

Comparative Study of Vaginitis and Candida in Sexually Active Women in Traditional Sprawling Town in the Niger Delta, South – South Nigeria

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Research Article

ABSTRACT

This study was carried out to investigate the aetiologic agents of vaginitis in sexually active women in Ikot Ekpene, Akwa Ibom State, Nigeria. The subjects were 150 randomly selected sexually active women attending antenatal, postnatal, gynaecology and family planning clinics in the Department of Obstetrics and Gynaecology of General Hospital Ikot Ekpene, Akwa Ibom State. Two high vaginal swab samples were taken from each pregnant and non-pregnant woman, which translated to 80 samples from pregnant and 70 samples from non-pregnant women. Microscopy and culture including biochemical tests were done for the isolation and identification of organisms. Questionnaires assessing socio-demographic characteristics of the patients were administered. The prevalence of various aetiologic agents was found to be higher in pregnant women than in non-pregnant women. *Candida albicans* was more prevalent in pregnant women. Twenty-eight samples (35.0%) from the pregnant women yielded *C. albicans* with $p < 0.05$. Age range of 16-30 years and ≥ 45 years had high prevalence of *C. albicans*, though more (40%) in pregnant women. While non-pregnant women in the age group 16-30 years had the highest prevalence of *G. vaginalis*, 8 (26.7%). Among pregnant women, the widowed had a higher prevalence rate of *C. albicans* infection (50%) which was not statistically significant. The divorced non-pregnant women had the highest prevalence of *C. albicans* with $p < 0.05$ which was statistically significant. This study also revealed that pregnant women in their second trimester of pregnancy had more *C. albicans* infection, 15 (46.9%) when compared to other trimesters of pregnancy. Women who were pregnant for the first time or primigravidae had higher prevalence of *C. albicans*, 13 (37.1%) when compared to others. Pregnant women who use contraceptives prior to their recent pregnancies were found to have more infections of *G. vaginalis*, 14 (93.3%). Non-pregnant women on antibiotics had increased prevalence of *C. albicans*, 44.4%. The highest prevalence of *C. albicans* and *T. vaginalis*, 5 (6.3%) coinfection was seen in pregnant women. Irrespective of the women's status, none were coinfecting with the three microbial agents.

INTRODUCTION

Vaginal infections are the most common women's health problem and have been increasingly linked to a growing array of serious health risks. It is known medically as vaginitis which is a common gynaecologic disorder that is responsible for 10 million office visits to physicians each year in Nigeria. Bacterial Vaginosis (BV) is usually caused by *Gardnerella vaginalis*, *Mycoplasma hominis* and various anaerobic bacteria including *Mobiluncus* species and *Prevotella* species (Sexually Transmitted Diseases Treatment Guidelines, 2010). The important causes of vaginal infection are trichomoniasis disease due to *Trichomonas vaginalis*, candidiasis due to *Candida albicans* and bacterial vaginosis due to proliferation of endogenous vaginal flora of *Gardnerella vaginalis*, anaerobes and *Mycoplasma hominis*. Infectious vaginitis is the most common cause of vaginal discharge. Bacterial vaginosis occurs when the normal lactobacilli of the vagina are replaced by mostly anaerobic bacteria (Hainer and Gibson, 2011).

Typical symptoms of vaginal infections are itching, burning, painful urination, pain during sexual intercourse and increased vaginal discharge (fluorine). The vast majority of women will experience vaginitis at some point during their lives, and the unpleasant symptoms usually send women to their doctors for treatment. Its prevalence increases during pregnancy and it facilitates infection with HIV and other sexually transmitted infection (World Health Organization, 2001).

Trichomoniasis is a common sexually transmitted disease (STD) that affects both women and men, although symptoms are more common in women. An estimated 7.4 million new cases occur each year in women and men in Africa (Centers for Disease Control

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and Prevention (CDC), 2010). Trichomonal infection has been encountered in every continent and climate and has no seasonal variability. It has been identified in all racial groups and socio-economic strata (Sood and Kapil, 2008).

Vaginal trichomoniasis infects 180 million individuals worldwide and it is one of the most frequent and widespread sexually transmitted infections/diseases. In Africa, it is estimated that 2-50% of the population carry the infection. The main signs of trichomonas infection in women are abdominal pain, itching, yellow or green frothy discharge and presence of foul-smelling discharge with abundant leucocytes. The vagina is the most common site of infection in women (Uneke et al., 2006).

Gardnerella vaginalis is a bacterium that can cause a wide diversity of diseases, including bacterial vaginosis (BV). Bacterial vaginosis is caused by a change or imbalance in the types of the bacteria normally found in the vagina and causes an overgrowth of organisms such as *Gardnerella vaginalis*. It is named after Hermann L. Gardner (1918-2005), an American Bacteriologist who discovered it in 1955. This disease used to be called non-specific vaginitis and is the most common cause of vaginitis. It occurs in (1/3) one-third of adult women.

Up to 95% of women with bacterial vaginosis harbour *Gardnerella vaginalis*. Normal vaginal flora consists of a dynamic equilibrium between Lactobacilli on one side and these opportunistic pathogens on the other. When the balance of the vaginal microflora is disturbed, the number of Lactobacilli declines and the amount of *Gardnerella vaginalis* and anaerobic bacteria increase (Mekkes, 2003). *Trichomonas vaginalis* and *Gardnerella vaginalis* have similar clinical presentations and can cause a frothy gray or yellow-green vaginal discharge, pruritus.

Candidiasis or yeast infections occur when the normal vaginal environment is disrupted, or the immune system is weakened and cannot stop the yeast from proliferating. Vaginitis is commonly caused by an overgrowth of yeast (*Candida albicans*), which occur when the delicate balance of organisms in the vagina is upset. *C. albicans* is the second most common cause of vaginitis, about 80% of yeast infections are caused by *Candida albicans* while *C. glabrata* and *C. tropicalis* are found less frequently (Physicians Desk Reference Health, 2012). Approximately 10%–20% of women harbour *Candida* species and other yeasts in the vagina. Women who experience chronic yeast infections often have a common history of being on repeated courses of antibiotics. While antibiotics may destroy harmful bacteria, they also have the potential to wipe out helpful normal flora such as *Lactobacillus* in the vagina, as well as in the urinary and digestive tracts. (Ries, 1997).

Nearly 75% of all adult women have had at least one genital yeast infection in their lifetime. Factors that may upset the balance and lead to yeast infection include pregnancy, obesity, diabetes, birth control pills, steroids, prolonged exposure to moisture and poor feminine hygiene. The vaginal discharge frequently appears as a thick cottage cheese-like discharge, containing epithelial cells and masses of budding yeasts, hyphae/pseudohypha. Common symptoms may include vaginal itch, soreness, burning, pain during intercourse and intense pruritus of the vulva and erythematous vagina and labia. (Ries, 1997). *Gardnerella vaginalis*, trichomoniasis, and candidiasis are the three most common types of vaginal infections. Of the millions of cases of vaginitis each year, most are caused by *G. vaginalis* (about 40% to 50%), followed by yeast infections (20% to 25%) and then trichomoniasis (15% to 20%) (Physicians Desk Reference Health, 2012).

METHODOLOGY

Location and Sample Collection

The study was conducted in General Hospital, Ikot Ekpene, Akwa Ibom State. It is located in the South-South geopolitical zone of Nigeria. Ikot Ekpene Local Government Area lies between latitudes 5° 10' and 5° 30' North and longitudes 7° 30' and 7° 45' East.

The study population consisted of women (pregnant and non-pregnant) seen in general out-patient, gynaecological out-patient clinics, family planning clinics, women of child bearing age ranges from sixteen (16) to fifty-five (55) years and most sexually active women. Participants sampled included all married women, pregnant and non-pregnant women. Women of childbearing age (16-55) years who gave informed consent were eligible for inclusion. Subjects excluded for this study included children (0-15 years) and menopausal women.

Sample was collected from randomly selected women attending the out-patients Gynaecology and Antenatal Clinic of General Hospital, Ikot Ekpene. Two high vaginal swabs (HVS) were collected from each woman; the first swab was for microscopical wet smear examination and gram staining technique while the second swab was used for culture, using a sterile speculum for HVS. Questionnaires were administered to collect data on socio-demographic and obstetric history, etc. This was done by the attending Clinician or Laboratory Scientist on duty.

Laboratory Analysis

Collection of vaginal discharge to detect *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Candida albicans* was carried out according to the method described by [8]. Sterile swab stick was used to collect a specimen from the vagina. Two high vaginal swab (HVS) were collected from each pregnant and non-pregnant women. One sample was put in a bijoux bottle containing Amies transport medium. Sterile swab stick was cut to allow the bottle top to be replaced tightly. The swab stick was protected from direct light and heat by putting the sample into improvised icepack and transfer to the laboratory within 24 hours. After inoculation into Amies medium, the sample was stored at 2-8°C.

Wet Mount Preparation to Detect Motile *T. vaginalis*

Each sample was transferred from a sterile swab stick to a clean grease free microscopic slide. A thin smear was made; a drop of normal saline was added and mixed to wet the swab. A coverslip was used to cover the slide. The slide was labelled and

examined microscopically for the characteristic jerking movement of *T. vaginalis* (Franklin and Monif, 2000).

Gram Staining

To another slide containing the specimen a thin smear was made. The smear was allowed to air-dry, protected from dust and insects. The slide was labelled for Gram staining. Using pasteur pipette the smear were flooded with crystal violet stain for one minute (1 min) and washed with water. The smear again were flooded with Grams iodine solution for 1 minute then washed with water. Ethyl alcohol was added to decolourize the smear for 20 seconds, and then washed the smear with water. The smear was counter stained with Safranin for 1 minute, then washed with water and allowed to air dry. A drop of oil immersion was placed on the smear and viewed under the microscope using x100 objective. The gram-positive organisms retained the purple colour of the crystal violet while gram negative appeared red, taking the colour of the safranin (Cheesbrough, 2004). Gram stain morphology showing Gram-variable bacillary forms indicate the present of *Gardnerella vaginalis*.

Microscopy

Trichomonas vaginalis was viewed using x10-x40 objectives for jerking movement of *T. vaginalis* trophozoites. *C. albicans* was viewed using x100 objective for ovoid budding yeast and pseudohyphae. *G. vaginalis* was viewed using x100 objective. A drop of oil immersion was placed on the smear to view gram variable rods, epithelial cells with adhering polymorphic bacteria (clue cells). Cheesbrough, (2004).

Culture and Identification of *Gardnerella vaginalis*

Bijou bottle containing Amies medium were brought out with HVS sample from improvised icepack and kept on a working bench to assume a temperature of 35-37 °C. Plate containing *Gardnerella* selective agar with 5% human blood was inoculated with HVS swab (Oxoid and Remel, 2013). Sterile wireloop was used to cross streak from primary to secondary and tertiary and incubate at 37 °C for 24-48 hours in an atmosphere containing 7% CO₂ (carbon IV oxide). The plate was examined for colonies showing diffused beta (β) haemolysis which indicate the presence of *Gardnerella vaginalis*. The plate was subcultured to obtain pure colonies in case of a mixed growth. The pure isolates were gram stained.

Isolation of *C. albicans*

Plate containing Sabouraud Dextrose Agar (SDA) was used. The plate was inoculated with HVS swab sample. Sterile wire loop was used to cross streak from primary to secondary and tertiary and incubated at 37 °C for 24-48 hours.

Simple Germ Tube Test (A Confirmatory Test for *C. albicans*)

A total of 0.5ml of human serum was pipetted into a small test tube. The serum was inoculated with a yeast colony from the culture plate using sterile wire loop. The test tube containing serum and yeast culture (inoculum) were incubated at 35-37 °C for 2-3 hours. Using a Pasteur pipette, a drop of inoculum was placed on a grease free microscopic slide. A drop of lactophenol cotton blue was added to stain the yeast cells, and then covered with a coverslip. The slide was labelled and examined microscopically using x10 - x40 objectives to give a good contrast. Any sprouting yeast-like cells with tube-like outgrowth (germ tubes) seen indicated the presence of *C. albicans*, and absence of germ tubes indicated the presence of yeast.

Biochemical Identification

Catalase Test for *G. vaginalis*

About 2-3ml of Hydrogen Peroxide (H₂O₂) solution was poured into a test tube. A sterile wooden stick was used to remove several colonies of the test organism and immersed in H₂O₂ solution. The presence of gas bubbles indicated catalase positive while absence of gas bubble indicated catalase negative (Cheesbrough, 2004).

Oxidase Test

A total of 1g of oxidase reagent (tetramethylphenylene diaminedehydrochloride) was weighed and dissolved in 100ml of distilled water. Whatman No.1 filter paper was soaked in 1% of the oxidase reagent. The isolates were streaked on the soaked filter paper and deep purple colouration within 10 seconds was taken as positive while the absence of deep purple colouration was oxidase negative (Agwung-Fobellah and Kemajou, 2007).

Statistical Analysis

The data was processed using SPSS (Statistical Product for Service Solution) version 17. Proportions were compared using Chi-square with values put at $p < 0.05$.

RESULTS AND DISCUSSION

A total of one hundred and fifty samples were investigated, 80 samples for pregnant women, 70 samples for non-pregnant women in South-South Nigeria. Out of the 80 samples for pregnant women, a total of 55 were positive isolates for *Candida albicans*, *Gardnerella vaginalis* and *Trichomonas vaginalis*, and out of 70 samples for non-pregnant women, a total of 28 isolates were positive for the three aetiologic agents of vaginitis respectively. Table 1 shows the prevalence of three aetiologic agents in pregnant and non-pregnant women. The table shows that the prevalence of *C. albicans* was 28 (35.0%) and 10 (14.3%), *G. vaginalis* 17 (21.3%) and 11 (15.7%) and *T. vaginalis* 10 (12.5%) and 7 (10.0%) for pregnant and non-pregnant women, respectively. The prevalence of the aetiologic agents was higher in pregnant than in the non-pregnant women. The pregnant women had the higher prevalence, 28 (35.0%) of *C. albicans*, with p-value (0.004) which was statistically significant (p-value <

0.05).

Table 2 shows the aetiologic agents among age groups in non-pregnant women. In non-pregnant women age group 16 – 30 years showed the higher prevalence of 20.0%, 26.7% and 16.7% for *C. albicans*, *G. vaginalis* and *T. vaginalis*, respectively. There was no statistically significant ($p > 0.05$) relationship between age and vaginitis aetiologic agent distribution in non-pregnant women. Figure 1 shows the distribution of aetiologic agents based on age groups among pregnant women. In pregnant women age group 16 – 30 years and ≥ 45 years showed equal prevalence of *C. albicans*, 40% respectively. Age group 31 – 44 years has the highest prevalence, 28.0% of *G. vaginalis* while the ≥ 45 years has the highest prevalence of 30.0% of *T. vaginalis*. The differences in the prevalence of these aetiological agents among the age groups were statistically not significant ($p > 0.05$).

Figure 2 shows the observed frequency of *C. albicans*, *G. vaginalis*, and *T. vaginalis* based on the marital status of the subjects. In pregnant women, 50% of the widowed were *C. albicans* positive which was the highest prevalence of *C. albicans* in the group, 33.3% of married women were *G. vaginalis* positive while widows showed the highest prevalence, 37.5% of *T. vaginalis*. The Chi-square analysis showed that there was no statistically significant difference ($p > 0.05$) in the prevalence of all the aetiologic agents in pregnancy.

Figure 3 shows the distribution of aetiologic agents based on marital status among non-pregnant women. In non-pregnant women, the divorced showed the highest prevalence, 62.5% and 37.5% of *C. albicans* and *G. vaginalis*, respectively. There was statistically significant difference ($p < 0.05$) in the prevalences of *C. albicans* and *T. vaginalis* by marital status. The prevalence of *T. vaginalis* was higher, 57.1% in widowed than other groups.

Table 3 shows the distribution of aetiologic agents based on trimesters of pregnancy. In the distribution of the *C. albicans*, second trimester, had the highest prevalence 46.9% followed by third trimester. The prevalence for the second and third trimesters were significantly greater than the first trimester. The odd of second and third trimester to be *C. albicans* positive were 8 and 6 times that of the first trimester. The third trimester showed the highest prevalence of *G. vaginalis*. The Chi-square analysis shows that the prevalence of *G. vaginalis* was significantly higher in the third trimester. The odd of third trimester to be *G. vaginalis* positive was 16 times that of the first trimester. The prevalence of *T. vaginalis* in first, second and third trimesters were 14.3%, 15.6% and 7.4%, respectively. The differences were statistically not significant ($p > 0.05$).

Table 4 shows the Co-infection of the three aetiologic agents in pregnant and non-pregnant women. In non-pregnant women, the only co-infection present was *C. albicans* and *T. vaginalis* and this was seen in 1.4% of non-pregnant women. The co-infections observed in pregnant women were 6.3%, 2.5%, 2.5% for *C. albicans* and *T. vaginalis*, *C. albicans* and *G. vaginalis*, *G. vaginalis* and *T. vaginalis*, respectively. Irrespective of the women’s status none were co-infected with the three microbial agents. The relationship between aetiologic agents of vaginitis and parity are shown in Table 5. Non-pregnant women had the highest prevalence of *C. albicans*, 15.4% while the prevalence of *T. vaginalis* and *G. vaginalis* were higher in primigravid women, 12.9% and 16.1%, respectively. In pregnant women, the prevalence of these vaginitis aetiologic agents were higher in primigravidae, *C. albicans* 37.1%, *T. vaginalis* 17.1% and *G. vaginalis* 31.4%, respectively.

The relationship between aetiologic agents of vaginitis and contraceptives use (Table 6). The result showed that women who use contraceptives have the highest prevalence, 15.8%, 10.5% and 36.8% for *C. albicans*, *T. vaginalis* and *G. vaginalis*, respectively. The prevalence of the *C. albicans*, 40.0% and *T. vaginalis*, 13.8% were higher in pregnant women who do not use contraceptives while 93.3% of women who use contraceptives were infected with *G. vaginalis*. The relationship between antibiotic intake and vaginitis in pregnant and non-pregnant women was shown in Table 7. Among non-pregnant women, those who took antibiotics had the highest prevalence of *C. albicans*, (44.4%) *T. vaginalis*, (22.2%) and *G. vaginalis*, (33.3%). In pregnant women, the prevalence of *T. vaginalis*, 14.0% was higher in those who do not take antibiotics.

Table 8 shows the relationship between douching and vaginitis in pregnant and non-pregnant women. Both in non-pregnant and pregnant women, those who applied douching had the highest prevalence of *C. albicans*, *T. vaginalis* and *G. vaginalis*. In pregnant women, 60.0%, 20.0% and 32.0% were the prevalences of *C. albicans*, *T. vaginalis* and *G. vaginalis*, respectively.

Agents	Pregnant Women	Non-Pregnant Women (n=70)	Total	Chi-square (x2)	P-value
	(n=80)	No. (%)	No. (%)		
	No. (%)				
<i>C. albicans</i>	28 (35.0)	10 (14.3)	38 (25.3)	8.47	0.004
<i>G. vaginalis</i>	17 (21.3)	11 (15.7)	28 (18.7)	0.75	0.39
<i>T. vaginalis</i>	10 (12.5)	7 (10.0)	17 (11.3)	0.23	0.63

Table 1. The prevalence of three aetiologic agents of vaginitis and Candida in pregnant and non-pregnant women.

Age group	No. tested	C. albicans	G. vaginalis	T. vaginalis
			No. (%)	No. (%)
16 – 30	30	6 (20.0)	8 (26.7)	5 (16.7)
31 – 44	25	4 (16.0)	2 (8.0)	2 (8.0)
≥ 45	15	0 (0)	1 (6.7)	0 (0)
Chi-square (χ^2)		3.36	4.28	2.73
p-value		0.19	0.12	0.23

Table 2. The distribution of aetiologic agents of vaginitis by age of non-pregnant women.

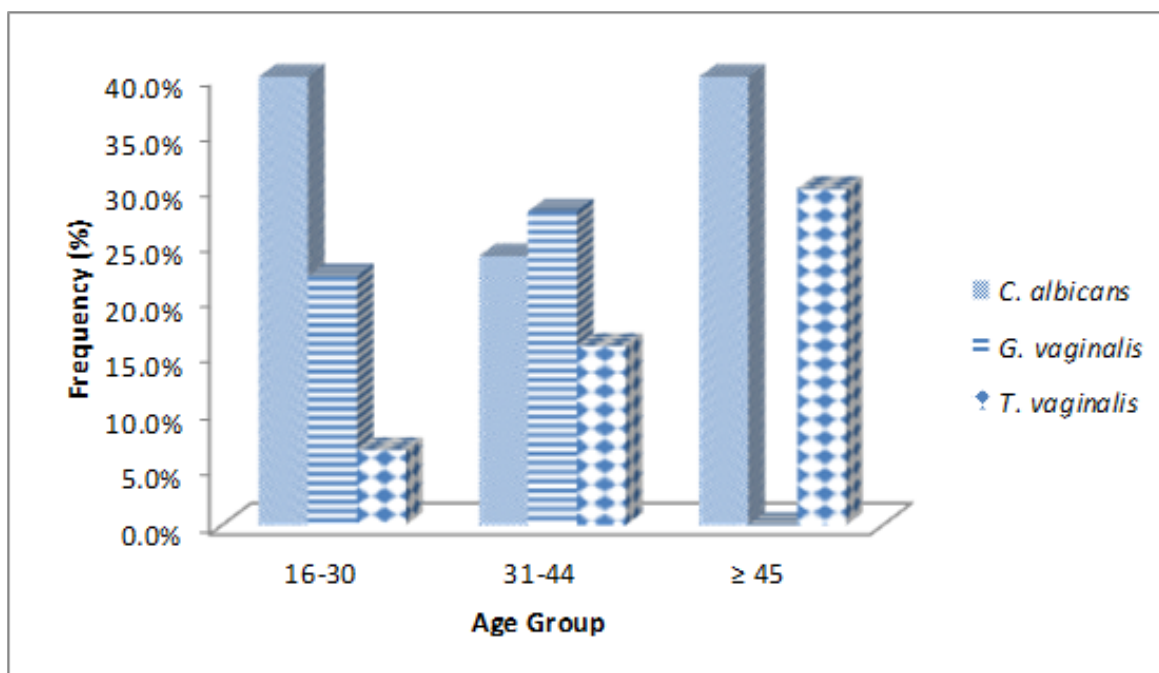


Figure 1. The distribution of aetiologic agents based on age groups among pregnant women.

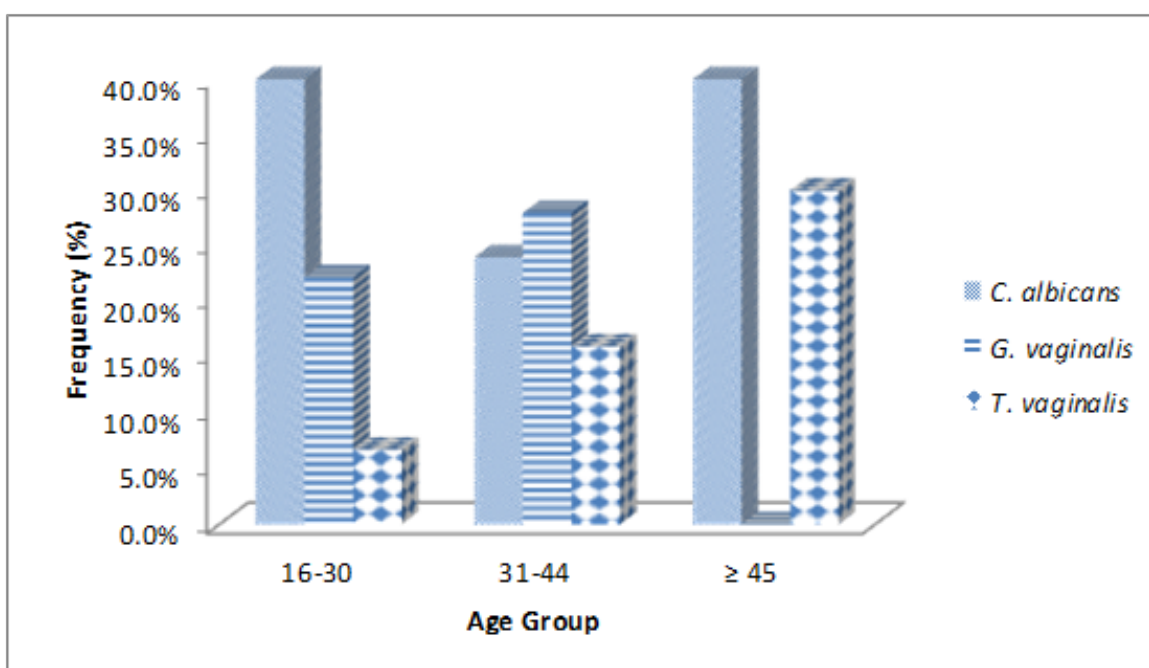


Figure 2. The distribution of aetiologic agents based on marital status among pregnant women.

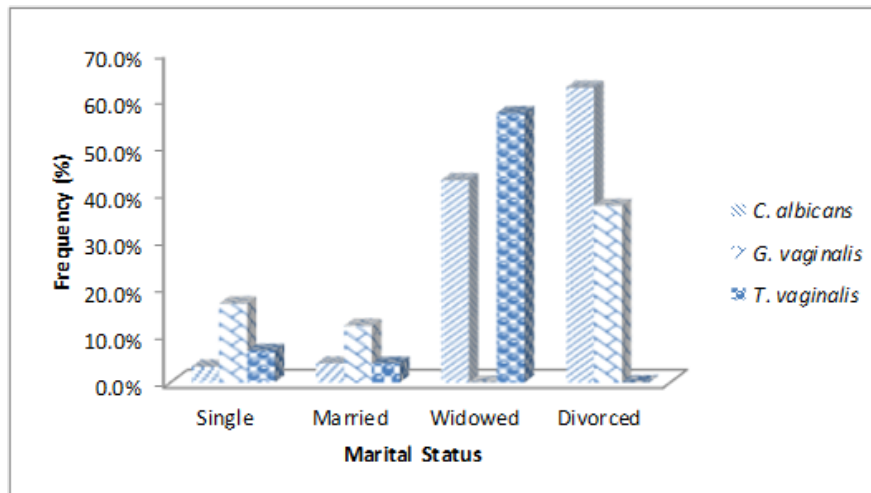


Figure 3. The distribution of aetiologic agents based on marital status among non-pregnant women.

Trimester status	No. tested	C. albicans	G. vaginalis	T. vaginalis
		No. (%)	No. (%)	No. (%)
First	21	2 (9.5)	1 (4.8)	3 (14.3)
Second	32	15 (46.9)	4 (12.5)	5 (15.6)
Third	27	11 (40.7)	12 (44.4)	2 (7.4)
Chi-square (x2)		8.37	13.56	1.05
p-value		0.02	0.001	0.69

Table 3. The distribution of aetiologic agents based on trimesters of pregnancy

Pregnancy Status	C. albicans and T.vaginalis	C. albicans and G. vaginalis	G. vaginalis and T. vaginalis
	No. (%)	No. (%)	No. (%)
Non-Pregnant	1 (1.4)	0 (0)	0 (0)
Pregnant	5 (6.3)	2 (2.5)	2 (2.5)

Table 4. Co-infections with the aetiologic agents in pregnant and non-pregnant women

Gravidity status	No. tested	Non-pregnant women			No. tested	Pregnant women		
		C. a.	T. v.	G. v.		C. a.	T. v.	G. v.
		No.(%)	No. (%)	No.(%)		No.(%)	No.(%)	No.(%)
Primigravid	31	4 (12.9)	4(12.9)	5 (16.1)	35	13 (37.1)	6 (17.1)	11(31.4)
Multigravid	39	6 (15.4)	3 (7.7)	6 (15.4)	45	16 (35.6)	4 (8.9)	6 (13.3)
Total			7(10.0)	11(15.7)	80	29 (36.3)	10(12.5)	17(21.3)

Table 5. The relationship between aetiologic agents of vaginitis and parity.

Key: C. a. = *Candida albicans*

T. v. = *Trichomonas vaginalis*

G. v. = *Gardnerella vaginalis*

Contraceptive	No. tested	Non-pregnant women			No. tested	Pregnant women		
		C. a.	T. v.	G. v.		C. a.	T. v.	G. v.
		No. (%)	No.(%)	No. (%)		No. (%)	No. (%)	No. (%)
Yes	19	3 (15.8)	2(10.5)	7 (36.8)	15	3 (20.0)	1 (6.7)	14(93.3)
No	51	7 (13.7)	5 (9.8)	4 (7.8)	65	26(40.0)	9 (13.8)	3 (4.6)
Total	70	10(14.3)	7(10.0)	11(15.7)	80	29(36.3)	10(12.5)	17(21.3)

Table 6. The relationship between aetiologic agents of vaginitis and contraceptives use.

Key: C. a. = *Candida albicans*

T. v. = *Trichomonas vaginalis*

G. v. = *Gardnerella vaginalis*

Antibiotic	No. tested	Non-pregnant women			No. tested	Pregnant women		
		C. a.	T. v.	G. v.		C. a.	T. v.	G. v.
		No. (%)	No.(%)	No. (%)		No. (%)	No. (%)	No. (%)
No	61	6 (9.8)	5 (8.2)	8 (13.1)	57	13(22.8)	8 (14.0)	10 (17.5)
Yes	9	4 (44.4)	2(22.2)	3 (33.3)	23	16(69.6)	2 (8.7)	7 (30.4)
Total	70	10(14.3)	7(10.0)	11(15.7)	80	29(36.3)	10(12.5)	17 (21.3)

Table 7. The relationship between antibiotic intake and vaginitis in pregnant and non- pregnant women.

Key: C. a. = *Candida albicans*

T. v. = *Trichomonas vaginalis*

G. v. = *Gardnerella vaginalis*

Douching	No. tested	Non-pregnant women			No. tested	Pregnant women		
		C. a.	T. v.	G. v.		C. a.	T. v.	G. v.
		No. (%)	No.(%)	No. (%)		No. (%)	No. (%)	No. (%)
No	55	7 (12.7)	4 (7.3)	7 (12.7)	55	14(25.5)	5 (9.1)	9 (16.4)
Yes	15	3 (20.0)	3(20.0)	4 (26.7)	25	15(60.0)	5 (20.0)	8 (32.0)
Total	70	10(14.3)	7(10.0)	11(15.7)	80	29(36.3)	10(12.5)	17(21.3)

Table 8. The relationship between douching and vaginitis in pregnant and non-pregnant women.

Key: C. a. = *Candida albicans*

T. v. = *Trichomonas vaginalis*

G. v. = *Gardnerella vaginalis*

Vaginitis originating from pathogens is a common and serious health problem among women of childbearing age. *Gardnerella vaginalis*, *Candida albicans* and *Trichomonas vaginalis* are the most common pathogens responsible for vaginitis especially in pregnant and non-pregnant women. These organisms may occur as single infection or as co-infections. The pregnant women had the higher prevalence of *C. albicans*, 35.0% ($p < 0.05$) which is in line with that reported by Hay and Czeizel (2007). The finding of this study establishes the fact that candidiasis is the highest cause of vaginitis among pregnant women of General hospital, Ikot Ekpene. This can be attributed to the weakened immune system due to pregnancy which leads to a decrease in acidity (higher pH) because of an increase in the amount of intracellular glycogen and also unhygienic facilities in the antenatal toilets and bathrooms.

Pregnant women within the age group, 16 - 30 and ≥ 45 years showed equal prevalence of *C. albicans*, (40%) while those within 31 - 44 years had the lowest prevalence, 24.0%. Age group, 31 - 44 years had the highest prevalence, 28.0% of *G. vaginalis* which was in contrast with the report of Inyang et al. (2012). The age group, ≥ 45 years had the highest prevalence of *T. vaginalis*, (30.0%). This was similar to the report of Shutter et al. (1998) who obtained a higher prevalence of trichomoniasis among older age groups of pregnant women in New York, U.S.A. This finding corroborates with the report of Usanga et al. (2010) who obtained a higher prevalence of trichomoniasis among age group 16 – 30 years of pregnant women in Cross River State, Calabar. Nigeria.

In non-pregnant women age group, 16 - 30 years had the higher prevalence 20.0%, 26.7% and 16.7% for *C. albicans*, *G. vaginalis* and *T. vaginalis*, respectively compared to that of 31 - 44 years and 45 years and above. The higher prevalence of infection observed among the younger age group, 16 - 30 years in this study corroborates the findings of Ulogu et al., 2007. The highest incidence of *T. vaginalis* was found in age group, 16 - 30 years which was in agreement with the research work carried out by Al-Samarra (2002) in Baghdad, Iraq and Al-Saeed (2011) in Dohok province, Iraq. The observed frequency of *C. albicans*, *G. vaginalis* and *T. vaginalis* based on the marital status of pregnant women were consistent with previous reports by Cotech (1991). In non-pregnant women, those divorced had the highest prevalence of *C. albicans* and *G. vaginalis*. The prevalence of *T. vaginalis* was significantly higher, 57.1% in widowed than other groups. This finding was in contrast with the report of Usanga (2010) and Okpara (2009). *C. albicans* and *T. vaginalis* was statistically significant in this group ($p < 0.05$).

With respect to infection and trimester of pregnancy, women within their second trimester had the highest prevalence of *C. albicans*, 46.9% followed by third trimester, 40.7% while those in their first trimester had the least of prevalence of the infections, 9.5%. This finding was in contrast with the report of other workers which had 32.41% as the highest prevalence of *C. albicans* (Okpara, 2009) and (Okonkwo, 2010). Women within their third trimester of pregnancy had the highest prevalence of *G. vaginalis*, 44.4%. The Chi-square analysis showed that *G. vaginalis* was significantly higher in the third trimester, which was statistically significant in this group ($p < 0.05$). This finding was in contrast with the report made by [16] who recorded 3.3% prevalence of

G. vaginalis in Akwa Ibom State.

The prevalence of *T. vaginalis* in second trimester was higher compared to first and third trimester. *T. vaginalis* diagnosed in the second trimester was associated with an increased risk of preterm delivery and premature rupture of the membranes. The prevalence of trichomoniasis recorded in this study was higher than those reported by Aboyeji and Nwabuisi (2003); Usanga (2010) who recorded 4.7% and 5.2%, respectively in Ilorin and Calabar. In pregnant women, the prevalence of vaginitis aetiologic agents were higher in primigravidae. In non-pregnant women multigravidae showed the highest prevalence of *C. albicans* while prevalence of *T. vaginalis* and *G. vaginalis* was higher in primigravid women, respectively. The prevalence of these aetiologic agents was in agreement with the findings of Inyang (2012) and Soper (2004).

Women who use contraceptives had the highest prevalence of the three aetiologic agents of vaginitis (*C. albicans*, *T. vaginalis* and *G. vaginalis*), when compared to women with no contraceptive use. The highest prevalence of *G. vaginalis* was found in women who use contraceptives. It was observed that the use of intrauterine contraceptive device (IUCD) increases the risk of premature rupture of membranes. This finding was in agreement with the study of Hay (2002). Intrauterine contraceptive device is a foreign object into the vagina, causing a proliferation of anaerobic bacteria in the cervix and increases the risk of vaginitis. IUCD increases vaginal epithelial thickness leading to an increase in vaginal pH, thus changing the vaginal flora. This suggests that certain IUCD might increase the risk of developing vaginosis (mostly *G. vaginalis*).

In pregnant women, the prevalence of *T. vaginalis* was higher in those who do not take antibiotics whereas those who took antibiotics in non-pregnant women had the highest prevalence of *C. albicans*, *T. vaginalis* and *G. vaginalis*. The use of antibiotics (especially broad spectrum) has an effect on vaginitis (Ferris et al., 2002). Antibiotics especially Penicillins, Cephalosporins and tetracyclines may temporarily destroy Lactobacilli and thus have a responsibility for infection. Both pregnant and non-pregnant women involved in douching had the highest prevalence of *C. albicans*, *T. vaginalis* and *G. vaginalis*. Douching (vaginal washes) increases the risk of vaginitis, so it is not recommended (Vaginal Health Organization, 2010). Douching lead to disturbance of vaginal flora leading to the onset of vaginitis by loss of protective hydrogen-peroxide (H₂O₂) producing Lactobacilli.

In non-pregnant women, the only co-infection present was *C. albicans* and *T. vaginalis*, 1.4%. The co-infections prevalence in pregnant women was *C. albicans* and *T. vaginalis*, 6.3% *C. albicans* and *G. vaginalis*, 2.5% and *G. vaginalis* and *T. vaginalis*, 2.5%. This finding was in contrast with that reported by Adeoye and Akande, (2007). Who recorded 0.6% coinfection of *C. albicans* and *T. vaginalis* in Lagos. The finding of this study also shows that candidiasis, trichomoniasis and *G. vaginalis* are implicated as the most frequent causes of vaginitis.

CONCLUSION

This study revealed that *Candida albicans* and *Trichomonas vaginalis* coexist in pregnant women using wet mount preparation whereas *Candida albicans* and *Gardnerella vaginalis* coexist in non-pregnant women using culture method. *Candida albicans* and *Trichomonas vaginalis* coexist in non-pregnant women using wet mount preparation. The socio-demographic factors such as age, marital status, trimesters, parity, douching, antibiotic and contraceptive use can cause changes in the vaginal environment allowing pathogens to proliferate. The findings from this study show that there is need to identify, control and treat these microbial agents (*Gardnerella vaginalis*, *Candida albicans* and *Trichomonas vaginalis*) among pregnant and non-pregnant women. Since these microbial agents can travel into the uterus causing serious damage to the fallopian tubes which may affect maternal health care delivery system, posing pregnant women at increased risk. This study provides information on the aetiologic agents of vaginitis among pregnant and non-pregnant women in Ikot Ekpene and could serve as a base-line data for further studies in the area. There is need to eradicate these infections by identifying the aetiologic agents of vaginitis among pregnant women in this area because of the risk of reproductive health implications. Increased antenatal/postnatal screening for pregnant women is therefore essential in preventing adverse pregnancy outcomes among sexually active women. The need for improved personal hygiene and other effective intervention programmes among these vulnerable groups of women is advocated.

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