Received: 8 April 2019 | Revised: 21 May 2019 | Accepted: 22 May 2019

DOI: 10.1111/myc.12946

ORIGINAL ARTICLE

mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases | WILEY

Epidemiology and antifungal susceptibility patterns of *Candida* isolates from Greek women with vulvovaginal candidiasis

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Summary

Vulvovaginal candidiasis (VVC) is a common infection of the genital tract affecting millions of women worldwide. Data on epidemiological trends of VVC in Greece are scarce. This study was undertaken to evaluate the prevalence of VVC among symptomatic women in Crete, Greece, identify the *Candida* species involved and determine their susceptibility to antifungals. Over a 6-year period (2012-2017), 10 256 symptomatic women with vaginitis were evaluated. Isolation of yeasts was performed on Sabouraud dextrose agar with chloramphenicol, and the isolates were identified using the API 20 C AUX and/or the Vitek 2 YST card. Susceptibility of the isolates to amphotericin, fluconazole, voriconazole and flucytosine was determined by the Vitek 2 automated system. The results were interpreted according to Clinical and Laboratory Standards criteria. Vaginal swab cultures of 1217 (11.9%) women yielded *Candida* species. Recurrent VVC was documented in 62 (5.1%) of them. *Candida albicans* was the most frequently isolated species (75.6%), followed by *Candida glabrata* (13.6%). Overall, resistance rates to amphotericin B, fluconazole, voriconazole and flucytosine were 0.2%, 6.6%, 1.4% and 2.1%, respectively. Fluconazole resistance of *C. albicans* significantly increased in the second period of the study (2015-2017) ($P = 0.031$). This study demonstrated that VVC is a common infection among women in our region, with *C. albicans* being the predominant species involved. Although resistance to antifungals was infrequent, resistance to fluconazole among *C. albicans* isolates was found to significantly increase with time. Continued surveillance of changes in species distribution and susceptibility to antifungals are necessary to guide treatment.

KEYWORDS

antifungal susceptibility, *Candida* species, epidemiology, vulvovaginal candidiasis

1 | INTRODUCTION

Candida is a commensal organism of the genitourinary tract with colonization rates of 11.6%-17%.[1,2] However, disruption of local host defense mechanisms limiting *Candida* growth lead to a non-invasive infection, called vulvovaginal candidiasis (VVC).[3] In particular, factors predisposing to VVC include pregnancy, hormone replacement, uncontrolled diabetes mellitus, long-term broad-spectrum antibiotic treatment, immunosuppression, contraceptives usage, and also poor personal hygiene and some sexual and clothing habits.[4] In Europe, VVC is one of the most common causes of vaginitis,[5] and in the United States, it is the second most frequent cause of infection after bacterial vaginosis.[4] It has been estimated that 75% of all women will experience at least one episode of VVC in their lives. Between

40% and 50% of initially infected women will experience a second episode, while 7%-8% will develop recurrent VVC (RVVC), which is defined as at least four confirmed episodes per year.[4,6]

In more than 70% of cases, VVC is caused by *Candida albicans*, followed by *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*.[7,8] During the last two decades, an increasing trend of VVC caused by non-*albicans Candida* species (NAC) has been reported due to the widespread use of antifungal agents that are frequently available over-the-counter.[9] Treatment failure is common in NAC vaginitis, since some species are intrinsically resistant or show low susceptibilities to the azoles, the most commonly prescribed class of antifungal agents.[10] For this reason, the identification of *Candida* to the species level and the determination of their susceptibility to antifungals are necessary for providing effective therapy.

This study was undertaken to determine the prevalence, species distribution and the antifungal susceptibilities of *Candida* species causing VVC, which have been isolated from Greek patients with symptomatic vaginitis.

2 | PATIENTS AND METHODS

The present study was conducted over a period of 6 years (2012-2017), at the University Hospital of Heraklion, Crete, Greece, a 650-bed tertiary care hospital serving a population of around 650 000 persons. Microbiological data obtained from women aged 18 years or over, who were admitted to the outpatient clinic of Obstetrics and Gynaecology with signs and symptoms suggestive of vulvovaginitis, were prospectively collected in the present study.

Specimens were taken from the posterior fornix of the vagina with sterile cotton-tipped swabs and promptly transferred to the microbiology laboratory for further processing. For each vaginal swab, wet mount preparations, Gram-stained smears and cultures were carried out following the laboratory protocols. For the isolation of *Candida* spp., specimens were inoculated on Sabouraud dextrose agar supplemented with chloramphenicol (50 mg/mL) (Oxoid) and incubated at 36°C for 72 hours. Identification to the species level was performed by sugar assimilation tests using the API 20 C AUX system and/or the Vitek 2 automated system (both products of BioMérieux).

Susceptibility to antifungal drugs was determined using the Vitek 2 system, according to the manufacturer's instructions. The antifungals tested included amphotericin B, fluconazole, voriconazole and flucytosine. Minimal inhibitory concentrations (MICs) were interpreted using the CLSI M27-S4 breakpoints.[11] If there were no breakpoints, the epidemiological cutoff values (ECVs) were used to discriminate wild-type (WT) and non-wild type isolates. For amphotericin B, isolates with MIC ≤ 2 μg/mL were considered to be WT.[12] For fluconazole, *C. albicans*, *C. tropicalis* and *C. parapsilosis* isolates with MIC ≤ 2 μg/mL were considered susceptible (S), with MIC = 4 μg/mL susceptible dose-dependent (SDD) and with MIC > 8 μg/mL resistant (R). *Candida glabrata* isolates with MIC ≤ 32 μg/mL were considered SDD and those with MIC ≥ 64 μg/mL R. *Candida krusei* isolates were considered all R to fluconazole. For voriconazole, *C. albicans*, *C. tropicalis* and *C. parapsilosis* isolates with MIC ≤ 0.12 μg/mL, MIC = 0.25-0.5 μg/mL and MIC ≥ 1 μg/mL, were categorised as S, I (intermediate) and R, respectively. *Candida krusei* isolates with MIC ≤ 0.5 μg/mL were considered S and those with MIC ≥ 2 μg/mL R, while *C. glabrata* isolates were considered WT when MIC ≤ 0.5 μg/mL, and non-WT when MIC > 0.5 μg/mL. For flucytosine, isolates with MIC values ≤ 4 μg/mL were considered S, 8-16 μg/mL I and ≥32 μg/mL R. *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6285 were used as control strains.

Statistical analysis was conducted by the chi-square and Fisher exact test, as appropriate. Statistical significance was set at $P < 0.05$. All statistical analyses were performed with Graphpad Prism, V.4 (GraphPad Software Inc).

Since our study was a laboratory-based study on microbiological data for clinical isolates of *Candida* species collected during the everyday clinical care of patients, it did not require approval, as per the guidelines of the Institutional Review Board and of the Ethics Committee of the University Hospital of Heraklion.

3 | RESULTS

During the study period, 10 256 patients with signs and symptoms of vaginal infection were evaluated. Of them, 1217 (11.9%) were diagnosed with VVC. The mean age of the patients was 32.7 years, ranging from 18 to 89 years. Younger women between 18 and 29 years had the highest prevalence rates of VVC (13.9%), while in the age groups 30-39, 40-49, 50-59 and ≥60 the prevalence rates were 12.3%, 11.3%, 6.5% and 5.9%, respectively.

A total of 1234 *Candida* isolates were obtained from the 1217 vaginal specimens. Isolates included 933 (75.6%) *C. albicans*, and 301 (24.4%) NAC represented by 168 (13.6%) *C. glabrata*, 34 (2.7%) *C. krusei*, 22 (1.8%) *C. tropicalis*, 17 (1.4%) *C. lusitaniae*, 12 (1%) *C. lipolytica*, 11 (0.9%) *C. parapsilosis*, 11 (0.9%) *C. kefyr*, 9 (0.7%) *C. dubliniensis*, 9 (0.7%) *C. ciferrii*, 5 (0.4%) *C. incospicua*, 1 (0.1%) *C. famata*, 1 (0.1%) *C. norvegiensis* and 1 (0.1%) *C. rugosa*. Species distribution of the isolates was relatively stable over the study period, with slight fluctuations (Table 1). Non-significant differences were observed in *C. albicans* and NAC isolations between the two study periods, 2012-2014 and 2015-2017 ($P = 0.14$). The overall recurrence rate was 5.1%, with *C. albicans* being more frequently involved (71%). The mean age of the patients with RVVC was 31.5 years, ranging from 18 to 50 years. Mixed infection with 2 species of *Candida* isolated from one specimen was observed in 17 episodes of VVC. The majority of them (14 out of 17) yielded *C. albicans* and *C. glabrata*. Two cultures yielded *C. albicans* and *C. krusei*, and one yielded *C. glabrata* and *C. tropicalis*. NAC species were isolated significantly more often among menopausal patients than from women of reproductive age ($P < 0.0001$) (Table 2).

Susceptibility test results for the 1234 isolates revealed that resistance to amphotericin B (0.2%), voriconazole (1.4%) or flucytosine

TABLE 1 Species distribution of *Candida* isolates from patients with vulvovaginal candidiasis during a 6-year period

Year, n (%)							
Species	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2012-2017**
C. albicans[a]	175 (76.7)	80 (79.2)	147 (78.2)	173 (71.5)	151 (74.8)	207 (75.8)	933 (75.6)
NAC[a]	53 (23.3)	21 (20.8)	41 (21.8)	69 (28.5)	51 (25.2)	66 (24.2)	301 (24.4)
C. glabrata	32 (14.0)	10 (9.9)	23 (12.2)	40 (16.5)	26 (12.8)	37 (13.6)	168 (13.6)
C. krusei	8 (3.5)	3 (3.0)	4 (2.1)	5 (2.1)	8 (3.9)	6 (2.2)	34 (2.7)
C. tropicalis	2 (0.9)	2 (2.0)	2 (1.1)	5 (2.1)	4 (2.0)	7 (2.6)	22 (1.8)
C. lusitaniae	2 (0.9)	1 (1.0)	2 (1.1)	3 (1.2)	2 (1.0)	7 (2.6)	17 (1.4)
C. lipolytica				8 (3.3)	2 (1.0)	2 (0.7)	12 (1.0)
C. parapsilosis	4 (1.8)	3 (3.0)	1 (0.5)	1 (0.4)	1 (0.5)	1 (0.4)	11 (0.9)
C. kefyr	1 (0.4)	1 (1.0)	3 (1.7)	4 (1.7)		2 (0.7)	11 (0.9)
C. dubliniensis	4 (1.8)		1 (0.5)	2 (0.8)		2 (0.7)	9 (0.7)
C. ciferrii			4 (2.1)	1 (0.4)	4 (2.0)		9 (0.7)
C. incospicua		1 (1.0)	1 (0.5)		2 (1.0)	1 (0.4)	5 (0.4)
C. famata					1 (0.5)		1 (0.1)
C. norvegiensis					1 (0.5)		1 (0.1)
C. rugosa						1 (0.4)	1 (0.1)

[a]Non-significant differences were observed in *C. albicans* and non-*albicans Candida* species isolations between the two study periods, 2012-2014 and 2015-2017 ($P = 0.14$).

TABLE 2 Species distribution of *Candida* isolates by age group of patients with vulvovaginal candidiasis

Species	18-29	30-39	40-49	50-59	≥60
C. albicans	453	325	102	31	22
C. glabrata	61	51	32	20	4
C. krusei	13	11	4	5	1
C. tropicalis	4	9	4	2	3
C. lusitaniae	6	9	2		
C. lipolytica	1	6	1	3	1
C. parapsilosis	2	7	2		
C. kefyr	1	5	2		3
C. dubliniensis	3	4	1		1
C. ciferrii	7	2			
C. incospicua	1	1	1	2	
C. famata		1			
C. norvegiensis	1				
C. rugosa				1	
Total	553	431	151	64	35

Note: Non-*albicans Candida* species species were isolated significantly more often among menopausal patients than from women of reproductive age ($P < 0.0001$).

(2.1%) was infrequent. Intermediate susceptibility to voriconazole occured among 0.4% of the isolates, and intermediate susceptibility to flucytosine occured among 3.5% of them. Resistance to fluconazole was observed among 6.6% of the isolates, while 15.8% were susceptible dose-dependent. The susceptibility test results for each species are presented in Table 3. Resistance to amphotericin B was observed only among *C. albicans* isolates (0.2%), while all other *Candida* species were uniformly susceptible. A total of 3.7% *C. albicans* isolates were resistant and 2.1% were susceptible dose-dependent to fluconazole, whereas 1.2% of *C. glabrata* isolates were resistant and 98.8% were susceptible dose-dependent to fluconazole. Fluconazole resistance of *C. albicans* was found significantly higher in the second period of the study (2015-2017) ($P = 0.031$). All *C. krusei* isolates were considered fluconazole resistant as per CLSI guidelines. Resistance to fluconazole was significantly higher among NAC species versus *C. albicans* ($P < 0.0001$). Voriconazole resistance was noted among *C. glabrata* (3%) and *C. albicans* (1.3%), while flucytosine resistance was observed among *C. albicans* (2.6%) and *C. glabrata* (1.2%).

4 | DISCUSSION

Vulvovaginal candidiasis is a common infection affecting millions of women annually, with considerable negative impact on the quality of their social and sexual lives and is associated with significant direct and indirect costs.[13,14]

Poor data are available of the frequency of VVC, since the disease is not reportable and is often self-diagnosed without clinical and laboratory confirmation. The prevalence of VVC in symptomatic women with microbiologic confirmation varies by country and population studied. Prevalence rates ranging from 5.3% to 60% have been reported, with higher rates found in Nigeria, Tunisia, Brazil and Australia.[2,5,15-17] In the present study, the prevalence rate of VVC

TABLE 3 In vitro susceptibility of the 1234 *Candida* isolates to four antifungal agents

Candida species (n)	Amphotericin B		Fluconazole			Voriconazole			Flucytosine		
	S (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Candida albicans (933)	931 (99.8)	2 (0.2)	879 (94.2)	20 (2.1)	34 (3.7)	918 (98.4)	3 (0.3)	12 (1.3)	905 (97)	4 (0.4)	24 (2.6)
Candida glabrata (168)	168 (100)	0 (0)	0 (0)	166 (98.8)	2 (1.2)	163 (97)	0 (0)	5 (3)	166 (98.8)	0 (0)	2 (1.2)
Candida krusei (34)	34 (100)	0 (0)	0 (0)	0 (0)	34 (100)	34 (100)	0 (0)	0 (0)	12 (35.3)	22 (64.7)	0 (0)
Candida tropicalis (22)	22 (100)	0 (0)	22 (100)	0 (0)	0 (0)	22 (100)	0 (0)	0 (0)	22 (100)	0 (0)	0 (0)
Candida lusitaniae (17)	17 (100)	0 (0)	17 (100)	0 (0)	0 (0)	17 (100)	0 (0)	0 (0)	14 (82.4)	3 (17.6)	0 (0)
Candida lipolytica (12)	12 (100)	0 (0)	2 (16.7)	3 (25)	7 (58.3)	11 (91.7)	1 (8.3)	0 (0)	5 (41.7)	7 (58.3)	0 (0)
Candida kefyr (11)	11 (100)	0 (0)	10 (90.9)	1 (9.1)	0 (0)	10 (90.9)	1 (9.1)	0 (0)	9 (81.8)	2 (18.2)	0 (0)
Candida parapsilosis (11)	11 (100)	0 (0)	11 (100)	0 (0)	0 (0)	11 (100)	0 (0)	0 (0)	11 (100)	0 (0)	0 (0)
Candida dubliniensis (9)	9 (100)	0 (0)	6 (66.7)	3 (33.3)	0 (0)	9 (100)	0 (0)	0 (0)	9 (100)	0 (0)	0 (0)
Candida spp. (17)	17 (100)	0 (0)	11 (64.7)	2 (11.8)	4 (23.5)	17 (100)	0 (0)	0 (0)	12 (70.6)	5 (29.4)	0 (0)

was 11.9%, similar to that of a previous Greek epidemiological survey comprising 4743 patients (12.1%).[8]

In our study, the highest VVC rate was observed among women 18-29 years old, a finding consistent with several studies.[18-20] This is attributed to the sexual activity, and the increased amounts of estrogens produced in this age group that promote yeast adhesion and penetration into the vaginal mucosa.[4]

Among the fourteen species identified in this study, *C. albicans* was the predominant one accounting for 75.6% of VVC cases. An earlier study conducted over the past decade, found a higher incidence of *C. albicans* (80.2%).[8] Most epidemiologic studies worldwide reported prevalence of *C. albicans* in patients with VVC. In our study, NAC species were isolated from 24.4% of the affected women. Higher recovery rates of 33%, 41.4%, 51.5%, 65% and 67.6% NAC species have been reported in Iran, Ethiopia, Nigeria, Egypt and India, respectively.[21-25] *Candida glabrata* was the most common among NAC species followed by *C. tropicalis*. This finding is consistent with data previously reported from Greece, although the recovery rate of these species is higher in the present study. It has been suggested that the indiscriminate use of both over-the-counter and prescribed antifungal treatments may lead to selection of NAC, which are more resistant to the commonly used antifungals than *C. albicans*.[26] *Candida glabrata* accounted for more than half of cases of NAC-VVC. In the majority of reports, *C. glabrata* is the most common NAC species accounting for half to two-thirds of NAC vaginitis.[26] NAC species distribution varies in women with VVC depending on the locations and the population studied. The second most common NAC species differ in different studies worldwide, with *C. tropicalis*, *C. parapsilosis* and *C. krusei* being isolated in varying proportions. Significantly higher percentages of NAC species have been isolated from women at menopause than those at reproductive age, probably due to hormonal changes. The association of increasing age with NAC infections has been reported by several investigators.[27-29]

In our study, 62 patients (5.1%) were diagnosed with RVVC. The actual prevalence of RVVC is difficult to determine. According to several investigators, the prevalence of RVVC in adult women ranges between 6% and 9%, depending on the study population, the geographic area and the methods used.[7,8,16,30,31] A web-based survey of 6010 women from 5 European countries and the USA found a prevalence rate of 9%.[32] Similarly, a 2006 VVC study of 576 Greek women found that 8.5% had RVVC.[8] According to more recent estimates, 142 337 Greek women and about 138 million worldwide are affected by RVVC every year.[33,34] Consistent with several studies, our results suggest that *C. albicans* is the predominant species in RVVC patients.[4,7,16,35]

The assessment of in vitro susceptibility against the vaginal *Candida* isolates is important due to the increasing recovery of isolates that exhibit inherent or acquired resistance to antifungals. In Greece, there is no information regarding the antifungal susceptibility of *Candida* isolates responsible for symptomatic vaginitis. In our study, the overall fluconazole susceptibility rate for *C. albicans* is 94.2%, the susceptibility dose-dependent rate is 2.1%, and

resistance was detected in 3.7% of the isolates. Our results are concurrent with the findings of Sobel et al.[36] Higher resistance to fluconazole was reported by other investigators.[30,37,38] In addition, our study demonstrated that only 26.3% of the NAC isolates were susceptible to fluconazole, 58.1% were susceptible dose-dependent and 15.6% resistant. Fluconazole resistance has been mostly reported among NAC species.[7,29,38] Voriconazole non-susceptibility was observed among 15 *C. albicans* isolates (1.6%). Ten out of the 15 isolates were also cross-resistant to fluconazole. Similarly, susceptibility to voriconazole was detected among 97.6% of the NAC isolates. The infrequent resistance to voriconazole of *C. albicans* isolates from patients with VVC was similar to that reported in other studies.[37,39] Earlier studies from Kuwait and Ethiopia reported 100% susceptibility to voriconazole among *C. albicans* vaginal isolates.[22,27]

In cases of azole-resistant *Candida* species, vaginal suppositories of amphotericin B have been successfully used.[40] In our study, 99.8% of the *C. albicans* and 100% of the NAC isolates were susceptible to amphotericin. Another alternative for treatment of azole-resistant VVC is flucytosine, which is rarely used alone because of its narrow spectrum of antifungal activity compared with azoles and polyenes and the high resistance rates of *Candida* species. Resistance to flucytosine develops rapidly during monotherapy,[41] so it is used in combination with amphotericin.[42]

In conclusion, our data indicate that in our region vulvovaginal candidiasis is a common infection affecting more frequently women at reproductive age. *Candida albicans* is still the most frequent species involved in either sporadic or recurrent VVC, followed by *C. glabrata*. Resistance of *C. albicans* to antifungals was infrequent, although susceptibility testing for other azoles (eg, miconazole) used for treatment of VVC was not performed. Since the epidemiology of vulvovaginal candidiasis is changing over time and vary among geographical locations, continuous surveillance of changes in the prevalence and susceptibility rates of *Candida* species are necessary to guide empirical treatment.

ACKNOWLEDGEMENTS

None.

DECLARATION OF INTEREST

None.

AUTHOR CONTRIBUTIONS

S.M and VE. M. conceived the ideas; D.S, A.K. and E.N. collected the data; VE. M., D.S and A.K. analysed the data; and S.M. and G.H. led the writing.

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REFERENCES

1. Tibaldi C, Cappello N, Latino MA, Masuelli G, Marini S, Benedetto C. Vaginal and endocervical microorganisms in symptomatic and asymptomatic non-pregnant females: risk factors and rates of occurrence. *Clin Microbiol Infect*. 2009;15:670-679.
2. Andrioli JL, Oliveira GS, Barreto CS, et al. Frequency of yeasts in vaginal fluid of women with and without clinical suspicion of vulvovaginal candidiasis. *Rev Bras Ginecol Obstet*. 2009;31:300-304.
3. Höfs S, Mogavero S, Hube B. Interaction of *Candida albicans* with host cells: virulence factors, host defense, escape strategies, and the microbiota. *J Microbiol*. 2016;54:149-169.
4. Sobel JD. Vulvovaginal candidosis. *Lancet*. 2007;369:1961-1971.
5. Jacob L, John M, Kalder M, Kostev K. Prevalence of vulvovaginal candidiasis in gynecological practices in Germany: a retrospective study of 954,186 patients. *Curr Med Mycol*. 2018;4:6-11.
6. Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *Am J Obstet Gynecol*. 1998;178:203-211.
7. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. *J Clin Microbiol*. 2005;43:2155-2162.
8. Grigoriou O, Baka S, Makrakis E, Hassiakos D, Kapparos G, Kouskouni E. Prevalence of clinical vaginal candidiasis in a university hospital and possible risk factors. *Eur J Obstet Gynecol Reprod Biol*. 2006;126:121-125.
9. Ferris DG, Nyirjesy P, Sobel JD, Soper D, Pavletic A, Litaker MS. Over-the-counter antifungal drug misuse associated with patient-diagnosed vulvovaginal candidiasis. *Obstet Gynecol*. 2002;99:419-425.
10. Bauters TG, Dhont MA, Temmerman MI, Nelis HJ. Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. *Am J Obstet Gynecol*. 2002;187:569-574.
11. Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement*. Wayne, PA: Clinical and Laboratory Standards Institute; 2012. (Document M27-S4).
12. Clinical and Laboratory Standards Institute (CLSI). *Epidemiological Cutoff Values for Antifungal Susceptibility Testing*, 1st edn. Wayne, PA: Clinical and Laboratory Standards Institute; 2016. (Document M59).
13. Aballéa S, Guelfucci F, Wagner J, et al. Subjective health status and health-related quality of life among women with recurrent vulvovaginal candidosis (RVVC) in Europe and the USA. *Health Qual Life Outcomes*. 2013; 11: 169.
14. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. *Candida* vaginitis: self-reported incidence and associated costs. *Sex Transm Dis*. 2000;27:230-235.
15. Okungbowa FI, Isikhuemhen OS, Dede AP. The distribution frequency of *Candida* species in the genitourinary tract among symptomatic individuals in Nigerian cities. *Rev Iberoam Micol*. 2003;20:60-63.
16. Amouri I, Sellami H, Borji N, et al. Epidemiological survey of vulvovaginal candidosis in Sfax, Tunisia. *Mycoses*. 2011;54:499-505.
17. Bradshaw CS, Morton AN, Garland SM, Morris MB, Moss LM, Fairley CK. Higher-risk behavioral practices associated with bacterial vaginosis compared with vaginal candidiasis. *Obstet Gynecol*. 2005;106:105-114.
18. Ahmad A, Khan AU. Prevalence of *Candida* species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. *Eur J Obstet Gynecol Reprod Biol*. 2009;144:68-71.
19. Vermitsky JP, Self MJ, Chadwick SG, et al. Survey of vaginal-flora *Candida* species isolates from women of different age groups by use of species-specific PCR detection. *J Clin Microbiol*. 2008;46:1501-1503.

20. Mtibaa L, Fakhfakh N, Kallel A, et al. Vulvovaginal candidiasis: etiology, symptomatology and risk factors. *J Mycol Med*. 2017;27:153-158.
21. Mahmoudi Rad M, Zafarghandi S, Abbasabadi B, Tavallaee M. The epidemiology of *Candida* species associated with vulvovaginal candidiasis in an Iranian patient population. *Eur J Obstet Gynecol Reprod Biol*. 2011;155:199-203.
22. Bitew A, Abebaw Y. Vulvovaginal candidiasis: species distribution of *Candida* and their antifungal susceptibility pattern. *BMC Womens Health*. 2018;18:94.
23. Jimoh O, Inabo HI, Yakubu SE, Ankuma SJ, Olayinka AT. Prevalence and speciation of non-albicans vulvovaginal candidiasis in Zaria. *J Nat Sci Res*. 2016;6:51-56.
24. Haleim MMA, El-Feky EAM, Sayed A, Ismail DK, Sayed AM, Abdella RMA. Provence of non-albicans species associated with vulvovaginal candidiasis in Egyptian women. *Int J Adv Int Health Sci*. 2015;12:304-313.
25. Kumari V, Banerjee T, Kumar P, Pandey S, Tilak R. Emergence of non-albicans *Candida* among candidal vulvovaginitis cases and study of their potential virulence factors, from a tertiary care center, North India. *Indian J Pathol Microbiol*. 2013;56:144-147.
26. Makanjuola O, Bongomin F, Fayemiwo SA. An update on the roles of non-*albicans Candida* species in vulvovaginitis. *J Fungi (Basel)*. 2018;4:121.
27. Alfuzan W, Dhar R, Ashkanani H, Gupta M, Rachel C, Khan ZU. Species spectrum and antifungal susceptibility profile of vaginal isolates of *Candida* in Kuwait. *J Mycol Med*. 2015;25:23-28.
28. Dan M, Segal R, Marder V, Leibovitz A. *Candida* colonization of the vagina in elderly residents of a long-term-care hospital. *Eur J Clin Microbiol Infect Dis*. 2006;25:394-396.
29. Liu XP, Fan SR, Peng YT, Zhang HP. Species distribution and susceptibility of *Candida* isolates from patients with vulvovaginal candidiasis in southern China from 2003 to 2012. *J Mycol Med*. 2014;24:106-111.
30. Gamarra S, Morano S, Dudiuk C, et al. Epidemiology and antifungal susceptibilities of yeasts causing vulvovaginitis in a teaching hospital. *Mycopathologia*. 2014;178:251-258.
31. Corsello S, Spinillo A, Osnengo G, et al. An epidemiological survey of vulvovaginal candidiasis in Italy. *Eur J Obstet Gynecol Reprod Biol*. 2003;110:66-72.
32. Foxman B, Muraglia R, Dietz JP, Sobel JD, Wagner J. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *J Low Genit Tract Dis*. 2013;17:340-345.
33. Gamaletsou MN, Drogari-Apiranthitou M, Denning DW, Sipsa NV. An estimate of the burden of serious fungal diseases in Greece. *Eur J Clin Microbiol Infect Dis*. 2016;35:1115-1120.
34. Denning DW, Kneale M, Sobel JD, Rautemaa-Richardson R. Global burden of recurrent vulvovaginal candidiasis: a systematic review. *Lancet Infect Dis*. 2018;18:e339-e347.
35. Sobel JD. Recurrent vulvovaginal candidiasis. *Am J Obstet Gynecol*. 2016;214:15-21.
36. Sobel JD, Zervos M, Reed BD, et al. Fluconazole susceptibility of vaginal isolates obtained from women with complicated *Candida* vaginitis: clinical implications. *Antimicrob Agents Chemother*. 2003;47:34-38.
37. Adjapong G, Hale M, Garrill A. A comparative investigation of azole susceptibility in *Candida* isolates from vulvovaginal candidiasis and recurrent vulvovaginal candidiasis patients in Ghana. *Med Mycol*. 2017;55:686-689.
38. Zhang JY, Liu JH, Liu FD, et al. Vulvovaginal candidiasis: species distribution, fluconazole resistance and drug efflux pump gene overexpression. *Mycoses*. 2014;57:584-591.
39. Luo X, Dong X, Pen Z. Distribution and drug susceptibility of *Candida* spp. associated with female genital tract infection, Chongqing, China. *Jundishapur. J Microbiol*. 2015;9:e19386.
40. Philips AJ. Treatment of non-albicans *Candida* vaginitis with amphotericin B vaginal suppositories. *Am J Obstet Gynecol*. 2005;192:2009-2013.
41. Kanafani ZA, Perfect JR. Antimicrobial resistance: resistance to antifungal agents: mechanisms and clinical impact. *Clin Infect Dis*. 2008;46:120-128.
42. White DJ, Habib AR, Vanthuyne A, Langford S, Symonds M. Combined topical flucytosine and amphotericin B for refractory vaginal *Candida glabrata* infections. *Sex Transm Infect*. 2001;77:212-213.

How to cite this article: Maraki S, Mavromanolaki VE, Stafylaki D, Nioti E, Hamilos G, Kasimati A. Epidemiology and antifungal susceptibility patterns of *Candida* isolates from Greek women with vulvovaginal candidiasis. *Mycoses*. 2019;62:692–697. https://doi.org/10.1111/myc.12946