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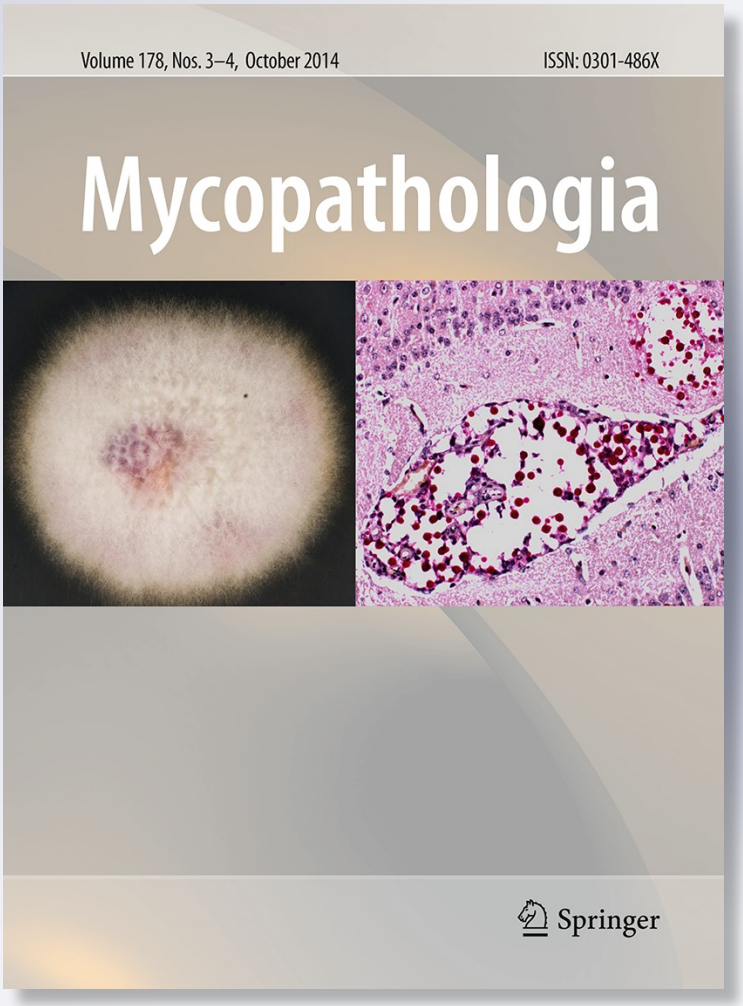

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Epidemiology and Antifungal Susceptibilities of Yeasts Causing Vulvovaginitis in a Teaching Hospital

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Abstract Vulvovaginal candidiasis is one of the most common mycosis. However, the information about antifungal susceptibilities of the yeasts causing this infection is scant. We studied 121 yeasts isolated from 118 patients with vulvovaginal candidiasis. The isolates were identified by phenotypic and molecular methods, including four phenotypic methods described to differentiate *Candida albicans* from *C. dubliniensis*. Antifungal susceptibility testing was performed according to CLSI documents M27A3 and M27S4 using the drugs available as treatment option in the hospital. Diabetes, any antibacterial and amoxicillin treatment were statistically linked with vulvovaginal candidiasis, while oral contraceptives were not considered a risk factor. Previous azole-based over-the-counter antifungal treatment was statistically associated with non-*C.albicans* yeasts infections. The most common isolated yeast species was *C. albicans* (85.2 %) followed by *C. glabrata* (5 %), *Saccharomyces cerevisiae* (3.3 %), and *C. dubliniensis* (2.5 %). Fluconazole- and itraconazole-reduced susceptibility was observed in ten and in only one *C. albicans* strains, respectively. All the *C. glabrata* isolates showed low fluconazole MICs. Clotrimazole showed excellent potency against all but seven isolates (three *C. glabrata*, two *S. cerevisiae*, one *C. albicans* and one *Picchia anomala*). Any of the strains showed nystatin reduced susceptibility. On the other hand, terbinafine was the less potent drug. Antifungal resistance is still a rare phenomenon supporting the use of azole antifungals as empirical treatment of vulvovaginal candidiasis.

Keywords Yeast · Vulvovaginitis · Identification · Susceptibility

S. Gamarra · C. Dudiuk · E. Mancilla · G. Garcia-Effron (✉)
Laboratorio de Micología y Diagnóstico Molecular, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, CONICET, Ciudad Universitaria UNL (Ruta 168), C.C. 242-S3000ZAA, CP 3000 Santa Fe, Santa Fe, Argentina
e-mail: ggarcia@unl.edu.ar

S. Gamarra · C. Dudiuk · E. Mancilla · G. Garcia-Effron
Catedra de Parasitología y Micología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, CP 3000 Santa Fe, Argentina

S. Morano · M. E. Nardin · E. de los Angeles Méndez
Sección Microbiología, Laboratorio Central, Hospital Dr. José María Cullen, CP 3000 Santa Fe, Argentina

Present Address:
E. Mancilla
Hospital San Carlos, Casilda, CP 2170 Santa Fe, Argentina

Introduction

Vulvovaginal candidiasis (VVC) is one of the most common reasons for seeking gynecological attention. Up to 75 % of all women will suffer at least one VVC episode during their lifetime and around 50 % of them will have at least a second episode. Moreover, VVC is

one of the most frequent infections during pregnancy, and recurrent VVC affects 5 % of the patients [1, 2].

The identification of yeasts to species level is essential as an aid in choosing the correct antifungal treatment [3]. More than 20 *Candida* species are considered human pathogens [4–7], but between 65 and 90 % of the VVC are attributed to *C. albicans* [8–10]. However, an important understudied issue about VVC is the prevalence of emergent cryptic yeast species (*C. orthopsilosis*, *C. metapsilosis*, *C. nivariensis*, etc.). This knowledge is important for clinicians since some of the emerging yeast species are associated with reduced in vitro susceptibility to many antifungal drugs [4, 6, 7, 11].

Antifungal susceptibility patterns are used as a tool for the selection of antifungal treatment for invasive candidiasis [4, 7]. However, little is known about antifungal susceptibility patterns in VVC, especially to antifungal drugs used as over-the-counter [10].

The aims of this work were (1) to describe the epidemiologic data on risk factors for VVC in Santa Fe city (Argentina), (2) to study the species distribution including those that required molecular tools for identification (3) to evaluate the susceptibility patterns of the antifungal drugs available in our region for VVC treatment. Moreover, a series of phenotypic methods able to differentiate *C. albicans* and *C. dubliniensis* were analyzed and compared with molecular identification.

Materials and Methods

Patients and Samples

A total of 510 vaginal specimens were obtained from the same number of patients with vaginal infection symptoms attending the Jose María Cullen Hospital (Santa Fe-Argentina) from June to October 2011. All samples were subjected to GRAM stain and direct microscopic examination. Vaginal pH was measured and amine test (Whiff test) was also performed. Afterward, vaginal specimens were plated on CHROMagar *Candida*™ (Medica-Tec SRL, Buenos Aires Argentina) and incubated for 48 hs at 35 °C.

Yeast Identification

All the yeasts isolates were derived to the "Micología y Diagnóstico Molecular" Laboratory (CONICET-UNL) where they were identified based on morphology and carbohydrate assimilation and fermentation [12] and by molecular procedures as described below.

Evaluation of Phenotypic Methods to Differentiate *C. albicans*/*C. dubliniensis*

All the isolates plus 20 control strains (12 *C. dubliniensis* and 8 *C. albicans*) were evaluated in their capacity for precipitate tween-80 (opacity test agar, OTA: 1 % peptone, 0.50 % sodium chloride, 0.01 % calcium chloride, 1.5 % agar and 1 % tween 80), chlamidoconidia formation on tobacco agar tween 80 (TAT80: 5 % tobacco, 2 % agar and 1 % tween 80), xylose assimilation (XA: 0.5 % SO4(NH4)2, 0.1 % PO4H2 K, 0.05 % SO4 Mg, 1.5 % agar and xylose 20 % in paper disks), and growth in hypertonic Sabouraud broth (HSB: 2 % glucose, 1 % peptone, 0.65 % sodium chloride). All the mentioned tests were performed following published protocols, and their results were compared with the molecular identification [13–16]. The control strains included *C. dubliniensis* NCPF3949, *C. albicans* ATCC 90028, *C. albicans* ATCC 36082, *C. albicans* Sc5314 and 16 clinical strains obtained from different clinical sources (8 *C. albicans* and 9 *C. dubliniensis* isolated from blood and 3 *C. dubliniensis* obtained from patients with oropharyngeal candidiasis). All the strains were identified by phenotypic and molecular methods (ITS sequencing or *ACT1* and *HWP1* genes amplification) [17–19].

Molecular Identification

Yeast DNA was extracted using a phenol-based procedure [20]. The phenotypic identifications of *Candida albicans*/*C. dubliniensis*/*C. africana*, *C. glabrata* sensu lato, *C. krusei*, *C. tropicalis*, *C. parapsilosis* sensu lato, and *Saccharomyces cerevisiae* were confirmed using species specific PCRs [21–23]. *C. parapsilosis* sensu lato and *C. glabrata* sensu lato species differentiation were performed using a PCR-based restriction endonuclease analysis [24] or by a single-tube PCR [25], respectively. *C. albicans* cryptic species (*C. dubliniensis* and *C. africana*) were discriminated using the *ACT1* and *HWP1* gene amplification [17, 19]. Sequencing of the 5.8S RNA gene and adjacent internal transcribed spacer 1 and 2 regions (*ITS1* and *ITS2*) was performed for strains

with inconclusive identification results or for the species not included above [18].

Antifungal Susceptibility Testing

Antifungal drugs tested were fluconazole (FLC), itraconazole (ITC), terbinafine (TRB), clotrimazole (CLT), anfotericin B (AMB), and nystatin (NYS) (all purchased from Sigma-Aldrich Química—Buenos Aires, Argentina). Drug selection was performed based on the treatment options available (topical, vaginal ovules, and systemic presentations) at JM Cullen Hospital (Santa Fe Argentina). AMB was added to the list of tested drugs to know whether it is possible to use it as an in vitro subrogate marker for NYS resistance since AMB susceptibility testing is standardized by CLSI [26, 27]. Inoculums of all the isolates were obtained according to CLSI document M27-A3. Result interpretations were performed according to CLSI documents M27-A3 and M27-S4. FLC, ITC, and AMB MIC microtitration plates were produced following the CLSI M27-A3 guidelines [26, 27]. On the other hand, TRB, CLT, and NYS were diluted in dimethyl sulfoxide, and the final concentrations ranged from 8 to 0.015 μg/ml. Since there is no MIC limit ranges for microdilution tests for these antifungal agents, the quality control strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were subjected to 20 MIC repetitions on different days, and the results are displayed in Table 1. Afterward, these values were used as a control of the produced plates.

Table 1 Terbinafine, clotrimazole, and nystatin MIC ranges for control strains obtained following the CLSI M27-A3, and M27-S4 document recommendations

	MIC range (μg/ml)[a]		
	Terbinafine	Clotrimazole	Nystatin
C. parapsilosis ATCC 22019	0.03–0.12	0.015–0.03	0.12–0.25
C. krusei ATCC 6258	>4–>4	0.015–0.06	0.50–1.00

[a] 24 h reading

Data Collection and Statistical Analysis

The epidemiological variables recorded were: patient's age, underlying illnesses, pregnancy weeks, previous systemic antibiotic treatment (4 weeks earlier or less), use of estrogen-based contraception, use of other contraception methods, number of previous VVC episodes, discharge (presence and characteristics), and other signs and symptoms. Statistical analyses were done using the Statistical Package for the Social Sciences software (SPSS version 17.0; IBM SPSS statistics Inc., Chicago, IL, USA). Categorical variables are expressed as percentages (proportions) and compared by Fisher's exact test (two-tailed) or by χ^2 test. For descriptive data as age and pregnancy weeks, the statistical dispersion was measured as median and interquartile ranges (IQR). Continuous variables are expressed as means ± standard deviations and as median and ranges and analyzed by unpaired Student's *t* test. The MIC data presented here are expressed as geometric means (GMs) of three experiments performed in different days. Off-scale MICs were converted to the next concentration up or down in order to be included in the analysis. MIC values were approximated to a normal distribution by transforming them to log2 values. GMs were used to statistically compare MIC values, and MIC log2 values were used to establish susceptibility differences between strains. To establish significant levels of MIC differences, a one-way ANOVA test with Bonferroni's correction for multiple comparisons was used. Multivariate stepwise logistic regression analysis was carried out to identify predictors of VVC. A P value ≤ 0.05 was considered significant. This study was approved by the Biochemistry school (Universidad Nacional del Litoral) and JM Cullen ethics committees.

Results

Epidemiology and Species Distribution

During the study period, 510 patients were evaluated for a vaginal infection (Table 2). One hundred eighteen were diagnosed with VVC (23.14 %) (acute VVC or symptomatic recurrent VVC). Patients with VVC were statistically younger than non-VVC patients ($P < 0.001$). Patient's median age was 27 years (interquartile range, IQR: 12 years), 24 years (IQR: 10 years), and 28 years (IQR: 12 years) for all the studied patients, VVC patients, and non-VVC patients, respectively. Among the

Table 2 Demographic and clinical characteristics of the patients with VVC included in this study

Age group	*N*	VVC	Pregnant
13–20	108	37	25
21–30	206	56	33
31–40	124	18	8
41–50	35	1	0
51–60	9	1	0
>60	3	0	0
Not recorded	25	5	1
Total	510	118	68

Underlying diseases/treatment	Pregnant	Not pregnant
VVC patients		
Corticosteroid treatment[a]	4	1
Diabetes[b]	8 (3)	3
Gestational diabetes	3	–
Antibacterial treatment (last month)[c]	9 (6)	5
Hematological malignancies	–	2
Cardiovascular disease	–	2
Recurrent VVC	2	5
Other	2	6
Nothing to record	40	26
Total	68	50

[a] Include treatment for rheumatoid arthritis, hidradenitis suppurativa, uncontroled asma, extensive psoriasis, multiple sclerosis

[b] Diabetes mellitus (in parenthesis gestational diabetes)

[c] Non-genital infection (in parenthesis amoxicilin)

VVC patients, 58 % were pregnant ($n = 68$) with a median age of 25 years and a median of 34 weeks of pregnancy (IQR 12.5 weeks). When a multivariate logistic regression analysis was performed in the pregnant group, diabetes (gestational and mellitus) (Odds ratio (OR) 0.30 95 % Coefficient interval, 95 % CI 0.11–0.84, $P = 0.02$), previous treatment with any antibacterial for non-genital infection (OR 0.12, 95 % CI 0.03–0.40, $P = 0.001$), and previous treatment with amoxicillin for urinary infection (OR 0.17, 95 % CI 0.05–0.67, $P = 0.02$) were statistically associated with VVC. Also, previous antibacterial treatment was linked as a risk factor of VVC in the non-pregnant group (OR 0.36, 95 % CI 0.18–0.72, $P = 0.005$). On the other hand, oral hormonal contraceptives were not considered as a risk factor of VVC in this group of patients (OR 0.79, 95 % CI 0.48–1.30, $P = 0.35$). Previous (a year before the actual episode of VVC) azole-based over-the-counter antifungal treatment were used at least one time by 38.13 % ($n = 45$) of the patients with VVC. Also, this topical treatment was statistically associated with VVC due to non-*C. albicans* yeast ($P = 0.002$).

Recurrent VVC, defined as four or more episodes per year, were observed in seven patients (5.93 % of the VVC). Out of these seven patients, two were pregnant.

Out of the 118 patients diagnosed as having VVC, three showed mixed fungal infections, leaving 121 yeast strains to study (Table 3). *Candida albicans* was the most isolated yeast ($n = 103$, 85.95 %), followed by *C. glabrata* ($n = 6$, 4.96 %), *Saccharomyces cerevisiae* (n = 4, 3.31 %), *C. dubliniensis* ($n = 3$, 2.47 %), *C. parapsilosis* sensu stricto ($n = 1$, 0.83 %), *C. tropicalis* ($n = 1$, 0.83 %), *C. krusei* ($n = 1$, 0.83 %), *Pichia anomala* (*C. pelliculosa*, $n = 1$, 0.83 %) and *P. norvegensis* (*C. norvegensis*, $n = 1$, 0.83 %).

All the recurrent VVC cases were produced by non-*C. albicans* yeasts. Mixed infections were produced by a combination of *C. albicans* and *C. glabrata*, *C. albicans* and *C. krusei,* or *C. albicans* and *S. cerevisiae* (one each). Three of the isolated *S. cerevisiae* were obtained from pregnant woman (one co-infecting with *C. albicans*).

Antifungal Susceptibility Testing Results

The in vitro activities of the six tested antifungal drugs are summarized in Table 3. The azole antifungal drugs tested showed good in vitro activity. CLT showed the lowest MIC values for all the isolates (GM = 0.03 μg/ml). Overall, 104 of the 121 yeast isolates tested (85.6 %) were inhibited by 0.06 μg/ml of CLT. On the other hand, *C. glabrata* showed 11-fold higher MIC values than *C. albicans* for this azole drug (GM = 0.17 and 0.015 μg/ml, respectively). Moreover, there were seven strains showing CLT MIC values higher or equal than 1 μg/ml (3 *C. glabrata*, 2 *S. cerevisiae*, 1 *C. albicans,* and 1 *P. anomala*). Turning to the oral triazoles, ITC showed very good in vitro potency against all the isolates (GM = 0.04 μg/ml and a MIC90 = 0.25 μg/ml). There was only one *C. albicans* strain showing very high ITC MIC value (>16 μg/ml). This particular strain showed also high FLC and CLZ MIC

Table 3 In vitro susceptibilities of the strains included in the study

Species	N (%)	AMB		NYS		FLC		ITC		CLZ		TRB	
		MIC[a]	Range	MIC[a]	Range	MIC[a]	Range	MIC[a]	Range	MIC[a]	Range	MIC[a]	Range
All	121	0.08	0.03–0.25	0.09	0.03–0.25	0.50	0.06–>64.0	0.04	0.015–>16.00	0.03	0.015–2.00	2.01	0.12–8.00
C. albicans	103 (85.1)	0.08 (0.25)	0.03–0.25	0.09 (0.25)	0.03–0.25	0.42 (4.00)	0.06–>64.0	0.04 (0.25)	0.015–>16.00	0.015 (0.06)	0.015–2.00	2.01 (8.00)	0.12–8.00
C. glabrata	6 (5.0)	0.12	0.12	0.14	0.06–0.25	11.31	8.00–16.00	0.09	0.02–0.50	0.17	0.03–1.00	6.35	4.00–8.00
S. cerevisiae	4 (3.3)	0.04	0.03–0.06	0.08	0.06–0.12	0.49	0.12–2.00	0.04	0.015–0.12	0.015	0.015	0.87	0.25–8.00
C. dubliniensis	3 (2.5)	0.04	0.03–0.06	0.06	0.06	1.00	1.00	0.03	0.03	0.02	0.015–0.03	0.40	0.25–1.00
Candida spp.[b]	3 (2.5)		0.12–0.25		0.12–0.25		1.00–32.00		0.03–0.12		0.015–0.06		0.06–4.00
Pichia spp.[c]	2 (1.6)		0.06–0.12		0.06		4.00–16.00		0.03–0.06		0.015–1.00		4.00–8.00

MIC values are expressed in μg/ml

AMB amphotericin B, *NYS* nystatin, *FLC* fluconazole, *ITC* Itraconazole, *CLZ* clotrimazole, *TRB* terbinafine

[a] Geometric means expressed in μg/ml. In parenthesis MIC_{90} for all the strains and *C. albicans*

[b] *C. parapsilosis* sensu stricto (lowest TRB MIC), *C. tropicalis* and *C. krusei* (highest FLC MIC) one each

[c] *Pichia anomala* (*C. pelliculosa*) and *P. norvegensis* (*C. norvegensis*—highest FLC and CLZ MIC) one each

values (>64 and 0.25 μg/ml, respectively). FLC was the tested drug with the widest range of MIC values (0.06–>64 μg/ml). FLC-reduced susceptibility was observed in ten *C. albicans* strains (four strains were FLC susceptible-dose-dependent while six were FLC resistant). Moreover, one of the FLC-resistant *C. albicans* strain showed the highest CLT MIC value of the studied strains (FLC MIC = 16 μg/ml and CLT MIC = 2 μg/ml). On the other hand, the six *C. glabrata* isolates showed low FLC MICs and were considered as susceptible dose dependent following the interpretive guidelines published in the CLSI M27-S4 document (GM = 11.31 μg/ml, ranging from 8.00 to 16.00 μg/ml), while the *P. novergensis* strain showed high FLC MIC (16 μg/ml).

Both tested polyenes showed no differences in in vitro activity ($P = 0.39$). None of the 121 strains showed high MIC values for NYS or for AMB. Also, both drugs showed a narrow range of MIC (0.03–0.25 μg/ml for both AMB and NYS).

Terbinafine was the less active drug against the tested isolates (*C. albicans* GM = 2.01 μg/ml and MIC90 = 8.00 μg/ml). It can be stressed that *C. glabrata* was less susceptible to TRB (GM = 6.35 μg/ml) when compared with *C. albicans*. On the other hand, *C. dubliniensis* and *S. cerevisiae* were more susceptible to TRB than the other studied species (GM = 0.40 and 0.24 μg/ml, respectively) ($P = 0.004$).

Any of the comorbid recorded conditions were associated with reduced susceptibility to any of the antifungal drugs tested ($P > 0.05$).

Evaluation of Four Phenotypic Methods Used to Differentiate *C. albicans* and *C. dubliniensis*

All the strains were grown in OTA, TAT80, and HSB. Also, XA capacity was evaluated. The results were analyzed for the strains identified as *C. albicans* or *C. dubliniensis*. All the tests showed optimum sensitivity and negative predictive value to identify yeasts as non-*C. dubliniensis*. On the other hand, the HSB test was the only test that showed 100 % specificity and 100 % positive predictive value for *C. dubliniensis* identification. The other tests showed lower specificity (85, 87 and 88 % for XA, TAT80 and OTA tests, respectively) and positive predictive value for *C. dubliniensis* identification (13, 27, and 17 % for XA, TAT80, and OTA tests, respectively).

Discussion

Complicated VVC was defined by Sobel et al. and Pappas et al. [3, 28] as VVC caused by non-*C. albicans* species, VVC associated with immunosuppression, uncontrolled diabetes, or pregnancy and recurrent VVC. Most studies have reported that complicated VVC cases never surpasses 10–20 % [29]. In our study, 63.6 % (75 out of 118) of the patients included in this study had at least one of the described conditions to be considered as a complicated VVC. The reason of our findings could be due the population attending JM Cullen hospital. The hospital has a large gynecological and maternity service and covers a low-income socio-demographic area of Santa Fe city. Thus, patients tend to use over-the-counter antifungals to treat uncomplicated VVC cases more often than patients of other areas of the city and attend to the hospital only when the VVC is complicated.

Our results show that in the studied population, any antibacterial treatment (for non-vaginal infections) was statistically linked with VVC as described before [30–32]. Moreover, in the pregnant group, the use of amoxicillin for urinary infection was also independently linked with VVC. About this subject, and after a comprehensive literature search, we could not find any published study relating the use of this particular antibacterial with VVC in pregnancy.

As described worldwide and in Argentina, *C. albicans* was the most common yeast species associated with VVC (85.1 %) followed by *C. glabrata* (5 %) [9, 33–42]. The overall incidence of non-albicans vaginitis was also similar to those obtained in Argentina in non-pregnant and HIV negative populations [41, 42] and in USA, Australia, and Europe [33, 34, 40, 43, 44] but lower than that obtained in Argentina in preadolescent girls and general population [37, 38]. A significant outcome was that *S. cerevisiae* and *C. dubliniensis* were the third and fourth isolated species, respectively, surpassing other *Candida* species commonly isolated in other Argentinian and worldwide hospitals [21, 36, 41, 42]. These species are not commonly isolated from VVC. For example, *S. cerevisiae* was isolated in one pregnant women out of 207 studied in Turkey and represent only the 0.57 and 1.5 % of the isolated yeasts causing VVC in hospitals in Spain (46 out of 8050 VVC episodes) and in USA (9 out of 593 VVC episodes), respectively [21, 36, 45]. On the other hand, in an Argentinian hospital, the frequency of *S. cerevisiae* isolation was 2.2 % [41] approaching the same percentage range of the frequency obtained in our study. Similarly, the isolation of *C. dubliniensis* from genital specimens is rare. In a recent report, the frequency of isolation of *C. dubliniensis* from VVC in the UK was 1.20 % [46], whereas in our study, the frequency of VVC caused by *C. dubliniensis* doubled what was obtained in UK, reaching 2.50 %.

The MIC values obtained for all the antifungal agents showed that antifungal-reduced susceptibility was rare, reinforcing the idea that yeasts isolated from VVC patients are usually susceptible to antifungal agents [10, 36, 47–49]. For azoles, CLT and ITC showed the highest potency, and only one strain showed cross-reduced susceptibility (and FLC resistance) to all azole agents, and only six strains showed high CLT MICs. FLC MIC GM values were similar to those obtained in previous reports for yeast causing VVC [36, 41, 42]. However, 6 *C. albicans* strains showed a FLC-resistant phenotype (and four dose dependent), and two strains belonging to species considered naturally resistant to FLC (1 *C. krusei* and 1 *P. norvegensis*) were isolated [36, 50]. This FLC resistance frequency in yeast isolated from VVC was higher than other published reports where there were no FLC-resistant isolates [10, 48, 49]. Interestingly, out of these eight FLC-resistant strains, six were susceptible to CLT (all the FLC susceptible-dose-dependent *C. albicans* strains were CLT susceptible).

Turning to polyenes, NYS MIC values were low. Our strains showed NYS MIC values similar to those obtained previously by Arikan et al. (NYS MIC90 for *C. albicans* 0.5 μg/ml) [51] but much lower than those obtained by other authors (NYS MIC90 for *C. albicans* ranging from 2.0 to 16.0 μg/ml) [32, 36, 52]. Moreover, NYS and AMB showed similar MIC values. Thus, AMB in vitro susceptibility could be used as surrogated marker of NYS MIC as AMB susceptibility testing is more commonly performed in clinical labs.

In this study, we also compared four phenotypic tests described to differentiate *C. albicans* from *C. dubliniensis*. In our hands, the HSB test was the only test showing 100 % specificity and positive predictive value for *C. dubliniensis* identification. This method was also the easiest and cheapest to perform being ideal to be used in a clinical lab.

In conclusion, *C. albicans* is still the most recovered yeast species by far. Antifungal resistant seems not to be a big problem at least in Santa Fe city (Argentina) supporting the use of azole as empirical treatment of VVC. Also, in the case of a FLC-resistant strain, topical CLT would be good therapeutic option. It has to be highlighted that antifungal susceptibility testing should be performed for non-*albicans Candida* species and all the yeasts isolated from complicated VVC.

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References

1. Sobel JD. Vulvovaginitis. When *Candida* becomes a problem. *Dermatol Clin* 1998;16:763–8, xii.
2. Nyirjesy P, Sobel JD. Vulvovaginal candidiasis. Obstet Gynecol Clin North Am. 2003;30:671–84.
3. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:503–35.
4. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133–63.
5. Pappas PG, Rex JH, Lee J, et al. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis. 2003;37:634–43.
6. Lockhart SR, Messer SA, Gherna M, et al. Identification of *Candida nivariensis* and *Candida bracarensis* in a large global collection of *Candida glabrata* isolates: comparison to the literature. J Clin Microbiol. 2009;47:1216–7.
7. Diekema DJ, Messer SA, Boyken LB, et al. In vitro activity of seven systemically active antifungal agents against a large global collection of rare *Candida* species as determined by CLSI broth microdilution methods. J Clin Microbiol. 2009;47:3170–7.
8. Bauters TG, Dhont MA, Temmerman MI, Nelis HJ. Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. Am J Obstet Gynecol. 2002;187:569–74.
9. Holland J, Young ML, Lee O, Chen A. Vulvovaginal carriage of yeasts other than *Candida albicans*. Sex Transm Infect. 2003;79:249–50.
10. Mathema B, Cross E, Dun E, et al. Prevalence of vaginal colonization by drug-resistant *Candida* species in college-age women with previous exposure to over-the-counter azole antifungals. Clin Infect Dis. 2001;33:E23–7.
11. Gomez-Lopez A, Alastruey-Izquierdo A, Rodriguez D, et al. Prevalence and susceptibility profile of *Candida metapsilosis* and *Candida orthopsilosis*: results from population-based surveillance of candidemia in Spain. Antimicrob Agents Chemother. 2008;52:1506–9.
12. Lachance MA, Boekhout T, Scorzetti G, Fell JW, Kurtzman CP. The yeasts a taxonomic study. London. Editorial: Elsevier; 2013.
13. Staib P, Morschhauser J. Chlamydospore formation in *Candida albicans* and *Candida dubliniensis* an enigmatic developmental programme. Mycoses. 2007;50:1–12.
14. Khan ZU, Ahmad S, Mokaddas E, Chandy R. Tobacco agar, a new medium for differentiating *Candida dubliniensis* from *Candida albicans*. J Clin Microbiol. 2004;42:4796–8.
15. Slifkin M. Tween 80 opacity test responses of various *Candida* species. J Clin Microbiol. 2000;38:4626–8.
16. Alves SH, Milan EP, de Laet SP, Oliveira LO, Santurio JM, Colombo AL. Hypertonic sabouraud broth as a simple and powerful test for *Candida dubliniensis* screening. Diagn Microbiol Infect Dis. 2002;43:85–6.
17. Donnelly SM, Sullivan DJ, Shanley DB, Coleman DC. Phylogenetic analysis and rapid identification of *Candida dubliniensis* based on analysis of *ACT1* intron and exon sequences. Microbiology. 1999;145(Pt 8):1871–82.
18. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego, Calif: Academic press, Inc.; 1990. p. 315–22.
19. Romeo O, Criseo G. First molecular method for discriminating between *Candida africana*, *Candida albicans*, and *Candida dubliniensis* by using *HPW1* gene. Diagn Microbiol Infect Dis. 2008;62:230–3.
20. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press; 1998.
21. Echeverria-Irigoyen MJ, Eraso E, Cano J, Gomariz M, Guarro J, Quindos G. *Saccharomyces cerevisiae* vaginitis: microbiology and in vitro antifungal susceptibility. Mycopathologia. 2011;172:201–5.
22. Haynes KA, Westerneng TJ. Rapid identification of *Candida albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* by species-specific PCR of large subunit ribosomal DNA. J Med Microbiol. 1996;44:390–6.
23. Li YL, Leaw SN, Chen JH, Chang HC, Chang TC. Rapid identification of yeasts commonly found in positive blood cultures by amplification of the internal transcribed spacer regions 1 and 2. Eur J Clin Microbiol Infect Dis. 2003;22:693–6.
24. Garcia-Effron G, Canton E, Peman J, Dilger A, Roma E, Perlin DS. Assessment of two new molecular methods for identification of *Candida parapsilosis* sensu lato species. J Clin Microbiol. 2011;49:3257–61.
25. Enache-Angoulvant A, Guitard J, Grenouillet F, et al. Rapid discrimination between *Candida glabrata*, *Candida nivariensis*, and *Candida bracarensis* by use of a single-plex PCR. J Clin Microbiol. 2011;49:3375–9.
26. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts. Third edition. Document M27-A3. 2008.

27. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast: fourth informational supplement—CLSI document M27-S4-Wayne, PA. 2012.
28. Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol. 1998;178:203–11.
29. Ilkit M, Guzel AB. The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: a mycological perspective. Crit Rev Microbiol. 2011;37:250–61.
30. Xu J, Schwartz K, Bartoces M, Monsur J, Severson RK, Sobel JD. Effect of antibiotics on vulvovaginal candidiasis: a MetroNet study. J Am Board Fam Med. 2008;21:261–8.
31. Sobel JD. Management of recurrent vulvovaginal candidiasis: unresolved issues. Curr Infect Dis Rep. 2006;8:481–6.
32. Blignaut E, Messer S, Hollis RJ, Pfaller MA. Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals. Diagn Microbiol Infect Dis. 2002;44:169–74.
33. 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;46:1501–3.
34. Spinillo A, Capuzzo E, Gulminetti R, Marone P, Colonna L, Piazzi G. Prevalence of and risk factors for fungal vaginitis caused by non-albicans species. Am J Obstet Gynecol. 1997;176:138–41.
35. Saporiti AM, Gomez D, Levalle S, et al. Vaginal candidiasis: etiology and sensitivity profile to antifungal agents in clinical use. Rev Argent Microbiol. 2001;33:217–22.
36. 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;43:2155–62.
37. Mujica MT, Finquelievich JL, Jewtuchowicz V, Iovannitti CA. Prevalence of *Candida albicans* and *Candida* non-albicans in clinical samples during 1999–2001. Rev Argent Microbiol. 2004;36:107–12.
38. Giusiano G, Rojas F, Toma-Vanacore S, Mangiaterra M. Frequency and antifungal profile of *Candida* isolated from vaginal exudates of preadolescent girls. Enferm Infecc Microbiol Clin. 2009;27:428.
39. Garcia HM, Garcia SD, Copolillo EF, et al. Prevalence of vaginal candidiasis in pregnant women. Identification of yeasts and susceptibility to antifungal agents. Rev Argent Microbiol. 2006;38:9–12.
40. 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.
41. Buscemi L, Arechavala A, Negroni R. Study of acute vulvovaginitis in sexually active adult women, with special reference to candidosis, in patients of the Francisco J. Muniz Infectious Diseases Hospital. Rev Iberoam Micol. 2004;21:177–81.
42. Arechavala AI, Bianchi MH, Robles AM, Santiso G, Negroni R. Identification and susceptibility against fluconazole and albaconazole of 100 yeasts' strains isolated from vaginal discharge. Rev Iberoam Micol. 2007;24:305–8.
43. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. Am J Med. 1991;91:72S–5S.
44. Paulitsch A, Weger W, Ginter-Hanselmayer G, Marth E, Buzina W. A 5-year (2000–2004) epidemiological survey of *Candida* and non-*Candida* yeast species causing vulvovaginal candidiasis in Graz, Austria. Mycoses. 2006;49:471–5.
45. Kalkanci A, Guzel AB, Khalil II, Aydin M, Ilkit M, Kustimur S. Yeast vaginitis during pregnancy: susceptibility testing of 13 antifungal drugs and boric acid and the detection of four virulence factors. Med Mycol. 2012;50:585–93.
46. Borman AM, Szekely A, Linton CJ, Palmer MD, Brown P, Johnson EM. Epidemiology, antifungal susceptibility, and pathogenicity of *Candida africana* isolates from the United Kingdom. J Clin Microbiol. 2013;51:967–72.
47. Cross EW, Park S, Perlin DS. Cross-Resistance of clinical isolates of *Candida albicans* and *Candida glabrata* to over-the-counter azoles used in the treatment of vaginitis. Microb Drug Resist. 2000;6:155–61.
48. Ribeiro MA, Dietze R, Paula CR, Da Matta DA, Colombo AL. Susceptibility profile of vaginal yeast isolates from Brazil. Mycopathologia. 2001;151:5–10.
49. Sobel JD, Wiesenfeld HC, Martens M, et al. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med. 2004;351:876–83.
50. Guitard J, Angoulvant A, Letscher-Bru V, et al. Invasive infections due to *Candida norvegensis* and *Candida inconspicua*: report of 12 cases and review of the literature. Med Mycol. 2013;51(8):795–799.
51. Arikan S, Ostrosky-Zeichner L, Lozano-Chiu M, et al. In vitro activity of nystatin compared with those of liposomal nystatin, amphotericin B, and fluconazole against clinical *Candida* isolates. J Clin Microbiol. 2002;40:1406–12.
52. Carrillo-Munoz AJ, Quindos G, Tur C, et al. In-vitro antifungal activity of liposomal nystatin in comparison with nystatin, amphotericin B cholesteryl sulphate, liposomal amphotericin B, amphotericin B lipid complex, amphotericin B desoxycholate, fluconazole and itraconazole. J Antimicrob Chemother. 1999;44:397–401.