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Vulvovaginal Candidiasis: Profile of Antifungal Susceptibility Test of *Candida* Strains to Antifungal Drugs from 2018 to 2022, Ouagadougou, Burkina Faso

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Abstract

Background: Vulvovaginal candidiasis (VVC) is a common cause of significant morbidity, affecting millions of women worldwide. It is estimated that approximately 75%of women of childbearing age will have at least one episode of candidiasis in their lifetime. In the last decades, resistance to azoles has become a public health problem. Although studies on vulvovaginitis have been done, there is lack of VVC studies in our area. The aim of this study was to describe the etiological and resistance profiles of vulvovaginal candidiasis to standard antifungus at the Saint Camille Hospital of Ouagadougou (HOSCO), Burkina Faso. **Methods:** We conducted a prospective study from January 2018 to December 2022. From vulvovaginal swabs, *Candida* species were identified using the ChromID® *Candida* Agar medium and the API® *Candida* gallery. Antifungal susceptibility testing was performed using Kirby-Bauer agar disk diffusion. **Results:** A total of 4789 women were sampled. The average age of sexually active women was 27.80+/−6.77 years, with extremes ranging from 15 to 64 years. Vaginal *Candida* infections accounted for 74.16% of the cases. The 20 - 29 age group was the most affected by vulvovaginal candidiasis. Pregnant women accounted for 28.76% of our study population. Women in the second (2nd) trimester of pregnancy had more *Candida* infections. *Candida albicans* was the most isolated species (55.12%), followed by *Candida glabrata* (27.64%), *Candida tropicalis* (6.91%), *Candida famata* (6.67%), *Candida krusei* (2.56%). All the *Candida* species isolated showed very high of resistance

to Fluconazole (45.2%), Miconazole (23.7%) and Clotrimazole (45.7%). **Conclusion:** Species-specific antifungal results should always be considered to avoid antifungal resistance associated with vulvovaginal candidiasis. Identifying the causative species using vaginal fungal cultures can help guide therapy and improve outcomes for these patients.

Keywords

Vulvovaginal Candidiasis, *Candida albicans*, Azole Resistance, Burkina Faso

1. Introduction

Vulvovaginal candidiasis (VVC) is a widespread vaginal infection primarily caused by *Candida* species. It is the second leading cause of vulvovaginal infection in women of childbearing age [1]. It is also a debilitating, long-term condition that can severely affect quality of life, especially in developing countries [2]. In Burkina Faso, a recent study estimated that 1.7 million women suffer from vulvovaginal candidiasis [3]. The number of vulvovaginal candidiasis episodes tends to be higher in women who are sexually active, pregnant, immunocompromised, taking the contraceptive pill, or taking corticosteroids [4].

Because of the vertical transmission of *Candida* from mother to child during childbirth, vulvovaginal candidiasis remains a serious problem for pregnant women [5]. Vulvovaginal candidiasis symptoms include pruritus with dyspareunia, abundant, malodorous and creamy leucorrhea with a "curdled milk" [6].

Candida albicans is the most commonly isolated species. There are also other non-*Candida albicans Candida* species (CNCA) that are increasingly found (around 10 - 45% of isolates): *Candida glabrata, Candida krusei, Candida tropicalis, Candida parapsilosis, Candida famata*… An estimation of around 20% of women have candidiasis caused by non-*albicans Candida* species. In October 2022, *Candida albicans* and *Candida glabrata* are classified as priority pathogens according to the first-ever fungal priority pathogens list (WHO FPPL) [7]. The first-line antifungal agents used for treatment are azoles and polyenes. Azoles act on *Candida* by inhibiting the activity of lanosterol 14-α-demethylase, a key enzyme in ergosterol biosynthesis. Ergosterol is the main lipid component of fungal cell membranes. Polyenes act on *Candida* by binding to ergosterol thereby affecting cell permeability [8]. In recent decades, azoles resistance has increased [9].

In Burkina Faso, *C. albicans* is most frequently isolated in over 60% of cases, followed by *Candida glabrata* [10]. The first-line antifungal agents are azoles and polyenes. Studies have shown the prevalence of *Candida* species resistant to azole to be > 59% [11] [12]. Considered Neglected Tropical Diseases (NTDs), vulvovaginal candidiasis receives little attention or resources; hence there is scarity of data on their distribution and the prevalence of resistance of *Candida* species to antifungal agents on vulvovaginal candidiasis. Therefore, our aim is to determine the rate of antifungal resistance among *Candida* species isolated from vulvovaginal

candidiasis in Ouagadougou, Burkina Faso.

2. Methods

2.1. Study Description

This descriptive and analytical cross-sectional study was conducted from January 2018 to December 2022 at the Saint Camille Hospital of Ouagadougou (HOSCO). The Saint Camille Hospital of Ouagadougou (HOSCO) is a reference confessional health center. The Camillian monks established it in 1967. Because of their social, non-profit nature, faith-based structures are often visited by even the most destitute populations who can afford quality health care at a lower cost [13]. This approach allowed us to interview several women from all walks of life. The study was approved by the Institutional Ethics Committee of HOSCO, Burkina Faso. The anonymity and confidentiality of the data were ensured.

2.2. Study Population

The study population consisted of any woman who presented to the HOSCO laboratory with a report for cytobacteriological examination of routine vulvovaginal swabs and agreed to participate in the study. There was no age limit.

2.3. Inclusion and Exclusion Criteria

Women who visited the laboratory for cytobacterial examination were included if they provided consent to participate in the study. These conditions were respected by women before:

- No intimate hygiene on the day of sampling.
- No sexual intercourse within 24 hours of admission.
- No current antibiotic therapy or discontinuation of antibiotic therapy for 72 hours.
- Not during menstruation.

The exclusion criteria were as follows: Any woman who did not meet the inclusion Sample collection.

2.4. Sample Collection

Once all conditions were met, the woman was gynecologically placed. After placing the speculum, a vaginal swab was taken using 2 sterile swabs: one for secretions from the posterior vaginal, swept from top to bottom, and the other for the cervix. Macroscopic examination was carried out: cervical condition, appearance, odor and color of leucorrhea and vaginal ph.

In virgin women, only the vulval swab is used.

The vulvovaginal swab was examined microscopically and then cultured.

2.5. Microscopic Examination

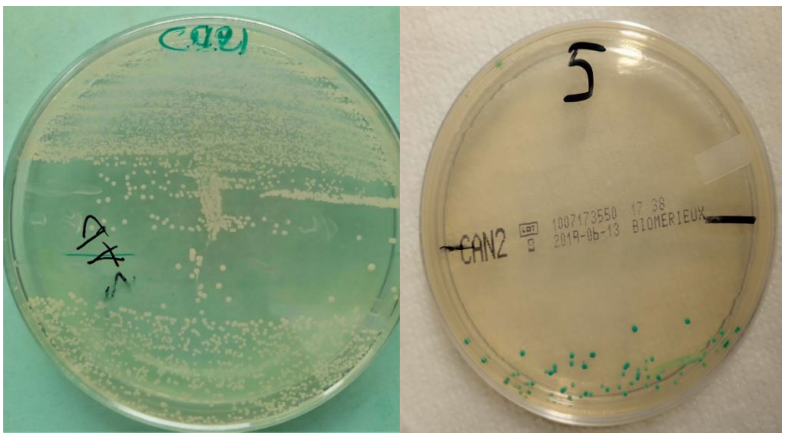
After sampling, the microscopy stage consisted of the fresh state (a few drops of physiological water added to the vaginal sample and the mixture mounted

between slide and coverslip) to examine the presence of epithelial cells, yeasts, and/or mycelial filaments; leukocytes (altered or intact); and red blood cells. Gram staining was used to detect and quantify yeast and mycelial filaments.

2.6. Identification of *Candida* Species

After fresh observation and GRAM staining, vulvovaginal swabs were inoculated on Sabouraud + chloramphenicol medium and incubated at 37˚C for 24 - 48 H. Once the culture was positive, the strains were purified and identified using ChromID® *Candida* Agar chromogenic medium (REF 43639, BioMérieux, Marcy l'Etoile, France), API® *Candida* Gallery (REF 10 500, BioMérieux, Marcy l'Etoile, France) and Apiweb Standalone version 1.3.2. for identification of other *Candida* species.

ChromID® Candid Agar is a chromogenic, selective culture medium for isolating yeasts and molds. It directly identifies *Candida albicans* and provides presumptive identification of other species. *Candida albicans* colony are stained blue-green by the specific hydrolysis of a chromogenic substrate by hexosaminidase (BioMérieux patent) (Figure 1).



(A) (B)

Figure 1. (A): Pure colonies of *Candida* grown on Sabouraud + chloramphenicol medium. (B): Blue-green colonies of *Candida albicans* on ChromID® *Candida* Agar chromogenic medium (BioMérieux, Marcy l'Etoile, France). (Source: DOVO Essi, 2019).

The API® *Candida* Gallery is a standardized system for identifying yeasts within 18 - 24 hours. Moreover, it allows yeasts to be identified by studying their biochemical characteristics, in particular, their ability to acidify sugars or their enzymatic reactions.

2.7. Antifungal Susceptibility Tests

Antifungal susceptibility testing was carried out using the Kirby-Bauer agar disk diffusion method, as recommended by CLSI M44-A/EUCAST for yeasts [14]. The inoculum suspension was prepared in 5 mL NaCl saline, turbidity was adjusted to

0.5 McFarland. Finally, inoculation was performed by swabb testing Sabouraud-Chloramphenicol agar plates. The antifungals used were all produced by Liofilchem Srl (Italy):

Azoles: imidazoles: clotrimazole CLO (50 µg), econazole ECN (10 µg), ketoconazole KCA (10 µg), miconazole MCL (10 µg) and triazoles: fluconazole FLU (100 µg), itraconazole ITR (50 µg).

Polyens: nystatin (100 IU) and amphotericin B (10 IU) were deposited in Petri dishes for incubation at 37˚C for 24 to 48 hours (Figure 1). Inhibition diameters were measured in millimeters and results were interpreted according to CLSI/EUCAST recommendations [15] validated for in vitro yeast susceptibility testing (Figure 2, Table 1).

Table 1. Antifungals interpretive breakpoint.

Antifungus	Interpretive breakpoint	Diameters
Nystatine	S	>10 mm
	R	<10 mm
Fluconazole, Itraconazole	S	≥19 mm
	SDD	15 - 18 mm
	R	14 mm
Econazole, Clotrimazole, Miconazole, Ketoconazole	S	≥20 mm
	SDD	10 - 20 mm
	R	≤10 mm

Legend: S: Sensitive; SDD: Susceptible Dose Dependent; R: Resistant.

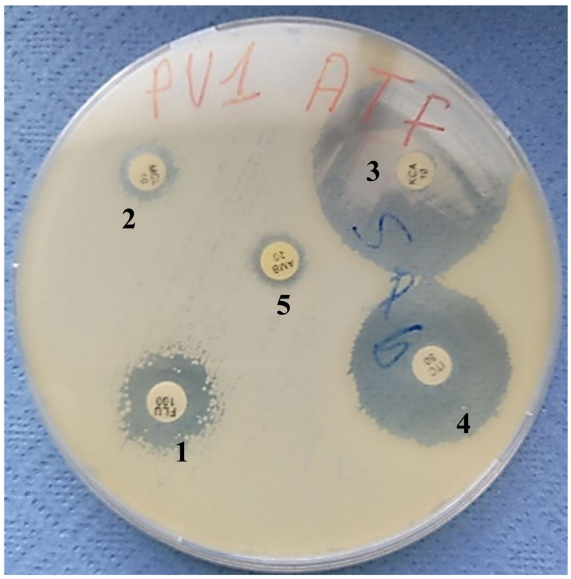


Figure 2. Antifungal Susceptibility test of *Candida albicans* on Sabouraud Chloramphenicol agar after incubation 37˚C/24H. 1—Fluconazole FLU (100 µg); 2—Miconazole MCL (10 µg); 3—Kétoconazole KCA (10 µg); 4—Itraconazole ITR (50 µg); 5—Amphotéricine B AMB (10 UI) (Source: DOVO Essi, 2020).

2.8. Data Analysis

Data was collected using Excel 2019 and analyzed using R software version 4.1.1. The tests used were the Chi2 test and the Fisher test with a 95% confidence level. A p value < 0.05 is considered statistically significant.

3. Results

3.1. Socio-Demographic Characteristics of the Study Population

From January 2018 to December 2022, we received a total of 4791 women aged between 3 and 64, including 02 girls aged 03 and 11. For 4789 women, the age range was 15 - 64 years, with an average of 27.80 + 6.77. Vaginal infections were found in 3275 women, *i.e.* a positivity rate of 68.4%. Vaginal *Candida* infections were found in 2429 women (74.16%). The 20 - 29 years age group was the most affected by vulvovaginal candidiasis. Pregnant women in our study population numbered 1378, *i.e.*, 28.76%. Pregnant women in their second (2nd) trimester were most affected by candidiasis (Table 2).

Table 2. Socio-demographic characteristics.

Age group	Number	Percentage
<20 ans	233	4.87
20 - 29ans	2988	62.40
30 - 39ans	1151	24.03
40 - 49ans	356	7.43
>50 ans	61	1.27
Pregnant women		
First Trimester	374	27.08
Second Trimester	561	40.62
Third Trimester	446	32.30

3.2. Correlation of Macroscopic Appearance of Leucorrhea and Cervix with *Candida* Occurrence

During vaginal sampling, aspects of the cervix and leucorrhoea (odour, color, abundance, vaginal ph) are noted in Table 3 below.

Table 3. Correlation of Macroscopic appearance of leucorrhea and cervix with *Candida* occurrence.

	Candida		OR	IC 95 %	p value
	Yes	No			
Cervix					
Normal	2	1974	Reference	-	-
Inflamed	2238	144	1119	279.68 - 4483.42	p < 0.0001

Continued

vagina pH					
4	1	1501	Reference		
5	2450	804	4573.94	642.25 - 32545.72	p < 0.0001
6	9	9	1501	171.81 - 13112.73	p = 1.2658
Appearance of Leucorrhea					
Minimal	17	1362	Reference		
Scanty	23	175	7.78	4.10 - 14.75	p = 3.783
Abundant	2404	770	212.25	135.49 - 332.51	p < 0.0001
Very abundant	16	15	90.80	40.72 - 202.46	p = 1.589
Odor					
Odorless	198	1679	Reference		
Foul	2228	602	31.52	26.52 - 37.46	p = 0.0006
Nauseating	25	47	4.51	2.71 - 7.49	p = 5.8792
Purulent	9	1	76.40	9.62 - 606.29	p = 4.587
Color					
Colorless	16	431	Reference		
Whitish	676	1889	9.63	5.80 - 16	p = 1.2587
Yellowish	1781	13	3692.50	1762.84 - 7734.43	p < 0.001
Reddish	2	1	53.87	4.64 - 625.45	p = 2.358

3.3. Identification of the Different *Candida* Species Isolated

In our study, *Candida albicans* was the most isolated species with a percentage of 55.12% (n = 1356), followed by *C. glabrata* 27.64% (n = 680), *C. tropicalis* 6.91% (n = 170), *Candida famata* 6.67% (n = 16), *Candida krusei* 2.56% (n = 63) and *Saccharomyces cerevisiae* 1.10% (n = 5) (Figure 3).

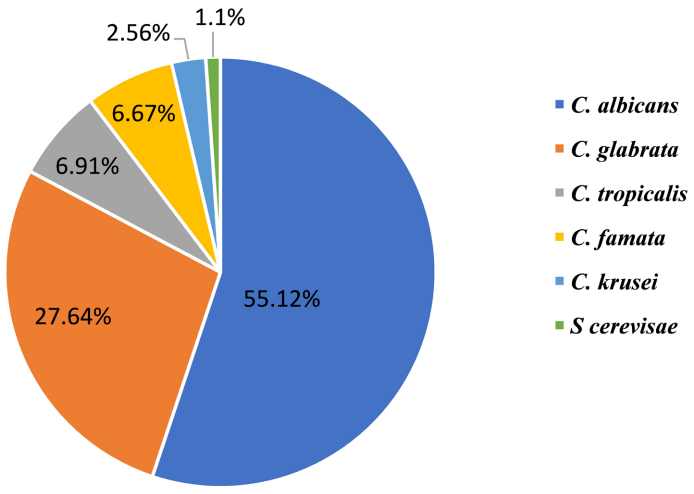


Figure 3. *Candida* species frequency.

3.4. Prevalence of *Candida* Species Resistance to Azole and Polyene Antifungals

In our study, Fluconazole, clotrimazole and Miconazole had the highest resistance in all species isolated, at over 40%. Amphotericin B was the most resistant, compared with nystatin. Similarly, Fluconazole was more resistant in *C. glabrata* (53.5%) than in *C. albicans*. Econazole and Ketoconazole were the most sensitive. Figure 4 below summarizes the resistance observed.

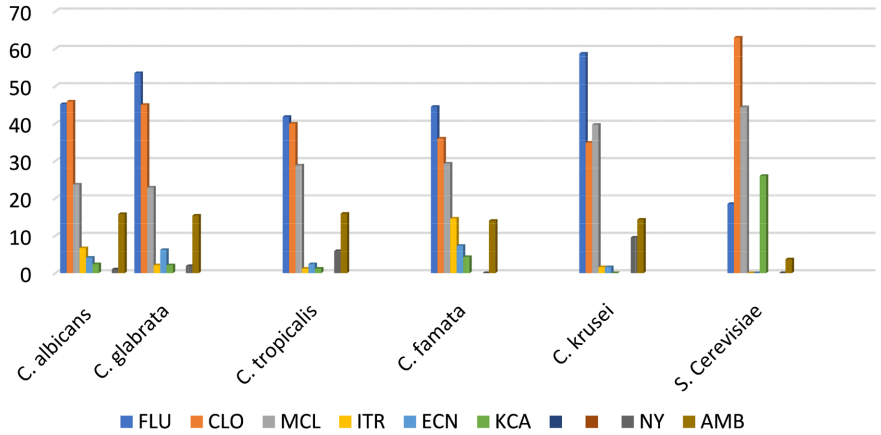


Figure 4. Prevalence of *Candida* species to antifungus.

4. Discussion

Vulvovaginal candidiasis, a neglected disease, has become a real public health problem. The aim of this study was to determine the prevalence of resistance of *Candida* species to common antifungals isolated in vulvovaginitis from 2018 to 2022 at HOSCO, Ouagadougou, Burkina Faso.

Vulvovaginal candidiasis affects an average of one in two women during their childbearing years. In our study, the age group most affected by candidiasis was 20 - 29 years. Our results corroborate those of other authors in Nigeria, Ghana, Ethiopia, Côte d'Ivoire, the United States and Europa [2] [16]-[18]. This could be due to various risk factors linked to the woman, such as clothing habits, sexual activity, hormonal changes… [19].

Similarly, among pregnant women, the women most affected by candidiasis were in the 2nd trimester,) (40.62%). Our results corroborate those of Sangare *et al.* in Burkina Faso and several other authors in Benin, Côte d'Ivoire, Mauritania, Kenya, Europe and the USA, who studied the cytobacteriology of vaginal swabs taken during the second and third trimesters, and the incidence of vaginal candidiasis during pregnancy. According to these studies, positive *Candida* cultures vary between 33.3 - 46% [2] [20]-[23]. Pregnancy is a risk factor for the onset of candidiasis, as it modifies the pH of the vagina, leading to the proliferation of numerous pathogenic germs, including *Candida*.

Vulvovaginal candidiasis is characterized by vulvar pruritus and abundant leucorrhea with a "curdled milk" appearance [6]. Our results show a correlation

between the occurrence of *Candida* and vaginal pH = 5, inflamed cervix, abundant and foul-smelling leucorrhea, where the p-value is less than 0.05. Inflamed cervix is not only specific to vulvovaginal candidiasis, but also to other genital infections such as vaginitis [24].

Our results show that *Candida albicans* are the most isolated species (55.12%). They concur with those of Nadembega *et al.* in their study on determining the prevalence of vaginal infections in women aged 15 to 24 in Ouagadougou from April 2010 to February 2011, where *C. albicans* was the most isolated species at 59% [25]; the same is true of Zida *et al.*, who obtained a prevalence of 59.36% of *C. albicans* in their study of sensitivity testing of *C. albicans* to usual antifungal agents isolated in vulvovaginitis and oral infections in Ouagadougou from January 2013 to December 2015, and a retrospective analysis (60.3%) of vaginal infections at HOSCO from June 2015 to June 2018 [9]. The same is true of various studies around the world, in which C *albicans* is the most isolated species. This can be explained by their ease of adhesion and colonization of the host [19].

Among non-*C. albicans Candida*, *C. glabrata* (27.64%) was the species most frequently found in our results. The other species were C *famata*, *C. tropicalis* and *C. krusei*. Although *C. glabrata* has been found to be the most prevalent between 22 - 35% in other studies, the prevalence of other species differs from one study to another [9] [10] [25].

Our results show high resistance to Fluconazole, Miconazole and Clotrimazole of 28.05 - 48%. This corroborates with various studies in Nigeria, Ivory Coast, East Africa, where the prevalence of Fluconazole resistance especially in *C. albicans* was 30 - 58% [26]-[30]. This may be due to the excessive use of these azoles. Amphotericin B was the most resistant up to 15.8%. This is in line with Dembele *et al.*, who also obtained Amphotericin B resistance in Ouagadougou from 2018-2019. Their prevalence of 93.75% could be explained by their small sample size [11]. Various resistance mechanisms could explain these resistances, and will be the subject of future research.

5. Conclusion

Vulvovaginal candidiasis is a real public health problem, affecting mainly young women of childbearing age. In our study, the age group most affected was 20 - 29 years. Among pregnant women, those in their second and third trimesters were the most affected. Although *C. albicans* is the most isolated species (55.12%), other non-*albicans* species such as *C. glabrata* are increasingly found and are highly resistant to the usual antifungal agents. Fluconazole, Miconazole and Clotrimazole are the most resistant of all the species isolated. It is important to sound the alarm on the excessive use of antifungal agents in order to prevent their ever-increasing resistance. The mechanisms of resistance will be the subject of future studies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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