensis isolates from a pregnant patient. Molecular identification using sequencing and MALDI-TOF

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Received July 4, 2016; Accepted July 15, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: *Candida nivariensis* is a recently described yeast, phenotypically indistinguishable from *Candida glabrata*. We report a vaginal isolation of *C. nivariensis* from an Indian pregnant woman, hospitalized for premature ruptured membranes. The isolate was initially incorrectly identified as *C. glabrata* using Vitek 2 system and then retested by MALDI-TOF. *Candida nivariensis* identification was then confirmed by sequencing. The current study revealed an high MIC to Fluconazole (2 mcg/mL), in according to previous studies. *Candida nivariensis* is a causative agent of vulvovaginal candidiasis, but its real incidence in our continent may be underestimated due to lack of adequate molecular or mass spectroscopy surveillance strategies. Antifungal susceptibility of this species requires further study.

Keywords: *Candida nivariensis*, vulvovaginal, candidiasis, pregnancy, new pathogen

Introduction

Candida species are frequently described as colonizers of the estrogenized vagina. Vulvovaginal candidiasis (VVC) affects up to 75% of women of child-bearing age at least once in their lifetime and is predominantly caused by *Candida albicans* [1-4]. An increase in the prevalence of non-*albicans* species in VVC has been reported, mainly *C. glabrata* [1]. Based on molecular analysis, two new species that are closely related and phenotypically strictly similar to *C. glabrata* have been described: *C. nivariensis* and *C. bracarensis* [5-13]. *Candida nivariensis* was first described in 2005; after it was isolated from different clinical samples (bronchoalveolar lavage, blood culture, and urine) from three patients in the Canary Islands (Spain) [5]. Few data of clinical significance regarding *C. nivariensis* and *C. bracarensis* in VVC are currently available [14, 15]. Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) analyses were demonstrated to be an efficient tool to differentiate these species [16]. Since then, cases of *C. nivariensis* and *C. bracarensis* in VVC have been reported in Asia [15, 17, 18]. To the best of our knowledge, the isolation of *C. nivariensis* from vaginal swabs has not been reported to date in Europe.

Several studies have shown that *C. nivariensis* and *C. bracarensis* are less susceptible than *C. glabrata* to azole antifungal agents, that are commonly used in the treatment of candidiasis (Fluconazole, Itraconazole and Voriconazole) [19, 20].

In this paper we report the isolation of *C. nivariensis* from a vaginal swab of a pregnant woman, hospitalized for premature ruptured membranes.

Materials and methods

A 25 years old Indian woman, secundigravida, was admitted to the Obstetrics Department of Papa Giovanni XXIII hospital in Bergamo, Italy at 25 weeks of gestation for premature ruptured membranes. Her personal and familiar anamnesis were negative and the previous pregnancy was uneventful. On admission she was asymptomatic and without fever. Laboratory tests were negative for inflammation and amni-

Table 1. Review of *C. nivariensis* VVC literature

Reference	Country	Total of isolates (n)	Source of isolates	Susceptibility antifungal profile		
				Clinical details	Low MIC	High MIC
Sharma et al. (2013)	India	4	Vaginal mucosa	Vulvovaginal candidiasis	VRC, ITC, AMB, POS, ISA	FLC
Li et al. (2014)	China	1	Vaginal mucosa	Vulvovaginal candidiasis	NYT	FLC, ITC
Tay et al. (2014)	Malaysia	1	Vaginal mucosa	Vulvovaginal candidiasis	Not stated	Not stated
Present paper (2015)	Italy	1	Vaginal mucosa	Premature ruptured membranes	AMB, CAS, ITC, POS,VRC, 5FC	FLC

AMB: Amphotericin B; CAS: Caspofungin; FLC: Fluconazole; ISA: Isavuconazole; ITC: Itraconazole; MIC: Minimal Inhibitory Concentration; NYT: Nystatine; POS: Posaconazole; VRC: Voriconazole; 5FC: 5-Fluorocytosine.

otic fluid was clear. The ultrasound examination revealed a viable fetus with intrauterine growth restriction and a reduced amniotic fluid.

The woman was treated for the membranes' rupture with an antibiotics prophylaxis with intravenous Ampicillin at a daily dose of 6 g for 10 days, Tractocile for tocolysis with Nifedipine 20 mg, Fluconazole 100 mg daily for 2 days. Corticosteroids for fetal lung development were also administrated to the patient.

A vaginal swab was performed to check the presence of any pathogens. Microscopic examination showed many leukocytes and parabasal cells, within lactobacilli and few conidia. White colonies with a smooth texture yielded on a Sabouraud agar plate incubated for 48 hours at 35±2°C in aerobic atmosphere. The isolate was initially identified using Vitek2 YST card (bioMérieux, Marcy-l'Etoile, France) as *C. glabrata*. The strain was retested by MALDI-TOF (VITEK-MS, bioMérieux, Marcy-l'Etoile, France) technique. Conventional formic acid protein extraction was performed and the isolate was identified as *C. nivariensis*. This identification was then confirmed through molecular sequencing of its ribosomal DNA coding for LSU rRNA and compared to GenBank (NBCI) sequences using the BLAST 2.2.32+ program. *In vitro* antifungal susceptibility profile by broth microdilution testing (Sensititre YeastOne, ThermoScientific, USA) revealed low MICs to all the azoles (Ketoconazole, 0.06 mcg/mL; Voriconazole, 0.03 mcg/mL; Itraconazole, 0.25 mcg/mL; Posaconazole, 0.25 mcg/mL) except for Fluconazole (2 mcg/mL). The MICs were low also to Amphotericin B (0.25 mcg/mL), 5-Fluorocytosine (0.125 mcg/mL) and Caspofungin (0.125 mcg/mL).

Laboratory tests and ultrasound examination to check amniotic fluid amount were performed regularly during all the recovery. The patient remained asymptomatic, blood tests showed no signs of inflammation; amniotic fluid was reduced but stable. Vaginal swabs after therapy resulted negative.

At 32 weeks of gestation a lower segment cesarean section was performed because of non-reassuring cardiotocography pattern and fetal growth restriction. A male infant of 1091 grams was delivered. Apgar score at 5 minutes was 9, pH on the umbilical artery was 7.35. Histological placenta examination revealed chorionitis. The patient had an uneventful post-delivery recovery and was discharged home on day 3 after surgery. Post-partum gynaecological check after one month was regular.

Results and discussion

Candida glabrata is the second most common yeast recovered from the genital tract of women with vaginitis. It accounts for approximately 5-10% of vaginal infections [1, 4]. *Candida nivariensis* and *Candida bracarensis* are two recently described yeasts that are phenotypically indistinguishable from *Candida glabrata* [5, 7]. Since phenotypic tests are not able to discriminate among the three species of the *Candida glabrata* complex, molecular methods were applied to confirm the identification of these clinical strains [6]. However, Polymerase Chain Reaction (PCR) and sequencing are time consuming and expensive. In recent years, MALDI-TOF has been introduced into diagnostic laboratories for the identification of cryptic *Candida* spp [16]. MALDI-TOF analysis is rapid (under 15 min) and inexpensive.

According to the current published literature [5, 8, 11, 12, 15-18], six isolates of *C. nivariensis* from vaginal swabs have been described in in Asia, but there are no cases of *C. nivariensis* involved in VVC in Europe. Hence, to the best of our knowledge, this is the first report of *C.*

nivariensis from a vaginal swab of a symptomatic woman in Europe, expecially in Italy (**Table 1**). The woman, however, was of Indian nationality: this anamnestic data could suggest the particular geographical origin of *C. nivariensis* in Asian countries.

Concerning the antifungal susceptibility profile, several studies have shown that *C. glabrata* and other non-*albicans* species are less susceptible to Fluconazole than most *C. albicans* [1]. Borman et al. [10] studied the minimum inhibitory concentration (MIC) ranges of 13 strains of *C. nivariensis* and 13 strains of *C. glabrata* isolates. *Candida nivariensis* exhibited significant *in vitro* resistance to Itraconazole, Voriconazole, and Fluconazole and was susceptible to Amphotericin B, 5-Fluorocytosine, Posaconazole, and Caspofungin. The current study revealed low MICs to all the azoles except for Fluconazole (2 mcg/mL). The MICs were low also to Amphotericin B, 5-Fluorocytosine and Caspofungin, in according to previous studies. Sharma et al. [15] reported that one of the four vaginal *C. nivariensis* isolates had a high Fluconazole MIC (16 mcg/mL), and the corresponding VVC patient did not respond to Fluconazole therapy. The three additional patients with VVC caused by *C. nivariensis* responded well to oral Fluconazole therapy. In our study the patient achieved mycological eradication with Fluconazole. This result is inconsistent with the *in vitro* antifungal susceptibility test results, and requires further studies. In conclusion, *C. nivariensis* is a causative agent of VVC, detected especially in Asia. However, we strongly believe that its real incidence in our continent may be underestimated due to lack of adequate molecular or mass spectroscopy surveillance strategies. Although *C. nivariensis* is rare, its correct identification is clinically important because of its azolic resistance. The limitations of the biochemical routine diagnostic methods for the identification of new pathogenic *Candida* species were demonstrated in this study. Other diagnostic approaches, such as ribosomal rRNA sequencing and MALDI-TOF mass spectroscopy, may be very usefull to identify cryptic *Candida spp*.

Disclosure of conflict of interest

None.

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