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Original article

Prevalence of vulvovaginal candidiasis among pregnant women in Akwa Ibom State, Nigeria

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ABSTRACT

Background: Vulvovaginal candidiasis is ranked next to bacterial vaginitis as the common and leading vaginal infection affecting greater number of women of childbearing age across the world. Hence, the objective of this study was to investigate the prevalence of vulvovaginal candidiasis among pregnant women in Akwa Ibom State, Nigeria. **Methods:** High vaginal swab (HVS) samples were collected from 360 pregnant women selected from public health facilities in Akwa Ibom State and tested for the presence of *Candida* species using standard microbiological methods. **Results:** The result revealed an overall prevalence of 87(24.16%), significantly (P = 0.05) associated with the socio-demographic characteristics of the pregnant women. Higher prevalence was obtained among pregnant women who were aged 26-30 years 24(27.58%), married 33(37.93%), illiterate 25(22.98%), engaged in businesses 22(25.28%), in their third trimester 42(48.27%), depended on junk nutrition 33(37.93%), had itchy signs/symptoms 39(44.82%), and leaving in urban residence 38(43.67%).The *Candida* species encountered were *C. albicans*68(78.2%), *C. tropicalis*12(13.8%), *C. krusei*6(6.9%), and *C. parapsilosis*1(1.1%). These were 100% susceptible to fluconazole, clotrimidazole, and nystatin, except *C. parapsilosis* with few resistant strains to fluconazole. Molecular analysis revealed resistance encoded genes CDR1 and CDR2 in the fluconazole resistant *C. parapsilosis*. **Conclusion:** The prevalence rate of VVC among the pregnant women in Akwa Ibom State is relatively high. Routine medical services, proper diagnosis before treatment, and avoidance of antifungal abuse are recommended. Further studies to include larger sample size and the susceptibility profile of the *Candida* sp. to other commonly used anti-fungal drugs are suggested.

Introduction

Vulvovaginal candidiasis (VVC) has been regarded as the second leading and common vaginal infection in about 70-75% of women of childbearing age [1, 2]. About 80-90% of vulvovaginal candidiasis is caused by *Candida albicans* while 10-20% of the cases may be caused by non-*albicans* species, including *C. glabrata*, *C. tropicalis, C. krusei and C. parapsilosis* [3, 4]. However, about 75% of women generally harbor these fungi without it causing harm to them [5].

Pregnant women are most susceptible to volvovaginal candidiasis and the infection can be acute, chronic, superficial or deep, with broad

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clinical spectrum [6]. Several risk factors can make vaginal conditions conducive for the growth of Candida species or make asymptomatic conditions symptomatic. These include the use of oral or hormonal contraceptives, trauma or damage to the vaginal mucosa and hygiene of the female genitalia [7-9]. Increased estrogen levels during pregnancy leads to the production of more glycogen in the vagina and encourages the proliferation of yeast cells on the wall of the vagina [6]. However, any physiological changes affecting the beneficial bacteria in the vagina can cause altered acidity of the vagina to about 5.0 - 6.5, encouraging the establishment of pathogenic organisms including *Candida* species [10-11].

Moreover, during normal pregnancy, candidiasis is frequently encountered without significant risk against the foetus [5]. Nevertheless, pregnancy may be negatively affected by volvovaginal candidiasis and if untreated can lead to chorioamnionitis with subsequent abortion and prematurity in pregnant women, congenital infection of the neonate and pelvic inflammatory disease (PID) resulting in infertility in non-pregnant women [5]. However, commonly experienced symptoms of Volvovaginal candidiasis include pain in the vaginal area, irritation, burning sensation, dyspareunia, and dysuria, which is preceded by acute pruritus, vaginal discharge, itching, as well as blisters and uncomfortable state during sex [12-14]. These detrimental impacts of *Candida* infection on the health of women, especially pregnant women, should be of great concern. Hence, the objective of this study was to investigate the prevalence of vulvovaginal candidiasis among pregnant women in AkwaIbom State, Nigeria.

Materials and Methods

Study design and sample size

A cross-sectional survey design was used in this investigation to determine the prevalence of volvovaginal candidiasis in the population of all the pregnant women on antenatal visit to public health facilities in Akwa Ibom State (latitudes 4º33′ and 5º33′ N and longitudes 7º35′ and 8º25′ E) between 17/05/2023 to 16/05/2024. A sample size of 360 pregnant women selected from six major public hospitals was used in the study and was calculated based on the prevalence rate of 37.4% established in a previous study [15] (equation 1).

$$n = \frac{z^2pq}{E^2} \qquad (1)$$

Where: n = minimum sample size for a statistically significant survey, z = standard normal deviate usually 1.96 at 95% confidence level, p = prevalence rate from previous study (37.4% for this study), E = Allowable error, 5% at 95% confidence interval, q = 1 – p

Ethical approval and consent

Ethical approval was obtained from the Akwa Ibom State Health Research Ethics Committee (AKSHREC) of the Ministry of Health to collect samples from public health facilities in the study areas (Appendix I). Selected hospitals were visited for further permission and to seek cooperation from the health workers. Participants were fully informed about the entire procedure and significance of the study; oral and written consent were obtained from all volunteers.

Inclusion and exclusion criteria

The investigation included all the pregnant women that had registered for antenatal services in the selected hospitals during the period of this study. The participants were divided into two groups. Group I consisted of participants with or without symptoms of vaginal candidiasis. Group II consisted of participants with symptoms of itching due to other illnesses. However, the study excluded participants from non-antenatal care unit, pregnant women under antifungal medication, and those without informed consent.

Recruitment of participants and data collection

A total of 360 eligible participants (60 per hospital) were randomly selected using hat-and-draw method to avoid bias. Folded papers with ‘yes’ or ‘no’ response written on them were presented to eligible participants to pick and only those who picked ‘yes’ were recruited for the study. Validated and approved structured interview-based questionnaire were administered to the selected participants. They were briefed on how to fill the questionnaires, which were retrieved immediately after completion for analysis.

Limitations of the study

This study was restricted to only six public hospitals and few participants. Antifungal disks were not readily available and as such, only three were used. Whether some of the information provided in the questionnaires by the participants was true or false could not be verified by authors.

Sample collection

After detailed explanation of the sampling procedure and willful acceptance by the included participants, duplicates high vaginal swab (HVS) samples were collected, with the help of health workers, from each participant using sterile cotton wool swap sticks [6, 11]. The participant were made to lie in a lithotomic position, the labia of the vagina opened using a sterile disposable speculum, and a sterile swab stick inserted into the high vaginal region to the posterior fornix and gently rotated two to three circles round the vaginal walls to soak fluid unto the sterile cotton. The swab sticks were aseptically removed and transported immediately in Amies transport medium on icepack container to the Microbiology Laboratory, AkwaIbom State University for analysis. Samples were properly labeled using generated ID numbers for participants.

Examination of samples

Whiff test and direct wet mount: Whiff test was carried out to identify participants with vulvoviginal candidiasis [6]. One set of swab stick from each participant was rolled on a clean slide, a drop of 10% KOH added to it, and held close to the nose. The samples of which amine-fishy smell was detected were considered vulvoviginal candidiasis positive. The slides were covered with cover slips and viewed under the microscope using x10 and x40 objective lenses with the diaphragm closed to give good contrast for the presence of yeast buds and pseudo-hyphae [5].

Isolation and identification of *Candida* species: Sampleswere inoculated on Sabouraud dextrose agar (SDA) medium supplemented with gentamicin (0.05mg/ml) by streaking technique and incubated at 37ºC for 72h. Colony characteristics of the isolates were recoded, the isolates purified, Gram's staining, and biochemical tests including sugar assimilation, fermentation, urease activity, were performed [16]. Germ tube test was carried out as confirmatory test using standard procedure [17]. Exactly 500 µl (0.5ml) of human serum will be pipette into test tubes and a loopful of 24h pure yeast colony inoculated using sterile wire loop. The tubes were incubated at 37º C for 2 - 3 h, after which time a drop of the serum-yeast broth was transferred to a clean glass slide, covered with a cover slip and viewed under the microscope with x10 objective for the formation of tube-like outgrowths from the cells.

Antifungal susceptibility test

Antifungal susceptibility of the *Candida* isolates to nystatin (100 µg), fluconazole (25 µg) and voriconazole (10 µg) was performed on Mueller-Hinton (MH) agar (Biotech Lab Ltd. UK) supplemented with 2% glucose and methylene blue (0.5µg/ml) using disk diffusion [16, 18]. Exactly 0.1 ml aliquot suspension of test isolates with turbidity equivalent to 0.5 McFarland standards was inoculated in duplicate on sterile Meuller-Hinton agar plates and aseptically spread using sterile swab stick to ensure even distribution and allowed to dry for 15 minutes at room temperature. Then, the antifungal discs were placed aseptically on each at some distance away from each other and the edge of the petri dishes using sterile forceps. The plates were allowed to stand for 15 minutes at room temperature for effective diffusion before incubation at 37ºC for 24h. After incubation, inhibition zone formed on the plates were measured and expressed in millimeter (mm) to determine the sensitivity of the isolates. Zones were recorded as sensitive (≥19, ≥17, and ≥25) or resistant (≤14, ≤13, and ≤12) for fluconazole, clotrimazole, and nystatin respectively [5].

Molecular Characterization of most resistant *Candida* isolates

DNA extraction was carried out using Zymo Research Quick-DNA Fungal and Bacteria Kit and procedure and quantified using a spectrophotometer (Gene Quant Pro). Amplification of the extracted genomic DNA was performed using PCR master mix composed of amplification buffer, MgCl, DmSO, DNTPs, Taq polymerase, and ITS1 F 5'TCCGTAGGTGAACCTGCGG3' and ITS4 R 5'TCCTCCGCTTATTGATATGC3' as forward and reverse primers respectively. Amplicons were separated on 1.5% agarose gel electrophoresis for 20 minutes at 120 V and viewed EV trans-illuminator, using 50 bp DNA ladder as molecular weight standard. The PCR products obtained were purified and sequenced in forward and reverse direction to determine the sequence of nucleotides for the ITS gene in each isolate. Sanger sequencing was carried out on ABI Prism 3130X1 Genetic Analyzer (Applied Biosystems) and BigDye terminator V3.1 kit (Applied Biosystems Inc.). The raw sequences obtained were edited by trimming both ends and removing bad chromatograms. Nucleotide sequences were aligned by ClustalW. BLASTn algorithm was conducted on NCBI with aligned sequences for each isolate to determine its sequence

similarity and identity, and the phylogenetic analysis performed using MEGA11 [19].

Statistical analysis

Data generated in this study were analyzed using descriptive statistics and Chi-square test of independence on IBM SPSS statistics v.23 at 5% significant level to determine the relationships between some socio-demographic/clinical data and prevalence rates of vulvovaginal candidiasis in AkwaIbom State.

Results

Prevalence of volvovaginal candidiasis among study population

Figure 1 shows the prevalence of volvovaginal candidiasis among the population of pregnant women in AkwaIbom State. Out of the 360 pregnant women, 273 (75.83%) and 87 (24.16%) were volvovaginal negative and positive respectively.

Prevalence of volvovaginal candidiasis according to socio-demographic characteristics

Table 1 shows the distribution of volvovaginal candidiasis among the study population according to socio-demographic characteristics. The prevalence was significantly ($P < 0.05$) higher among pregnant women of age group 26-30 years (27.58%), the married pregnant women (37.93%), pregnant women with no basic educational background (28.73%), those engaged in business (25.28%), those on junk food/drinks (37.93%), the pregnant women from urban residence (43.67%).

Prevalence of volvovaginal candidiasis according to gestation period

There was significant ($P = 0.006$) difference in the prevalence of volvovaginal candidiasis among the pregnant women at different gestational periods (Figure 2). This was highest (48.27%) among the pregnant women under 42 weeks of pregnancy and lowest (22.98%) among those under 13 weeks of pregnancy.

Prevalence of volvovaginal candidiasis according to signs/symptoms

Figure 3 shows the prevalence of volvovaginal candidiasis among the pregnant women based on signs/symptoms. Pregnant women with itching had the highest prevalence (44.82%) while those with vaginal discharge had the least (18.39%). However, there was no significant ($P = 0.05$) difference between the development of signs/symptoms and the prevalence of volvovaginal candidiasis among the pregnant women.

Frequency of distribution of *candida* isolates

A total of 87 *Candida* isolates were encountered and out of this, 68 (78.2%) were *Candida albicans*, 12 (13.8%) *Candida tropicalis*, 6 (6.9%) *Candida krusei*, and 1 (1.1%) *Candida parapsilosis*(Figure 4).

Antifungal susceptibility profile and resistance gene of *Candida* isolates

Table 2 shows the result of the antifungal susceptibility profiles of the *Candida* species isolated among the pregnant women with volvovaginal candidiasis to fluconazole, clotrimazole and nystatin. *Candida albicans* were 100% susceptible to Fluconazole, Clotrimazole and Nystatin with large inhibition zones ranged 19 – 21 mm, 19 – 22 mm, and 26 – 28 mm, respectively. However, among the non-*albicans*species, 84.2% and 15.8% were sensitive and resistant to fluconazole respectively, while 100% were sensitive to both Clotrimazole and nystatin.

Molecular analysis of the fluconazole resistant *candida* isolates yielded amplicons of 450bp specific for ITS1 and ITS4 genes region and 50bp specific for CAN1 gene region (Figure 5). The multiple sequence alignment of the isolates tested shows identical nucleotide in the ITS1 and ITS4 gene region (Figure 6). The blast and phylogenetic analysis of the aligned nucleotide sequences of the isolates matched 100% with *Candida parapsilosis* strain ER87 (Figure 7).

Table 1. Prevalence of volvovaginal candidiasis among pregnant women in AkwaIbom State according to socio-demographic characteristics.

Socio-demographic characteristics	Total number of respondent	Number positive	Percentage positive	Chi-square value	*P*-value
Age group (year)					
15-20	68	19	21.83		
21-25	81	20	22.98		
26-30	78	24	27.58	14.04	*0.0171
31-35	60	13	14.94		
36-40	54	13	14.94		
≥41	19	10	11.49		
Marital status					
Single	73	22	25.28		
Married	127	33	37.93		
Divorced	44	04	4.59	3.422	*0.027
Widow	39	09	10.34		
Co-habiting	77	19	21.83		
Educational status					
No education	72	25	28.73		
Primary	87	20	22.98		
Secondary	82	16	18.39	7.04	*0.0383
Nomadic	61	17	19.54		
Tertiary	58	09	10.34		
Occupation					
Housewife	53	21	24.13		
Farming	38	05	5.74		
Trading	49	10	11.49	15.76	*0.0107
Business	104	22	25.28		
Civil servant	83	12	13.79		
Unemployed	33	17	19.54		
Nutrition					
Balanced diet	94	22	25.28		
Carbohydrates	80	18	20.68	18.64	*0.0048
Veggies/fruits	74	14	16.09		
Junk foods/drinks	112	33	37.93		
Residence					
Urban	151	38	43.67		
Rural	137	26	29.88	6.328	*0.024
Semi-urban	72	23	26.43		

Table 2. Antifungal susceptibility profiles of the *Candida* isolates.

Antifungal drug	*Candidaalbicans*(n = 68)		Non-albicans (n = 19)		Inhibition zone (mm)	
	S	R	S	R	S	R
Fluconazole	68 (100%)	0.00	16 (84.2%)	3 (15.8%)	≥ 19	≤ 14
Clotrimazole	68(100%)	0.00	19 (100%)	0.00	≥ 17	≤ 13
Nystatin	68(100%)	0.00	19 (100%)	0.00	≥ 25	≤ 12

R = Resistance, S = Sensitive, % = Percentage, mm = millimeter

Figure 1. Prevalence of volvovaginal candidiasis among the population of pregnant women in AkwaIbom.

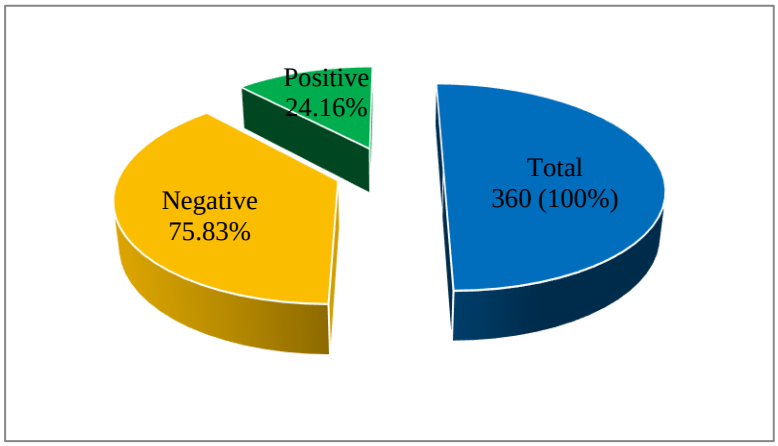


Figure 2. Prevalence of volvovaginal candidiasis among pregnant women in AkwaIbom State according gestation period.

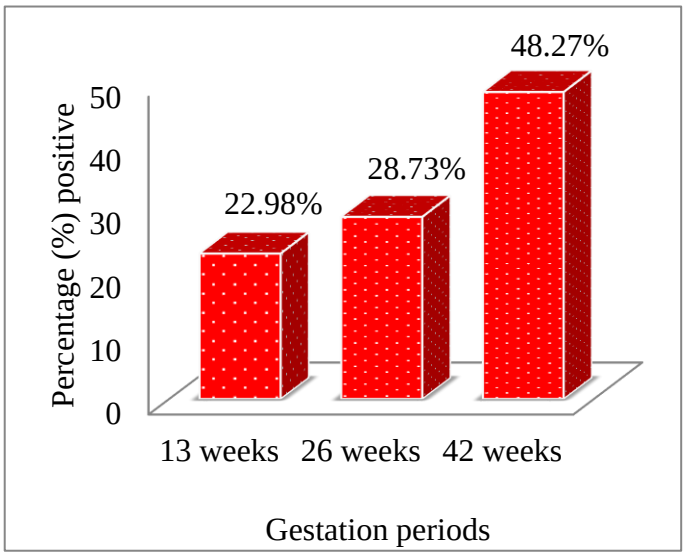


Figure 3. Prevalence of volvovaginal candidiasis among pregnant women in Akwa Ibom State according to sign/symptoms.

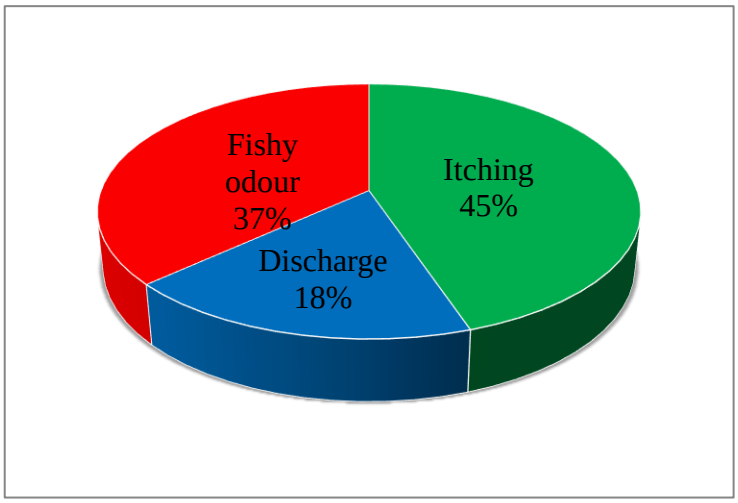


Figure 4. Distribution of *Candida* species among volvovaginal candidiasis positive pregnant women in Akwa Ibom State.

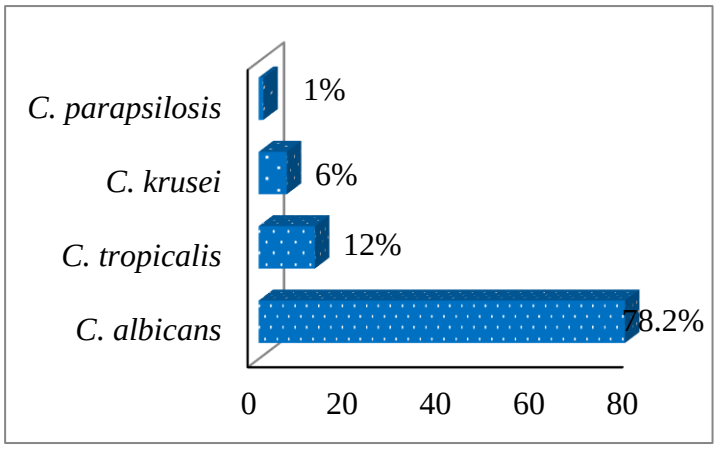


Figure 5. PCR amplification of the CDR 1 gene and CDR2 genes, run against a 400bp DNA ladder. M = Molecular weight standard (DNA ladder); 1 and 2 = PCR product from fungi ITS primers.

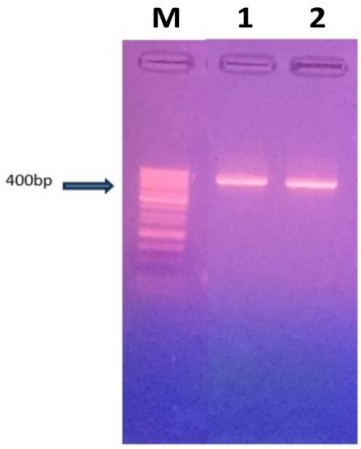


Figure 6. Multiple sequence alignment of the isolates which shows that they have exactly the same nucleotide in the CDR1 and CDR2 gene region.

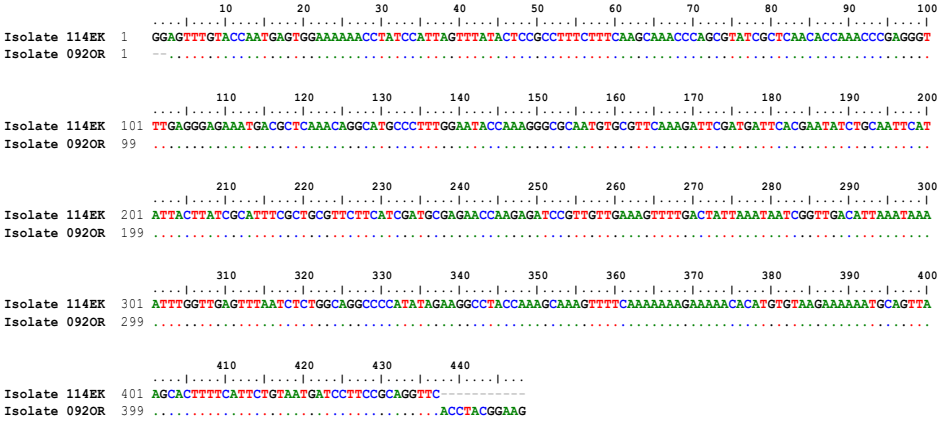
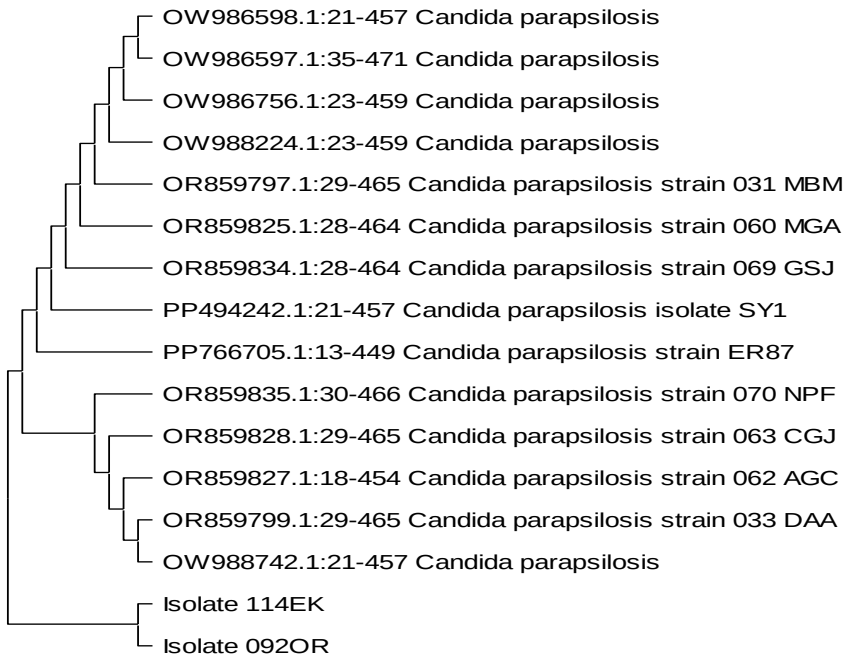


Figure 7. Phylogenetic analysis of the nucleotide sequences isolates 114EK and 092OR.



Discussion

Investigation on the prevalence of vulvovaginal candidiasis among pregnant women selected across six different hospitals in Akwa Ibom State was undertaken and the result revealed overall prevalence rate of 24.16%. Several previous studies had reported similar prevalence rate of volvovaginal candidiasis in Akwa Ibom Sate and other locations across Nigeria [11, 17, 20]. However, this is lower compared to the prevalence rate reported in other studies [6, 21-22]. The relatively low prevalence rate in Akwa Ibom State is likely due to good personal hygiene and normal levels of estrogens among the pregnant women [17, 23].

The prevalence rate is significantly ($P < 0.05$) associated with the socio-demographic characteristics of the participants. Highest prevalence was observed among the pregnant women of age group 26-30 years and lowest among the pregnant women of age above 40 years. This result agrees with similar studies on the prevalence of vulvovaginal candidiasis in pregnant women attending antenatal clinic in Abak L.G.A, Akwa Ibom State, South-South Nigeria and other locations within and outside Nigeria [5, 11, 17]. The high prevalence of volvovaginal candidiasis among the pregnant women of age group 26-30 may be due to high sexual activity, use of contraceptives especially the emergency pills to prevent pregnancy, and abuse of antibiotics/antifungal drugs for the treatment of such infections, which may cause reduced immunity to infection [17, 23]. The prevalence was higher among the pregnant women who were married, had no basic educational background, engaged in businesses, feed on junk food/drinks, and leaved in urban residence. These findings correlate with similar studies [5, 11, 24]. However, the findings contrast with similar study which reported highest prevalence rate of volvovaginal candidiasis among the pregnant women who were unemployed [6, 16]. Low educational background may correlate with poor personal hygiene and low economic status, which may in turn, make the pregnant women prone to volvovaginal candidiasis [24]. The role of dietary habits in volvovaginal candidiasis has been suggested as a risk factor because of the change in Candida virulence in response to the increased available sugar substrates [25]. Altayyar *et al*. [26] had mentioned poor dietary habit as a cause of higher prevalence of volvovaginal candidiasis among pregnant women and may be associated with elevated intake of milk, yogurt, cottage cheese and artificial sweeteners.

The percentage of positive cases of volvovaginal candidiasis among the respondents was significantly ($P = 0.006$) related with gestation periods. The rate obtained was higher among the pregnant women under 42 weeks gestation period than those under 26 and 13 weeks. This agrees with

Waikhom *et al.* [5] which had reported higher prevalence among pregnant women in their trimester. However, studies had reported higher prevalence during second trimester [6, 8, 11].

There was no significant (P = 0.05) association between signs/symptoms and the prevalence of volvovaginal candidiasis among the pregnant women. This is in contrast with the reports by Yadav and Prakash [6] that there exist significant association between the prevalence of volvovaginal candidiasis and the development of signs/symptoms. However, in this study, pregnant women with itching showed relatively higher prevalence, followed by those with fishy odour, while the least prevalence was obtained among the pregnant women who had vaginal discharge. This result again is in contrast with that which was obtained by Christopher *et al.* [11] who had reported higher positive cases among the pregnant women who had vaginal discharge, followed by those with itching and burning symptoms.

Among the *Candida* species identified, *Candida albicans* was most prevalent. Yadav and Prakash [6] had also reported similar findings. However, according to literature, about 80 - 90% of vulvovaginal candidiasis is caused by *Candida albicans*, with only about 10 - 20% cases attributed to non-*albicans* species [17]. The hormonal environment of the vagina during pregnancy enhances *Candida albicans* colonization and serves as risk factor [6].

Candida albicans were most susceptible to Fluconazole, Clotrimazole and Nystatin, with high inhibition zones. However, among the non-*albicans* species, higher percentage was sensitive to both Clotrimazole and nystatin while lower percentage was resistant to fluconazole. Studies have reported similar results [5, 27]. Previous reports suggested that yeasts are universally susceptible to nystatin [5]. The resistant encoding genes in the resistant *Candid* Fluconazole resistance can occur through different mechanisms involving mutations in the drug target enzyme and sterol 14a-demethylase (14DM), alterations in sterol biosynthesis, increased expression of the ERG11 gene, as well as overexpression of genes coding membrane transport proteins of the ABC transporter (CDR1/CDR2) or the major facilitator (MDR1) superfamilies (29). Previous studies also illustrated that developing efflux pumps is the most frequent mechanism for azole resistance in Candida species (Sanglard *et al.*, 1997). Efflux pumps coded by two carrier gene families include CDR-1 and CDR-2 genes belonging to the ATP-binding cassette superfamily, as well as MDR-1 genes from the major facilitator superfamily (28). *C. parapsilosis* were identified as CDR1 and CDR2.

Conclusion and recommendations

The prevalence rate of volvovaginal candidiasis obtained in this study among the selected pregnant women attending antenatal facilities in Akwa ibom State is relatively high and has significant association with the socio-demographic characteristics of the pregnant women. Among the *Candida* species identified, *Candida albicans* was the most frequently encountered species and cause over 78.2% of VVC among the pregnant women. All the strains of the *Candida* species, except *C. parapsilosis* with few strains resistant to fluconazole,were 100% susceptible to the three antifungal (fluconazole, clotrimidazole, and nystatin) drugs tested. The fluconazole resistant profile of the *C. parapsilosis* is due to the presence of resistance encoded genes CDR1 and CDR2. We recommend routine medical check-up and antenatal care services for pregnant women and treatment of suspected VVC using nystatin and clotrimidazole or combination with fluconazole after a proper anti-fungal test.

Conflict of interest: The authors declare no conflict of interest

Data availability: Data are available upon request from the corresponding author.

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Authors contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Peace S. Icha], [Maria E. Bassey] and [Etanguno E. Owowo]. Samples collection was provided by [Peace S. Icha], [Idorenyin O. Jacob] and [Emem I. Ntekpere]. Statistical analysis was performed by [Idorenyin O. Jacob], [Maria E. Bassey] and [Etanguno E. Owowo]. The first draft of the manuscript was written by [Peace S. Icha] [Maria E. Bassey] and [Etanguno E. Owowo] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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