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Prevalence and Antifungal Susceptibility of *Candida* spp. Amongst Pregnant Women in Two Hospitals in N'Djamena (Chad)

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ABSTRACT

Background and Objective: The lack of epidemiological data on vulvovaginal candidiasis in pregnant women in Chad is a real public health problem. This study aimed to provide baseline information on the existence of vulvovaginal candidiasis in pregnant women. **Materials and Methods:** A cross-sectional analytical study was conducted at the Mother and Child University Teaching Hospital and the Hospital Our Lady of the Apostles in N'Djamena (Chad). The cervicovaginal swab was taken and used for mycological examination. A total of 168 samples were cultured on Chrom Agar Candida medium for isolation and identification of *Candida* species. Five antifungal agents including amphotericin B (AMB), ketoconazole (KTC), fluconazole (FLU), nystatin (NY), and miconazole (MCZ) were used to test the sensitivity of the field isolates. The dilution technique was used for antifungal susceptibility testing on the Sabouraud medium. The data were analyzed using SPSS 26 software and potential risk factors were evaluated with the administration questionnaire using a binary logistic regression model. Statistical signi cance was measured at $p<0.05$. **Results:** Of the 168 vaginal swabs taken, 79 were positive for *Candida* spp., presenting a prevalence of 47.02% of vulvovaginal candidiasis in pregnant women in Chad with the Hospital Our Lady of the Apostles in N'Djamena and the Mother and Child University Teaching Hospital presenting a prevalence of 89.60 and 11.39%, respectively. Identification of yeast isolates showed that *C. albicans* was the majority species (65.82%), followed by *C. krusei* (27.64%), *Candida* spp. (5.06%), and *C. tropicalis* (1.26%). Risk factors for vulvovaginal candidiasis were primiparous women, multiparous women, and women aged 15-22 years. The antifungal sensitivity of the isolates showed total resistance to Amphotericin B 100% in all species. **Conclusion:** Multispecies vulvovaginal candidiasis was found in pregnant women in this study. Amphotericin B showed resistance to contact with isolated Candida. It is therefore important that amphotericin should not be prescribed for the treatment of vulvovaginal candidiasis.

KEYWORDS

Vulvovaginal candidiasis, pregnancy, fungal disease, *Candida* spp., amphotericin B

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INTRODUCTION

The world's population is beginning to recognize fungal diseases as a significant health problem. An estimated 1 billion individuals are considered infected with fungal infections annually, which causes about 1.5 million deaths[1]. In the United States, the estimated annual cost of diagnosing and treating VVC, including productivity losses and absenteeism, is $1 billion[2]. Vulvovaginal candidiasis (VVC) is an infection of the vulva and/or vagina caused by Candida yeast, characterized by profuse vaginal discharge, dyspareunia, burning during urination, vaginal itching, and unpleasant odors[3]. Vaginitis is the most prevalent complaint in women visiting General practitioner (GP) clinics, with most women expected to experience at least one episode of the condition in their entire lives[4]. After bacterial vaginosis, which is reported at gynecological clinics, vulvovaginal candidiasis (VVC) is the second most common causes of vaginal infections[2]. Pregnant women are the most vulnerable to CVV because of certain risks. In addition, their offspring are also at risk of premature delivery and congenital infections[5].

In Africa, the overall prevalence of CVV has been put at 29.2%[6]. In Cameroon, a studies in Maroua found a prevalence of 55.40% for CVV in pregnant women[7]. Pregnancy is One of the main factors favoring VVC, due in particular to the hormonal changes observed during this period. It creates favorable conditions for the development of Candida yeasts in the vagina and encourages lower genital infections, which are all easier when environmental hygiene is poor[8]. In Chad, vulvovaginal candidiasis is endemic in all 119 districts, meaning that at least one species of Candida are present there. Most of those touched are either migrant nomads or live in rural areas. Regretfully, there are no published data available to the scientific community that illustrates this scenario. The lack of epidemiological data on vulvovaginal candidiasis in pregnant women in Chad are a real public health problem. It is therefore imperative to seek information about the health state of the population of N'Djamena concerning vulvovaginal candidiasis, and the factors that predispose them to the disease. This study was therefore carried out to provide baseline information on the existence of vulvovaginal candidiasis in pregnant women treated at the Mother and Child University Teaching Hospital and the Hospital Our Lady of the Apostles in N'Djamena (Chad) to develop a realistic and adapted control strategy.

MATERIALS AND METHODS

Study site: This study took place over 3 months from March 13 to June 2, 2023. A cross-sectional analytical study was conducted at the Mother and Child University Teaching Hospital and the Hospital Our Lady of the Apostles in N'Djamena (Chad). Both sites are located in the capital of Chad, which is N'Djamena.

Study population and sample size: The study population consisted of pregnant women attending antenatal clinics at the Mother and Child University Teaching Hospital and the Hospital Our Lady of the Apostles in N'Djamena (Chad), who freely consented to take part in the study. Using Bongomin *et al.*[1] formula and the EPI Info software's StatCalc Version 7.2., the sample size was determined. Using the prevalence of 27.65% from a study on Vulvovaginal Candidiasis in Pregnant Women Attending the Garoua Regional Hospital (Cameroon) and Antifungals Susceptibility Profile of Isolates[9], with an 80% power to detect significant associations or differences and a 5% accepted margin of error, the minimal sample size estimate was 350.

Inclusion and exclusion criteria: Included were pregnant women over the age of twenty who attended ANC at these two hospitals and signed an informed consent form. All pregnant women using antibiotics and those who did not live in N'Djamena were not included.

Cervicovaginal and mycological examination and identification: For personal protection, gloves were worn while the patient was positioned in a gynecological posture on the bed. To visualize the cervix for a macroscopic examination that aims to characterize the appearance of the vaginal wall and then evaluate the amount, appearance, color, and odor of leucorrhoea, a sterile disposable speculum was placed into the vagina and the screw tightened. The culture 1and isolation were carried out on CHROMagar™ Candida medium poured into Petri dishes. The technique used was seeding using tight streaks and the incubation temperature was 37°C for 24-48 hrs. When reading the culture media after incubation for 24 hrs, if there is a pure culture, an antifungigram is done according to the germ identified; in the absence of pure culture, purification is done. The antifungigram was carried out using the Sabouraud+Chloramphenicol medium.

Sensitivity to antifungal agents: Five antifungal agents including amphotericin B (AMB), ketoconazole (KTC), fluconazole (FLU), nystatin (NY), and miconazole (MCZ) were bought from Gibco/Invitrogen (Carlsbad, California, USA) and were used to test the sensitivity of isolates.

Parameters studied

Prevalence: The prevalence (P) was calculated using the formula[2]:

$$\text{Prevalence} = \frac{\text{Number of individuals infected}}{\text{Number of individual examined}} \times 100$$

Resistance profile using an antifungigram: The rate of resistance of the *Candida* species detected in the cervicovaginal samples antifungal drugs were evaluated using the antifungigram.

Ethical considerations: The National Committee on Research Ethics for Human Health provided ethical clearance. The Declaration of Helsinki was followed in the completion of this work. All ethical guidelines about doing research with marginalized populations, like inmates, have been adhered to.

There were no external restrictions on the patient's ability to attend the research.

Statistical analysis: Before being analyzed, the data were first entered into Excel and then imported into SPSS version 16.0 for coding. The tables and graphs were created using Microsoft Word and Excel, respectively. Percentages were used to express the data. Only factors with a $p<0.05$ (Chi-square test) were significant.

RESULTS

Table 1 shows the population's distribution according to sociodemographic characteristics. It follows from the analysis of Table 1 that the age of the study participants ranged from 17 to 38 years. Half of the study population was in the 23-30 age bracket. In terms of education, the majority had secondary education (35.7%) and a minority had primary education (7.1%). Of the participants, 63.7% were housewives. As 96.4% of the women were married. In terms of religion, 61.3% of participants were Muslim.

Vaginal swabs were taken from 168 pregnant women attending consultations in the two target health facilities. Of these women, 79 tested positive after mycological examination, giving a prevalence of 47.02% for vulvovaginal candidiasis.

Table 2 shows the prevalence of vulvovaginal candidiasis according to hospital. It appears that out of the 146 women who were admitted to the Hospital Our Lady of the Apostles in N'Djamena and the Mother and Child University teaching hospital, 70 and 9 of them were positive, with a prevalence of 88.60 and 11.39%, respectively.

Table 1: Population distribution based on sociodemographic characteristics

Parameters	Number examined	Percentage
Age		
15-22	46	27.4
23-30	84	50.0
31-38	38	22.6
>38		00.0
Level of education		
Primary	12	7.1
Secondary	60	35.7
Higher	52	31.0
No schooling	44	26.2
Occupation		
Civil servant	14	8.3
Housewife	107	63.7
Shopkeeper	4	2.4
Pupils	20	11.9
Student	23	13.7
Marital status		
Single	2	1.2
Married	162	96.4
Cohabiting	4	2.4
Religion		
Christian	65	38.7
Muslim	103	61.3
Parity		
Primiparous	32	19.0
Multiparous	131	78.0
Nulliparous	5	3.0
Gestational age		
First trimester	46	27.4
Second trimester	69	41.1
Third trimester	53	31.5
Total	168	100.0

Table 2: Prevalence of vulvovaginal candidiasis according to hospital

Site	Number examines	Number positive	Prevalence	p-value
MCUTH	146	70	88.60	0.538
HOLA	22	9	11.39	
Total	168	79	100.0	

MCUTH: Mother and Child University Teaching Hospital and HOLA: Hospital our lady of the apostles

Table 3: Overall prevalence of different *Candida* species

Species	Number	Prevalence	p-value
Candida albicans	52	65.82	0.000
Candida krusei	22	27.84	
Candida tropicalis	1	1.26	
Candida spp.	4	5.06	
Total	79	100.00	

The overall prevalence of different *Candida* species seen in Table 3 shows that *C. albicans* is the most frequently isolated species, accounting for 65.82% of the total yeast isolates, followed by *C. krusei* 27.84%, *C. tropicalis* 1.26%, and *Candida* spp. 5.06%.

Table 4 shows the prevalence of CVV according to socio-demographic data. Table 4 shows the age group with the highest infection rate (48.10%) was 23-30 years old. The age range of 15-22, with a prevalence of 30.37, comes next. Christians were the least affected in terms of religion (34.17). The most infected patients were those with only a secondary level of education (35.44), followed by those with a university level of education (31.65). The prevalence rates for married and individuals cohabiting were 3.79 and 96.20, respectively.

Table 4: Prevalence of CVV according to socio-demographic data

Parameters	Number examined	Number positive	Prevalence	p-value
Age				
15-22	46	24	30.37	0.713
23-30	84	38	48.10	
31-38	38	17	21.51	
Level of education				
Primary	12	7	8.86	0.825
Secondary	60	28	35.44	
University	52	25	31.64	
Illiterates	44	19	24.05	
Occupation				
Civil servant	14	6	7.59	0.974
Housewife	107	49	60.02	
Shopkeeper	4	2	2.52	
Student	20	10	12.65	
Student	23	12	15.18	
Marital status				
Single	2	00	00.00	0.220
Married	162	76	96.20	
Cohabiting	4	3	3.79	
Religion				
Christian	65	27	34.17	0.258
Muslim	103	52	65.82	
Parity				
Primiparous	32	15	18.98	0.054
Multiparous	131	59	74.68	
Nulliparous	5	5	6.32	
Gestational age				
First trimester	46	21	26.58	0.577
Second trimester	69	30	37.79	
Third trimester	53	28	35.44	
Total	168	79	100.00	

Table 5: Prevalence of VVC according to clinical signs

Clinical signs	Number examine	Number positive	Prevalence (%)	p-value
Leucorrhoea	123	58	73.41	0.993
Dysuria	2	1	1.26	
Pelvic pains	16	7	8.86	
Vaginal itching	27	13	16.45	
Total	168	79	100.00	

Table 5 shows the prevalence of VVC according to clinical signs. From this Table 5, 73.41% of the women had leucorrhea, 16.45% had vaginal itching, 8.86% had pelvic pain, and 1.26% had dysuria.

Figure 1 shows the resistance profile of *Candida* spp., to antifungal. It appears from this figure that Amphotericin B was resistant to all species. Fluconazole was 100% resistant to *C. tropicalis*, 72.72% to *Candida krusei*, 36.53% to *C. albicans*, and 25% to *Candida* sp. Nystatin was 50% resistant to *C. albicans*, 22.72% to *C. krusei*, and 5.76% to *C. albicans*. As 13.63% of *Candida krusei* and 11.53% of *C. albicans* were resistant to Ketoconazole. Miconazole was 13.63% resistant to *C. krusei* and 1.92% resistant to *C. albicans*.

Table 6 shows the risk factors associated with vulvovaginal candidiasis. It shows that primiparous women [1.004 (0.666-1.512); 0.985], multiparous women [1.200 (0.844-1.707); 0.332] and women aged 15-22 years [1.165 (0.440-3.081); 0.759] were more likely to develop the disease compared with nulliparous women [0.454 (0.384-0.537); 0.016] and the 23-30 age group [0.919 (0.410-2.059); 0.838].

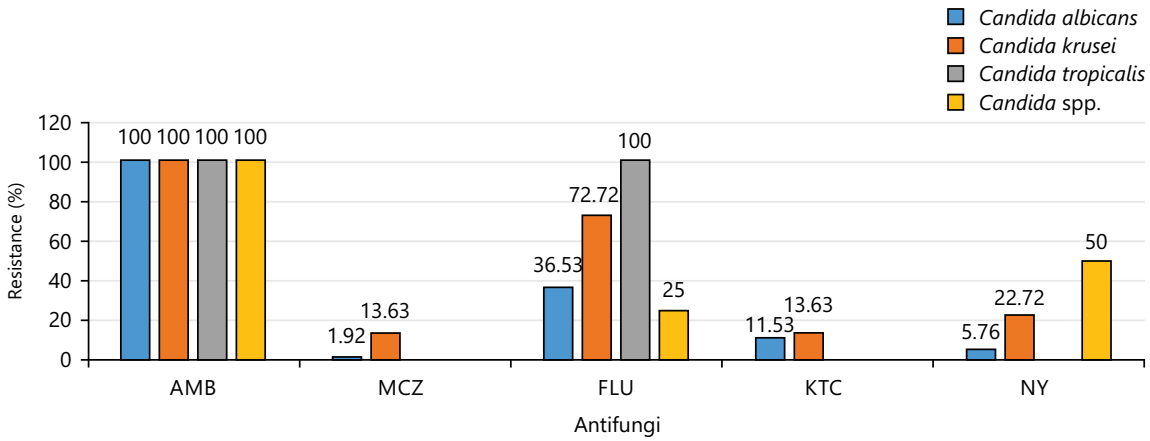

Fig. 1: Resistance profile of *Candida* spp., to antifungals
AMB: Amphotericin B, MCZ: Miconazole, FLU: Fluconazole and KTC: Ketoconazole

Table 6: Risk factors associated with vulvovaginal candidiasis

Risk factors	Number examined	Number positive	Odds ratio (%)	95% confidence interval	p-value
Products used for intimate hygiene					
Water	132	60	0.667	0.039-11.416	0.780
Soap	34	18	0.999	0.055-18.274	0.999
Other	2	1	-	-	-
Parity					
Primiparous	32	15	1.004	0.666-1.512	0.985
Multiparous	131	59	1.200	0.844-1.707	0.332
Nulliparous	5	5	0.454	0.384-0.537	0.016
Gestational age					
First trimester	46	21	0.695	0.305-1.585	0.387
Second trimester	69	30	0.649	0.308-1.366	0.255
Third trimester	53	28	-	-	-
Age					
15-22	46	24	1.165	0.440-3.081	0.759
23-30	84	38	0.919	0.410-2.059	0.838
31-38	38	17	-	-	-
>38	00	00	-	-	-

DISCUSSION

Mycological analysis of cervicovaginal swabs from these patients revealed 79 patients with vulvovaginal candidiasis, representing a prevalence of vulvovaginal candidiasis of 47.02%. This high prevalence is close to that obtained in a systematic review and meta-analysis of the prevalence of VVC in pregnant women in Africa, which was 45.4% in Uganda[5].

Similar results were obtained by Nadia *et al.*[10] were they had a prevalence of vulvovaginal candidiasis (VVC) of 53.07 and 51.33%, respectively, at the Maroua Regional Hospital and the Domayo Catholic Private Integrated Health Centre. This prevalence was much higher than in the present study because the authors were interested exclusively in pregnant women with suspicious vaginal leucorrhoea.

The most predominant *Candida* species in this study were *C. albicans* with a prevalence of 65.82%, followed by *C. krusei* at 27.84%, *C. tropicalis* at 1.26% and *Candida* sp., at 5.06%. These prevalences are close to those obtained in a study in Ethiopia on the prevalence, risk factors and antifungal susceptibility profile of *Candida* species in pregnant women, where the prevalences were 56.3% for *C. albicans*, 21.9% for *C. krusei*, 1% for *C. tropicalis*, and 3.1% for *Candida* spp.[11]. Similarly, a study on the prevalence and antifungal resistance profile of vulvovaginal candidiasis infections among pregnant women in the city of Maroua also identified four species of Candida were isolated with a predominance of *C. albicans* (63.78%),

followed by *C. glabrata* (26.78%), *C. krusei* (7.87%), and *C. tropicalis* (1.57%)[10]. The predominance of *C. albicans* in all these studies is considered due to its dimorphism, in that it creates pseudo filaments to enable it to adhere easily to mucous membranes; the yeast form is the saprophytic form, whereas the filamentous form is the pathogenic form, making this dimorphism is an essential virulence factor[12].

Pregnant women, because of their various hormonal changes, have a greater susceptibility to VVC and many authors report that pregnancy is a factor favoring the occurrence of VVC in women[6,13]. In the present study, women with secondary education were more likely to be affected (35.44%), followed by women with a university level of education (35.44%). Regardless of age, parity or gestational age, all these women were likely to develop the disease. Primiparous and multiparous women were more likely to develop the disease than nulliparous women and women in the age group 23-30. These risk factors are similar to those obtained in Nigeria, where primiparous and multiparous women were significant risk factor[14]. The high rate among primiparous women may be explained by the fact that they were not in contact with midwives during early prenatal visits. The age group 15-22 years was a risk factor, with an Odds ratio of (1.165). This risk factor was also found in a study on vaginal infections in southern Poland, where the authors showed that candidiasis predominated in women, with 32.3 and 25%, respectively, in the age groups 15-24 and 25-35[15]. These age groups correspond to the peak of sexual activity, suggesting the possibility of Candida transmission by sexual means[16]. Vaginal colonization by Candida varies according to the age of pregnancy. According to the literature, a high frequency of VVC is observed in the last trimester of pregnancy due to the deposition of glycogen, which favors the development of Candida. However, this study found that VVC was more frequent in the second trimester (37.97%) than in the third and first trimester. This is in agreement with the results of a recent study in Tunis in 2010[17]. This high frequency in the second trimester in This study is due to the high participation of women who were in their second trimester.

Antifungal susceptibility testing in this study revealed that Amphotericin B was 100% resistant in all species. This resistance of all species to Amphotericin B was in agreement with that were obtained in a study conducted in Cameroon on the species distribution and antifungal susceptibility profile of *Candida* spp., isolated from the urine of patients hospitalized at the Dschang District Hospital[18]. This resistance of isolates to Amphotericin B is contrary to the results obtained in a study of the microbiological profile of lower genital infections in women of childbearing age in Burkina Faso, in which Amphotericin B was 97.8% sensitive[19]. This difference may be explained by the geographical location of the regions. Fluconazole was 100% resistant to *C. tropicalis*. It has been reported that the non-albicans species, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* showed higher rates of resistance to Fluconazole than *C. albicans* in a study in Jordan on the isolation and characterization of *Candida* spp., in Jordanian cancer patients presented Nystatin with 50% resistance to *Candida* sp., 22.72% to *C. krusei* and 5.76% to *C. albicans*[20]. This resistance is contrary to that obtained in a study on species distribution and sensitivity of Candida isolates from patients with vulvovaginal candidiasis in southern China, in which nystatin was more sensitive to Candida isolates[21]. The resistance of the isolates to fluconazole and nystatin is considered due to self-medication and the low cost of these drugs, which are accessible to everyone in Chad. It should be noted that in this study, 13.63% of *C. krusei* and 11.53% of *C. albicans* were resistant to Ketoconazole. This resistance of the isolates to Ketoconazole is very similar to the results obtained in Bafoussam, Cameroon, for vulvovaginal candidiasis in pregnant women, where resistance to Ketoconazole was 68.83%[14]. Miconazole was 13.63% resistant to *C. krusei* and 1.92% resistant to *C. albicans*. The low rate of resistance of miconazole indicates that it was highly sensitive, making it the antifungal agent best indicated for the treatment of CVV in these pregnant women. It was relatively active against 100% sensitive *C. tropicalis*, 78.84 and 19.23%, respectively, sensitive and intermediate to *C. albicans*, 75 and 25%, respectively, sensitive and intermediate to *Candida* sp. This sensitivity of isolates to Miconazole is similar to that was obtained in Iran where *C. albicans* and *C. glabrata* were all sensitive to Miconazole[15].

Ketoconazole was the most effective drug against *Candida* spp. and *C. tropicalis* had no resistant strains Amphotericin B was resistant in all *Candida* species identified. Isolates of *C. albicans*, *C. krusei*, *C. tropicalis*, and *Candida* spp., showed susceptibilities different with respect to Miconazole and Ketoconazole, however, all the isolates obtained have shown resistance to Amphotericin B.

Doctors and midwives should not prescribe Amphotericin B for the treatment of VVC. Pregnant women should practice better general and genital hygiene in particular to reduce the incidence of CVV. As a limitation of this study, the delay in obtaining authorization from health structures did not make it possible to reach a large number of pregnant women. Furthermore, we were unable to carry out the molecular identification of the isolates obtained.

CONCLUSION

According to this study, amphotericin showed complete resistance. This is a medication that is frequently taken. Thus, ongoing treatment surveillance of VVC in pregnant women is of paramount importance. However, it will be important to carry out molecular identification of the isolates obtained during this study and to carry out a comparative study with non-pregnant women and pregnant women for a better diagnosis of vulvovaginal candidiasis in all women.

SIGNIFICANCE STATEMENT

The lack of epidemiological data on vulvovaginal candidiasis in pregnant women in Chad is a real public health problem. This study was therefore carried out to provide baseline information on the existence of vulvovaginal candidiasis in pregnant women treated at the Mother and Child University Teaching Hospital and the Hospital Our Lady of the Apostles in N'Djamena (Chad) to develop a realistic and adapted control strategy. Identification of yeast isolates showed that *C. albicans* was the majority species followed by *C. krusei*, *Candida* spp., and *C. tropicalis*. As further studies, it will be important to carry out the molecular identification of the isolates obtained during this study.

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