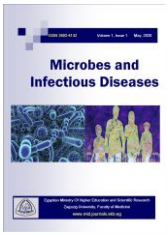
Microbes and Infectious Diseases 2023; 4(4): 1435-1445



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Microbiological and molecular investigation of *Candida* spp infection among women accessing antenatal care at Prince Abubakar Audu University Teaching Hospital Anyigba, North-Central Nigeria

Martin-Luther Oseni Okolo [1], Adebayo Zainab Alaba [1], Sunday Ocholi Samson [1,2], Cornelius Arome Omatola*[1], Idache Benjamin Mudi [3], Julius Akor Omatola [4], Ugbane Eleojo Okolo [5]

1- *Department of Microbiology, Prince Abubakar Audu University, Anyigba, Nigeria.*
2- *Faculty of Chemistry, Department of Biochemistry, Molecular Biology, and Biotechnology, Wroclaw University of Science and Technology, 50-370 Wrocław, Wybrzeże Wyspiańskiego 29, Poland.*
3- *Department of Human Kinetics, Prince Abubakar Audu University, Anyigba, Nigeria.*
4- *Department of statistics, Kogi State Polytechnic, Lokoja, Nigeria.*
5- *Federal Medical Center, Lokoja, Nigeria.*

ARTICLE INFO

Article history:
Received 15 January 2023
Received in revised form 14 February 2023
Accepted 17 February 2023

Keywords:
Pregnancy
Candida spp.
Prevalence
Infant mortality
Nigeria

ABSTRACT

Background: *Candida* is an opportunistic pathogen that is common in humans, especially pregnant women. The fungus is a frequent cause of oral candidiasis, candidemia, cutaneous candidiasis, systemic infections, and vaginitis with high mortality and morbidity rates globally. Although several studies have been conducted on vaginal candidiasis, there is currently no documented report of the infection in the study area. Therefore, the current study was designed to assess the prevalence, characterize *Candida* species (*Candida* spp.) with a molecular approach, and further assess the predisposing risk factors in pregnant women attending antenatal clinics in Anyigba, Kogi State, Nigeria. **Method:** High vaginal swab (HVS) samples were collected from 50 pregnant women between the month's May to July 2022. The sample was cultured using Sabouraud dextrose agar (SDA) and cultured, identification was carried out with morphological characteristics, germ tube test was used for further identification, and their DNA was extracted for molecular analysis. **Result:** Out of the 50 pregnant women sampled, 36 (72%) tested positive for *Candida* spp. Further identification using a germ tube test was carried out in which 12(33.33%) tested positive for *Candida albicans*. The age group 26-35 years had the highest prevalence rate of 16(44.44%) while 36-45 recorded the least prevalence rate of 6(16.66%). The result also showed that women in their third trimester recorded the highest prevalence rate of 22(61.11%). In terms of the effect of the level of education, there was a high prevalence at the primary level 16 (44.44%). Based on their occupation, traders recorded the highest prevalence rate of 20(55.55%) while civil servants had the least 6(16.66%). However, Internal Transcribed Spacer (ITS) gene extracted for molecular analysis revealed *Candida albicans* (CBS:6362; 8.33%, IMAN-25; 16.66%, IMAN-225; 8.3% and IMAN-22, 11.11%), *Candida akabanensis*; 27.77%, *Pichia kudriazevii* (*Candida krusei*) 16.66% and *Candida glabrata* (11.11%). **Conclusion:** Based on the observations made, there is a need for routine surveillance and education of pregnant women on *Candida* spp. infection as a holistic procedure in antenatal care. In addition, the use of molecular characterization will enhance the proper identification of *Candida* spp. in hospital settings.

DOI: 10.21608/MID.2023.187584.1452
* *Corresponding author:* Cornelius Arome Omatola
E-mail address: omatolac@gmail.com

Introduction

Vaginal candidiasis is the most frequent and global form of fungal infection that affects the female genital tract [1, 2]. They are majorly responsible for vaginitis with symptoms like itching, vaginal pruritis, dyspareunia, thick white vaginal discharge, and inflammation of the vulva [3]. Vaginal candidiasis can be categorized into complicated and uncomplicated forms depending on the clinical appearance and the response to antifungal therapy. Complicated vaginal candidiasis is common among pregnant women and immunodeficiency individuals and they are generally caused by non-*albicans Candida* species, whereas uncomplicated vaginal Candidiasis is often mostly instigated by *Candida albicans* with mild to moderate symptoms [1,3].

Among the several pathogenic *Candida* species that are responsible for human infection include, *Candida krusei, Candida kyfe, Candida glabrata, Candida dubliniensis, Candida albicans, Candida tropicalis, Candida parapsilosis,* and *Candida guillermondii* [4]. Previous research showed that females at their reproductive age experience at least one episode of vaginal candidiasis in their entire life [5]. Factors such as HIV infection, pregnancy (multigravida and the stage of pregnancy like 3rd trimester), diabetes mellitus, broad-spectrum antibiotics, and the use of contraceptives could elevate vaginal candidiasis development in patients [6,7]. The outcomes of vaginal candidiasis could be more severe in pregnant women as the colonization by *Candida* could result in life-threatening, psychological imbalance and pain [8]. It could also result in a high infant mortality rate due to fetal contamination associated with invasive neonatal candidiasis. Approximately 65% of neonatal colonization in the first week of life attributed to the vertical transmission had been observed [9]. It is important to address the fact that the occurrence of candidiasis infection is more in pregnant women than in non-pregnant women because of some physiological changes (for instance, a significant increase in reproductive hormones like estrogen and progesterone) in pregnant women that encourage the growth and proliferation of *Candida* spp. in their genitourinary areas. Such physiological changes could retard the effects on the anti-*Candida* actions of neutrophils [2].

Several antifungal therapies are available such as nucleoside analogs, allylamines, echinocandins, and polyenes (Amphotericin B and nystatin), but azoles (such as Fluconazole, FLZ) are the most commonly used drugs for *Candida* infections treatment [10]. The application of antifungal drugs for the treatment of candidiasis depends on the type and sensitivity profile of *Candida* infection, and the anatomical site [10]. However, there are several literature about the resistance of some *Candida* species against antifungal agents including azoles [11-13]. Therefore, there is an urgent need to develop new classes of antifungal agents which are more effective with limited toxicity for the treatment of *Candida* infections, especially in pregnant women.

In North-central Nigeria, there is a dearth of information regarding *Candida* infection in pregnancy. *Candida* diagnostic methods based on phenotypes such as blood culture, microscopic examination, and biochemical identification [14], are time-consuming and labor-intensive with low sensitivity. The long period of waiting time required to diagnose the infection often leads to a delay in the start of treatment with antifungal drugs. Thus, molecular [15] and serological methods [16,17] have been applied for detailed characterization. Therefore, using the PCR technique, the current study was designed to assess the prevalence, characterize *Candida* species with a molecular approach, and further assess the predisposing risk factors in pregnant women attending antenatal clinics in Anyigba, Kogi State, Nigeria.

Methods

Study sites and population

The study was carried out at Anyigba, situated in Dekina Local Government Area. The occupation of the people is heterogeneous with farming as a major occupation [18,19]. The ethical approval for the current study was obtained from the Ethical Board of Antenatal Clinic, Prince Abubakar Audu University teaching hospital, Anyigba, written informed consent form was provided to each of the pregnant women. The study population consisted of fifty (50) pregnant women that attended the antenatal clinic in the Hospital from May to July 2022. The inclusion criteria include pregnant women who were clinically pregnant and aged between 15-45 years accessing antenatal care at the study site; while the exclusion criteria were non-pregnant women, pregnant women who did not

consent to participate in the study, those not accessing antenatal care at the study site, women outside the age group (<15 or > 45 years), and pregnant women with the history of antibiotic therapy.

Data and sample collection

Fifty (50) high vaginal swab (HVS) specimens were aseptically collected from consented pregnant women attending antenatal clinic of Prince Abubakar Audu University teaching hospital, Anyigba, with the help of Laboratory scientists using sterile swab sticks. Demographic, social and risk factor information relevant to the study was obtained from each consented patient employing a structured questionnaire. The samples were labelled appropriately and taken to the Laboratory immediately for analysis.

Sample cultivation and isolation for identification

One swab was used for culture and the other for direct Gram smear for Gram positive oval-shaped organisms with buds and/or pseudohyphae/hyphae. The HVS samples were respectively streaked onto Sabouraud dextrose agar plates containing Chloramphenicol and the sample was incubated at 37°C for 48 hours while a second plate was incubated at room temperature. The cultural identification of suspected colonies was done by gram staining and the germ tube test was further employed to confirm presence of yeast-like cells.

Germ tube test

Germ tube experiment was used as a rapid tool for identification of *C. albicans.* Using a sterile wire loop, a small portion of pure growth of *Candida* was harvested and inoculated in a sterile test tube containing 0.5ml of human serum. The resulting suspension was incubated aerobically at 37°C for 3 hours. A drop of yeast serum suspension was placed on a clean slide with 1 drop of cotton blue lactophenol stain, covered with a cover slip, and examined microscopically, using the x10 and x40 objective lenses. The appearance of small sprouting tube –link outgrowths projecting from the cell surface confirmed the production of germ tubes [20].

Molecular identification

DNA extraction and quantification

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. The extraction procedure was based on the kits manufacturers specifications. The ultra-pure DNA was stored at -20ºC for the downstream reaction. Prior to PCR amplification and other downstream applications, the extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer.

Internal transcribed spacer (ITS) amplification

The ITS region of the isolates was amplified using the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'- TCCTCCGCTTATTGATATGC-3, primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 53°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualization on a UV transilluminator showed band sizes of 500bp against the 100 bp molecular ladder **(Figure 1).**

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. Final volume of 10 ul was used, and the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4 min.

Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit. Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN **(Figure 2).** These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 [21]. The bootstrap consensus tree inferred from 500 replicates[22] was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [23].

Statistical analysis

Data were analyzed using the InStat Graph pad version 18 and test statistics such as Chi-square and student T-test were used were appropriate to assess for level of association. Statistical precision was achieved at the 0.05 probability limit.

Results

Fifty high vaginal swabs (HVS) samples were obtained from pregnant women attending the antenatal clinic in Prince Abubakar Audu Teaching Hospital Anyigba, Kogi State, for the prevalence of *Candida* spp. Thirty-six 36(72%) of the samples were positive for *Candida* spp. **Table 1** shows the molecular characterization of *Candida* spp among pregnant women. The result (**Table 1**) showed that 3(8.33%) were *Candida albicans* strain CBS:6362, 4 (11.11%) *Candida albicans* strain IMAN-22, 6 (16.67%) *Candida albicans* strain IMAN-25 and 3 (8.33%) *Candida albicans* strain IMAN-225 were identified. The non-albican species identified were *Candida akabanensis (*PMM09-2528L; 10(27.77%), *Pichia kudriazevii* (OOSF20; 6(16.66%) and *Candida glabrata* 4(11.11%).

Table 2 reveals the age group of the women attending an ante-natal clinic in Prince Abubakar Audu Teaching Hospital Anyigba, Kogi State enrolled in this study. The age group ranged from 15-45 years. The highest prevalence rate was observed in the age group of 26-35(44.44%). There was no significant difference in the prevalence of *Candida* spp within the age group at 0.05 probability levels (Chi-square 0.4582, degree of freedom 2, *p* value 0.7952). **Table 3** shows the prevalence of *Candida* spp among pregnant women in relation to socio-demographic factors. The results show that traders (55.55%) had a higher prevalence rate compared to housewives (27.77%) and civil servants (16.66%). However, there was no significant difference in the prevalent rate with respect to the occupation of the women (Chi-square 0.007, degree of freedom 2, *p* value 0.9986). **Table 3** also shows the level of education of the women attending the ante-natal clinic. The prevalence rate of primary education (44.44%) was higher compared to women who had attained secondary and tertiary certificates (27.77%) which are the same. There was no significant difference in the prevalence rate as it relates to the attainment of educational certificates (Chi-square 0.7513, degree of freedom 2, *p* value 0.6863). Prevalence of *Candida* spp. was higher in the third trimester (61.11%) compared to first 3 (8.33%) and second trimester (30.55%), **(Table 3)**. Statistical analysis revealed that there was no significant difference in the prevalence rate as it relates to Trimester phase (Chi-square 0.1122, degree of freedom 2, *p* value 0.9454). The prevalence rate of *Candida* spp. was higher among married women (94.4%) compared to unmarried ones (5.5%) **(Table 3)**. There was no significant difference in the prevalence rate as it relates to marital status (Chi-square 0.1142, degree of freedom 2, *p* value 0.7354). **Table 4** shows the prevalence of *Candida* spp. among pregnant women in relation to predisposing risk factors. There were no significant differences in all the risk factors considered during the study.

Table 1. Molecular characterization of *Candida* spp among the pregnant women.

***Candida* spp**	**Number positive (%)**
Candida albicans strain CBS:6362	3 (8.33%)
Candida albicans strain *IMAN*-22	4 (11.11%)
Candida albicans strain IMAN-25	6 (16.67%)
Candida albicans strain *IMAN*-225	3 (8.33%)
Candida akabanensis strain *PMM*09-2528L	10 (27.78%)
Pichia krudriavzevii strain OOSF20	6(16.67%)
Candida glabrata	4 (11.11%)

Table 2. Prevalence of *Candida* spp among the pregnant women according to age group.

Value name (age groups (years))	Samples number	Number of positive (%)	X^2	*P*-value	D.F
15-25	22	14 (38.88%)			
26-35	22	16 (44.44%)			
36-45	6	6 (16.66%)	0.4582	0.7952	2

Table 3. Prevalence of *Candida* spp among the pregnant women in relation to socio-demographic factors.

Variables	Number of Samples	Number of positive (%)	X^2	*P*-value	D.F
Occupation					
Civil servant	8	6(16.66%)			
Traders	28	20 (55.55%)			
House wives	14	10 (27.77%)	0.0068	0.9966	2
Education					
Primary	19	16 (44.44%)			
Secondary	17	10 (27.77%)			
Tertiary	14	10 (27.77%)	0.7513	0.6863	2
Trimester					
1-3 months	5	3 (8.33%)			
3-6 months	14	11 (30.55%)			
6-9 months	31	22 (61.11%)	0.1122	0.9454	2
Marital status					
Separated	2	2 (5.5%)			
Married	48	34 (94.4%)	0.1142	0.7354	1

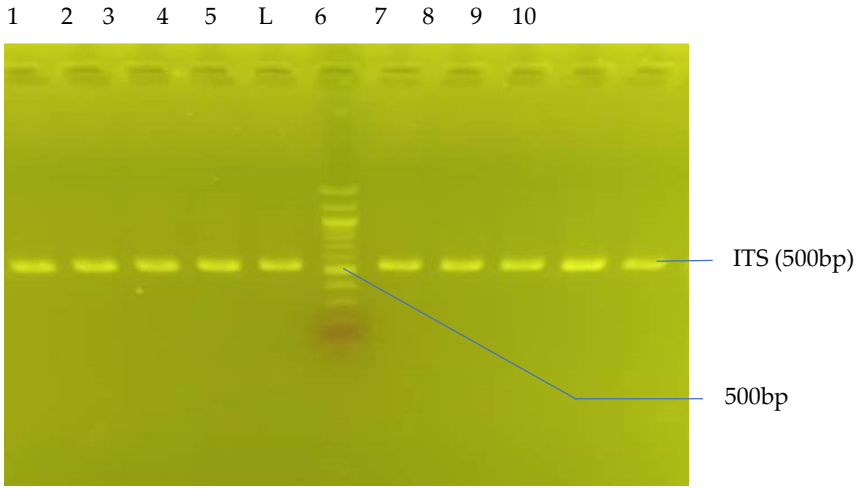
Note: total number of cases studied is 50.

Table 4. Prevalence of *Candida* spp among the pregnant women in relation to predisposing risk factors.

Variables		Number of samples	Number positive (%)	X^2	*P*-value	D.F
Was there previous use of antifungal	Yes	20	15 (41.66%)	3.500	0.1772	1
	No	30	21 (58.33%)			
Are you free from any other virginal infection	Yes	32	25 (69.44%)	0.7071	0.5528	1
	No	18	11 (30.55%)			
Are you currently experiencing whitish vaginal discharge	Yes	28	22 (61.11%)	1.400	0.2965	1
	No	22	14 (38.88%)			
Have you been previously diagnosed with candidiasis	Yes	12	10 (27.77%)	1.400	0.3949	1
	No	38	26 (72.22%)			
Have you been diagnosed of sexually transmitted infection previously	Yes	5	3 (8.33%)	1.400	0.3949	1
	No	45	33 (91.66%)			
Was there any invasive procedure before conceiving	Yes	6	6 (16.66%)	1.000	0.500	1
	No	44	30 (83.33%)			
Has your partner ever been diagnosed with candidiasis	Yes	12	4 (11.11%)	1.00	0.500	1
	No	38	32 (88.88%)			

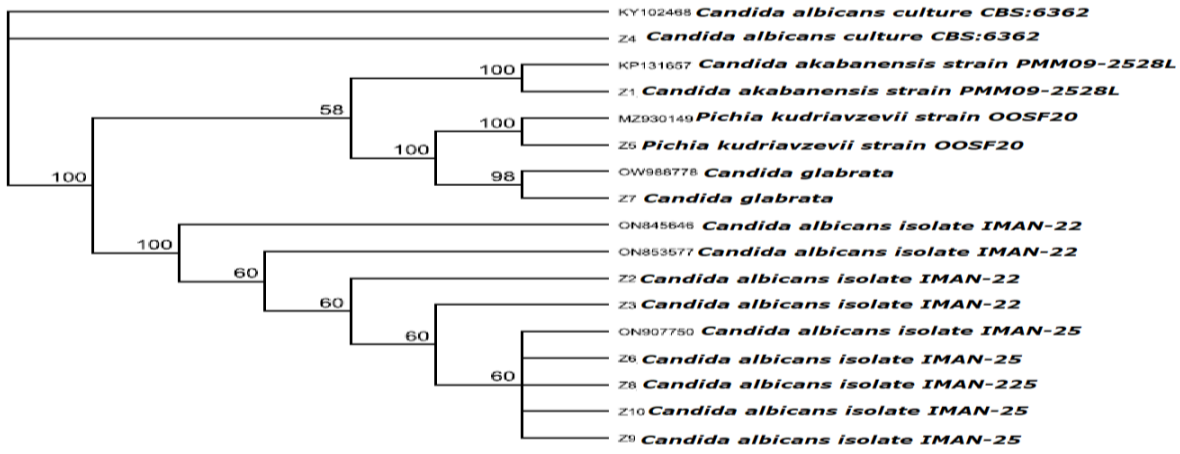
Note: total number of cases studied is 50

Figure 1. Agarose gel electrophoresis showing the amplified ITS of the fungal isolates. Lane 1-10 represent the ITS bands at 500 bp while lane L represents the 100 bp molecular ladder.



The amplified ITS of the isolates when resolved on 1.5% agarose gel electrophoresis and visualized on a transilluminator showed band sizes of 500bp using the 100 bp molecular ladder as tracker before sending for sequencing

Figure 2. Phylogenetic tree showing the evolutionary distance between the fungal isolates.



The obtained ITS sequence from the isolates produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The ITS of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method showed that the ITS of the isolates were within the *Candida* and *Pichia* spp and revealed a close relationship with *Candida glabrata*, *Candida albicans*, *Candida akabanensis* and *Pichia kudriavzevii*.

Discussion

This study focused on the status of vaginal Candidiasis among pregnant women attending the antenatal clinic of Prince Abubakar Audu Teaching Hospital, Anyigba, Kogi State Nigeria. A higher frequency of vaginal candidiasis within different age ranges of the pregnant women was observed within the age ranges 26 - 35 years (44.44%). The infection was at a higher frequency in this age group than the other age groups and reason could be attributed to the fact that women in this age group are likely to use drugs indiscriminately and contraceptives to prevent pregnancy [24]. This observation is consistent with reports of **Okungbowa et al.** [25] who reported a 54% prevalence rate within age bracket 20 - 30 years in Northern Nigeria. Fifty five percent (55%) and 57% prevalence rates was previously reported within age group 26 - 35 years in Benin City [26]. The authors attributed the age vulnerability to sexual promiscuity, drug abuse and use of contraceptives. A slightly lower rate of vaginal candidiasis infection in the pregnant women was observed in this study within the age ranges 15

- 25 years (38.88%). This age group was second in prevalence of infection from age ranges 26 - 35 years in this study. The age group contains women who are younger and are sexually active. They also have the habit of using contraceptives especially the emergency pills to prevent pregnancy and misuse of antibiotics for treatment of such infections. A lower rate of prevalence of the infection in pregnant women was reported within the age ranges 36 - 45 years age group with prevalence rates of (16.66%). Women in the age group 36 - 45 are nearing their menopause age and are becoming less sexually active. This may be the reason why vaginal candidiasis was recorded at a low prevalent rate in this age group. The finding is in line with a previous report by **Okungbowa et al.** [25] who reported a prevalence rate of 10% within the age groups of 36 - 45 and they reported that it was probably due to the possible increase in vaginal immunity with age.

In the current study, vaginal candidiasis was higher in the 3rd trimester than the 2nd (30.55%) and 1st (8.33%) trimesters and reason could be that pregnant women in the 3rd trimester of pregnancy have suppressed immune system than those in the 2nd and 1st trimesters which steps up the risk of *Candida* spp. to become pathogenic. This is due to the emotional stress, which increases as one is expecting a child. At this trimester, an increased level of estrogen and corticoids hormones decreases the level of vaginal defense mechanisms against such opportunistic infections as *Candida* [27]. These factors contributed to the highest prevalence of vaginal candidiasis in the 3rd trimester of pregnancy. The results agree with a previous study by **Sobel et al.** [28] who reported the highest prevalent rate of 67% in the 3rd trimester of pregnancy. He reported that it was due to increased emotional stress as a pregnant woman is expecting a child resulting to suppression of the immune system that steps up the risk of *Candida* species to become pathogenic. The 1st and 2nd trimesters of pregnancy recorded low prevalent rates of vaginal candidiasis infection. The pregnant women in these two trimesters could have less emotional stresses, high levels of vaginal defense mechanisms against *Candida* infections because of low levels of estrogen and corticoids hormones. Therefore, they have strong immune system against *Candida* spp. infections [27]. These factors could have contributed to the low prevalence of the infection in these two trimesters of pregnancy. The findings in this study shows prevalence of *Candida* spp was higher in the third trimester compared to first and second trimester (**Table 3**). Statistical analysis revealed that there was no significant difference in the prevalence rate as it relates to trimester phase. This was due to the difference in the status of the immune system of the pregnant women in the trimesters of pregnancy. It was also due to difference in the levels of vaginal defense mechanisms against *Candida* infections because of low levels of estrogen and corticoids hormones. This observation is consistent with other reports by **Gonzalez et al.** [27] who reported prevalence rates of 11% and 20% respectively. It was reported that pregnant women in these two trimesters of pregnancy still have strong immune system and high levels of vaginal defense mechanisms against *Candida* infections [27].

On the occupational status, traders recorded the highest prevalence rate of (55.55%) compared to housewives (27.77%) and civil servants (16.66%) respectively, a result that is in agreement with the report of **Emeribe et al.** [29]. Traders may not have enough time for laboratory tests therefore may result to wrong self-medication thereby making them carriers of the infection. Due to the nature of their activities, they are prone to urinate or defecate in public toilet or nearby bushes, not minding the poor hygiene. This study also revealed that pregnant women had higher prevalence rate at the primary level (44.44%) when compared to secondary and tertiary with the same prevalence rate (27.77%). This indicates that the level of education played an important role in candidiasis infection. Been fully educated helps reduce various misconceptions about many illnesses including Candidiasis and encourages preventive practices [30]. On the other hand, this result is lower than previous study by **Akah et al.** [31] which reported high prevalence of Candidiasis with prevalence rates 60% and 62.2% respectively among pregnant women in Jos and Enugu State, Nigeria. It was found that (94.4%) of the married women were infected with *Candida* (candidiasis), compared to single with (5.5%), this presupposed that sexual activities have some effect on candidiasis. The presumption agreed with report by **Oladimeji et al.** [32].

The high prevalence of vaginal candidiasis may be due to many different reasons in relation to predisposing risk factors in **table (4)** above; suppression of the immune system due to the pregnancy as it is among the contributing factors of

vaginal candidiasis, whitish vaginal discharge, sexually transmitted infection, prolonged and misuse of antibiotics and antifungal drugs, which leads to the destruction of good and beneficial bacteria resulting to reduction of vaginal immunity could have also contributed to the increase of the prevalence of the infection [26]. Hormones during pregnancy can play role of enhancing *Candida* colonization and serve as risk factor of the vagina infection, progesterone has suppressive effect on the anti-*Candida* activity of neutrophils while estrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida albicans* [33]. Inadequate knowledge, poor personal hygiene, limited diagnostic facilities, poor dietary habits have also been attributed to high prevalence vaginal candidiasis [26].

Molecular characterization carried out in this study showed four different strains of *Candida albicans* (CBS:6362; 3(8.33%), IMAN-25; 6(16.66%), IMAN-225; 3(8.3%) and IMAN-22, 4(11.11%), *Candida akabanensis (*PMM09-2528L; 10(27.77%), *Pichia kudriazevii* (OOSF20; 6(16.66%) and *Candida glabrata* 4(11.11%). The observed results demonstrated lower percentage of *Candida albicans* strain compared to the work of **Azike et al.** [34] who reported the phylogeny of Candida species as *Candida albicans* 45%, *Candida glabrata* 25%, *Candida akabanensis* 8.4% and *Picha kudrazevii* 4.2%) respectively. **Azike et al.** [34] also isolated and confirm the identity of *Candida akabanensis* strain PMM09-2528L, which was also identified in this study. Other investigations by **Fornari et al.** [35] and **Alhussaini et al.** [36] all reported higher percentage of *Candida albicans* in their study (82.5%, 54% and 45.8%, respectively).

Conclusion

The prevalence of vaginal candidiasis in the pregnant women was high especially in the age ranging 26 to 35 years and at the third trimester of pregnancy. From the investigation, there is an indication that proper awareness in terms of the control, prevention, and treatments of candidiasis is needed among pregnant patients. With adequate pharmacotherapy, avoidance of contributing factors such as use of contraceptive, improvement of personal hygiene and sex discipline, the incidence and prevalence of candidiasis can be greatly reduced. In addition, this study also revealed that molecular characterization of *Candida* spp. is very important in identifying the different strains that may be responsible for candidiasis amongst pregnant women in the study area.

Acknowledgments

Author sincerely thank the laboratory technicians of Microbiology Department, Prince Abubakar Audu University, for their support and assistance during the microbiological studies. Authors also acknowledge members of NUCLEOMETRIX LAB in Bayelsa State for providing technical support and enabling environment during the during the PCR assay.

Author Contributions

Conceptualization, M-L.O. O. and A.Z.A., Data source preparation, M-L.O.O., A.Z.A, S.O.S, C.A.O, I.B.M, J.A.O and U.E.O. All authors have read, edited, and agreed to the published version of the manuscript.

Funding

The study was not in receipt of any funding.

Ethical consideration

This study was carried out in a resource semi-urban centre in West Africa in compliance with ethical considerations for use of human subjects (authorization no. KSUTH/CMAC/ETHICAL/005/VOL.1/16).

Competing interests

Authors declare that there was no conflict of interest.

References

1-**Esmaeilzadeh S, Omran SM, Rahmani Z.** Frequency and Etiology of Vulvovaginal Candidiasis in Women Referred to a Gynecological Center in Babol, Iran. 2009; 3: 74-77

2-**Kamath P, Pais M, Nayak MG.** Risk of vaginal candidiasis among pregnant women. Int. J. Curr. Microbiol. App. Sci 2013; 2(9):141-146

3-**Hillier SL, Austin M, MacIo I, Meyn LA, Badway D, Beigi R.** Diagnosis and Treatment of Vaginal Discharge Syndromes in Community Practice Settings. Clin. Infect. Dis 2021; 72: 1538–1543.

4-**Sutaria P, Cholera M, Donga SB.** A Prevalence Study of Vaginal Candidiasis among Pregnant Women. Int. J. Adv. Med 2019; 6: 922–926.

5-**Lionakis MS, Netea MG.** Candida and Host Determinants of Susceptibility to Invasive Candidiasis. PLoS Pathog 2013; 9(1): e1003079.

6-**Konadu DG, Owusu-Ofori A, Yidana Z, Boadu F, Iddrisu LF, Adu-Gyasi D, et al.** Prevalence of Vulvovaginal Candidiasis, Bacterial Vaginosis and Trichomoniasis in Pregnant Women Attending Antenatal Clinic in the Middle Belt of Ghana. BMC Pregnancy Childbirth 2019; 19: 1–10.

7-**Salehei Z, Seifi Z, Mahmoudabadi AZ.** Sensitivity of Vaginal Isolates of Candida to Eight Antifungal Drugs Isolated From Ahvaz, Iran. Jundishapur J. Microbiol 2012; 5: 574–577.

8-**Alonso-Monge R, Gresnigt MS, Román E, Hube B, Pla Js.** Candida Albicans Colonization of the Gastrointestinal Tract: A Double-Edged Sword. PLOS Pathog 2021; 17: e1009710.

9-**Bliss JM, Basavegowda KP, Watson WJ, Sheikh AU, Ryan RM.** Vertical and Horizontal Transmission of Candida Albicans in Very Low Birth Weight Infants Using DNA Fingerprinting Techniques. Pediatr. Infect. Dis. J 2008; 27: 231–235.

10-**Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al.** Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis 2016; 15:62(4):e1-50.

11-**Zhang MR, Zhao F, Wang S, Lv S, Mou Y, Yao CL, et al.** Molecular Mechanism of Azoles Resistant Candida Albicans in a Patient with Chronic Mucocutaneous Candidiasis. BMC Infect. Dis 2020;20: 1–6.

12-**Pristov KE, Ghannoum MA.** Resistance of Candida to Azoles and Echinocandins Worldwide. Clin. Microbiol. Infect 2019; 25: 792–798.

13-**Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD**. Azole Antifungal Resistance in Candida Albicans and Emerging Non- Albicans Candida Species. Front. Microbiol 2017; 7:2173.

14-**Alam MZ, Alam Q, Jiman-Fatani A, Kamal MA, Abuzenadah AM, Chaudhary AG, et al.** Candida identification: a journey from conventional to molecular methods in medical mycology. World J Microbiol Biotechnol 2014;30(5):1437-51.

15-**Vahidnia A, Bekers W, Bliekendaal H, Spaargaren J.** High throughput multiplex-PCR for direct detection and diagnosis of dermatophyte species, Candida albicans and Candida parapsilosis in clinical specimen. J Microbiol Methods 2015;113:38-40.

16-**Zehm S, Schweinitz S, Würzner R, Colvin HP, Rieder J.** Detection of Candida albicans by mass spectrometric fingerprinting. Curr Microbiol 2012;64(3):271-5.

17-**Gunasekera M, Narine M, Ashton M, Esfandiari J.** Development of a Dual Path Platform (DPP®) immunoassay for rapid detection of Candida albicans in human whole blood and serum. J Immunol Methods 2015;424:7-13.

18-**Omatola CA, Iyeh SD, Abuh SJ, Mofolorunsho CK, Okolo MLO, Akoh PQ.** High Rate of Sexually Transmitted Infections (STIs) among Asymptomatic Pregnant Women in a Resource-Poor Setting in the Middle Belt Zone of Nigeria. Hosts and

Viruses 2020; 7(1): 10-.19.

19-**Omatola CA, Okolo MO.** Hepatitis B and Asymptomatic Malaria Infection among Pregnant Women in a Semiurban Community of North-Central Nigeria. J Environ Public Health 2021;(2):1-7.

20-Moya-Salazar J, Rojas R. Comparative Study for Identification of Candida Albicans with Germ Tube Test in Human Serum and Plasma. Clin. Microbiol. Infect. Dis 2018; 3(3): 1-4

21-**Saitou N, Nei M.** The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. Mol. Biol. Evol 198; 4: 406–425.

22-**Felsenstein J.** Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; 39: 783–791.

23-**Jukes TH, Cantor CR.** Evolution of Protein Molecules. Mamm. Protein Metab 1969; 21–132.

24-**Asmamaw DB, Eshetu HB, Negash WD.** Individual and Community-Level Factors Associated With Intention to Use Contraceptives Among Reproductive Age Women in Sub-Saharan Africa. Int. J. Public Health 2022; 67: 107.

25-**Okungbowa FI, Isikhuemhen OS, Dede AP.** The distribution frequency of Candida species in the genitourinary tract among symptomatic individuals in Nigerian cities. Rev Iberoam Micol 2003;20(2):60-3.

26-**Akortha EE, Nwaugo VO, Chikwe NO.** Antifungal Resistance among Candida Species from Patients with Genitourinary Tract Infection Isolated in Benin City, Edo State, Nigeria. African J. Microbiol. Res 2009; 3: 694–699.

27-**González GM, Elizondo M, Ayala J.** Trends in Species Distribution and Susceptibility of Bloodstream Isolates of Candida Collected in Monterrey, Mexico, to Seven Antifungal Agents: Results of a 3-Year (2004 to 2007) Surveillance Study. J. Clin. Microbiol 2008; 46; 2902.

28-**Sobel JD.** Epidemiology and Pathogenesis of Recurrent Vulvovaginal Candidiasis. Am. J. Obstet. Gynecol 1985; 152: 924–935.

29-**Emeribe AU, Nasir IA, Onyia J, Ifunanya AL.** Prevalence of Vulvovaginal Candidiasis among Nonpregnant Women Attending a Tertiary Health Care Facility in Abuja, Nigeria. Res. Rep. Trop. Med 2015; 6: 37–42.

30-**Sahoo B, Bhandari H, Sharma M, Malhotra S, Sawhney H, Kumar B.** Role of the male partner in the lower genitourinary tract infection of female. Indian J Med Res 2000;112:9-14.

31-**Akah PA, Nnamani CE, Nnamani PO.** Prevalence and Treatment Outcome of Vulvovaginal Candidiasis in Pregnancy in a Rural Community in Enugu State, Nigeria. J. Med. Med. Sci 2010; 1: 447–452.

32-**Idowu MO, Makinde GI, Oluranti OO, Adebayo MA, Adekunle OA.** Prevalence of Vulvo-Vaginal Candidiasis among Women Attending Clinics in Selected Hospitals in Oyo State, Southwest, Nigeria. J. Public Heal 2022; 14: 45–52.

33-**Nwadioha SI, Egah DZ, Alao OO, Iheanacho E.** Risk Factors for Vaginal Candidiasis among Women Attending Primary Health Care Centers of Jos, Nigeria. J. Clin. Med. Res 2010; 2: 110–113.

34-**Azike CA, Nwokah EG, Abbey SD.** Molecular Characterization and Phylogeny of Candida Species Isolated from High Vaginal Swab Samples among Patients Presenting with Vulvovaginal Candidiasis in Port Harcourt, Nigeria; Port Harcourt, Nigeria,

2019. Corpus ID: 212440727: 1-15

35-**Fornari G, Vicente VA, Gomes RR, Muro MD, Pinheiro RL, Ferrari C, et al.** Susceptibility and molecular characterization of Candida species from patients with vulvovaginitis. Braz J Microbiol 2016 47(2):373-80.

36-**Alhussaini MS, El-Tahtawi NF, Moharram A.** Phenotypic and Molecular Characterization of Candida Species in Urine Samples from Renal Failure Patients. Science Journal of Clinical Medicine 2013; 2: (1): 14-25.

Okolo MLO, Alaba AZ, Samson SO, Omatola CA, Mudi IB, Omatola JA, Okolo UE. Microbiological and molecular investigation of *Candida* spp infection among women accessing antenatal care at Prince Abubakar Audu University Teaching Hospital Anyigba, North-Central Nigeria. Microbes Infect Dis 2023; 4(4): 1435-1445.