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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Identification of *Candida* species in vaginal flora using conventional and molecular methods

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Summary Vaginal swab samples were collected both from women with signs of acute vulvovaginal candidiasis (VVC; $n = 270$) and from healthy controls ($n = 100$). The microscopic examination revealed a significant difference in the proportion of positive cultures of *Candida* between the VVC (33%) and control (21%) groups. To determine the frequency of different species, positive cultures were analyzed using the germ tube test, CHROMagar medium, API 20 AUX System and DNA analysis. CHROMagar and API 20 AUX System tests identified 67% of samples as *C. albicans*. Out of these, 42% appeared to be *C. dubliniensis* when the specific primers in the polymerase chain reaction (PCR) were used. We observed the prevalence of *Candida* species isolated from the vagina in descending order as follows: *C. albicans* (38—39%), *C. glabrata* (32—33%), *C. dubliniensis* (28—29%) and *C. tropicalis* (0—1%) for both the VVC and the control group.
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Introduction

The infection caused by *Candida*, the vulvovaginal candidiasis (VVC), affects ca. Seventy-five percent of women at least once during their lives and 5—8% of adult women have recurrent VVC [1,2]. The susceptibility of *Candida* species to antifungal agents varies. While *C. albicans* is sensitive to azoles, non-*albicans Candida* species have shown resistance to azoles [1,3,4]. Moreover, it has been observed that the susceptibility of all species to fluconazole treatment has

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decreased in less than ten years, during 2006—2013 [2]. In order to avoid the increase of drug resistance, the exact identification of the species would be important.

The most common species causing an acute sporadic VVC has been *C. albicans* in most studies worldwide. *C. albicans* has been reported to be responsible even for 90% of the VVC infections [1,2,5]. The frequency of *C. galabrata* has varied between 15—30%, however, during 2006—2013, its frequency has been observed to be increased from 11% to 22% [2]. Epidemiologic data on the distribution of *Candida* species are limited and geographic differences in *Candida* species distribution have been reported [5].

The conventional identification methods of *Candida* species are based upon a combination of morphological and biochemical criteria [6]. However, these methods do not separate all species reliably and new molecular methods provide more accurate identification of the species [7,8].

We aim to increase the knowledge of the *Candida* species colonizing healthy women's vaginas as well as of the species that cause an acute VVC. We isolated *Candida* species from vaginal swab samples collected both from asymptomatic and symptomatic women and identified the species using some conventional methods as well as using the molecular method of polymerase chain reaction (PCR) in order to increase the accuracy of the result.

Materials and methods

Vaginal swab samples were collected from women suffering from acute symptoms of vulvovaginal candidosis (VVC; $n = 270$) as well as from healthy women that were chosen randomly among university students (control; $n = 100$) in Riyadh, Saudi Arabia. The symptoms of VVC were pruritus, vaginal discharge, soreness and dyspareunia. Specimens were collected on a cotton-tipped swab that was inserted about 5 cm in the vagina, rotated for 10 to 30 s, air-dried and stored in screw capped tubes. Samples were transported immediately using an icebox in the laboratory and stored at −80 °C until processed. According to the procedure of Hobbs et al. [9], the recovery of vaginal secretions was performed by placing each swab in a sterile tube containing 3.0 mL phosphate buffered saline. The tubes were incubated at room temperature for 15—30 min for elution, centrifuged at 250 × *g* for 10 min and the supernatants were collected.

To check the presence of regular blastopores, as well as pseudopyphae, vaginal swab samples were stained with methylene blue and examined microscopically at 40 × magnification. Vaginal swab samples were cultivated separately on Sabouraud Dextrose agar (SDA, Oxoid Ltd, Basingstoke) where antibiotics were added in order to inhibit the bacterial growth. Plates were incubated for 48 h at 37 °C. The appearance of yeast colonies was monitored to determine positive and negative cultures. Positive samples were stored in glycerol at −80 °C for further analysis.

We performed the germ tube test, which is indicative of *C. krusei* ATCC 6258, *C. albicans* ATCC 24433 and *C. dubliniensis* ATCC MY 646 [10]. The species were identified also by CHROMagar [11,12], and API 20 AUX System (bioMerieux Marcy l'Etoiole, France — Appendix) [13], according to the manufacturer's instructions.

DNA was extracted from the positive samples. *Candida* isolates were grown individually on SDA medium and incubated for 24—48 h. Suspensions of each *Candida* species were prepared and the DNA was extracted with a MagNA Pure LC instrument using a MagNA Pure LC DNA isolation kit III (Roche Applied Science, Indianapolis, IN) as recommended by the manufacturer. A puReTaq Ready-To-Go PCR Beads kit (GE Healthcare companies, UK) was used and the PCR were performed immediately. Thermo cycling conditions (primus96plus-MWG-AG, Biotech) consisted of an initial denaturation stage of 94 °C for 4 min, followed by 35 cycles of 94 °C for 20 s, 67 °C for 60 s, and 72 °C for 20 s, and a final stage of 72 °C for 4 min. The product was analyzed in agarose gel. Oligonucleotide primers presented in Table 1 were used to distinguish *Candida* species. Fisher's exact test was performed to study the difference in the prevalence of positive cultures as well as *Candida* species between the VVC and control groups.

Results

Microscopic examination of *Candida* species revealed a significant (Fisher's test; $P = 0.02$) difference between the VVC and the control group. In the VVC group, 33% of the samples (90 samples out of 270) formed a positive culture of *Candida* sp. (Table 2). Only 21% of the samples were positive out of 100 control group samples.

According to the germ tube test, 67% of the positive samples were identified as *C. albicans* or *C. dubliniensis*. Out of 90 positive cultures in the VVC group, 60 were identified as *C. albicans* or *C. dubliniensis* and 14 out of 21 positive samples in the control group (Table 3).

Table 1 Oligonucleotide primers used to distinguish the *Candida* species.

Candida species	Primer	Sequence (5′→3′)	Amplicon size (bp)
Candida sp	CWO2	GCATCAGTTTGGGCGGTAGGACG	132
	NL4EL1	AGATCATTATGCCAACATCCTAGGC CG	
C. glabrata	CGL1	TGGGCTTGGGACTCTCGCAGCTC	173
	NL4CGL1	TAACCATTATGCCAGCATCCTAGAT AAC	
C. tropicalis	CTR22	TGGGCGGTAGGAGAATTGCGTTA	126
	NL4CTR1	TAAGATCATTATGCCAACATCCTAGGTATA	
C. albicans	CAL5	TGTTGCTCTCTCGGGGGCGGCCG	175
	NL4CAL	AAGATCATTATGCCAACATCCTAGG TAAA	
C. dubliniensis	CDU2	AGTTACTCTTTCGGGGGTGGCCT	175
	NL4CAL	AAGATCATTATGCCAACATCCTAGG TAAA	

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