



Vulvovaginal Candidiasis in Pregnant Women Attending the Garoua Regional Hospital (Cameroon) and Antifungals Susceptibility Profile of Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IME and YSI were involved in the conception and design of the study. Authors FNK, YSI and AA were involved in experiments. Authors IME and TTY were involved in the data analysis. Author YSI drafted the manuscript, then authors IME, FNK and TNN corrected and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Both vaginal colonization and infection by yeast (a type of fungus) are most likely to occur in Pregnant women. Considering this fact and owing to the lack of data on this subject in North Cameroon, the main objective of this study was to determine the prevalence of vulvovaginal candidiasis (VVC) in pregnant women at the Garoua Regional Hospital and the susceptibility of isolates to some commonly used antifungal agents.

Methods: We conducted a cross-sectional study during a period of 6 months (January to June 2023) at the Regional Hospital of Garoua. A total of 282 vaginal swabs collected were cultured on ChromAgar Candida medium for isolation and identification of the *Candida* species. The microdilution technique was used for the susceptibility testing of the isolates.

Results: Mycological examination revealed that, out of 282 vaginal swab samples collected, 78 (27.65%) were positive for *Candida* spp culture. The identification of yeast isolates showed that only two *Candida* species were involved in VVC in the studied population. *Candida albicans* was the major species with 71.79%) and *Candida krusei* was 28.20%. The most affected age groups were those of [26-30] and [31-35] with 35.9% each. Abundant leucorrhoea (69.23%) and pruritus (66.67%) were the most common symptoms in pregnant women with VVC. Wearing tight clothes and being under antibiotic treatment were the most prominent risk factors associated with VVC among the study participants. *Candida albicans* showed 100% resistance towards nystatin and amphotericin B while the highest of *Candida krusei* was observed with itraconazole (72.41%) and amphotericin b (96.55%). Both species showed sensitivity towards miconazole and econazole.

Conclusion: The present study has revealed that the prevalence of vulvovaginal candidiasis cases among pregnant women at the Garoua Regional Hospital is 27.65%. Only *C. albicans* and *C. krusei* were involved in VVC in the studied population. The antifungal susceptibility profile confirmed the efficacy of econazole and miconazole for the treatment.

Keywords: Vulvovaginal candidiasis; pregnant woman; *Candida* species.

1. INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common infection affecting women of reproductive age worldwide [1]. Worldwide, recurrent vulvovaginal candidiasis affects about 138 million women annually, with a global annual prevalence of 0.39%; 372 million women are affected by recurrent vulvovaginal candidiasis over their lifetime [2,3]. It is caused primarily by the overgrowth of *Candida* species, most commonly *Candida albicans*, in the vaginal region [4]. While VVC is prevalent in the general population, pregnant women are particularly susceptible to this condition due to hormonal changes and immune system alterations that occur during pregnancy [5]. Pregnancy is associated with numerous physiological changes that can contribute to an increased risk of vulvovaginal candidiasis [6]. Hormonal fluctuations, such as elevated oestrogen levels, alter the vaginal microbiota and create an environment more conducive to *Candida* overgrowth [7]. Additionally, immunological changes, including decreased cell-mediated immunity, further weaken the body's defence mechanisms against *Candida* species [8]. Consequently, the prevalence of VVC in pregnant women is

relatively high compared to the general population.

VVC during pregnancy not only causes discomfort and distress to affected women but also poses potential risks to both the mother and the developing foetus [9]. Uncontrolled or recurrent infections may lead to complications such as preterm labour, low birth weight, and an increased risk of acquiring other infections [10]. Therefore, understanding the prevalence, risk factors, and antifungal susceptibility profile of *Candida* isolates in pregnant women is vital for timely diagnosis, appropriate treatment, and effective prevention strategies.

Studies conducted in Morocco revealed that 20.64% of women were affected by vaginal candidiasis with a predominance of *Candida albicans* infections (47.22%) followed by *Candida glabrata* (34.21%) [11]. In Cameroon, few studies had focused on vaginal candidiasis in pregnant women. Toua *et al.* in 2013 found a prevalence of 55.40% of vulvovaginal candidiasis in pregnant women in a study conducted in Maroua in the far north of Cameroon [12]. Another study conducted in Yaoundé by Kechia *et al.* revealed 35.52% of cases of vulvovaginal candidiasis in

pregnant women, with a dominance of *Candida albicans* candidiasis (80.52%) followed by *Candida glabrata* (8.51%) [13].

Garoua Regional Hospital, located in Cameroon, serves as a significant healthcare facility in the region, catering for a diverse population. It provides comprehensive medical care, including obstetric and gynaecological services, to a large and diverse population. The study had focused on pregnant women attending the hospital's antenatal clinic, where they received routine check-ups, counselling, and necessary interventions to ensure a healthy pregnancy. The unique population served by Garoua Regional Hospital presents an opportunity to investigate the epidemiology of VVC in pregnancy and assess the susceptibility patterns of *Candida* isolates in this setting. Understanding the prevalence and antifungal susceptibility patterns of *Candida* isolates in pregnant women is crucial for the effective management and prevention of VVC-related complications [14].

It, therefore, seemed necessary to us to conduct this present study at the regional hospital of Garoua to investigate the occurrence of vulvovaginal candidiasis in pregnant women attending Garoua Regional Hospital and to determine the antifungal susceptibility profile of *Candida* isolates. By examining the prevalence, risk factors, and susceptibility patterns, this research had sought to provide valuable insights into the epidemiology of VVC in the context of pregnancy, aiding healthcare providers in developing appropriate treatment strategies and enhancing patient care.

2. MATERIALS AND METHODS

2.1 Type and Duration of the Study and Study Population

It was a cross-sectional study, spreading over a period of six months from January 2023 to June 2023, at the Garoua Regional Hospital in the North region of Cameroon.

2.2 Study Population

Our study population consisted of pregnant women (282), attending the Garoua Regional Hospital. Pregnant women undergoing antifungal therapy or having a history of taking antifungals were excluded.

2.3 Sampling and Mycological Examination and Identification

After cleansing the vaginal margins with Dakin's solution, vaginal swabbing (at the cervix margins) was performed using specula and sterile swabs. Each sample collected was preceded by a questionnaire.

The mycological examination consisted of culturing on the CHROMagar *Candida* medium (CHROMagar, Paris, France), the various samples. This permitted us to isolate and identify (depending on the color) *Candida* colonies after 24 to 48 hours of incubation. When the number of colonies counted after incubation was greater than $>10^4$ CFU/mL, this was associated with vulvovaginal candidiasis infection [15]. After identification, the *Candida* species were subjected to an antifungal susceptibility test.

2.4 Antifungal Susceptibility Testing of the Isolates

The susceptibility of the isolates was tested against eight (08) antifungal medicaments including: nystatin, itraconazole, miconazole, ketoconazole, fluconazole, clotrimazole, econazole, amphotericin B. Diameter of inhibition was determined by the agar diffusion technique described by the NCCLS protocol [16]. In brief, yeast inoculum was adjusted to 1.0×10^5 were spread on Sabouraud Dextrose Agar (SDA) plates. Disks containing the test agents were applied to the surfaces of inoculated plates. Plates were inverted and incubated at 30°C for 2 to 3 days to allow for fungal growth. Inhibition zone diameters (IZD) were measured in millimeters.

2.5 Ethical considerations

This study was approved by the Institutional Ethics Committee of the University of Garoua Research on Human Health with Reference Number 011/2023/L/ISSTSM/GRA. The samples were collected following international safety rules and ethical standards. Informed and written consent and assent were obtained from the study participants before data collection.

3. RESULTS

3.1 Characteristics of Study Participants

The socio-demographic subgroup's prevalence of vulvovaginal candidiasis is presented in

Table 1. Socio-demographic data and clinical presentations were collected from all 282 consenting participants (Table 1). Participants' age range was 15-45 years with 31.91% being in the group of [26-30 years]. Most (50%) of the participants were in their second trimester. The majority, 86.52% were married, with 52.48% having acquired a high school education. Only 2.84% had no formal education while 56.03 were unemployed. Out of the 282 pregnant women, 4.5% had at least one of the six associated symptoms. The main symptoms observed were abundant leucorrhoea in 104 (36.88%) pregnant women, followed by pruritus (26.24%) and dysuria (11.35%). Among the identified risk factors, wearing tight clothes had the highest number of participants, with 138 individuals (48.94% of the total) reporting this factor while under antibiotic treatment, 66 participants (23% of the total) were identified. None of the participants reported having diabetes.

3.2 Prevalence of Vulvovaginal Candidiasis

In the present study, we assessed yeast carriage associated with vaginal candidiasis symptoms, and the results are presented in Table 1. Out of the 282 pregnant women who participated in the study, 78 were found to be positive for vulvovaginal candidiasis infection giving a global prevalence rate of 27.65% in the study population. The age group of [26-30 years] had the highest percentage of candidiasis cases, with 28 individuals testing positive (35.9%) while the group of [41-45-year-olds] did not have any individuals testing positive for candidiasis. The percentage of participants who tested positive for candidiasis was highest among those with a high school education, with 44 out of 148 (56.41%) testing positive. Among participants with higher education, 28 out of 86 (35.9%) tested positive for candidiasis. Participants with no schooling or primary school education had lower percentages of positive candidiasis cases, with 2.56% and 5.13%, respectively. The percentage of participants who tested positive for VVC candidiasis was highest in the third pregnancy semester (third trimester), with 40 out of 70 participants (51.29%) being positive. The first pregnancy trimester had the lowest percentage of positive cases, with only 2.56% of participants testing positive for VVC candidiasis. The highest percentage of positive cases for VVC was observed among participants reporting pruritus

(itching), with 52 out of 74 individuals (66.67%) testing positive. Other symptoms such as dyspareunia, dysuria, vaginal dryness, and vulvar burning also showed a percentage of positive cases, ranging from 15.38% to 20.51%. Among the identified risk factors, wearing tight clothes had the highest percentage of positive cases, with 52 out of 138 (66.67%) participants testing positive for VVC. The presence of HIV infection was reported by 6 participants (2.13% of the total), and 2 out of those 6 individuals (2.56%) tested positive for VVC. There were no positive cases associated with diabetes mellitus.

3.3 Species Identification

In the present investigation, two *Candida* species were isolated from vaginal specimens and identified on Chromagar medium. The result of the distribution of *Candida* species is presented in Fig. 1. *C. albicans* was the most prevalent species in the study, accounting for 56 out of 78 isolates (or 71.79%). This species is commonly found in the human microbiota and can cause various infections, including oral thrush, vaginal yeast infections, and systemic candidiasis. *Candida krusei* was the second most abundant species, representing 22 isolates (or 28.2%) in the sample. This species is less common than *Candida albicans* but is known to be associated with certain infections, particularly in immunocompromised individuals. *Candida krusei* can exhibit some resistance to antifungal drugs, which may have implications for treatment.

3.4 Antifungal Susceptibility Outcome

Table 2 shows the susceptibility of *Candida albicans* and *Candida krusei* isolates to some commonly used antifungal agents. Both *Candida albicans* and *Candida krusei* isolates showed high susceptibility to nystatin, itraconazole, and amphotericin B while miconazole showed varying susceptibility, with *Candida albicans* isolates predominantly susceptible and *Candida krusei* isolates showing more resistance. Ketoconazole exhibited a mix of susceptibility and resistance in both *Candida albicans* and *Candida krusei* isolates. Fluconazole and clotrimazole displayed varying susceptibility patterns, with *Candida krusei* isolates showing more resistance compared to *Candida albicans*. Econazole shows lower susceptibility overall, particularly for *Candida krusei* isolates.

Table 1. Distribution of candidiasis in the study population according to socio-demographical characteristics

Variables		n=282	%	Positive to candidiasis	
				n=78	%
Age groups	[15-20]	22	7.8	2	2.56
	[21-25]	62	21.99	16	20.51
	[26-30]	90	31.91	28	35.9
	[31-35]	68	24.11	28	35.9
	[36-40]	30	10.64	4	5.13
	[41-45]	10	3.55	0	0
Occupation	Student	34	12.06	10	12.82
	Civil servant	36	12.77	16	20.51
	Trader	54	19.15	20	25.64
	Unemployed	158	56.03	32	41.03
Marital status	Single	38	13.48	12	15.38
	Married	154	54.60	16	20.51
	Divorce	90	31.91	50	64.10
Level of education	Illiterate	148	52.48	44	56.41
	Primary school	40	14.18	4	5.13
	High school	8	2.84	2	2.56
	Higher education	86	30.5	28	35.9
Pregnancy semester	First	72	25.53	2	2.56
	Second	140	50	36	46.15
	Third	70	24.46	40	51.29
Associated symptoms	Pruritus	74	26.24	52	66.67
	Abundant leucorrhoea	104	36.88	54	69.23
	Dyspareunia	18	6.36	16	20.51
	Dysuria	32	11.35	12	15.38
	Vaginal dryness	8	2.84	2	2.56
Risk factors	Vulvar burning	28	9.84	14	17.94
	HIV patient	6	2.13	2	2.56
	Diabetes	0	0	0	0
	Wearing tight clothes	138	48.94	52	66.67
	Under antibiotics	66	23	40	51.28

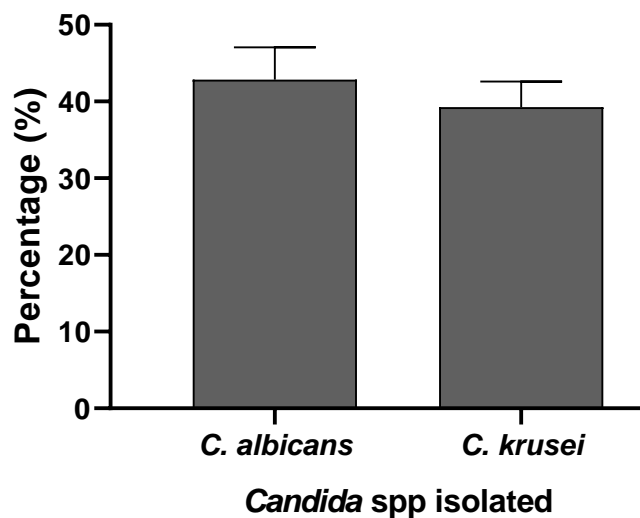


Fig. 1. Distribution of *Candida* species involved in vulvovaginal candidiasis in the studied population

Table 2. Susceptibility patterns of *Candida* species isolated to antifungals

Antifungals	<i>Candida krusei</i> (n=58)			<i>Candida albicans</i> (n=58)		
	R	I	S	R	I	S
Nystatin	22 (100%)	0 (0%)	0 (0%)	36 (62,06%)	10 (17,24%)	612 (20,69%)
Itraconazole	22 (00%)	0 (0%)	0 (0%)	42 (72,41%)	0 (0%)	16 (27,59%)
Miconazole	4 (18,18%)	0 (0%)	18 (81,81%)	8 (13,79%)	0 (0%)	50 (86,20%)
Ketonazole	16 (72, 72%)	4 (18,18%)	2 (9,1%)	12 (20,69%)	14 (24,13%)	32 (55,17%)
Fluconazole	20 (90,90%)	0 (0%)	2 (9,1%)	46 (79,31%)	2 (3,45%)	10 (17,24%)
Clotrimazole	10 (45,45%)	0 (0%)	12 (54,55%)	22 (37,93%)	4 (6,90%)	32 (55,17%)
Econazole	2 (9,1%)	0 (0%)	20 (90,90%)	2 (3,45%)	16 (27,59%)	40 (68,96%)
Amphotericin B	22 (100%)	0 (0%)	0 (0%)	56 (96,55%)	2 (3,45%)	0 (0%)

R= resistance, I = intermediary, S = sensitive

4. DISCUSSION

Vulvovaginal candidiasis (VVC) represents one of the most frequent gynaecological disorders caused by the overgrowth of *Candida* species affecting the genital tract as an opportunistic pathogen whereby *C. albicans* is the most common species [17,18]. This work was a cross-sectional study conducted to ascertain the prevalence of VVC, species identification and antifungal susceptibility of *Candida* species isolates from pregnant women at the Garoua Regional Hospital in North Cameroon. We also investigated the relationship between people's socio-demographic factors (i.e., age groups, occupation, marital status, level of education, pregnancy semester, associated symptoms, and risk factors). This study was important as a large body of evidence has shown that socio-demographic factors have a significant influence on health-related quality of life as it is a multi-dimensional concept that is affected by different variables [19].

In our study, the overall frequency of VVC among pregnant women was 27.65% while the prevalence of vaginal candidiasis reported by different studies was 16.5%, 21.31%, and 19%. A study by Toua et al. indicated a higher VVC prevalence amongst pregnant (55.4%) women compared with non-pregnant women (35.4%) in Maroua (Cameroon) [12]. No estimated prevalence or lifetime incidence of VVC had been reported in North Cameroon. To the best of our knowledge, this is the first study reporting the

prevalence of VVC in the Garoua locality in Cameroon. The prevalence of vaginal candidiasis reported by different studies was 39.76%, 30.70%, and 31.60% [20–22].

VVC was observed mostly among the age group [26-30 years] (35.9%) and least among those of [15-20 years] (2.6%). These findings do not align with the findings of Edrees et al, who reported a higher prevalence of *Candida*-positive mostly among the age group 28–37 years (54.48%) and followed by a group aged between 38-47 years (36.57%) [23]. However, this outcome agreed with Emeribe, who reported peak vaginal infections between ages 20 and 30 years as well as Waikhom et al. who reported it in the range of 20 to 29 years [22,24]. Worldwide, the group with the highest prevalence was women aged 25–34 years [2]. This finding could be explained by high sexual activity among this age group. Advancement in age, on the other hand, reduces the effect of oestrogen hormone in women, which could lead to lower infection rates as women advance in age. Notably, the age group [41-45 years] did not have any individuals testing positive for candidiasis. Most women aged over 46 years have reached menopause and are less or not sexually active. They also have a possible increase in vaginal immunity, as they have decreased levels of oestrogen and corticoids, and thus are resistant to *Candida* infections [25]. Therefore, these factors probably contributed to the absence of VVC occurrence in the age group [41-45 years]. Education level showed that there was a higher percentage of candidiasis cases

among individuals with a high school education compared to those with no schooling, primary school education, or higher education. Previous studies showed that low educational background may correlate with poor personal hygiene and/or low economic status, which may, in turn, make the pregnant women prone to VVC [10]. Most of the VVC-confirmed participants in our study were divorced. This was in accordance to what Bitew and Abebaw [10] reported; the majority of their VVC study participants were divorced or unmarried.

Regarding the pregnancy trimester, the third trimester showed the highest percentage of positive cases, followed by the second trimester. Based on these findings, it appeared that there may be an increasing trend in the percentage of VVC candidiasis cases as the pregnancy progresses. A similar trend was obtained in a previous study [26]. From clinical symptom findings, it appeared that pruritus (itching) and abundant leucorrhoea are the most common symptoms associated with VVC among the study participants. It was previously found that,

From the risk factors of VVC, it appeared that wearing tight clothes and being under antibiotic treatment were the most prominent risk factors associated with VVC among the study participants. Although diabetes mellitus and previous encounters with candidiasis had been found in several studies as potential factors contributing to vaginal colonization during pregnancy [5], it was not the case in this study.

Our study revealed that *C. albicans* was the dominant species in the studied population, comprising the majority of isolates. *Candida albicans* had been recognized as the primary causative agent of VVC [27]. *Candida krusei*, although less prevalent, still represents a significant proportion of the isolates. It has also been found to cause infection in approximately 1% of VVC cases [28]. The presence of both species highlights the importance of identifying and understanding the distribution of different *Candida* species for proper diagnosis and treatment strategies.

Our study has also highlighted differences in antifungal susceptibility patterns between *Candida albicans* and *Candida krusei* isolates. It is important to note that susceptibility patterns can vary geographically and over time, and clinical decision-making should consider local guidelines and patient-specific factors [29,30].

Findings from this work has highlighted the importance of considering antifungal susceptibility patterns when selecting appropriate treatment options for *Candida* infections. It is crucial to consult current clinical guidelines and individual patient factors when making treatment decisions. Additionally, local surveillance of antifungal resistance can help inform therapeutic approaches and guide the development of effective treatment strategies.

5. CONCLUSION

This research aims to investigate the prevalence of vulvovaginal candidiasis in pregnant women attending Garoua Regional Hospital and assess the antifungal susceptibility profiles of *Candida* isolates. The findings from this study will contribute to the understanding of VVC in pregnancy, providing valuable insights into the epidemiology, risk factors, and optimal treatment strategies. Ultimately, this research aims to improve the management and prevention of VVC-related complications in pregnant women, thereby promoting maternal and fetal health at Garoua Regional Hospital and beyond.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

This study was approved by the Institutional Ethics Committee of the University of Garoua Research on Human Health with Reference Number 011/2023/L/ISSTSM/GRA. The purpose of this study was well explained to pregnant women and strict adherence to confidentiality was maintained.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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