Original Research Article

PREVALENCE AND CORRELATESOF *GARDNERELLA VAGINALIS* AND *TRICHOMONAS VAGINALIS* AMONG FEMALE STUDENTS IN BINGHAM UNIVERSITY.

ABSTRACT

Aim: To determine the prevalence of *Gardnerellavaginalis* and *Trichomonasvaginalis* among female students in Bingham University.

Place and Duration of Study: The study was carried out in the Department of Biological Sciences, Bingham University, Karubetween the months of March to June 2016.

Study design: A cross-sectional study design was utilized.

Methodology: Low vaginal swabs were collected from 200 female students. *G.vaginalis was identified* using wet mount, whiff test and culture in chocolate media. *Trichomonasvaginalis*was identified by making wet mounts of vagina swabs and viewing for viable organism under x10 and x40 objectives of the light microscope.

Results: The results from this study reveals a prevalence rate of 39.0% comprising of 34.5% for *G. vaginalis* infection and 4.5% for *T. vaginalis.*Coinfection rate of (4.5%) was also recorded. Age,symptoms and predisposing factors were significantly associated with G. *vaginalis* infection ($p<0.05$).Bivariate analysis using correlation coefficient of 0.2065, 0.5863 and 0.4086for age, symptoms and predisposing factors shows a moderate positive correlation between these factors considered and the pathogens under study. Similarly, age was significantly associated with *T.vaginalis* ($p<0.05$). However,symptoms and predisposing factorsshowed no significant relationship($p>0.05$) with *T.vaginalis.*A prevalence rate of (24.5%) and (4.0%) was recorded in the age group 15-20yrs for *G.vaginalis* and *T.vaginalis* infection respectively.

Conclusion: The findings of the entire study revealsthat the prevalence of *T. vaginalis*is quite low however; the prevalence of 34.5%for *G. vaginalis* is quite alarming. These infections are associated with adverse pregnancy outcomes therefore; there is a need for increased provision of health information concerning *G.vaginalis*and *T.vaginalis* in the school peripheral. Screening and treatment of these infections will go a long way to eradicate these infections among female students in the University.

Keywords: Bacterial vaginosis, *Gardnerellavaginalis*, *Trichomonasvaginalis,* Prevalence.

1. INTRODUCTION

Bacterial vaginosis (BV) is a vaginal infection that occurs when the equilibrium of the natural flora in the vagina is altered. It is the most common cause of abnormal vaginal discharge [1,2] affecting millions of women of reproductive age annually.

Thevaginal flora are the microorganisms that colonize the vagina. The amount and type of bacteria present have significant implications for a woman's overall health. The primary colonizing bacteria of a healthy individual are of the genus *Lactobacillus* e.g. *Lactobacillus crispatus* [3].It is generally accepted that the production of lactic acid by these bacteria protect females against infection by pathogenic species [4,5,6].

Approximately, 95% of vaginitis is caused by one of the three organisms; *Candida albicans*, *Trichomonasvaginalis* and *Gardnerellavaginalis*[7] and these organisms are among the leading infectious agents causing vaginitis among young women in urban communities in Nigeria [8].

The key player in the pathogenesis of Bacterial vaginosis and the development of a biofilm is *Gardnerellavaginalis*. It was shown in a study of microbiota on the epithelial surfaces of vaginal biopsy specimens from women with bacterial vaginosis, that a biofilm adhered to parts of the epithelium and *Gardnerellavaginalis* comprised 90% of bacteria in that biofilm [9]. *Gardnerellavaginalis* is a gram-variable facultative anaerobe characterized by fastidious, beta-hemolytic growth. It is non-motile and un-encapsulated in nature. The bacilli produces a pore forming toxin, vaginolysin which affects only human cells and has been demonstrated by many studies to have a relationship with other bacteria such as *Lactobacillus* species, *Prevotella*, and anaerobes like*Mobiluncus* and*Bacteroides* [10].

Common symptoms associated with vaginosis in women, caused by *G vaginalis* include increased foul smelling (usually smells like fish) vaginal discharge. The discharge is often white or grey in colour. The smell is particularly noticeable after intercourse. Semen is alkaline and it reacts with the bacteria thereby causing the release of chemicals, hence the fishy smell. There may be burning sensation with urination [11].

*Trichomonasvaginalis*is an anaerobic, parasitic, flagellate protozoan. It is also a member of the Parabasalia, and is the causative agent of trichomoniasis [12].*Tvaginalis*, principally infects the squamous epithelium in the urogenital tract: vagina, urethra, and paraurethral glands [13]. Other less common sites include the cervix, bladder, Bartholin glands, and prostate. Humans are the only known host with the trophozoite transmitted principally via vaginal sexual intercourse, and rarely via fomites [14].

T. vaginalis which is the causative agent of trichomoniasis, can cause vaginitis, cervicitis, urethritis and is the most prevalent non-viral sexually transmitted infection worldwide [12], with an estimated 180 million infection acquired annually worldwide [15]. Coexistence of *T vaginalis* and bacterial vaginosis pathogens is common, with coinfection rates of 60 to 80 percent [16].

Bacterial vaginosis and trichomoniasis are responsible for different infections and have been linked to premature labour, preterm delivery, low birth weight, increase prenatal mortality in pregnant women. They also predispose their patients to HIV/AIDS [17]. Some recent studies also provide evidence that trichomoniasis predispose many to certain malignant disease such as prostate and cervical cancer [18].

Nearly half of all women with *G. vaginalis* and *T. vaginalis*are asymptomatic [19, 20] and they may not be aware that they have the infection. It is important that more effective and safe diagnosis and therapeutics be made to ensure that *G vaginalis* which could be present as a normal microflora in some women, does not exceed its number or be found in other sites as it is not just confined to the lower genital tract in bacterial vaginosis [21].

2. METHODOLOGY

2.1 STUDY AREA

The study was carried out in Bingham University, Karu, Nasarawa state. The school is located in AutaBalefi; a community situated in the middle belt of Nigeria at Longitude 8°32ˈN 8°18ˈE and Latitude 8.533°N 8.300° E. It is characterized by a tropical sub-humid climate with two distinct seasons; wet and dry seasons. Monthly temperature ranges from 20ºC to 34ºC and annual rainfall ranging from 1100 mm to about 2000 m [22]. It occupies a land mass of over 200 square meters, and it is 23 kilometers away from Abuja [23].

2.2 STUDY POPULATION

Two hundred female students of Bingham University between the age group of 15-35 years were randomly recruited for the study.

2.3 EXCLUSION CRITERIA

Students who were currently on their period at the time of the study were excluded from the study.

2.4 ETHICAL APPROVAL

Ethical approval was obtainedfrom the ethical committee of Bingham University.

2.5 SAMPLE COLLECTION

Vaginal swabs were collected from 200 female students. They students were enlightened on the causative agents of the diseases,and the appropriated method of collecting the required sample was demonstrated by the nurses.Sterile cotton tipped swab was inserted into the exterior vaginal fornix and low vaginal swabs (LVS) was taken.

2.6 LABORATORY IDENTIFICATION OF MICROORGANISMS

Whiff-amine test was used to test for the production of a fishy smell as described by Amselet al [24]. Microscopic examination was carried out to determine the presence of clue cells using X40 objective.A swab containing vaginal discharge was inoculated on Chocolate agar and incubated at 33℃ in a 5-10% CO_2 incubator. The plates were examined macroscopically for *Gardnerellavaginalis*.Biochemical tests (Catalase and Indole tests) were carried out.Antibiotic sensitivity testwas carried out using 5mg metronidazole as control.
*Trichomonasvaginalis*was identified by making wet mounts of vagina swabs and viewing for viable organism under x10 and x40 objectives of the light microscope.*Trichomonasvaginalis*was identified by its characteristic pear shape [25].

2.7 STATISTICAL ANALYSIS

The data obtained was subjected to bivariate analysis using correlation coefficient.A confidence interval of 75% was also used and Chi square test was used to determine if there is a significant relationship between the infections and the factors considered.

3. RESULTS

Two hundred female students were screened for *Gardnerellavaginalis* and *Trichomonasvaginalis* infection.There were 69 positive samples for *G.vaginalis* and 9 positive samples *for T.vaginalis,* thus, constituting a prevalence rate of 34.5% and 4.5% respectively as shown on Table 1.The prevalence in this study is 39.0% at 75% confidence index.

Table 2 shows the age distribution of *G. vaginalis*and *T.vaginalis.* A high prevalence rate was recorded within the age group of 15-20yrs (24.50%), (4.0%) for *G. vaginalis* and *T. vaginalis* respectively. Similarly, a low prevalence rate was recorded at 26-30yrs for *G. vaginalis* (1.0%) and 21-25yrs for *T. vaginalis* (0.5%). Statistical analysis shows that prevalence of *G. vaginalis*and *T.vaginalis* infection is associated with the age of the participants ($p<0.05$).

Table 3 shows the prevalence rate of *G. vaginalis* and *T.vaginalis*with respect to presence of symptoms. 73 of the participants in this study were symptomatic, 127 were asymptomatic. The peak of the infection in symptomatic individuals with G. *vaginalis* was recorded with those who had discharge alone; 12(16.43%) and those who had itching alone had the least prevalence of 5.47%. With respect to *T. vaginalis*, the prevalence was similar (1.36%) for those who had Itching alone, discharge alone and discharge with itching.

Table 4 shows the prevalence rate of *G.vaginalis* and *T.vaginalis*with respect to predisposing factors that may enhance the infection transmission. Those that douched had a higher prevalence (20.8%) of *G. vaginalis*followed by those that were sexually active (4.69%). For *T.vaginalis*, prevalence (0.67%) was similar for all the risk factors.

Table 5 shows the coinfection rate of *G. vaginalis* with *T. vaginalis* on the basis of age. A coinfection rate of 4.5% was recorded. Coinfection was greater among the age group 15-20yrs; 4.0%.

Table 6 shows the results of bivariate analysis using correlation coefficient. Tables 7 and 8 represents the Chi square values for *Gardnerellavaginalis and Trichomonasvaginalis*with respect to age, symptoms and risk factors.

Table 1.Prevalence of *Gardnerellavaginalis* and *Trichomonasvaginalis.*

Organisms	No. screened	No. of positive	No. of negative	Prevalence (%)
Gardnerellavaginalis	200	69	131	34.50
Trichomonasvaginalis	200	9	191	04.50
Total	**200**	**78**	**322**	**39.0**

Table 2 Prevalence of *G.vaginalis* and *T.vaginalis* on the basis of the age of the students.

	Gardnerellavaginalis			***Trichomonasvaginalis***	
Age (yrs)	**No. screened**	**No. of positive**	**Prevalence (%)**	**No. of positive**	**Prevalence (%)**
15-20	124	49	24.50	8	04.00
21-25	70	18	09.00	1	00.50
26-30	6	2	01.00	-	-
31-35	-	-	-	-	-
Total	**200**	**69**	**34.5**	**9**	**4.5**

Table 3.Prevalence of *G.vaginalis* and *T.vaginalis* on the basis of symptoms acquisition.

	Gardnerellavaginalis			***Trichomonasvaginalis***	
Symptoms	**No. indicated**	**No. of positive**	**Prevalence (%)**	**No. of positive**	**Prevalence (%)**
Itching only	9	4	05.47	1	01.36
Itching + Discharge	21	10	13.69	1	01.36

Discharge only	43	12	16.43	1	01.36
Total	**73**	**26**	**35.59**	**3**	**4.08**

Table 4.Prevalence of *G. vaginalis* and *T. vaginalis* on the basis of predisposing factors that may enhance the infections.

	Gardnerella vaginalis			***Trichomonas vaginalis***	
Risk factors	**No. indicated**	**No. of positive**	**Prevalence (%)**	**No. of positive**	**Prevalence (%)**
Douching	109	31	20.80	1	0.67
Smoking	4	2	01.34	1	0.67
STI acquisition	8	2	01.34	-	-
Sexual activity	28	7	04.69	1	0.67
Total	**149**	**42**	**28.17**	**3**	**2.01**

Table 5.Coinfection rate of *G. vaginalis* and *T. vaginalis* with respect to age.

Age (yrs)	No. screened	Coinfection of *G. vaginalis* and *T. vaginalis*	Prevalence (%)
15- 20	124	8	4.00
21-25	70	1	0.50
26-30	6	-	-
31-35	-	-	-
Total	**200**	**9**	**4.5**

Table 6.Bivariate analysis showing correlation coefficient results

Factors considered	**Calculated correlation coefficient**
Age	0.2065
Symptoms	0.5863
Risk Factors	0.4086

Table 7. Chi square values for *Gardnerella vaginalis* with respect to age, symptoms and risk factors.

Factors considered	**Chi square value (χ^2)**	**P Values**
Age	89.193	0.00001
Symptoms	4.002	0.28569

Risk Factors	54.949	0.00001

Table 8.Chi square values for *Trichomonasvaginalis*with respect to age, symptoms and risk factors.

Factors considered	Chi square value (χ^2)	P Values
Age	19.888	0.000139
Symptoms	0	0.5
Risk Factors	0.999	1.95708

4. DISCUSSION

The prevalence of *G.vaginalis* in the vaginal swab samples collected and examined in this study is 34.5%. The high prevalence in this study may be related to the physiology and abnormal changes that occur in women of reproductive age at some point in their lifetime. It could also be, probably due to improper hygienic conditions that range from the use of medicated and highly caustic soap to wash the vagina and use of sanitary pads for long period of time during menstruation which could increase the risk of acquiring the infection. The prevalence of *G.vaginalis*in this study is higher than the findings ofIsiaka-Lawalet al [26], Okonko et al[27] and Usanga et al[28] who recorded prevalence rates of 9.7%, 10.5% and 0.9% in three states of Nigeria which include Ilorin, Ibadan and Calabar respectively. However, the prevalence is slightly lower than the findings ofNsagha et al[29],Schwebke et al [30] and Andrea et al[31] who recorded prevalence rates of 41.0%, 53.0% and 41.3% in Cameroon, Nigeria and Rhode Island, USA respectively.

A high prevalence of 24.50% was recorded amongst female students between the ages of 15-20yrs. This prevalence rate correlates with the findings ofAdinma et al [32] who also recorded a high prevalence of 26.0% among pregnant women between the ages of 16-20yrs. The high prevalence among these young ladies is perhaps due to sexually activities or probably due to little or inadequate knowledge on how their vagina environment should be, hence a higher incidence of *G.vaginalis* infection. This supports the report byAdekanle et al[33] who reported that pregnant women between the age group of 15-30yrs were the most infected (100%) by one STD or the other.

Although *G.vaginalis* is not necessarily called a sexually transmitted infection, it cannot be disputed that sexual activity can enhance its acquisition. Prevalence of positive individuals 35.61% symptomatic for *G.vaginalis*was higher than those asymptomatic 33.92%. This supports the general consensus that higher isolation rate of G. *vaginalis* occurs in symptomatic compared to asymptomatic women as reported by Adinma et al[32].

Certain risk factors could enhance the rate at which *G. vaginalis* is transmitted or acquired. A high prevalence rate of 50.0% was recorded for those that smoke than those that douche. Although the number of those that douched was more than those that smoked, the high prevalence rate obtained from those that smoked backs up report byHellberg et al[34]that smoking increases the risk of acquiring bacterial vaginosis. TheBritish Association for Sexual Health and HIV[35]reportedthat vaginal douching could disrupt vaginal environment and increase the number of anaerobic bacteria there, thereby allowing opportunistic infections to thrive.

The prevalence of *T.vaginalis* obtained in this study is 4.5%. This prevalence agrees with the separate findings ofMahmoud et al[36],Isiaka-Lawal et al [26] and Esheteet al [37]who reported prevalence rates of 5.0%, 5.6%, 4.98% in Egypt, Nigeria and Ethiopia respectively.However, other studies in Nigeria

demonstrated a higher prevalence of13.67%, 17.5% in Zaria and OnitshabyAkafyi et al[38] and Iwueze et al [39]recorded a lower prevalence of 1.2% in Yaounde, Cameroon Nsagha et al [29]. The low prevalence of *T. vaginalis* infection observed in this study when compared to other studies may be partly explained by the difference in sensitivity of methods of diagnosis; microscopy rather than culture which has a comparatively high sensitivity and is considered as the gold standard. It may also be due to the difference in study population and hygienic practices as supported byMaufi et al [40].

A prevalence rate of 4.0% for *T.vaginalis* was recorded among female students within the age group of 15-20 years. This supports the high prevalence of 71.4% reportedamong pregnant women between the age group of 15-20yrs in a study conducted by Oyeyemiet al [41].However, Mahmoud et al [36] and Iwueze et al [39]recorded high prevalence rates in ages 21-40yrs and 30-39yrs respectively.

In this study, symptoms acquisition was not significantly associated with *T. vaginalis*infection.

A prevalence rate of 0.67% was recorded for those that douched, smoked, and had one sexually transmitted infection and those that were sexually active. This supports the finding of [19] which states that smoking and sexual activity increases the risk of acquiring *T. vaginalis.*

The coinfection of *Gardnerellavaginalis* and *Trichomonasvaginalis* in this study is 4.5%. This corresponds to the prevalence obtained for *Trichomonasvaginalis* alone. All the individuals that tested positive for *T.vaginalis* had G. *vaginalis*. This supports the findings of Piperakiet al [42] who reported that *G.vaginalis*was more frequent in women infected with *T. vaginalis.*

Bivariate analysis showing correlation coefficient results on Table 6 reveals that the factors considered in this study (age, symptoms and predisposing factors) had the following correlation coefficients 0.2065, 0.5863 and 0.4086 respectively. This result suggests that the factors considered have a moderate positive correlation as the Pearson's correlation coefficient ranges from -1 to 1.

4.2 CONCLUSION

The result of this study reveals that the prevalence of *G. vaginalis* is relatively high compared to that of *T. vaginalis.* This supports the factthe replacement of vaginal lactobacilli with*Gardnerellavaginalis* is associated with bacterial vaginosis. On the other hand, *T. vaginalis*is a sexually transmitted disease which is acquired during unprotected sex. These infections can be eradicated or reduced by improved personal hygiene, abstinence from sex before marriage and adequate treatment.If left untreated these infections may cause untold damage to young ladies during pregnancy.

REFERENCES

[1] Wilson J, Shann SM, Brady SK, Mammen-Tobin AG, Evans AL, Lee RA. Recurrent bacterial vaginosis: The use of maintenance acidic vaginal gel following treatment. International Journal of STD AIDS.2005; 16: 736-738.

[2] Donders GG. Diagnosis and management of bacterial vaginosis and other types of abnormal vaginal bacterial flora: A review. *Obstetrics Gynecology Survey*.2010; 65(7): 462-473.

[3] Vasquez A, Jakobsson T, Ahrne S, ForsumU and Molin G."Vaginal Lactobacillus Flora of Healthy Swedish Women". *Journal of Clinical Microbiology.*2002;40 (8): 2746–2749.

[4] Strus M, Malinowska M. Heczko PB. (2002). In vitro antagonistic effect of Lactobacillus on organisms associated with bacterial vaginosis. *Journal of Reproduction Medicine.*2002; 47: 41-46.

[5] Matu M. Orinda GO, Njagi ENM, Cohen CR. Bukusi EA. In vitro inhibitory activity of human vaginal lactobacilli against pathogenic bacteria associated with bacterial vaginosis in Kenyan women. *Anaerobe.*2010;(16) 210-215.

[6] Graver M, Wade J. The role of acidification in the inhibition of Neisseria gonorrhoeae by vaginal lactobacilli during anaerobic growth.*Ann. Clinical Microbiology of Antimicrob*ials.2011;10: 8.

[7] Andrist LC. Vaginal health and infections.*Journal of Obstetric, Gynecologic and Neonatal Nursing*. 2001;30: 306-315.

[8] Olawuyi O.The prevalence of bacterial vaginosis among young women in urban areas in Nigeria and its major risk factors.*Sexually Transmitted Infections*.2011;87:299-300.

[9] Swidsinski A, Mendling W, Loening-Baucke V, Ladhoff A, Swidsinski S, Hale LP, Lochs H. Adherent biofilms in bacterial vaginosis. *Obstetrics Gynecology*. 2005; 106(1): 1013–1023.

[10] Philippe HG.Bacterial vaginosis. 2015.

[11] Jane R,Schwebke MD. Asymptomatic bacterial vaginosis; Response to therapy.*American Journal of Obstetrics and Gynecology*.2000;183(6):1434-1439.

[12] Shira CS, Frank JS.Viability of *Trichomonasvaginalis* in urine: epidemiologic and clinical implications. *Journal of Clinical Microbiology.*2006; 44: 3787-3789.

[13] Kissinger P. Epidemiology and Treatment of Trichomoniasis. *Current Infectious Disease Rep*. 2015;(17) 484.

[14] Wilkerson RG.Trichomoniasis in Emergency Medicine.2011.

[15] World Health Organization (WHO). Integrating care for reproductive health, sexually transmitted and other reproductive tract infections; A guide to essential practice, Morbidity Mortality Weekly Recommendation Report.2004; 51(2): 1-118.

[16] Sobel J, SubramanianC, Foxman B. Mixed vaginitis more than coinfection and with therapeutic implications. *Current Infectious Diseases Rep.*2013; 15: 104.

[17] Turovskiy Y, Noll SK, ChikindasML.Theaetiology of bacterial vaginosis.*Journal of Applied Microbiology.*2011; 110: 1105–1128.

[18] Schwebke J, Burgess D. Trichomoniasis. *Clinical Microbiology Review.*2004; 17: 794 –803.

[19] Johnston V, Mabey DC.Global epidemiology and control of *Trichomonasvaginalis.Current Opinion on Infectious Disease.*2008; 21: 56-64.

[20] Donbraye E, Donbraye-Emmanuel OOB, Okonko IO, Okedeji IO, Alli JA, NwanzeJC.Detection and prevalence of *Trichomonasvaginalis* among pregnant women in Ibadan, Southwestern Nigeria. *World Applied Science Journal.*2010; 11(12): 1512 – 1517.

[21] Swidsinski A, Verstraelen H, Loening-Baucke V, Swidsinski S, Mendling W, Halwani Z. Presence of a polymicrobial endometrial biofilm in patients with bacterial vaginosis. *Plos One.*2013; 8(1) e53997.doi: 10.1371/journal.pone.0053997.

[22] National Bureau of Statistics. Population census.Federal Republic of Nigeria. (2006).

[23] Obiekeze SO, Odun NN, Ogwu D. Aerobe microbiological quality of Nono sold in Keffi metropolis. *International Journal of Chemical Science.*2012;5(2): 157-162).

[24] Amsel R, Otten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK.Nonspecific vaginitis.Diagnostic criteria and microbial and epidemiologic associations.*American Journal of Medicine.*1983;74(1): 14–22.

[25] Cheesebrough M. District Laboratory Practice inTropical Countries. 2nd Edition.CambridgeUniversity Press Part 1. 2005: 218-239.

[26] Isiaka-Lawal S, NwabuisiC, Fakeye C, Saidu R, Adesina KT, Ijaiya MA, Jimoh, AA and Omokanye LO. Pattern of sexually transmitted infections in human immunodeficiency virus positive women attending antenatal clinics in north-central Nigeria. *Sahel Medical Journal* 2014;17(4): 145-150.

[27] Okonko IO, Akinpelu AO, Okerentugba PO. Prevalence of Sexually Transmitted Infections (STIs) among Attendees of AFRH Centre in Ibadan, Southwestern Nigeria.*Middle-East Journal of Scientific Research*.2012;11(1): 24-31.

[28] Usanga V, Abia-Bassey L, Inyang-etohP,Udoh S, Ani F, Archibong E. *Trichomonasvaginalis* infection among pregnant women in Calabar, Cross River State, Nigeria.*The Internet Journal of Gynecology and Obstetrics*.2009;14: 2.

[29] Nsagha DS,Zofou D, Assob JC, Njunda AN, Nchang CD, MvoNgum N, Patrick WE, Marcelin NN."The epidemiology of *Trichomonasvaginalis, Gardnerellavaginalis* and *Candida albicans* Co-Infections in women attending the Yaounde University Teaching Hospital."*American Journal of Epidemiology and Infectious Disease*.2015;3(2): 28-31.

[30] Schwebke JR, Muzny CA,Josey WE. Role of *Gardnerellavaginalis* in the pathogenesis of bacterial vaginosis: a conceptual model. *Journal of InfectiousDiseases*.2014; 210: 338– 343.

[31] Andrea SB, Chapin KC. Comparison of Aptima*Trichomonasvaginalis* Transcription-Mediated Amplification Assay and BD Affirm VPIII for Detection of *T. vaginalis* in Symptomatic Women: Performance Parameters and Epidemiological Implications. *Journal of Clinical Microbiology.* 2011;49(3)866–869. doi: 10.1128/JCM.02367-10.

[32] Adinma J, Okwoli NR, Agbai, Unaeze N. Prevalence of *Gardnerellavaginalis*in pregnant Nigerian women. *African Journal of Reproductive Health*.2001;5(1): 50- 55.

[33] Adekanle DA, Adeyemi AS,Odu OO. Teenage and non-teenage pregnant women in south western Nigeria.A descriptive study.*Calicut Medical Journal*.2008;6(3): 5.

[34] Hellberg, D., Nilsson, S and Mardh, P.A. (2000). Bacterial vaginosis and smoking. *International Journal of Sexually Transmitted Diseasesand AIDS*.11: 603-606.

[35] British Association for Sexual Health and HIV (BASHH).UK National Guideline for the Management of *Trichomonasvaginalis* infection.2014.

[36] Mahmoud A, Sherif NA,Abdella R, El-Genedy AR, El KatebAY, AskalaniANH. Prevalence of *Trichomonasvaginalis* infection among Egyptian women using culture and Latex agglutination: cross-sectional study. *BMC Women's Health*.2015; 15:7.doi: 10.1186/s12905-015-0169-2.

[37] Eshete A, Mekonnen Z, Zeynudin A. Trichomonasvaginalis infection among pregnant women in Jimma University specialized Hospital, Southwest Ethiopia. *Journal of Infectious diseases*. 2013;Article ID 485439.doi.org/10.5402/2013/485439.

[38] AkafyiD,OkoJO, Umar M, Obafemi A, Micheal R.Prevalence of Bacterial, Trichomonas and candidalvaginosis among females in angwanfulani, PalladanIn Zaria, Nigeria. *Journal of Applied Life Sciences International*.2016; 5(2): 1-6.

[39] Iwueze MO, Ezeanyanwu LN, Okafor FC, Nwaorgu OC, Ukibe SC. Prevalence of *Trichomonas vaginalis*infection among women attending hospitals/health centres in Onitsha community, Onitsha north local government area of Anambra state. *The Bioscientist*. 2014;2(1): 54- 64.

[40] Maufi AJ, Mazigo HD, Kihunrwa A. Prevalence and factors associated with *Trichomonas vaginalis* infection among pregnant women attending public antenatal clinics in Mwanza city, North-western Tanzania. *Tanzania Journal of Health Research*. 2016; 18(2). ISSN: 0856-6496; eISSN 1821-9241.

[41] Oyeyemi O, Fadope O, Oyeyemi IT. *Trichomonasvaginalis* infection in Nigerian pregnant women and risk factors associated with sexually transmitted diseases. *International Journal of Sexually Transmitted Diseases*.2015. DOI:10.1177/0956462415611292.

[42] Piperaki ET, Theodora M, Mendris M, Barbitsa L, Pitiriga V, Antsaklis A, Tsakris A. Prevalence of *Trichomonasvaginalis* infection among women attending a major gynecological hospital in Greece: a cross-sectional study. *Journal of Clinical Pathology*. 2010; 63(3): 249-53.