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Research Article

Vulvovaginal Candidiasis among Pregnant Women at the University Hospital of Angré, Abidjan-Côte d'Ivoire

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Abstract

Vulvovaginal candidiasis is a frequent reason for consultation in gynecology. Few studies have been conducted in pregnant women, even though they may be asymptomatic. This study aimed to determine the epidemiological profile of vulvovaginal and antifungal susceptibility patterns among pregnant women at the university teaching hospital of Angré in Abidjan. This cross-sectional study was carried out in the gynecology-obstetrics department of the Angré University Hospital from June to October 2024. Swabs were taken from Pregnant women for mycological analyses. Each sample was examined directly and cultured at 37°C on Sabouraud-Chloramphenicol medium. *Candida* yeasts were identified using Chromagar *Candida* medium or Vitek 2. Antifungal susceptibility was determined using the agar diffusion method with discs. Of total of 402 women included, 70 were positive on culture, representing an overall vaginal candidiasis carriage rate of 19.2%. The yeast species identified were *Candida albicans* (48.1%), *Candida krusei* (27.8%), *Candida tropicalis* (12.7%), and *Candida glabrata* (11.4%). Symptoms such as vaginal discharge, vulvar pruritus, and dyspareunia were statistically linked to *Candida* carriage ($p<0.05$). Non-*albicans* species showed low sensitivity to antifungal agents. *Candida krusei* was only 68% susceptible to amphotericin B and econazole while *Candida tropicalis* had low susceptibility.

This study showed a relatively high frequency of *Candida* yeasts in pregnant women. The emergence of non- *albicans* with less susceptibility to the antifungal drugs highlights the importance of systematically screening pregnant women to better manage vulvovaginal candidiasis.

Keywords: Vulvovaginal candidiasis; Pregnant women; Antifungal; Abidjan

Abbreviations

AMB: Amphotericin B; CLSI: Clinical And Laboratory Standards Institute; FCZ: Fluconazole; SDA: Sabouraud Dextrose Agar; VVC: Vulvovaginal Candidiasis.

Introduction

Vulvovaginal candidiasis is a common reason for gynecological consultations and ranks second after bacterial vaginosis [1,2]. It is estimated that 75% of women experience at least one episode of candidiasis during their lifetime. Among these women, some will have several episodes and around 5 to 8% will develop recurrent vulvovaginal candidiasis (VVC), characterized by at least four confirmed episodes in a year [3]. Certain risk factors for VVC are related to sexual activity, recent antibiotic use, immunosuppression attributable to conditions such as poorly controlled HIV infection or diabetes, and pregnancy [4,5]. All of these factors contribute to an imbalance in the vaginal flora and the onset of clinical and biological symptoms. Pregnancy is the leading factor promoting CVV due to the hormonal changes observed [6,7]. *Candida* species are part of the normal flora of the genital tract. In healthy asymptomatic non-pregnant women, these yeasts have been found in 20–30% [3,8]. This situation only poses a danger to the fetus and newborn when the manifestations occur in a context of prematurity or when the prognosis may be life-threatening [9,10]. When vaginal candidiasis is not asymptomatic, it can lead to vulvar pruritus and characteristic vaginal discharge. These symptoms may be associated with dysuria, dyspareunia, vaginal dryness, or vulvar burning [11].

Untreated, vaginal candidiasis can lead to chorioamnionitis with subsequent miscarriage and prematurity in pregnant women, or congenital infection of inflammatory disease in newborns and pelvic inflammatory disease in infertile women [12,13].

Vulvovaginal candidiasis is most often caused by the overabundance of an opportunistic pathogenic yeast, *Candida albicans* (approximately 90%), which is a common member of the vaginal flora [14]. Moreover, the emergence of *Candida* species isolated from clinical samples, indicated that non-*albicans* species were considered emerging fungal pathogens in pregnant women [13,14]. These yeasts are commensal species of the skin and gastrointestinal tract, but may be present in the vaginal tract of 20 to 30% of asymptomatic healthy women at any given time. If the balance between the colonizing yeast and the host is temporarily disrupted, *Candida* can cause infections such as vaginal candidiasis, associated with clinical signs of

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inflammation [3]. *Candida albicans* is known to be resistant to certain antifungal drugs and is generally treated with azole antifungals due to their low toxicity and availability [15,16]. Vulvovaginal candidiasis is a public health issue due to the morbidity associated with symptoms in women [17]. The prevalence varies from one country to another. In Côte d'Ivoire, some studies in women reported that the prevalence rate was higher than 28% from non-pregnant women [2,18]. Few studies have focused on vaginal candidiasis in pregnant women. Therefore, the aim of this study was to determine the prevalence of vulvovaginal and antifungal susceptibility pattern among pregnant women at the university teaching hospital of Angré in Abidjan.

Material and Methods

Study Design and Setting

This descriptive cross-sectional study was carried out from June to October 2024 in the gynecological consultation service of the Angré University Hospital for sampling and in the parasitology-mycology unit of the medical biology department for vaginal candidiasis mycological analyses. This hospital receives patients from all districts of Abidjan city and others neighboring municipality. It has high-quality technical facilities, modern healthcare infrastructure, and a wide variety of services. We also note the presence of the Parasitology-Mycology Laboratory in the Medical Biology Department, which has the necessary equipment to carry out our study.

Study Population and Sample Size

The population consisted of all pregnant women consulting the Gynecology-Obstetrics service of University Hospital of the Angré for prenatal care during the study period. All pregnant women who attended prenatal consultations and gave their informed consent were included in our study. Pregnant women undergoing antibiotic or antifungal treatment during the 15 days prior to their consultation; or with vaginal bleeding were not included in this study.

The sample size for this study was determined using the single population proportion formula.

$$n=Z^2\times p\times (1-p)/d^2$$

When the proportion of vaginal candidiasis (p = 38%) was taken from a previous study [2]. (Konaté et al 2014), with the assumption of precision or degree of error 0.05, the confidence interval was 95%. Where n = Sample size, Z = value corresponding to 95% level of significance = 1.96, P = proportion of prevalence vaginal candidiasis in pregnant women = 38%, d = marginal error assumed to be 5%. The final sample size was 365 pregnant women.

Sampling and Data Collection

All pregnant women attending prenatal consultations were included in the sample until the total sample size for this study was obtained in the hospital. Based on the client's sequences, which were used as a sampling frame using systematic random sampling. The process was continued throughout data collection until the required sample size was achieved. Midwives and gynecologists were selected to collect data. Training was provided to the data collector and supervisor. Sociodemographic information, clinical factors, and behavioral factor data were collected using structured questionnaires. A vaginal swab was taken from each pregnant woman using two sterile swabs moistened with physiological saline solution, which were then inserted and gently rotated to collect the sample from the posterior cul-de-sac after insertion of a speculum. Samples collected from each pregnant woman were immediately transported to the parasitology-mycology laboratory for analysis.

Mycological Analyses

Direct Examination and Culture Procedures:

For each sample, a swab was used for direct examination between a slide and coverslip in physiological saline to look for spores and mycelial filaments. The other swab was then used for culture on Sabouraud Dextrose Agar (SDA) containing 2% chloramphenicol, incubated at 37°C and examined after 24 hours for cream-colored colonies and budding yeast cells suggesting the presence of *Candida* species.

Identification of *Candida* Species and Antifungal Susceptibility Testing:

The SDA isolates were inoculated in *Candida* selective agar Chromogenic medium (Chromagar *Candida*) using an inoculating needle and incubated at 37 °C for 24h to ensure detection of mixed cultures by colon colors such as C. *albicans* (light green), C. tropicalis (blue to metallic blue), C. glabrata (cream to white), and C. krusei (purple-pink). The method is based on the differential release of chromogenic breakdown products from various substrates by *Candida* species after differential exoenzyme activity [19]. When difficulties arose in identifying yeasts using the Chromoagar *Candida* medium, the VITEK 2 automated system was used.

Antifungal susceptibility testing for all *Candida* isolates was performed using a agar diffusion method with antifungal discs, in accordance with the 2018 guidelines of the Clinical Laboratory Standards Institute (CLSI). The test was performed by suspending a young yeast colony in saline solution and comparing it to the 0.5 McFarland standard. The casitone medium poured into Petri dishes was then flooded with the yeast suspension and dried. Antifungals discs, comprising amphotericin B 100, fluconazole 10 µg, Clotrimazole 10 µg, miconazole, ketoconazole and econazole 30 µg, were placed on the agar using a disc dispenser. The Petri dishes were then incubated at 37°C for 24 hours. The inhibition zones (zone diameters) were then measured and interpreted in accordance with CLSI guidelines [20].

Data Analysis

Data were entered into Microsoft Office Excel 2007 and exported to STATA version 14.2 software for statistical analysis. Univariate analysis was performed (Chi-square and Fisher's exact test) to compare the groups. Frequency, percentages, and odds ratio (OR) were computed to describe population. Bivariate and multivariate logistic regression was used to assess factors associated with vulvovaginal candidiasis in study population. Associations and differences with a p-value less than 0.05 were considered statistically significant.

Ethical Aspects

The study was based entirely on routine clinical and laboratory data. The consent to participate wasn't so obligatory. Approval was obtained from the medical and scientific management of the CHU of Angré (reference n° 067/MSHPCMU/CHUA/DMS/akad). Samples

were taken from pregnant women who had given their informed consent. Each sample was assigned an identification number to preserve the anonymity and confidentiality of these pregnant women.

Results

Socio-demographic Characteristics of Pregnant Women

A total of 418 pregnant women were interviewed and 402 were eligible and consented to be included in this study with inclusion rate of 96.2%. The remaining 16 pregnant women were either not eligible or verbally declined to be part of the study (Figure 1). Complete socio-demographic data, clinical presentations and vaginal swabs were taken from 420 consenting participants. Pregnant women mean age was 29 years, with a range of 15 – 42 years. The age of the study participants ranged from 14 to 46 years and the mean age was 30.83 with a standard deviation of ± 5.8 years. The age group between 31 and 46 year was the most represented, accounting for 53.48%. According marital status, 93.1% of pregnant women were married, 14.4% were single, widow and divorced women accounting for 2% and 2.5% respectively. Among pregnant women included in our study, most of them were in their third trimester of pregnancy (55.97%); multigravida women accounted for 71.6% while nulliparous women accounted for 38.6%. Regarding the number of prenatal visits and the type of residence, pregnant women who had consulted less than 4 times were the most represented at 54.98%, and the majority lived in villa or apartments. Table 1 shows the sociodemographic characteristics of pregnant women included in the study.

Symptoms and Risky Behavior of Pregnant Women

Of the 317 pregnant women included the current in the study, 23.9% had vaginal discharge, vulvar pruritus in 6.2%, dyspareunia and vaginal burning were observed at 8.2% and 2.à% respectively. Regarding risky behavior of pregnant women proportion of participants 25.62% used public toilets, and 5.0% used antiseptic gel for their toilet (Table 2).

Table 1: Sociodemographic characteristics of pregnant women included in the study (n= 402).

Variables	Category	Frequency (n=402)	Percentage (%)
Women age group (years)	<20	10	2.5
	20-29	155	38.6
	30-46	237	58.9
Marital status	Single	58	14.4
	Married	326	81.1
	Widow	8	2.0
	Divorced	10	2.5
Gestation period	1st trimester	79	19.6
	2nd trimester	98	24.4
	3rd trimester	225	56.0
Number of pregnancies	Primigravida	114	28.4
	Multigravida	228	71.6
Number of births	Nulliparous	155	38.6
	Primiparous	128	31.8
	Multiparous	119	29.6
Prenatal visits	≤ 3	221	56;0
	4-6	175	43.5
	> 6	6	1.5
Type of housing	Residence common	25	6.2
	Villa/Apartment	377	93.8

Table 2: Symptoms and risky behavior of pregnant women included in the study (n= 402).

Variables	Category	Frequency (n=402)	Percentage (%)
Leucorrhea	Yes	96	23.9
	No	306	76.1
Vulvar pruritus	Yes	25	6.2
	No	377	93.8
Vaginal burning	Yes	8	2.0
	No	400	98.0
Dyspareunia	Yes	33	8.2
	No	369	91.8
Use of public toilet	Yes	103	25.6
	No	299	74.4
Use of toilet gel	Yes	20	5.0
	No	282	95.0

Prevalence of Vaginal *Candida* Species

In the current study, the overall prevalence of vulvovaginal candidiasis among pregnant women during the study period was attended 19.2% (77/402) with (95% CI. 15.4.9 - 23.4). According to age, the highest prevalence was observed in the age range of 20–30 years with 24.5%, followed by those above 30 years at 14.8%. Among 162 (40.3%) women presenting clinical signs, vaginal candidiasis was found in 57, accounting for 35.2%. Of the total *Candida* species isolated from pregnant women in our study, the predominant was *Candida albicans* at 48.1%. Non-*albicans Candida* species accounted for 51.9%, and the identified was C. krusei at 27.8%, followed by C. tropicalis (12.7%) and C. glabrata (11.4%). Figure 2 shows the frequencies of *Candida* species isolated among pregnant women

Factors Associated with the Prevalence of Vulvovaginal Candidiasis

In our study, the pregnant women who had gestational period in the 3rd trimester were at higher risk when compared to pregnant women in the 1st trimester (AOR = 2.11, 95% CI: 1.50–5.98). Regarding symptoms, the pregnant women who had leucorrhea were 4 times more likely to have an infection with vaginal candidiasis than those who had not clinical signs (AOR = 4.49, 95% CI: 1.84–6.63). The number of births was significantly associated with vulvovaginal candidiasis infection, where multiparous pregnant women were 3 times more likely to be infected by vulvovaginal candidiasis than primiparous pregnant women (AOR = 3.43, 95% CI: 1.92–8.90). Table 3 shows the associated factors with vulvovaginal candidiasis among pregnant women.

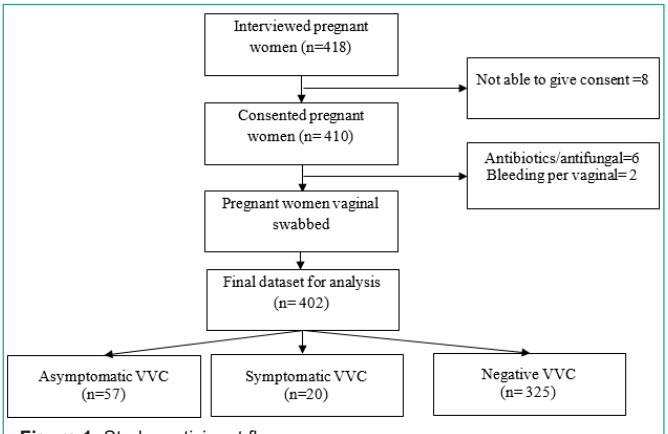

Figure 1: Study participant flow.

Table 3: Bi-variable and multivariate analysis of associated factors with vulvovaginal candidiasis among pregnant women.

Variables	Category	VVC (+)	VVC (-)	COR [95% CI]	p-value	AOR [95% CI]	p-value
Age group (years)	<20	4	6	1		1	
	20-29	38	117	0.48 [0.13-1.82]	0.285	1.57 [0.28-8.76]	0.907
	30-46	25	202	0.26 [0.07-0.97]	0.045	0.87 [0.15-5.07]	0.879
Marital status	Widow	1	7	1		1	
	Divorced	4	6	4.67 [0.40-53.9]	0.217	4.33 [0.25-74.9]	0.313
	Single	14	44	2.2 [0.25-19.7]	0.472	3.74 [0.29-48.2]	0.312
	Married	58	268	1.51 [0.18-12.5]	0.70	3.36 [0.27-42.1]	0.347
Gestational period	1st trimester	17	62	1		1	
	2nd trimester	22	76	1.05 [0.52-2.16]	0.882	2.11 [1.50-5.98]	0.037
	3rd trimester	38	187	0.74 [0.39-1.40]	0.359	0.80 [0.34-1.88]	0.435
Pregnancies	Primigravida	31	83	1		1	
	Multigravida	46	242	0.51 [0.30-0.86]	0.011	0.31 [0.11-0.92]	0.076
Number of births	Nulliparous	34	121	1		1	
	Primiparous	22	106	0.74 [0.41-1.34]	0.319	2.03 [0.68-6.10]	0.205
	Multiparous	21	98	0.76 [0.42-1.40]	0.380	3.43 [1.92-8.90]	0.039
Prenatal visits	≤ 3	50	171	1		1	
	4-6	26	149	0.60 [0.35-1.00]	0.053	0.70 [0.33-1.46]	0.341
	> 6	1	5	0.68 [0.08-5.60]	0.732	0.94 [0.09-9.55]	0.960
Type of housing	Residence common	12	13	1		1	
	Villa/Apartment	65	312	0.22 [0.10-0.52]	0.001	0.20 [0.07-0.56]	0.062
Leucorrhea	No	43	263	1		1	
	Yes	34	62	3.35 [1.98-5.69]	<0.0001	4.49 [1.84-6.63]	<0.0001
Vulvar pruritus	No	67	310	1		1	
	Yes	10	15	3.08 [1.33-7.16]	0.009	1.96 [1.30-3;03]	0.044
Vaginal burning	No	72	322	1		1	
	Yes	5	3	7.45 [1.74-31.9]	0.007	4.42 [0.69- 28.3]	0.017
Dyspareunia	No	69	300	1		1	
	Yes	8	25	1.40 [0.60-3.21]	0.440	0.77 [0.27-2/13]	0.610
Use of public toilet	No	56	243	1		1	
	Yes	21	82	1.11 [0.63-1.94]	0.712	0.78 [0.41-1.51]	0.465
Use of toilet gel	No	73	309	1		1	
	Yes	4	16	1.06 [0.34-3.26]	0.921	0.40 [0.08-1.88]	0.245

* indicates statistically significant at p < 0.05, Reference = 1. VC (+) is vulvovaginal *Candida* positive and VC (-) is vulvovaginal *Candida* negative. COR is crude odds ratio and AOR is adjusted odds ratio. CI is confidence interval.

Table 4: Antifungal susceptibility patterns of *Candida* species isolated from pregnant women.

Antifungal drugs	Phenotypic profile	Global susceptibility	*C. albicans* Number (%)	*C. krusei* Number (%)	*C. tropicalis* Number (%)	*C. glabrata* Number (%)
Amphotericin B	S	60 (75.9%)	32 (84.2%)	15 (68.2%)	6 (60.0%)	7 (77.8%)
	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	19 (24.1%)	6 (15.8%)	7 (31.8%)	4 (40.0%)	2 (22.2%)
Fluconazole	S	77 (97.5%)	38 (100%)	12 (54.5%)	10 (100%)	9 (100%)
	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	2 (2.5%)	0 (0.0%)	10 (45.5%)	0 (0.0%)	0 (0.0%)
Clotrimazole	S	66 (83.6%)	30 (78.9%)	20 ‘(90.9%)	9 (90.0%)	7 (77.8%)
	I	2 (2.5%)	2 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	11 (13.9%)	6 (15.8%)	2 (9.1%)	1 (10.0%)	2 (22.2%)
Ketoconazole	S	72 (91.1%)	34 (89.5%)	20 ‘(90.9%)	10 (100%)	8 (88.9%)
	I	1 (1.3%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	6 (7.6%)	3 (7.9%)	2 (9.1%)	0 (0.0%)	1 (11.1%)
Miconazole	S	73 (92.4%)	37 (97.4%)	20 ‘(90.9%)	7 (70/0%)	9 (100%)
	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	6 (7.6%)	1 (2.6%)	2 (9.1%)	3 (30.0%)°	0 (0.0%)
Econazole	S	62 (78.5%)	35 (92.1%)	14 (63.6%)	6 (60.0%)	7 (77.8%)
	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	17 (21/5%)	3 (7.9%)	8 (36.4%)	4 (40.0%)	2 (22.2%)

S: sensitive profile. I: Intermediate profile. R: resistant profile.

Antifungal Susceptibility of *Candida* Species from Pregnant Women

In the current study, a total of six antifungal drugs were tested. The global proportions of resistance profile were high for amphotericin B at 24.1% and econazole with 21.5%. Furthermore, fluconazole, ketoconazole and miconazole were the most effective antifungal drugs for all. Regarding antifungal susceptibility testing by *Candida* species, *C. tropica*lis was to resistant to amphotericin B, miconazole and econazole respectively at 40;0%, 30.0% and 40.0%. Most of *C. krusei*

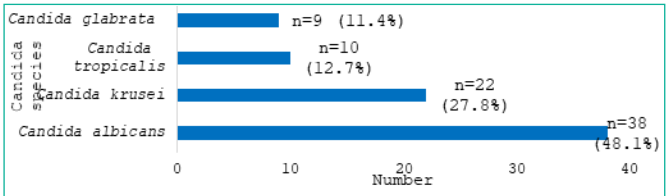

Figure 2: Frequencies of *Candida* species isolated among pregnant women.

species were resistant at 45.5% to fluconazole, 31.8% to amphotericin and 36.4% to econazole. The clotrimazole had low resistant profile to all *Candida* species identified among pregnant women in this study. Table 4 the antifungal susceptibility patterns of *Candida* species isolated from pregnant women.

Discussion

The current study aimed to describe the epidemiological and mycological profile of vaginal candidiasis in pregnant women attending prenatal consultations at the University Hospital of Angré in Abidjan. The prevalence of *Candida* species causing vaginitis is pregnant women vary from one population to another. In our study, we found an overall prevalence rate of vulvovaginal candidiasis of 19.15%. This prevalence rate is slightly lower than compared to a study conducted in University Hospital of Bouaké, which found a rate of 39.7% [21]. Similarly, studies in other African countries showed a high of vulvovaginal candidiasis [22,23]. Pregnant women are more susceptible to develop vulvovaginal candidiasis due to various hormonal changes, and many authors report that pregnancy is a contributing factor [9]. This difference in prevalence rates could be explained by the fact that the occurrence of vulvovaginal candidiasis varies according to geographical area and sample size [24]. In the current study, 40.3% of pregnant women developed clinical signs. A statistically significant difference was observed between leucorrhea, vulvar itching, burning during urination, and the occurrence of vulvovaginal candidiasis. Similar results were reported elsewhere [11,18,25]. The findings of this study revealed that leucorrhea were 4 times significantly associated with vulvovaginal candidiasis infection and multiparous pregnant women were 3 times more likely to be infected by vulvovaginal candidiasis than primiparous one [8]. The current study showed that the gestational period pregnant women in the 3rd trimester was at higher risk compared to pregnant women in the 1st trimester. Our result is in line with to the study conducted in others African countries [19,26].

Regarding yeast species identified in our study, *Candida albicans* was the most commonly identified species at 48.1%, followed by *Candida* krusei (27.8%), *Candida* tropicalis (12.7%), and *Candida* glabrata with lower frequencies of 11.4%. A study conducted in Benin showed that *C. albicans* was the most observed yeast species in vulvovaginal candidiasis [21]. These results corroborate those reported in other studies [18,27]. The predominance of *C. albicans* could be explained by its significant ability to adhere to the vaginal mucosa using specific vaginal cell receptors. This can lead to the expression of virulence factors, germination patterns and transformation from a saprophytic blastospores forms into pathogenic filamentous [13,14,27]. Although *C. albicans* remains the most species involved in the development of disease, vulvovaginal candidiasis due non-albicans species is increasingly being reported [28,29]. The frequency of these non-albicans species varies from 10% to 30%, with *C.* grabrata being the most common [30,31]. These data do not agree with those of our study, in which *C. krusei* was the most non-albicans species identified.

Regarding the antifungal susceptibility, our study reveals a relatively high sensitivity of *C. albicans* for econazole and miconazole. These sensitivity rates are consistent with those reported in other studies in which good sensitivity of *C. albicans* to had good susceptibility to azoles drug [6,32]. The current study reported that *C. krusei* showed a high resistance to econazole (31.8%). These data are completely different from those in which a high sensitivity of *C. krusei* to miconazole [33]. As for *C. tropicalis,* we note a good sensitivity to econazole and miconazole. Our results are in line with those from a study which showed very good sensitivity of this species to miconazole [25]. From all above findings, we observe a decrease in the sensitivity of the *Candida* species to azoles antifungal class. This could be explained by the overuse and Self-medication of these drugs.

In our study, amphotericin B showed good efficacy against all *Candida* species identified. The literature reports similar efficacy for amphotericin B [2,18,25]. For these non- albicans species, several authors have reported results very similar to ours. All these results show that amphotericin B is the most effective antifungal agent among the molecules in our study. However, there may be slight resistance [34]. The limitations of the study stem from the fact that its cross-sectional design makes it difficult to establish causal relationships. Molecular identification of species was not performed due to budgetary constraints. In addition, not all antifungal sensitivities could be determined due to budget constraints, and no other reagents were available in the country, limiting the number of drugs that could be tested.

Conclusion

The current study reported a moderate frequency of vulvovaginal candidiasis in pregnant women. The most commonly isolated yeast species were C. albicans and C. krusei. Factors such as gestational age, number of deliveries, leucorrhea, and other symptoms were significantly associated with vulvovaginal candidiasis. In terms of antifungal susceptibility, ketoconazole and miconazole were found to be the most effective for all *Candida* species. However, most of C. krusei species were resistant to the majority of antifungal drugs tested. It is therefore advisable to screen all pregnant women for candidiasis in order to enhance infection prevention strategies during admissions to healthcare facilities.

Author Contributions

AK.E. and N.M.A. contributed to the study design. A.A., O.M. and E.N. contributed to the data collection and verification. A.A. and A.K.E. analyzed the data. The first draft of the manuscript was written by A.K.E. D.V., K.S. and M.E.H. extensively reviewed the manuscript. The study was supervised by K.N.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts Interests: The authors have no relevant financial or nonfinancial interests to disclose.

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