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Epidemiology of Vulvovaginal Candidiasis in Greece: A 2-Year Single-Centre Study

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ABSTRACT

Background: The epidemiology of vulvovaginal candidiasis (VVC) in Greece remains poorly reported and outdated.

Objectives: We therefore conducted a 2-year retrospective survey to assess the epidemiological aspects of the infection among symptomatic Greek patients.

Patients/Methods: High vaginal swab samples were collected from adult women with clinically suspected VVC attending a private diagnostic laboratory in Athens. VVC was confirmed through microscopic examination of a wet mount preparation revealing yeasts and *Candida*-positive culture. Species were identified by MALDI-ToF MS, and in vitro susceptibility was determined according to the EUCAST-E.Def 7.4. Predisposing host factors were associated with the occurrence of the infection and isolated *Candida* spp. using Fisher's exact test, and epidemiological changes over time were analysed with the χ^2 test for trend.

Results: Among 1300 women screened, 283 VVC episodes were recorded among 233 (18%) patients, whereof 11 (5%) had recurrent VVC (RVVC) and 19 (8%) had mixed *Candida* infections. Coinfection with other pathogens and recent prior use of antifungals were associated with RVVC. *Candida albicans* was the most prevalent pathogen (50%), followed by *Candida parapsilosis sensu stricto* (SS) (35%), *Nakaseomyces glabratus* (former *Candida glabrata*) (10%), *Pichia kudriavzevii* (former *Candida krusei*) (3%), *Candida orthopsilosis* (1.5%) and *Clavispora lusitaniae* (former *Candida lusitaniae*) (0.5%). Regarding the RVVC cases, 54% were attributed to *C. albicans*, 37% to *N. glabratus* and 9% to *C. parapsilosis* SS. Resistance to fluconazole was found in 4% of *C. albicans* and 23% of *N. glabratus* strains with cross-resistance to other azoles. Fluconazole-resistant isolates were recovered from 5 of 11 RVVC patients, whereof 4 of 5 had previous exposure to azoles. During the study period, an increase in *N. glabratus* VVC and fluconazole resistance was noted.

Conclusions: VVC is common in our region, with *C. albicans* as the predominant species, followed by *C. parapsilosis* SS and *N. glabratus*. Fluconazole resistance is low in *C. albicans* but high in *N. glabratus*, emphasising the need for targeted antifungal strategies.

The last two authors share authorship.

1 | Introduction

Vulvovaginal candidiasis (VVC) is the second most common vaginal infection globally, caused by opportunistic pathogens of the *Candida* genus [1]. Approximately 75% of women will suffer from VVC at least once in their lifetime [2], while an estimated 9% will develop recurrent vulvovaginal candidiasis (RVVC) [3], which is defined as three or more confirmed episodes of VVC within a year [4]. VVC is a multifactorial disorder caused by a disruption of the host's local defence mechanisms, leading to an imbalance in the vaginal microbiota and *Candida* overgrowth [5]. Several predisposing factors that increase the risk of developing VVC have been described, including oestrogens and hormone replacement, pregnancy, diabetes mellitus, coinfection with bacteria or viruses, and prolonged use of broad-spectrum antibiotics or antifungal medications [6, 7].

Although *Candida albicans* remains the predominant pathogen in VVC, a rising shift to non-*albicans Candida* spp. (NAC) has been reported over the last two decades [8–10]. Of note, treatment failure in NAC vaginitis is frequent, as certain species are inherently resistant or display reduced susceptibility to azoles, the first-line treatment for VVC [11, 12]. To date, the therapeutic approach to the disease is primarily empirical, as species-specific clinical breakpoints (CBPs) or epidemiological cut-off values (ECOFFs) for most of the antifungals administered are not available [13]. Given that empirical treatment relies heavily on local epidemiological data, it is essential to regularly monitor regional variations in prevalence rates, species distribution, and susceptibility profiles of VVC isolates over time.

We therefore conducted a 2-year retrospective study among symptomatic women who visited a Greek private diagnostic laboratory. The study aimed to provide valuable insights into the demographic characteristics, predisposing factors, treatment patterns, pathogen prevalence and antifungal susceptibility profiles among women with VVC.

2 | Materials and Methods

2.1 | Study Setting and Population

A retrospective surveillance study was conducted at the private diagnostic laboratory 'MycoLab' (Athens, Greece), specialising in fungal infection testing and diagnostics, from October 2019 to November 2021. The study involved adult women with VVC signs (such as oedema, excoriation/fissures, and thick curdy vaginal discharge) and symptoms (such as burning, itching and irritation) referred for culture after gynecologic consultation. Data on the date of sample collection and patients' age, underlying host factors, microbiological findings, and response to antifungal treatment, were collected removing identifying information. No ethics approval was required as patients' data were anonymised.

From each patient, two high vaginal swab samples were collected aseptically, one for direct wet mount microscopy using 10% potassium hydroxide solution and the other for culture. Samples were inoculated onto blood agar, MacConkey agar, and chocolate agar (all three media from Bioprepare, Athens, Greece) incubated at 37°C, and Sabouraud glucose agar with gentamicin and chloramphenicol (SGC; Oxoid, Athens, Greece) and chromogenic agar (*Brilliance*^{TM} *Candida* Agar; Oxoid, Athens, Greece) incubated at 30°C for up to 48 h. The pH of the samples was also measured using pH indicator strips. A confirmed VVC episode was defined through microscopic detection of yeast structures (budding yeasts and/or pseudohyphae) in fresh vaginal smears, followed by positive *Candida* spp. culture [4]. Patients with positive for *Candida* microscopy and/or culture but without corresponding clinical symptoms/signs were not included in the study. RVVC was considered as the occurrence of ≥ 3 symptomatic VVC episodes within 1 year [4]. Patients with mixed VVC, identified as the isolation of ≥ 2 different *Candida* spp. from a single specimen, and mixed vaginitis due to VVC and bacterial vaginosis or trichomoniasis were included.

2.2 | Species Identification

VVC isolates were initially identified at species level by the colorimetric yeast identification Auxacolor^{TM} 2 commercial system (Bio-rad, Athens, Greece). All isolates were stored at −70°C in normal sterile saline with 10% glycerol (AppliChem, Athens, Greece) and were retrospectively identified by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF MS; Bruker Daltonics, Bremen, Germany).

2.3 | In Vitro Antifungal Susceptibility Testing (AFST)

AFST was conducted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) E.Def 7.4 protocol [14]. Briefly, laboratory-grade pure powders of fluconazole, itraconazole, ketoconazole, econazole, clotrimazole, boric acid (all powders from TCI, Athens, Greece), miconazole (Fluorochem, Athens, Greece) and fenticonazole (Sigma-Aldrich, Athens, Greece) were used. The microtitre plates were incubated at 35°C ± 2°C for 24 h, and the MICs were determined spectrophotometrically (540 nm) as the lowest drug concentration at which a ≥ 50% fungal growth inhibition, compared with the drug-free control, was observed. Inoculum density checks were performed by spread plate counts on SGC agar plates, whereas the recommended *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality control strains.

To classify the isolates, species-specific EUCAST CBPs were applied for fluconazole and itraconazole [15]. For other drugs, recently estimated local ECOFFs for the EUCAST E.Def 7.4 method were used to discriminate between wild-type (WT) and non-WT strains [16].

2.4 | Statistical Analysis

The incidence of VVC was expressed as the ratio of patients diagnosed with VVC to the total number of women visiting the laboratory during the study period. The incidence of RVVC was

expressed as the ratio of patients that developed RVVC to the number of women diagnosed with VVC. Medians and interquartile ranges (IQR) were calculated for continuous variables, while numbers and percentages were calculated for categorical parameters. Categorical variables (various predisposing host factors) were associated with the occurrence of VVC/RVVC and the isolation rate of the different *Candida* spp. using Fisher's exact test. The prevalence of mixed VVC, RVVC, different *Candida* species and resistance to fluconazole together with coinfection rates and treatment modalities (combination therapy, use of fluconazole, topical treatment, previous exposure to antifungals or antibiotics) were evaluated over the study period broken in 6 months intervals using the χ^2 test for trend. For both tests, a two-tailed p value of <0.05 was considered indicative of a statistically significant difference. The modal minimum inhibitory concentrations (MICs), MIC ranges, geometric mean (GM) MICs, MIC_{50}s and MIC_{90}s (the concentrations that inhibited 50% and 90%, respectively, of the isolates), and resistance/non-WT rates were also estimated. Data analysis was conducted using the statistics software package GraphPad Prism, version 10.0, for Windows (GraphPad Software, San Diego, CA, USA).

3 | Results

3.1 | Demographic Data and Predisposing Factors

During the 2-year study period, 1300 symptomatic adult women visited the laboratory. In total, 233 (18%) patients were diagnosed with VVC (overall 283 episodes), 19 (8%) had mixed *Candida* infections, and 11 (5%) suffered from RVVC (3–6 episodes/year). Their median (range, IQR) age was 37 (20–69, 31–45) years, with the majority (193/233; 83%) being of reproductive age (≤49 years).

Pregnancy was reported in a small proportion of patients (7/233; 3%). Disturbance in the normal vaginal flora, characterised by the detection of a limited number of lactobacilli during the microscopic examination of vaginal smears (<10% of the optical field, considering at least 10 optical fields), was recorded in more than half of the patients (156/233; 67%). Additionally, a large proportion of patients (168/233; 72%) had mixed vaginitis (Table 1). The pH of vaginal samples had a median (range, IQR) of 6.0 (3.5–9.0, 5.5–6.5). Notably, samples from VVC caused by *C. albicans* and NAC had similar pH (median 5.5 and 6.0, respectively). Despite *C. albicans* being the dominant species in both VVC and RVVC patients, the number of patients with pH >4.5 was significantly higher among women with RVVC (82 vs. 36%, $p=0.003$).

Moreover, half of the patients (130/233, 56%) had received antibiotics, predominantly metronidazole, during the past month. The use of antifungal agents, for longer than 1 week, up to 1 month before visit—with or without a medical prescription—was recorded for 44 of 233 (19%) patients. Considering the RVVC cases, 6 of 11 (55%) patients reported previous systemic antifungal treatment, with either fluconazole (3; 50% as monotherapy and 2; 33% in combination with topical azoles) or itraconazole (1; 17%) (Table 1). Previous exposure to fluconazole or itraconazole or a combination of oral and topical treatment was significantly higher among patients with RVVC ($p\leq0.047$).

Among the predisposing host factors, only the frequency of recent antifungal use and coinfection was significantly higher in patients with RVVC than those with VVC (55% vs. 17%, $p=0.007$ and 100% vs. 71%, $p=0.037$, respectively), specifically mixed vaginitis with *U. urealyticum* (55% vs. 20%, $p=0.016$) and *C. trachomatis* (36% vs. 12%, $p=0.044$) (Table 1). Over the 2-year study period, the reported previous exposure to antifungals increased (from 17% to 38%, p for trend $=0.013$).

3.2 | Species Distribution

Among the 302 isolates recovered from 283 VVC episodes, *C. albicans* accounted for the majority of them (152/302; 50%), followed by *C. parapsilosis sensu stricto* (SS) (105/302; 35%) and *Nakaseomyces glabratus* (31/302; 10%). Other species, including *Pichia kudriavzevii* (8/302; 3%), *C. orthopsilosis* (5/302; 1.5%) and *Clavispora lusitaniae* (1/302; 0.5%), were rare.

Among the 38 isolates recovered from 19 episodes with mixed infections, *C. albicans* was the species most frequently isolated (34/38; 90%). Particularly, there were 11 of 19 (58%) episodes with *C. albicans* and *C. parapsilosis* SS, 5 of 19 (27%) with *C. albicans* and *N. glabratus*, 1 of 19 (5%) with *C. albicans* and *P. kudriavzevii*, 1 of 19 (5%) with *C. parapsilosis* SS and *N. glabratus* and 1 of 19 (5%) with *C. parapsilosis* SS and *C. orthopsilosis*.

Concerning the 39 isolates recovered from 11 RVVC cases, *C. albicans* remained the predominant species (21/39, 54%). However, the isolation rate of *N. glabratus* (10/39, 26%) was significantly higher than VVC (26% vs. 10%, $p=0.015$), as opposed to that of *C. parapsilosis* SS that was reduced (8/39, 20%). Overall, the most common species in 11 RVVC cases was *C. albicans* (6/11, 54%), followed by *N. glabratus* (4/11, 37%) and *C. parapsilosis* SS (1/11, 9%).

The dispersion of *C. albicans* and NAC isolates did not appear to significantly differ according to predisposing factors, such as the balance of the vaginal flora, recent antibiotic use and pregnancy ($p\geq0.126$). However, NAC infections were considerably less common in women of reproductive age ($p=0.024$), whereas in coinfected patients *C. albicans* was the mainly isolated species ($p<0.001$) (Table 1). We also observed a significant increase in *N. glabratus* VVC (from 8% to 24%, p for trend $=0.002$) over the study period.

3.3 | MALDI-ToF MS Versus Auxacolor

The agreement between MALDI-ToF MS and Auxacolor in identifying different *Candida* spp. was 91% (274/302). In particular, 8 of 31 (26%) *N. glabratus* strains, 7 of 105 (7%) *C. parapsilosis* SS, 4 of 152 (3%) *C. albicans*, and 1 of one (100%) *C. lusitaniae* were misidentified by Auxacolor (Table 2).

3.4 | Antifungal Susceptibility Testing

The in vitro antifungal susceptibilities of the 302 VVC isolates against 8 antifungals are summarised in Table 3. Overall, varying resistance/non-WT phenotype rates were observed among

TABLE 1 | Predisposing host factors for VVC ($n=222$) and RVVC ($n=11$) cases and cases caused by *Candida albicans* ($n=126$) and NAC species ($n=107$).

	No of patients (%)			No of patients with VVC + RVVC (%)		
Host factor	**VVC**	**RVVC**	***p*[a]**	***C. albicans*[b]**	**NAC**	***p*[a]**
Reproductive age (≤49 years)	182 (82)	11 (100)	0.219	111 (88)	82 (77)	**0.024**
Pregnancy	7 (3)	0 (0)	>0.999	4 (3)	3 (3)	>0.999
Disturbed vaginal flora	149 (67)	7 (64)	0.755	90 (71)	66 (62)	0.126
Vaginal pH > 4.5	79 (36)	10 (91)	**0.003**	51 (41)	38 (36)	0.499
Coinfection	157 (71)	11 (100)	**0.037**	105 (83)	63 (59)	**<0.001**
Ureaplasma urealyticum	45 (20)	6 (55)	**0.016**	31 (25)	20 (19)	0.341
Anaerobic bacteria	45 (20)	3 (27)	0.701	29 (23)	19 (17)	0.335
Enterobacterales	43 (19)	3 (27)	0.458	25 (20)	21 (20)	>0.999
Streptococcus agalactiae	39 (18)	3 (27)	0.422	21 (17)	21 (20)	0.610
Chlamydia trachomatis	27 (12)	4 (36)	**0.044**	18 (14)	13 (12)	0.701
Staphylococcus spp.	21 (9)	1 (9)	>0.999	7 (6)	15 (14)	**0.041**
Enterococcus spp.	13 (6)	2 (18)	0.152	7 (6)	8 (7)	0.600
Mycoplasma genitalium	5 (2)	1 (9)	0.254	4 (3)	2 (2)	0.690
HPV	2 (0.9)	0 (0)	>0.999	0 (0)	2 (2)	0.210
HSV-2	1 (0.5)	0 (0)	>0.999	1 (0.8)	0 (0)	>0.999
UTI[c]	10 (5)	2 (18)	0.103	7 (6)	5 (5)	>0.999
Endometriosis	24 (11)	1 (9)	>0.999	10 (8)	15 (14)	0.144
Diabetes mellitus	2 (0.9)	1 (9)	0.136	0 (0)	3 (3)	0.095
Recent antibiotic use[c]	121 (55)	9 (82)	0.118	70 (56)	60 (56)	>0.999
Recent antifungal use[c]	38 (17)	6 (55)	**0.007**	28 (22)	16 (15)	0.181
Fluconazole per os	14 (37)	3 (50)	**0.037**	12 (43)	5 (3)	0.208
Itraconazole per os	0 (0)	1 (17)	**0.047**	0 (0)	1 (6)	0.484
Fluconazole + itraconazole per os	6 (16)	0 (0)	>0.999	5 (18)	1 (6)	0.217
Topical treatment with azoles	12 (32)	0 (0)	>0.999	9 (32)	3 (19)	0.147
Topical treatment with boric acid	2 (5)	0 (0)	>0.999	0 (0)	2 (13)	0.214
Topical + oral treatment	4 (10)	2 (33)	**0.028**	2 (7)	4 (25)	0.417

Abbreviations: HPV, human papillomavirus; HSV-2, herpes simplex virus type 2; NAC, non-*albicans Candida* spp.; RVVC, recurrent vulvovaginal candidiasis; VVC, vulvovaginal candidiasis.
[a]Significant differences ($p<0.05$) are indicated with bold numbers.
[b]Mixed infections with *C. albicans* + NAC were also included.
[c]Over the past month.

the species, with *N. glabratus* exhibiting the highest resistance level. The fluconazole resistance rate across all species was 4.3% (13/302 strains), with 1% (3/302) categorised as susceptible increased exposure. An increase in fluconazole resistance in VVC cases over time was observed (from 5% to 24%, p for trend = 0.054). For itraconazole, 5% of *C. albicans* and 19% of *N. glabratus* strains were resistant, with all *C. parapsilosis* SS, *C. orthopsilosis* and *P. kudriavzevii* strains being susceptible. For the other azoles, non-WT rates ranged from 5% to 16% for *C. albicans*, from 0% to 1% for *C. parapsilosis* SS, from 0% to 35% for *N. glabratus* and from 0% to 40% for *C. orthopsilosis*. Lastly, no *Candida* species were classified as non-WT for boric acid and the substance exhibited its highest in vitro activity against *P. kudriavzevii*.

Cross-resistance emerged among azole drugs, particularly notable with miconazole, where *C. albicans* exhibited the highest rate of non-WT strains among the species. Among the 24 miconazole non-WT *C. albicans* strains, 11 of 24 were also non-WT for other two azoles, 11 of 24 were resistant/non-WT for >2 other azoles, and only 2 of 24 did not show cross-resistance. Similarly, for *N. glabratus*, which exhibited

TABLE 2 | Identification of the different *Candida* species using MALDI-ToF MS and Auxacolor.

MALDI-ToF MS (no isolates)	% of isolates identified with Auxacolor (no isolates/total number per MALDI-TOF species ID)
Nakaseomyces glabratus (31)	74% *C. glabrata* SC (23/31)
	19.5% *P. kudriavzevii* (6/31)
	6.5% *C. albicans* (2/31)
Candida parapsilosis SS (105)	93% *C. parapsilosis* SC (98/105)
	2% *N. glabratus* (2/105)
	2% *P. kudriavzevii* (2/105)
	2% *C. albicans* (2/105)
	1% *C. tropicalis* (1/105)
Candida albicans (152)	97% *C. albicans* (148/152)
	1% *P. kudriavzevii* (1/152)
	2% *C. parapsilosis* SC (3/152)
Pichia kudriavzevii (8)	100% *P. kudriavzevii* (8/8)
Clavispora lusitaniae (1)	100% *P. kudriavzevii* (1/1)
Candida orthopsilosis (5)	100% *C. parapsilosis* SC (5/5)
Mixed cultures (*C. albicans* + *C. parapsilosis* SS) (11)	73% *C. albicans* + *C. parapsilosis* SC (8/11)
	27% Single culture of *C. albicans* (3/11)

Note: Maldi-ToF MS: matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry.

the highest rate of fluconazole-resistant strains, 1 of 7 were non-WT for other two azoles and the remaining 6 of 7 were non-WT for > 2 other azoles. Cross-resistance to fluconazole and itraconazole was noted for 5 of 152 (3.3%) *C. albicans* and 6 of 31 (19.4%) *N. glabratus*. All those strains were non-WT for ≥ 2 other azoles, as well.

3.5 | Fluconazole Resistance in RVVC Cases

Among the 39 strains isolated from RVVC cases, 9 (23%) were fluconazole-resistant (5 *C. albicans* and 4 *N. glabratus*). These were recovered from 5 of 11 (45%) RVVC patients, whereof 4 of 5 (80%) had previously received oral azoles for ≥ 3 weeks (3 fluconazole and 1 itraconazole), while the remaining patient had no prior antifungal exposure. According to the available follow-up, clinical and mycological cure were recorded for 4 of 5 patients after concurrent administration of boric acid and probiotics (*Bacillus coagulans*). However, the remaining patient, who had 6 VVC episodes in 9 months involving 4 fluconazole-resistant and 2 fluconazole-susceptible *C. albicans* strains, did not respond to oral fluconazole, oral itraconazole, fenticonazole and the combination of boric acid and probiotics (*B. coagulans*).

4 | Discussion

During this two-year study, the prevalence of VVC and RVVC was 18% and 5%, respectively. Coinfection and prior antifungal use were more common among patients with RVVC. *C. albicans* was isolated more frequently, accounting for 50% of cases, followed by *C. parapsilosis* SS and *N. glabratus*, found in 35% and 10% of cases, respectively. Compared with NAC species, *C. albicans* was more prevalent among patients of reproductive age and those with coinfections. During the study period, an increase in *N. glabratus* VVC and fluconazole resistance was noted. AFST revealed varying degrees of resistance to azole antifungals among the isolated species, with *N. glabratus* exhibiting the highest resistance level. Overall, fluconazole resistance was observed in 4.3% of cases, and cross-resistance among azole drugs was also noted.

Our findings align with previous Greek data (Table S1) but offer important insights into VVC in our country. Existing literature was limited with over 50% of the studies performed before 2010. The incidence of VVC and RVVC across all age groups ranged 7%–32% (median 19.5%) and 5%–8%, respectively, as found in the present cohort. Over the years, *C. albicans* has been the major cause of VVC in Greece, with a frequency ranging 76%–99.6%, followed by *C. glabrata* SC, *P. kudriavzevii* and *C. parapsilosis* species complex (SC). We estimated a higher prevalence of NAC, which may reflect the increasing incidence of NAC in VVC, described in recent epidemiological studies [8, 17] or may be attributed to the use of identification methods with lower accuracy (API 20 or Vitek 2, BioMérieux) in previous studies [18]. Regarding RVVC, the isolation rate of *C. albicans* ranged 39%–71%, while existing studies do not provide data about the NAC species involved in RVVC. The previously reported resistance rate to fluconazole was 4%–7% for *C. albicans*, 0% for *C. parapsilosis* SC, and 1%–45% for *C. glabrata* SC. In vitro susceptibility of Greek VVC strains to topical antifungals has not been reported. Previous studies used nonstandard methods, focussed on fluconazole or applied arbitrary resistance breakpoints, while in this study, local ECOFFs recently determined using state-of-the art approaches were used [16]. Specifically, 4% of *C. albicans* isolates were resistant to fluconazole, aligning with the only Greek study reporting susceptibility data [19] Greek study[]. The global literature reports similar or higher rates (3.5%–8.8%) [12, 20–22]. The estimated resistance to itraconazole (5%) is consistent with studies from Turkey [23], Iran [22] and Vietnam [24] (2.4%–6.5%). Furthermore, we found 16% non-WT *C. albicans* strains for miconazole and ketoconazole, which were estimated at 3.5%–6.5% and 25.8%–27.7%, respectively, using Neo-Sensitabs (miconazole, $R \leq 11$ mm and

TABLE 3 | Profile of in vitro susceptibility to eight antifungals of VVC isolates.

Species (no isolates)	Antifungal agent	Modal (range) MIC (mg/L)	GM MIC (mg/L)	MIC_{50}/MIC_{90} (mg/L)	% resistant/ non-WT[a]
Candida albicans (152)	Fluconazole	0.25 (≤0.06 to 16)	0.29	0.25/2	4%
	Itraconazole	0.016 (≤0.004 to 0.125)	0.01	0.008/0.016	5%
	Miconazole	0.016 (≤0.004 to 2)	0.03	0.016/0.5	16%
	Ketoconazole	0.008 (≤0.004 to > 2)	0.01	0.004/0.125	16%
	Fenticonazole	0.5 (0.125 to > 2)	0.39	0.5/1	5%
	Econazole	0.03 (0.008 to 2)	0.03	0.016/0.25	15%
	Clotrimazole	0.008 (≤0.004 to 0.125)	0.01	0.008/0.016	8%
	Boric acid	1600 (400 to 3200)	1459	1600/1600	0%
Candida parapsilosis SS (105)	Fluconazole	1 (0.25 to 1)	0.80	1/1	0%
	Itraconazole	0.016 (0.008 to 0.125)	0.02	0.008/0.03	0%
	Miconazole	0.25 (0.125 to 0.5)	0.31	0.25/0.5	0%
	Ketoconazole	0.03 (0.016 to 0.125)	0.03	0.016/0.016	1%
	Fenticonazole	1 (0.5 to > 2)	1.2	1/2	1%
	Econazole	0.5 (0.125 to 2)	0.43	0.5/0.5	1%
	Clotrimazole	0.03 (0.016 to 0.06)	0.03	0.03/0.06	0%
	Boric acid	1600 (200 to 3200)	1160	1600/1600	0%
Nakaseomyces glabratus (31)	Fluconazole	8 (2 to > 64)	13.68	16/> 64	23%
	Itraconazole	0.25 (0.125 to > 2)	0.51	0.25/> 2	19%
	Miconazole	0.5 (0.03 to 1)	0.22	0.25/0.5	0%
	Ketoconazole	0.5 (0.03 to > 2)	0.48	0.5/> 2	23%
	Fenticonazole	1 (0.5 to > 2)	1.1	1/2	10%
	Econazole	0.125 (0.03 to 1)	0.21	0.25/1	0%
	Clotrimazole	0.25 (0.6 to 4)	0.64	0.5/4	35%
	Boric acid	3200 (400 to 6400)	2691	3200/6400	0%
Pichia kudriavzevii (8)	Fluconazole	32 (8 to 64)	26.91	32/64	0%
	Itraconazole	0.03 (0.03 to 0.25)	0.07	0.06/0.25	0%
	Miconazole	2 (0.125 to 2)	0.92	2/2	NA
	Ketoconazole	0.125/0.5 (0.125 to 0.5)	0.25	0.25/0.5	NA
	Fenticonazole	2 (1 to 2)	1.68	2/2	NA
	Econazole	1 (0.5 to 2)	0.92	1/2	NA
	Clotrimazole	0.06 (0.03 to 0.25)	0.08	0.06/0.25	NA
	Boric acid	800 (200 to 1600)	800	800/1600	NA
Candida orthopsilosis (5)	Fluconazole	2 (0.5 to 2)	1	1/2	0%
	Itraconazole	0.06 (0.008 to 0.06)	0.03	0.03/0.06	0%
	Miconazole	0.5 (0.5 to 2)	0.87	0.5/2	40%
	Ketoconazole	0.016 (0.016 to 0.03)	0.02	0.016/0.03	0%
	Fenticonazole	2 (1 to > 2)	2.3	2/> 2	40%
	Econazole	0.5 (0.25 to 1)	0.57	0.5/1	40%
	Clotrimazole	0.125 (0.3 to 0.125)	0.08	0.125/0.125	60%
	Boric acid	1600 (1600 to 3200)	1600	1600/3200	0%

(Continues)

TABLE 3 | (Continued)

Species (no isolates)	Antifungal agent	Modal (range) MIC (mg/L)	GM MIC (mg/L)	MIC_{50}/MIC_{90} (mg/L)	% resistant/ non-WT[a]
Candida lusitaniae (1)	Fluconazole	0.25	NA	NA	NA
	Itraconazole	0.008	NA	NA	NA
	Miconazole	0.016	NA	NA	NA
	Ketoconazole	≤0.004	NA	NA	NA
	Fenticonazole	0.5	NA	NA	NA
	Econazole	0.016	NA	NA	NA
	Clotrimazole	0.016	NA	NA	NA
	Boric acid	ND	NA	NA	NA

Abbreviations: GM, geometric mean; MIC, minimum inhibitory concentration; NA, not available; ND, not determine; non-WT, non-wild type.
[a]According to the EUCAST CBPs of fluconazole and itraconazole [15] and local ECOFFs of the other drugs [16].

ketoconazole, $R \leq 20$ mm) [25] and the CLSI method (for both drugs, $R \geq 4$ mg/L) [26]. However, higher rates of resistance to miconazole have also been reported (25%) [27]. For clotrimazole, a resistance rate of 10% (versus 8% non-WT in this study) has been estimated with the CLSI method ($R \geq 1$ mg/L) [28]. Lower rates of resistance (1.6%–7.3%) for these drugs have also been reported with the CLSI method [23]. Notably, the same study reported only 0.8% fluconazole-resistant *C. albicans* strains [23], supporting the observation that resistance among these azoles is correlated and can be shared [16]. We also observed that 92% of miconazole-non-WT *C. albicans* were non-WT to multiple azoles. Similarly, 84% of miconazole-resistant strains of the species were reported as resistant to fluconazole, itraconazole and econazole [29].

For *N. glabratus*, the MICs of the tested antifungals were comparatively higher than those of *C. albicans*. Specifically, 23% of the strains were resistant to fluconazole. A similar Greek–Belgian study reports 45% resistance for the species [30]. However, studies on VVC in Turkey [31] and China [32] identified resistance rates of 25% and 10%, respectively. According to our data, 19% of the *N. glabratus* strains were itraconazole-non-WT. Similar data were published after a study in Iran (24%) [22]. We also reported the highest resistance for clotrimazole and no resistance for miconazole and econazole. Similar trends in antifungal resistance of *C. glabrata* were described using the CLSI method for itraconazole ($R \geq 1$ mg/L), ketoconazole ($R \geq 16$ mg/L), miconazole ($R \geq 4$ mg/L) and clotrimazole ($R \geq 0.5$ mg/L), 8.3%, 5.6%, 2.8% and 14%, respectively [23]. However, we estimated higher non-WT rates (19%–35%) for all these drugs, except miconazole. The resistance rates of the species in this study may have been underestimated, as non species specific cutoffs were used . Our research also found that all fluconazole-resistant *N. glabratus* isolates exhibited cross-resistance to other azoles. According to a five-year study in Michigan, USA, the clinical response and mycological eradication rates of *C. glabrata*, related to treatment with topical and systemic azoles, were < 50% [33]. In contrast, we observed that fluconazole-resistant *N. glabratus* strains remained WT for miconazole and econazole, and similar data were provided by a study performed AFST with the CLSI method [34]. As reported, 26.9% were resistant to fluconazole, no resistance to miconazole (MICs < 0.6 mg/L) was observed, but econazole was not tested [34].

The present study aligns with existing literature indicating that while *C. parapsilosis* SS exhibits higher MICs to azoles than *C. albicans*, resistance remains rare in *C. parapsilosis* VVC cases [35, 36]. A previous study reported that strains of *C. parapsilosis* SS, *C. orthopsilosis* and *C. metapsilosis*, isolated from VVC patients were all susceptible to fluconazole and itraconazole, with no significant differences in treatment response [37]. However, the in vitro susceptibility data for these species remain limited. Regarding *P. kudriavzevii*, all strains tested were WT to fluconazole and itraconazole, as in a previous study from Turkey [38]. Nevertheless, *P. kudriavzevii* is considered intrinsically resistant to fluconazole and might exhibit resistance to all azoles since the species have MICs higher than the recently proposed local ECOFFs by our group [16]. These findings are supported by clinical trials reporting poor response to azole therapy [16].

Our research showed that boric acid, recommended by the Centers for Disease Control and Prevention as a therapeutic option for persistent VVC caused by NAC [4], was effective for all *Candida* spp. tested (0% non-WT for *C. albicans*, *C. parapsilosis* SS and *N. glabratus*). A recent retrospective study based on clinical charts estimates 77.1% satisfaction from treating RVVC with boric acid and 80% from treating both RVVC and recurrent bacterial vaginosis [39]. The antimicrobial properties of boric acid may contribute to alleviating symptoms in women with vaginal infections, as supported by our data indicating a high frequency of coinfections with *Candida* and bacteria. Nevertheless, resistance in VVC is not limited to NAC species; fluconazole resistance in *C. albicans* has been an ongoing concern for over a decade [40, 41], with an estimated increase of 7.1% from 2012 to 2021 [41]. Following the global epidemiological trends, this study shows a growing trend in both azole exposure and fluconazole resistance in Greece.

In conclusion, this study offers valuable insights into the epidemiology of VVC. We assessed disease prevalence, identified risk factors, described the latest epidemiological trends and evaluated the responsible pathogens, along with their susceptibility to azoles used in both topical and systemic treatment. While these findings are important, ongoing surveillance is essential to develop comprehensive resistance patterns and effective treatment protocols, particularly through multicentre studies. In the

meantime, the current data can guide clinicians in managing VVC, underscoring the importance of incorporating resistance trends in treatment decisions and considering alternative treatment options.

Author Contributions

Vasiliki Kroustali: writing – original draft, formal analysis, data curation, investigation, visualization. **Esmeralda Resoulai:** writing – review and editing, data curation, investigation. **Lamprini Kanioura:** investigation, writing – review and editing, validation, data curation. **Maria Siopi:** writing – review and editing, methodology, validation, formal analysis, supervision. **Joseph Meletiadis:** conceptualization, funding acquisition, writing – review and editing, validation, project administration, resources, supervision, software. **Stavroula Antonopoulou:** resources, supervision, data curation, project administration, writing – review and editing, validation, funding acquisition, methodology.

Conflicts of Interest

J.M. has received research grants paid to the institute from Gilead, Vircell, Mundipharma and Pfizer. The other authors have nothing to declare.

Data Availability Statement

Data available upon request from authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.