

Original Research Article

<https://doi.org/10.20546/ijcmas.2024.1311.002>

Coinfection of Bacterial Vaginosis and Candidiasis in Expectant Mothers with Vaginal Discharge attending Primary Health Center at Edo Central Senatorial District, Nigeria

Adewuyi Gbolagade Morufu^{1,2}, Iyevhobu Kenneth Oshiokhayamhe^{3*},
Adewuyi Bolanle Toyin^{4,5}, Momoh Abdul-Razak Mcsionel¹, Samuel Olowo Sunday¹,
Obowemu Kennedy Oberhiri⁶ and Edo Elvis⁷

¹Department of Medical Microbiology and Parasitology, Ambrose Alli University,
Ekpoma, Edo State, Nigeria

²Department of Medical Microbiology and Parasitology, Irrua Specialist Teaching Hospital,
Irrua, Edo State, Nigeria

³Department of Medical Microbiology, Faculty of Medical Laboratory Science,
Ambrose Alli University, Ekpoma, Edo State, Nigeria

⁴Department of Family Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria

⁵Department of Family Medicine, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria

⁶Department of Health, Wellbeing and Social Care, Global Banking School/Oxford Brookes University,
Birmingham, United Kingdom

⁷National Ear Care Centre, Kaduna, Kaduna State, Nigeria

*Corresponding author

ABSTRACT

Bacterial vaginosis (BV) is a condition caused by an overgrowth of normal vaginal flora in women of childbearing age. In pregnant women Infections occurring during pregnancy may be bacterial, viral, parasitic or fungal in origin, and could affect any part of the female genital tract. Some of these infections can be attributed to organisms which are present as normal flora in apparently health subjects, and this is the case of both vulvovaginal candidiasis (VVC) and BV in pregnancy, both of which frequently manifest with vaginal discharge. This study is carried out to investigate the prevalence of co-infection of bacterial vaginosis and candidiasis among pregnant women attending the Antenatal Clinic of Primary Healthcare Centre, Esan West, Edo State, Nigeria. A sample size of 220 subjects comprising of 151 pregnant women and 69 non-pregnant women were selected for this study. Subject's data were obtained verbally directly from the patient after consent for sample collection w given. The data collected included those on maternal age, gestational age, marital status and occupation. The sample was collected by means of a sterile swab stick by the attending physician. The swab sticks were properly labelled with identification number with which they will be processed, and sent immediate to the laboratory for analysis. From this study, pregnant women had a significantly ($p\text{-value} \leq 0.05$) higher prevalence of bacterial vaginosis (BV) (31.3%), compared to their non-pregnant counterparts (0%). The overall prevalence of BV among reproductive-aged women (pregnant and non-pregnant) was 21.36%. Although not significant ($p\text{-value} 0.05$), the prevalence of vulvovaginal candidiasis (VVC) was higher among pregnant women (38.41% compared to non-pregnant women (26.09%). The overall prevalence of VVC in the study population was 34.55%. The prevalence of BV and BV/VVC co-infection was significantly ($p\text{-value} \leq 0.05$) higher among pregnant women (13.91%) when compared to their non-pregnant counterparts (0%). The overall prevalence of co-infection among reproductive aged women was 9.55%. In conclusion, bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) and their co-infection are notable conditions in pregnant women with the potential of negatively affecting both maternal, foetal, and neonatal health. The overall prevalence of BV, VVC, and BV/VVC co-infection in this study was 21.36, 34.55 and 9.55% respectively.

Keywords

Co-infection,
Bacterial Vaginosis,
Candidiasis,
Candida,
Pregnant

Article Info

Received:
15 September 2024
Accepted:
22 October 2024
Available Online:
10 November 2024

Introduction

Bacterial vaginosis (BV) is a condition caused by an overgrowth of normal vaginal flora in women of childbearing age (Greenbaum *et al.*, 2019). BV is a disturbance in the vaginal microbiome and its associated with an increased risk of sexually transmitted infection, human immunodeficiency virus (HIV) and adverse pregnancy outcomes (Iyevhobu *et al.*, 2021). The most severely affected experience is an offensive fishy smelling discharge which frequently occur around the time of menstruation. It is characterized by an overgrowth of predominantly anaerobic organism in vaginal leading to a replacement of *Lactobacilli* and an increase in the vaginal pH (Iyevhobu *et al.*, 2021). These aerobic organisms include *Gardnerella vaginalis*, *Mobiluncus species*, *Bacteroides*, *Prevotella species* and *Mycoplasma species* (Iyevhobu *et al.*, 2021). BV often subsides after treatment with 50% of women having return of symptoms within 12 months (Iyevhobu *et al.*, 2021; Russo *et al.*, 2018). In Nigeria, the prevalence rate of 17%, 17.3%, and 25% for BV have been reported from separate studies done in South East, North-East, and South-West, Nigeria respectively (Adesiji *et al.*, 2007; Ibrahim *et al.*, 2014; Nigeeen *et al.*, 2015).

In pregnant women BV also increase the risk of miscarriage (Hay *et al.*, 1994; Animasaun *et al.*, 2023) pre-term labour, pre-term delivery and post-partum complication such as endometritis (Iyevhobu *et al.*, 2021; Minkoff *et al.*, 1984). These conditions are particularly of high incidence in pregnancy, due to the attendant reduction in maternal immunity to accommodate the growing foetus (Iyevhobu *et al.*, 2021; Kourtis *et al.*, 2014). Infections occurring during pregnancy may be bacterial, viral, parasitic or fungal in origin, and could affect any part of the female genital tract (Chan and Smith, 2018). Some of these infections can be attributed to organisms which are present as normal flora in apparently health subjects, and this is the case of both vulvovaginal candidiasis (VVC) and BV in pregnancy, both of which frequently manifest with vaginal discharge (Iyevhobu *et al.*, 2021).

The high prevalence of bacteria in vaginal disease or infection and concomitant lower genital tract infection among symptomatic and asymptomatic pregnant women is a confirmation of the resultant adverse pregnancy outcome associated with bacterial vaginosis and Candida infection (Iyevhobu *et al.*, 2021; Animasaun *et al.*, 2023; Govender *et al.*, 1996). A number of studies have

investigated the co-infection of bacterial vaginosis with vulvovaginal candidiasis among reproductive-aged women (Rivers *et al.*, 2011; Aubyn and Tagoe, 2013; Iyevhobu Kenneth Oshiokhayamhe, 2020; Gupta *et al.*, 2021; Oparaugo *et al.*, 2022; Huang *et al.*, 2023). However, in all of the reviewed literature, there was scarcity of information concerning co-infection of bacterial vaginosis with vulvovaginal candidiasis among pregnant women in Edo State, Nigeria. Hence, this study is justified by the need to fill this research gap by investigating the prevalence of co-infection of bacterial vaginosis and candidiasis among pregnant women attending the Antenatal Clinic of Primary Healthcare Centre, Esan West.

Materials and Methods

Geographical Description of the Study Area

In Ekpoma, the Esan West Local Government Area of Edo State, Nigeria, this study was conducted. With a surface area of 17,450 sq. km and a population of 3.1 million, Edo state is situated in the South-South geopolitical zone of Nigeria, between longitudes 06° 04'E and 06° 43'E and latitudes 05° 44'N and 07° 34'N. In the semi-urban town of Ekpoma, the main industries are agriculture, trade, public service, and education.

Sample Size

The number of subjects required in this research was guided by upper limit to give 95% level of confidence at an expected prevalence of about 37% from a previous study (Oparaugo *et al.*, 2022).

$$\text{Sample Size} = \frac{Z^2 Pq}{d^2}$$

Where:

Z= confidence level (95%)= 1.96

P=expected prevalence=37%=0.37

q= 1.0-P=1-0.37=0.63

d= 0.065

$$\text{Sample size} = \frac{1.96^2 \times 0.37}{0.065^2} \times x$$

Sample size= 211.95~212 samples

To further accommodate for sampling error, a sample size of 220 subjects comprising of 151 pregnant women and 69 non-pregnant women were selected for this study.

Inclusion Criteria

The inclusion criteria for this study are: all consenting pregnant and non-pregnant women indicated for high vaginal swab culture attending Primary Health Care, Esan West, with no history of antibiotics usage in the last two months.

Exclusion Criteria

The excluded groups in this study are: non-consenting women; women not indicated for high vaginal swab culture: women not attending Primary Health Care, Esan West women with history of antibiotics usage in the last two months.

Data and Sample Collection

Subject's data were obtained verbally directly from the patient after consent for sample collection was given. The data collected included those on maternal age, gestational age, marital status and occupation.

The sample was collected by means of a sterile swab stick by the attending physician. The swab sticks were properly labelled with identification number with which they will be processed, and sent immediately to the laboratory for analysis.

Ethical Consideration

Approval for this study was obtained from the Ethical Committee of the Ministry of Health in Esan West Local Government Area, Edo State, Nigeria. Verbal informed consent was obtained from the study participants.

Laboratory Analysis

Vulvovaginal Candidiasis (VVC)

Culture: Culturing was done using both SDA and CAC.

Principle: SDA contains chloramphenicol, gentamycin, and tetracycline which inhibit the growth of most bacterial organisms, thus favouring fungal growth.

Furthermore, the high sugar content of SDA promotes fungal growth.

CHROMagar™ Candida (CAC) is a selective medium for the isolation of fungi that simultaneously provides direct differentiation and identification of several *Candida* species. The yeasts produce enzymes that react with chromogenic substrates in the CAC medium, producing colonies of different colors. These enzymes are specific, allowing some yeasts to be identified to the species level by their colour and colony characteristics.

Method: The sample on the swab stick was used to make a primary inoculum on the surface of a prepared SDA. A wire loop was used to streak out the inoculum on the agar surface to obtain discrete colonies, followed by incubation of the plate at 37°C for 48 hrs. In the presence of growth, the isolates were identified as *Candida* using Gram staining technique, followed by sub-culturing into CAC to identify the *Candida* species.

Interpretation of Result

SDA: *C. albicans* shows white coloured, smooth, and yeast-like appearance; *Candida parapsilosis* colonies is white to creamy, shiny, and smooth; *C.dubliniensis* is cream to white; *Candida glabrata* colonies is smooth and cream coloured; while, *C.krusei* shows rough colonies (Hadi and AlSultany, 2020).

CAC: On CHROMagar™ Candida (CAC), *C. albicans* carry green colour, *C. glabrata* is pink-purple, *C.parapsilosis* is white to pale pink and *Candida krusei* appear as pink colour colonies (Hadi and AlSultany, 2020).

Data Analysis

The data obtained for this study were recorded and subjected to statistical analysis using the chi-square test performed by Microsoft Excel (Microsoft Corporation) version 2016. The socio-demographic characteristics of the study population and the prevalence of BV, VVC, and BV/VVC co-infection according to pregnancy status, maternal age, gestational age, and maternal occupation was determined.

In addition, the distribution of the various *Candida* species according to pregnancy status was also determined. The prevalence were calculated as the number of positive samples divided by the total number of samples tested. The

p-values ≤ 0.05 were considered statistically significant, while those with values > 0.05 were considered statistically insignificant.

Results and Discussion

The present study investigated the co-existence of bacterial vaginosis and candidiasis among pregnant women attending Primary Health Centre in Esan West Local Government Area of Edo State.

Table 1 shows the socio-demographic characteristics (age, occupation, and pregnancy status) of the study population. From the table, most of the respondents were within the ages of 21-30 years (68.64%) followed by >30 (18.18%), and 0-20 yrs (13.18%). Most of the respondents were merchants (32.73%) followed by students (30.45%), tailor (10.45%), housewives (10%), hairstylists (9.09%), teachers (2.73%) food vendors (2.27%), farmers (0.91%), and others (1.36%). The study population comprised of 15 (68.64%) pregnant women and 69 (31.36%) non-pregnant women respectively. Most of the pregnant women were in their second trimester (35%), followed by the third (26.82%) and first trimesters (6.82%).

Table 2 shows the distribution of vaginal infections according to pregnancy status. From the table, pregnant women had a significantly (p-value ≤ 0.05) higher prevalence of bacterial vaginosis (BV) (31.3%), compared to their non-pregnant counterparts (0%). The overall prevalence of BV among reproductive-aged women (pregnant and non-pregnant) was 21.36%. Although not significant (p-value 0.05), the prevalence of vulvovaginal candidiasis (VVC) was higher among pregnant women (38.41% compared to non-pregnant women (26.09%). The overall prevalence of VVC in the study population was 34.55%. The prevalence of BV and BV/VVC co-infection was significantly (p-value ≤ 0.05) higher among pregnant women (13.91%) when compared to their non-pregnant counterparts (0%). The overall prevalence of co-infection among reproductive aged women was 9.55%.

Table 3 shows the distribution of vaginal infections in pregnant women according to age. Although not significant (p-value > 0.05), the prevalence of BV among pregnant women was found to be decreasing with age. Pregnant women aged 0-20 years had a prevalence of 45%, followed by those aged 21-30 years (30.53%), and those >30 years (25%). There was also non-significant

variation (p-value > 0.05) in the prevalence of VVC with age, but the prevalence was found to be increasing with age. Pregnant women aged: 0-20 years had a prevalence of 35%, 21-30 years (36.84%), and >30 years (44.44%). There was also no significant variation (p-value > 0.05) in the prevalence of co-infection of BV and VVC with age. The highest prevalence was recorded among pregnant women aged 0-20 years (15%), followed by those aged >30 (13.89%), and 21-30 years (13.68%).

Table 4 shows the distribution of vaginal infections in pregnant women according to gestational age (trimester). There was no significant variation (p-value > 0.05) in the prevalence of BV across the three trimesters, but this prevalence was found to be increasing with gestational age (trimester): first trimester=26.67%, second=29.87%, and third trimester=33.90%. The prevalence of VVC varied significantly (p value ≤ 0.05) with trimester. The second trimester recorded the highest prevalence of 48.05%, followed by the first (40%), and third trimester (25.42%). There was no significant variation (p-value > 0.05) in the prevalence of BV and VVC co-infection with gestational age, but the prevalence was highest among those in the second trimester (16.88%), followed by those in the third (11.86%), and first trimester (6.67%).

Table 5 shows the distribution of vaginal infections in pregnant women according to occupation. There was no significant variation in the prevalence of any of the infections according to occupation, but co infection was more common among food vendors (20%), followed by hair stylists and house wives (15.79%), merchants (14.93%), tailor (14.29%), and students (9.09%). There was no co-infection among pregnant women who were farmers, teachers, and secretary by occupation.

Table 6 shows the distribution of *Candida* (species and intensity of growth) according to pregnancy status. There was no significant variation (p-value > 0.05) in the species of *Candida* isolated from pregnant and non-pregnant women. *Candida albicans* was the most common species isolated from pregnant women and non-pregnant women-75.86 and 77.78% respectively. In the pregnant women, this was followed by *C. krusei* (10.34%), *C. dubliniensis* and *C. glabrata* (5.17% each), and *C. parapsilosis* (1.72%), which mixed infection, both *C. dubliniensis* and *C. parapsilosis* was found in 1.72%. Among the non-pregnant women *C. albicans* was the most common species isolated followed by *C. dubliniensis* and *C. glabrata* (11.11% each). *C. krusei*

and *C.parapsilosis* was not isolated from non-pregnant women. Figure 1 shows the appearance of various species of *Candida* on CHROMagar*Candida*. There was also significant variation (p -value > 0.05) in the intensity of *Candida* growth according to pregnancy status but the non-pregnant women had more heavy growth (22.22%) compared to their pregnant counterparts (18.97%).

Several micro-organisms cause vaginal infections, and the most common vaginal infections are vulvovaginal candidiasis (VVC), bacterial vaginosis (BV), and trichomoniasis (TV) (Iyevhobu *et al.*, 2021; Olowe *et al.*, 2014). Among pregnant women in Africa, the prevalence of VVC is pooled at 29.2% (Mohamed *et al.*, 2022). Worldwide, almost one-third of women are positive for BV and a higher prevalence has been found in pregnant women from developing countries, with the highest prevalence of BV reported from several African countries (Animasaun *et al.*, 2023; Yalew *et al.*, 2022). Vaginal infections in pregnancy are associated with considerable discomfort and adverse pregnancy outcomes including pre-term delivery, low birth weight and increased infant mortality and also predisposition to HIV/AIDS (Olowe *et al.*, 2014).

Mixed vaginitis is due to the simultaneous presence of at least two vaginal pathogens, and approximately 20-30% of women with BV are co-infected with *Candida* species (Iyevhobu *et al.*, 2021; Iyevhobu Kenneth Oshiokehayamhe, 2020; Sobel *et al.*, 2013). There is wide scarcity of information concerning BV/VVC co-infection globally. Bearing this in mind, the present study sought to investigate the prevalence of BV, VVC, and BV/VVC co-infection among pregnant women attending Primary Health Care Centre, Esan West Local Government Area, Edo State.

The socio-demographic characteristics of the study population shows that most of the subjects were: within 21-30 years of age (68.64%); merchants (32.73%); and pregnant (68.64%). Most of the pregnant women were in their second trimester (35%), followed by the third (26.82%) and first trimesters (6.82%).

The age characteristics of the respondents is good because, biologically, the best period for bearing children is between 20 and 35 years of age (Iyevhobu *et al.*, 2021; Animasaun *et al.*, 2023). In addition, the median maternal age at birth in Nigeria is 26 years (Ayotunde *et al.*, 2009). The predominance of merchants as the most common occupation in the region disagrees with the

studies of Babatope *et al.*, (2018) that showed that most pregnant women in Ekpoma are civil servants (Iyevhobu *et al.*, 2021). The high occurrence of second trimester pregnant women in this study could simply be as a result of the timing of the study. In addition, Fagbamigbe *et al.*, (2021), reported that most pregnant women in Edo State (63.0%) initiate antenatal care during the second trimester.

In this study, the prevalence of BV and VVC among reproductive aged women were 21.36 and 34.55% respectively. The prevalence of VVC among the reproductive-aged women in this study is similar to the 38 and 36% earlier reported by Abdullahi and Danyaya (2021) and Olowe *et al.*, (2014) respectively, but was higher than the 10% reported in Abakaliki, South-Eastern Nigeria by Alo *et al.*, (2012), and this may be due to variation in the study period. The prevalence of VVC in this study (35.55%) collaborates with the notion that at least one in every three women with vaginitis is infected with vaginal candidiasis (Iyevhobu *et al.*, 2021; Jeanmonod and Jeanmonod, 2022). The prevalence of VVC reported in this study is a little lower than the 40% reported by Idowu *et al.*, (2022): but far lower than the 84.5% reported by Ugwa (2015). Furthermore, the prevalence of BV in this study (21.36%) is lower than the 74 % reported in AbiaState (Lawrence *et al.*, 2014), 51 % in Rivers state (Wariso *et al.*, 2017), and 39.5% in Ethiopia (Bitew *et al.*, 2021). The variation in women health hygiene in the various studies may account for these discrepancies (Iyevhobu *et al.*, 2021).

In this study, pregnant women had a significantly (p -value ≤ 0.05) higher prevalence of BV and VVC (31.3 and 38.41% respectively) compared to their non-pregnant counterparts (0 and 38.41% respectively). The higher prevalence of BV and VVC among pregnant women compared their non-pregnant counterpart have also been reported in a study conducted in Denmark (Thorsen *et al.*, 2006). However, a study conducted in Zaria, Nigeria, by Iyevhobu *et al.*, (2021) and Abdullahi & Danyaya (2021) reported a higher incidence of VVC in non-pregnant women compared to pregnant women.

Furthermore, Sabour *et al.*, (2018) and Achdiat *et al.*, (2018) in Iran and Indonesia respectively, had also noted a higher prevalence of BV among non-pregnant women compared to their pregnant counterpart. Population variations and individual characteristics of the non-pregnant women in the present study may account for the discrepancies with the aforementioned studies.

Table.1 Socio-demographic Characteristics of the Study Population

Variables		Frequency (n=220)	Percentage (%)
Age(years)	0-20	29	13.18
	21-30	151	68.64
	>30	40	18.18
Occupation	Merchant	72	32.73
	Student	67	30.45
	Tailor	23	10.45
	Housewives	22	10.00
	Hairstylists	20	9.09
	Teacher	6	2.73
	Food vendors	5	2.27
	Farmers	2	0.91
	Others (Nurse, Secretary,Soldier)	3	1.36
	1 st	15	6.82
	2nd	77	35.00
Pregnancy status	3rd	59	26.82
	Non-pregnant	69	31.36

Table.2 Distribution of Bacterial Vaginosis (BV), Vulvovaginal Candidiasis (VC), and Mixed Infection according to Pregnancy Status

	N	BV (%)	VVC(%)	BV and VVC(%)
Pregnant	151	47(31.13)	58(38.41)	21(13.91)
Non-Pregnant	69	0(0)	18(26.09)	0(0)
Total	220	47(21.36)	76(34.55)	21(9.55)
X ²		27.312	3.181	10.609
p-value		0.000*	0.074	0.001*

Key:N=Number examined; BV%=Bacterial Vaginosis percentage;

VVC %= Vulvovaginal Candidiasis percentage; BV and VVC %=Bacterial vaginosis and Vulvovaginal Candidiasis percentage

Table.3 Distribution of Bacterial Vaginosis (BV), Vulvovaginal Candidiasis (VVC), and Mixed Infection in Pregnant Women According to Maternal Age

Age Range (years)	N	BV (%)	VVC (%)	BV and VVC (%)
0-20	20	9(45)	7(35)	3(15)
21-30	95	29(30.53)	35(36.84)	13(13.68)
>30	36	9(25)	16(44.44)	5(13.89)
X ²		2.442	0.751	0.024
p-value		0.295	0.687	0.988

Key:N=Number examined; BV%=Bacterial Vaginosis percentage;

VVC %= Vulvovaginal Candidiasis percentage; BV and VVC %=Bacterial vaginosis and Vulvovaginal Candidiasis percentage

Table.4 Distribution of Bacterial Vaginosis (BV), Vulvovaginal Candidiasis (VVC), and Mixed Infection in Pregnant Women According to Gestational Age (Trimester)

Trimester	N	BV (%)	VVC (%)	BV and VVC (%)
1 st	15	4(26.67)	6(40)	1(6.67)
2 nd	77	23(29.87)	37(48.05)	13(16.88)
3 rd	59	20(33.90)	15(25.42)	7(11.86)
X ²		0.407	7.248	1.432
p-value		0.816	0.027*	0.489

Key:N=Number examined; BV%=Bacterial Vaginosis percentage;

VVC %= Vulvovaginal Candidiasis percentage; BV and VVC %=Bacterial vaginosis and Vulvovaginal Candidiasis percentage

Table.5 Distribution of Bacterial Vaginosis (BV), Vulvovaginal Candidiasis (VVC), and Mixed Infection in Pregnant Women According to Occupation

Occupation	N	BV (%)	VVC (%)	BV and VVC (%)
Merchant	67	16(23.88)	28(41.79)	10(14.93)
Food vendor	5	2(40)	2(40)	1(20)
Farmer	2	2(100)	0(0)	0(0)
Hairstylist	19	6(31.58)	8(42.11)	3(15.79)
Housewife	19	9(47.37)	7(36.84)	3(15.79)
Secretary	1	1(100)	0(0)	0(0)
Student	11	4(36.36)	4(36.36)	1(9.09)
Tailor	21	6(28.57)	9(42.86)	3(14.29)
Teacher	6	1(16.67)	0(0)	0(0)
X ²		11.592	6.266	1.995
p-value		0.170	0.617	0.981

Key:N=Number examined; BV%=Bacterial Vaginosis percentage;

VVC %= Vulvovaginal Candidiasis percentage; BV and VVC %=Bacterial vaginosis and Vulvovaginal Candidiasis percentage

Table.6 Distribution of *Candida* species according to Pregnancy Status in the study

Variables	Category	Pregnant (n=58) (%)	Non-pregnant (n=18) (%)	X ²	p-value
Species	<i>C. albicans</i>	44(75.86)	14(77.78)	3.962	0.555
	<i>C. dubliniensis</i>	3(5.17)	2(11.11)		
	<i>C. glabrata</i>	3(5.17)	2(11.11)		
	<i>C. krusei</i>	6(10.34)	0(0)		
	<i>C. parapsilosis</i>	1(1.72)	0(0)		
	Mixed	1(1.72)	0(0)		
Intensity of Growth	Heavy	11(18.97)	4(22.22)	0.092	0.762
	Moderate	47(81.03)	14(77.78)		

VVC is more common in pregnant women due to high concentration of reproductive hormones that increase the glycogen content in the vaginal tissue thereby providing a carbon source for *Candida* organism (Idowu *et al.*,

2022). Furthermore, although pregnant women have higher incidence for VVC, pregnant women are more often suffering from asymptomatic/-subclinical *Candida* spp. colonization than their non-pregnant counterparts,

thus making their diagnosis relatively difficult and late (Iyevhobu *et al.*, 2021; Leli *et al.*, 2013). In addition, as noted by Amalokwu *et al.*, (2019), in pregnancy, there is a rise in the overall numbers of vaginal flora compared to the non-pregnant state due mainly to an increase in *Lactobacilli* by approximately 10-fold. BV is characterized by a lower prevalence of *Lactobacilli* and a higher prevalence of anaerobic bacteria (Ravel *et al.*, 2021).

In this study, the overall (pregnant and non-pregnant) prevalence of BV/VVC co-infection was 9.55%. This prevalence is similar to the 10.7% prevalence in Nepal earlier reported by Iyevhobu (2020) and Gupta *et al.*, (2021) but lower than the 16% prevalence in Ghana; 33.1% in America; and 37.1% in Lagos; earlier reported by Rivers *et al.*, (2011); Aubyn and Tagoe (2013) and Oparaugo *et al.*, (2022) respectively. However, it is higher than the 5.0% reported by Huang *et al.*, (2023) in Taiwan. As noted by Oparaugo *et al.*, (2022), the differences observed can be as a result of type of study population, changes in vaginal flora and sexual activity. Furthermore, a major difference between this study and the fore mentioned studies is that in this case, all incidences of BV/VVC co-infection occurred among the pregnant women, who had a statistically significant prevalence rate of 13.91% (Iyevhobu *et al.*, 2021). This is higher than the 9.1% prevalence reported by Kamga *et al.*, (2019) in Cameroon, but lower than the 57.7% prevalence reported by Gaddar *et al.*, (2019) in Lebanon. Physiological changes in pregnancy may account for this higher prevalence of co-infection in pregnant women (Iyevhobu *et al.*, 2021).

In this study, there was no significant variation (p -value > 0.05) in the prevalence of BV, VVC, and BV/VVC co-infection with age in pregnancy. Other studies have also noted no significant variation of BV (Iyevhobu Kenneth Oshiohayamhe, 2020; Kamga *et al.*, 2019; Bhakta *et al.*, 2021) with age in pregnancy. However, some studies have noted a significant variation in BV (Ibrahim *et al.*, 2014; Aduloju *et al.*, 2019), and VVC (Okonkwo & Umeanaeto, 2010) infection in pregnant women. However, in this study the highest prevalence of BV and BV/VVC co-infection was among those aged 0-20 years (45% and 15% respectively), while VVC was more common in those >30 years (44.44%).

The higher prevalence of BV and VVC in young pregnant women could be because most women 0-20 years of age are having their first pregnancy and may not

have the necessary information to properly manage the case compared to their older counterpart (Iyevhobu *et al.*, 2021).

In the present study, the prevalence of BV, VVC, and BV/VVC co-infection was highest in the third (33.90%), second (48.05%), and second (16.88%) trimesters respectively. However, these differences with other trimesters were not statistically significant except for the prevalence of VVC with age which was found to be statistically significant. This is similar to the studies of Iyevhobu *et al.*, (2021) and Oviasogie & Okungbowa (2009) in Edo State, that also showed a higher prevalence of VVC during the second trimester. The higher prevalence of VVC and BV/VVC co-infection in pregnant women during the second trimester may be as a result of the balancing effect of both depleting immunity during the course of pregnancy (Abu-Raya *et al.*, 2020), as well as the role of antenatal care. As noted by Fagbamigbe *et al.*, (2021), most pregnant women in Edo State (63.0%) initiate antenatal care during the second trimester, and it is possible that cases of vaginal infections are properly managed before this trimester. The high prevalence BV/VVC during the second trimester may be as a result of the significant high prevalence of VVC in the same trimester. The higher prevalence of BV was also reported during the third trimester by Bhakta *et al.*, (2021). However, some studies have also noted a higher prevalence of BV and VVC during the second (Ibrahim *et al.*, 2014; Yalew *et al.*, 2022) and third trimester (Nelson *et al.*, 2013; Waikhom *et al.*, 2020) respectively. The differences in the study population may account for these discrepancies.

The present study showed that there was no significant variation in the prevalence of BV, VVC, and BV/VVC co-infection with occupation. However, co-infection was more common among food vendors (20%), followed by hair stylists and house wives (15.79%), merchants (14.93%), tailor (14.29%), and students (9.09%). The higher prevalence among food vendors, may be an artefact caused by their low number in the study population.

C. albicans was the most prevalent *Candida* species causing VVC in both pregnant and non-pregnant women- 75.86 and 77.78% respectively. *C. albicans* having the highest prevalence in this study corroborate with the findings of Idowu *et al.*, (2022) in Oyo State. However, in the studies of Emeribe *et al.*, (2015) in Abuja, a higher prevalence of *C. glabrata* was found. As noted by

Makanjuola *et al.*, (2018), *C. albicans* is known to be the primary pathogen responsible for the VC both in pregnant and non-pregnant women, however, an epidemiological shift toward non-albicans *Candida* (NAC) is now observed across the globe.

Other species of *Candida* isolated from pregnant women in this study include *C.krusei*, *C.dubliniensis*, *C. glabrata*, and *C. parapsilosis*; while non-pregnant women had only *C. dubliniensis* and *C. glabrata* in addition to *C. albicans*.

In conclusion, bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) and their co-infection are notable conditions in pregnant women with the potential of negatively affecting both maternal, foetal, and neonatal health. The overall prevalence of BV, VVC, and BV/VVC co-infection in this study was 21.36, 34.55 and 9.55% respectively. In pregnant women, it was 31.3 and 38.41, and 13.91% respectively. Pregnant women had a significantly higher prevalence of BV, VVC and BV/VVC co-infection compared to their non-pregnant counterparts. Only the prevalence of VVC varied significantly with trimester, with the second trimester recording the highest prevalence (48.05%). There was no significant variation in the prevalence of BV, VVC, and BV/VVC co-infection in pregnant women according to maternal age and occupation. *Candida albicans* was the most common species causing VVC in both pregnant (75.86%) and non-pregnant (77.78%) subjects, followed by *C. krusei* in pregnant and both *C. dubliniensis* and *C. glabrata* in non-pregnant subjects. Routine screening of bacterial vaginosis, vulvovaginal candidiasis and other vaginal infections should be conducted during antenatal clinic visit to enable early detection and treatment. Pregnant women in Esan West LGA and Edo State at large are encouraged to make early visit to antenatal care clinics, so as to diagnose any abnormal condition that could affect the normal course of pregnancy and delivery.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

Acknowledgements

The authors would like to acknowledge the management and all the technical staff of St Kenny Research Consult, Ekpoma, Edo State, Nigeria for their excellent assistance and for providing medical writing/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

Author Contributions

Adewuyi Gbolagade Morufu: Investigation, formal analysis, writing—original draft. Iyevhobu Kenneth Oshiokhayamhe: Validation, methodology, writing—reviewing. Adewuyi Bolanle Toyin:—Formal analysis, writing—review and editing. Momoh Abdul-Razak Mcsionel: Investigation, writing—reviewing. Sunday Olowo Samuel: Resources, investigation writing—reviewing. Obohwemu Kennedy Oberhiri: Validation, formal analysis, writing—reviewing. Edo Elvis: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

- Abdullahi, B. & Danyaya, R. (2021). Prevalence of *Candida albicans* and *Trichomonas vaginalis*. *Journal of Medical Laboratory Sciences (B.JNLS)*, 6(2),69-77.
- Abu-Raya, B., Michalski, C., Sadarangani, M.. & Lavoie, P. M. (2020). Maternal immunological adaptation during normal pregnancy. *Frontiers in Immunology*,

- 11, 2627.
<https://doi.org/10.3389/fimmu.2020.575197>
- Achdiat, P. A., Rowawi, R., Fakhrosa, I., Gunawan, H., Hindritiani, R., Suwarsa, O.,...Hastjarjari, A. D. (2018). High prevalence in asymptomatic females. *Serbian Journal of Dermatology and Venereology*, 11(2), 53-59.
- Adesiji, Y. O., Taiwo, S. S., Adekanle, D. A., Oboro, V. O., Fayemiwo, S. A., & Opaleye, O. O. (2007). Bacterial vaginosis and pregnancy outcome in Osogbo, Nigeria. *Research Journal of Medical Sciences (RJMS)*, 1(4), 195-198.
- Aduloju, O. P., Akintayo, A. A., & Aduloju, T. (2019). Prevalence of bacterial vaginosis in pregnancy in a tertiary health institution, south western Nigeria. *The Pan African Medical Journal*, 33, 9. <https://doi.org/10.11604/pamj.2019.33.9.17926>
- Alo M, Anyim C, Onyebuchi A, Okonkwo E. (2012). Prevalence of asymptomatic co-infection of candidiasis and vaginal trichomoniasis among pregnant women in Abakaliki, South-Eastern Nigeria. *J Nat Sci Res.*, 2(7), 87–91.
- Amalokwu, S., Okonta, P. I., & Ebinu, E. (2019). Prevalence of bacterial vaginosis among antenatal Nigeria. *Tropical Journal of Obstetrics and Gynaecology*, 36(1), 85-87. https://doi.org/10.4103/TJOG.TJOG_29_18
- Animasaun, O. P., Animasaun, O. S., Oladunni, O., Akomolafe, B. K., Iyevhobu, K. O., Bello, L.A., Olurinde, O.J., Oyebami, O.T., Ogundokun, O., Omolumen, B.A., Airhomwanbor, K.O., Ade-Balogun, T.D., Courage, O., Alonge, A.S., Ayodele, F.L., Amoo, E.D., Akanmu, M.O., Adepoju, O.T, Ameh, D.A. & James, D. B. (2023). Spousal and culture/religion influence on dietary pattern and the linkage between the dietary pattern and some biochemical parameters of pregnant women under PHCs in Oluyole LGA, Ibadan, Oyo State. *International Journal of Science Academic Research*, 04(11), 6545-6554.
- Aubyn, G. B. and Tagoe, D. N. A. (2013). Prevalence of Vaginal Infections and Associated Lifestyles of Students in the University of Cape Coast, Ghana. *Asian Pacific Journal of Tropical Disease*, 3, 267-270. [https://doi.org/10.1016/S2222-1808\(13\)60068-7](https://doi.org/10.1016/S2222-1808(13)60068-7)
- Ayotunde, T., Mary, O., Melvin, A. O., & Faniyi, F. F. (2009). Maternal age at birth and under-5 mortality in Nigeria. *East African Journal of Public Health*, 6(1), 11-14. <https://doi.org/10.4314/eajph.v6i1.45735>
- Babatope, I.O., Isabu, P.A., Imarenezor, E.P. K., Adesanya, T. M., & Ikimiukor, A.P. (2018). Normal CD4, CD8 T-lymphocytes and leucocyte baseline in healthy HIV-seronegative pregnant women in Ekpoma, Edo State, Nigeria. *International Journal of Basic, Applied and Innovative Research*, 7(1), 18-28.
- Bhakta, V., Aslam, S., & Aljaghawani, A. (2021). Bacterial vaginosis in pregnancy: prevalence and outcomes in a tertiary care hospital. *African Journal of Reproductive Health*, 25(1), 49–55. <https://doi.org/10.29063/ajrh2021/v25i1.6>.
- Bitew, A., Mengist, A., Belew, H., Aschale, Y., & Reta, A. (2021). The prevalence, antibiotic resistance from felegehiwot referral hospital, Ethiopia. *Infection and Drug Resistance*, 14, 2685-2696. <https://doi.org/10.2147/IDR.S305329>.
- Chan, M.Y. & Smith, M.A. (2018). Infections in pregnancy. *Comprehensive Toxicology*, 11, 232-249. <https://doi.org/10.1016/B978-0-12-801238-3.64293-9>
- Emeribe, A. U., Nasir, I. A., Onyia, J., & Ifunanya, A.L. (2015). Prevalence of vulvovaginal candidiasis among non-pregnant women attending a tertiary health care facility in Abuja, Nigeria. *Research and Reports in Tropical Medicine*, 6, 37-42. <https://doi.org/10.2147/RRTM.S82984>
- Fagbamigbe AF, Olaseinde O, Fagbamigbe OS. (2021). Timing of first antenatal care contact, its associated factors and state-level analysis in Nigeria: a cross-sectional assessment of compliance with the WHO guidelines. *BMJ Open*, 11(9), e047835. <https://doi.org/10.1136/bmjopen-2020-047835>
- Gaddar, N., El Roz, A., Ghsein, G., & Ibrahim, J. N. (2019). Emergence of vulvovaginal candidiasis among Lebanese pregnant women: prevalence, risk factors, and species distribution. *Infectious Diseases in Obstetrics and Gynaecology*, 2019, 78-84.
- Govender, L., Hoosen, A.A., Moodley, J., Moodley, P. & Sturm, A.W. (1996) Bacterial Vaginosis and Associated Infections in Pregnancy. *International Journal of Gynecology and Obstetrics*, 55, 23-28. [https://doi.org/10.1016/0020-7292\(96\)02744-0](https://doi.org/10.1016/0020-7292(96)02744-0).
- Greenbaum, S., Greenbaum, G., Moran-Gilad, J., & Weintraub, A. Y. (2019). Ecological dynamics of the vaginal microbiome in relation to health and disease. *American Journal of Obstetrics and Gynecology*, 220(4), 324-335. <https://doi.org/10.1016/j.ajog.2018.11.1089>
- Gupta, R. S., Bhargav, D., & Deep, J. P. (2021). Common types of vaginitis (bacterial vaginosis, vulvovaginal candidiasis, trichomoniasis) and their association with urinary tract infection among women visiting NMCTH, Birgunj. *International Journal of Current Microbiology and Applied Sciences*, 10(01), 996-

1010.
<https://doi.org/10.20546/ijcmas.2021.1001.121>
- Hadi, H. S., & AlSultany, S. J. (2020). Isolation and identification Candida species among renal failure Iraqi patients. *Drug Invention Today*, 14(6).
- Hay, P.E., Lamont, R.F., Taylor-Robinson, D., Morgan, D.J., Ison, C., & Pearson, J. (1994). Abnormal bacterial colonisation of the genital tract and subsequent pre-term delivery and late miscarriage. *British Medical Journal*, 308(6924), 295-298.
<https://doi.org/10.1136/bmj.308.6924.295>
- Huang, S.H., Hsu, H.C., Lee, T. F., Fan, H. M., Tseng, C. W., Chen, I. H.,... Hung, C. C. (2023). Bacterial vaginosis, and vulvovaginal candidiasis among women with vaginitis. *Microbiology Spectrum*, 11(3), e0016123.
<https://doi.org/10.1128/spectrum.00161-23>
- Ibrahim, S.M., Bukar, M., Galadima, G.B., Audu, B.M., & Ibrahim, H.A. (2014). Prevalence of bacterial vaginosis in pregnant women in Maiduguri, North-Eastern Nigeria. *Nigerian Journal of Clinical Practice*, 17(2), 154-158.
<https://doi.org/10.4103/1119-3077.127424>
- Idowu, M. O., Makinde, G. I., Oluranti, O. O., Adebayo, M. A., & Adekunle, O. A. (2022). Prevalence of vulvovaginal candidiasis among women attending clinics in selected Hospitals in Oyo State, Southwest, Nigeria. *Journal of Public Health and Epidemiology*, 14(1), 45-52.
<https://doi.org/10.5897/JPHE2021.1303>
- Iyevhobu Kenneth Oshiokhayamhe (2020). Assessment of the Co-existence of Typhoidal Antibodies and Malaria Parasitaemia among Pregnant Women. *Gulf Journal of Clinical Medicine and Medical Research*, 1(1), 06-11.
- Iyevhobu, K.O., Airefetalor A.I., Turay, A.A., Usoro, E.R. and Ken-Iyevhobu, B.A. (2021). Assessment of the Incidence of Candidiasis among Single and Married Women. *Scientific Research Journal of Medical Sciences*, 1(2), 16-20.
<https://doi.org/10.47310/srjms.2021.v01i02.004>
- Jeanmonod, R., & Jeanmonod, D. (2022). Vaginal candidiasis. In *StatPearls (Internet)*. StatPearls Publishing.
- Kamga, Y.M., Ngunde, J. P., & Akoachere, J. F. K. (2019). Prevalence of bacterial vaginosis and (KHD), Cameroon. *Biomedical Central Pregnancy and Childbirth*, 19(1), 1-8.
- Kourtis, A. P., Read, J. S., & Jamnison, D. J. (2014). Pregnancy and infection. *New England Journal of Medicine*, 370(23), 2211-2218.
<https://doi.org/10.1056/NEJMra1213566>
- Lawrence, U.C., Achi, O.K., Ifeanyi, O.E., & Queen, E. (2014). Prevalence of bacterial vaginosis among female students of Michael Okpara university of Agriculture, Umudike, Abia State, Nigeria. *Journal of Pharmacy and Biological Sciences*, 9(5), 39-52. <https://doi.org/10.9790/3008-09523952>
- Leli, C., Mencacci, A., Meucci, M., Bietolini, C., Vitali, M., Farinelli, S., ... Bistoni, F. (2013). Association of pregnancy and Candida vaginal colonization in women with or without symptoms of Vulvovaginitis. *Minerva Ginecologica*, 65(3), 303-309.
- Makanjuola, O., Bongomin, F., & Fayemiwo, S. A. (2018). An Update on the Roles of Non-*albicans* Candida Species in Vulvovaginitis. *Journal of fungi (Basel, Switzerland)*, 4(4), 121.
<https://doi.org/10.3390/jof4040121>
- Minkoff, H., Grunebaum, A. N., Schwarz, R. H., Feldman, J., Cummings, M., Crombleholme, W., Clark, L., Pringle, G. & McCormack, W.M. (1984). Risk Factors for Prematurity and Premature Rupture of Membranes: A Prospective Study of the Vaginal Flora in Pregnancy. *American Journal of Obstetrics and Gynecology*, 150, 965-972.
[https://doi.org/10.1016/0002-9378\(84\)90392-2](https://doi.org/10.1016/0002-9378(84)90392-2)
- Mohamed AO, Mohamed MS, Mallhi TH, Hussain MA, Jalloh MA, Omar KA, *et al.*, (2022). Prevalence of vulvovaginal candidiasis among pregnant women in Africa: a systematic review and meta-analysis. *J Infect Developing Ctries.*, 16(08), 1243–1251.
<https://doi.org/10.3855/jidc.15536>.
- Nelson, M., Wanjiru, W. & Muturi, M.W. (2013). Prevalence of Vaginal Candidiasis and Determination of the Occurrence of Candida Species in Pregnant Women Attending the Antenatal Clinic of Thika District Hospital, Kenya. *Open Journal of Medical Microbiology*, 3, 264-272.
<http://dx.doi.org/10.4236/ojmm.2013.34040>
- Nigean W, Bhat AS, Gulzar K, Taing S. (2015). Correlation of bacterial vaginosis with preterm labour: a case control study. *Int J Reprod Contracept Obstet Gynecol*, 4, 1868-1874.
<http://dx.doi.org/10.18203/2320-1770.ijrcog20151276>
- Okonkwo N.J. & Umeanaeto P.U. (2010). Prevalence of Vaginal Candidiasis among Pregnant Women in Nnewi Town of Anambra State, Nigeria. *African Research Review*, 4 (4), 539-548.
<https://doi.org/10.4314/afrev.v4i4.69250>
- Olowe, O.A., Makanjuola, O. B., Olowe, R., & Adekanle, D. A. (2014). Prevalence of vulvovaginal in Southwestern Nigeria. *European Journal of*

- Microbiology & Immunology*, 4(4), 193-197.
<https://doi.org/10.1556/EUJMI-D-14-00027>
- Oparaugo, C. T., Iwalokun, B. A., Nwaokorie, F. O., Okunloye, N. A., Adesesan, A. A., Edu-Muyideen, I.O.,...Deji-Agboola, M. A. (2022). Occurrence and clinical characteristics of vaginitis among women of reproductive age in Lagos, Nigeria. *Advances in Reproductive Sciences*, 10(4), 91-105.
<https://doi.org/10.4236/arsci.2022.104009>
- Oviasogie, F. E., & Okungbowa, F.I. (2009). Candida species amongst pregnant women in Benin city, Nigeria: effect of predisposing factors. *African Journal of Clinical and Experimental Microbiology*, 10(2),92-98.
<https://doi.org/10.4314/ajcem.v10i2.7511>
- Ravel, J., Moreno, I., & Simón, C. (2021). Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. *American Journal of Obstetrics and Gynaecology*, 224(3), 251-257.
<https://doi.org/10.1016/j.ajog.2020.10.019>.
- Rivers CA, Adaramola OO, Schwebke JR. (2011). Prevalence of bacterial vaginosis and vulvovaginal candidiasis mixed infection in a southeastern American STD clinic. *Sex Transm Dis.*, 38(7), 672-674.
<https://doi.org/10.1097/OLQ.0b013e31820fc3b8>.
- Russo, R., Edu, A., & De Seta, F. (2018). Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. *Archives of Gynecology and Obstetrics*, 298, 139-145. <https://doi.org/10.1007/s00404-018-4771-z>.
- Sabour, S., Arzanlou, M., Vaez, H., Rahimi, G., Sahebkar, A., & Khademi, F. (2018). Prevalence of bacterial vaginosis in pregnant and non-pregnant Iranian women: a systematic review and meta-analysis. *Archives of Gynaecology and Obstetrics*, 297(5), 1101-1113. <https://doi.org/10.1007/s00404-018-4722-8>.
- Sobel, J. D., Subramanian, C., Foxman, B., Fairfax, M., & Gyax, S. E. (2013). Mixed vaginitis-more of vaginal fluid. *Journal of Clinical Microbiology*, 18(1), 170-177.
- Thorsen, P., Vogel, I., Molsted, K., Jacobsson, B., Arpi, M., Møller, B. R., & Jeune, B. (2006). Risk factors for bacterial vaginosis in pregnancy: a population-based study on Danish women. *ActaObstetricia et Gynecologica Scandinavica*, 85(8),906-911.
<https://doi.org/10.1080/00016340500432655>.
- Ugwa, E.A. (2015). Vulvovaginal candidiasis in Aminu Kano Teaching Hospital, North-West Nigeria: Hospital-Based Epidemiological Study. *Annals of Medical and Health Sciences Research*, 5(4),274-278. <https://doi.org/10.4103/2141-9248.160185>
- Waikhom, S.D., Afeke, I., Kwawu, G. S., Mbroh, H. K., Osei, G. Y., Louis, B.,...Opintan, J.A. (2020). Prevalence of vulvovaginal candidiasis among pregnant women in the Ho municipality, Ghana: species identification and antifungal susceptibility of Candida isolates. *Biomedical Central Pregnancy and Childbirth*, 20, 1-14.
- Wariso, K., A. Igunma, J., L. Oboro, I., A. Olonipili, F., & Robinson, N. (2017). Prevalence of Bacterial Vaginosis among Patients with Vulvovaginitis in a Tertiary Hospital in Port Harcourt, Rivers State, Nigeria. *Asian Journal of Medicine and Health*, 7(4), 1-7.
<https://doi.org/10.9734/AJMAH/2017/36736>
- Yalew, G.T., Muthupandian, S., Hagos, K., Negash, L., Venkatraman, G., Hagos, Y.M.,...Saki, M. (2022). Pregnant women from northern Ethiopia: A cross-sectional study. *PloS One*, 17(2), e0262692.
<https://doi.org/10.1371/journal.pone.0262692>.

How to cite this article:

Adewuyi Gbolagade Morufu, Iyevhobu Kenneth Oshiokhayamhe, Adewuyi Bolanle Toyin, Momoh Abdul-Razak Mesionel, Samuel Olowo Sunday, Obohwemu Kennedy Oberhiri and Edo Elvis. 2024. Coinfection of Bacterial Vaginosis and Candidiasis in Expectant Mothers with Vaginal Discharge attending Primary Health Center at Edo Central Senatorial District, Nigeria. *Int.J.Curr.Microbiol.App.Sci*. 13(11): 9-20.

doi: <https://doi.org/10.20546/ijcmas.2024.1311.002>