Asian Journal of Research in Medical and Pharmaceutical Sciences

2(2): 1-8, 2017; Article no.AJRIMPS.36127

Prevalence of Bacterial Vaginosis amongst Female Students of the University of Calabar, Calabar, Cross River State

U. O. Edet[1,2*], C. I. Mboto[1], E. N. Mbim[1*], U. E. George[3], C. F. Umego[1] and J. Okon[1]

[1]Deparment of Microbiology, Faculty of Biological Sciences, University of Calabar, Calabar, Cross River State, Nigeria.
[2]Deparment of Microbiology, Faculty of Natural and Applied Sciences, Obong University, Obong Ntak, Etim Ekpo LGA, Akwa Ibom State, Nigeria.
[3]Department of Virology, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors UOE, CIM, ENM, UEG, JO and CFU designed the study. Author UOE performed the statistical analysis while authors CIM, ENM and JO wrote the protocol and the first draft of the manuscript. All the authors managed the analyses of the study. Authors UOE, JO and CIM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2017/36127
Editor(s):
(1) Davina Hijam, Professor, Department of Biochemistry Regional Institute of Medical Sciences, India.
Reviewers:
(1) Akobi Oliver Adeyemi, Nigeria.
(2) Sivalingam Nalliah, Surgery International Medical University, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/22186

Original Research Article

Received 14th August 2017
Accepted 14th November 2017
Published 7th December 2017

ABSTRACT

Introduction: Bacterial vaginosis (BV) is one of the most prevalent vaginal infections among women in Africa and is caused by several behavioral, hormonal and sexual factors.
Aim: The aim of this study was to determine the prevalence of BV among female students of the University of Calabar and to make recommendation on modifiable risk factors based on administered questionnaires.
Materials and Methods: One hundred and fifty high vaginal swab (HVS) samples were collected from female students of the University of Calabar following informed consent. Samples collection and microbiological processing were done using standard techniques. BV was analysed using

**Corresponding author: E-mail: uwemedet27@gmail.com, mbimelizabeth@gmail.com;*

Amsel's and Nugent's criteria. Open ended questionnaires were administered to the respondents to obtain information about their sociodemographic factors and risk factors to BV. Simple descriptive statistics were used to analyse the data collected and these were done using Microsoft Excel 2007.

Results: Twenty three percent (35) of female students were identified as BV positive with Amsel's Criteria while twenty-seven percent (41) were positive for BV using Nugent's criteria. Age range of 21-25 years had the highest prevalence rate of BV of 16 (45.70%). Prevalent microbial isolates were *Staphylococcus aureus, Escherichia coli* and *Candida* species in descending order.

Conclusion: This study reveals that Nugent's Criteria have a higher positive predictive value (PPV) than Amsel's criteria in the diagnosis of BV. Furthermore, the data obtained suggests that the prevalence rate of BV is relatively high and thus suggest the need for a comprehensive health care education program aimed at reducing BV prevalence.

Keywords: Bacterial vaginosis; females; prevalence.

1. INTRODUCTION

Bacterial vaginosis (BV) often described as a polymicrobial syndrome is characterized by a shift in vaginal flora from a predominant population of *Lactobacilli* to an overgrowth of anaerobes with associated symptoms [1,2]. Lawrence et al. [3] observed that the infection is the most common cause of vaginal odour, discharge and itching especially among women of reproductive age. The incidence of bacterial vaginosis has been reported by Van-der Wijgert et al. [4] to be on the increase especially in sub-Saharan Africa where knowledge of this infection is limited. This infection is known to be caused by the proliferation of a number of organisms including *Gardneralla vaginalis*, *Mobilincus* species*, Prevotella species*, *Porphyromonas species*, *Bacteroides species*, *Peptostreptococcus*, *Mycoplasma hominis* and *Ureaplasma urealyticum* [1]. This imbalance in vaginal flora may lead to the characteristic symptoms of homogenous, malodorous vaginal discharge. However, over half of those with BV report no symptoms [5-6]. Furthermore, BV is one of the most frequent conditions encountered in sexually transmitted diseases (STD), genitourinary medicine (GUM) or other reproduction health clinics throughout the world [7].

The overall prevalence of BV varies greatly depending on the population with estimates ranging from 4% among asymptomatic college students to 61% among women attending sexually transmitted disease clinics [8-9]. Among reproductive age women in the general population, the estimated prevalence rate varies from 10% to 25% [10]. Among women of child bearing age in the United States, BV is reported to be the most common vaginal infection accounting for 40% to 50% of vaginal infection [1]. BV has been reported in 15 to 19% of ambulatory gynaecologic patients, 10-80% of pregnant patients and 20 to 41% of patients in sexually transmitted diseases clinics [11]. The vulnerability of pregnant women to this infection is as a result of increased levels of oestrogen during pregnancy, which creates a climate for the growth of these agents. Race and ethnicity, education, income and age are significant correlates of BV [12]. Some studies have reported an association between chronic stress and prevalence of BV in both pregnant and non-pregnant population [13]. The relationship of chronic stress to poor health outcomes maybe mediated by stress induced immune function changes leading to increased susceptibility to bacterial vaginosis. Some reports have revealed an association of BV with pelvic inflammatory disease, gynaecologic post-operative infection, human papilloma virus and HIV infection [10,14-16].

Most documented studies on BV in Nigeria and other countries are limited to pregnant women [17-19]. Furthermore, studies on BV among apparently healthy females in the country are rare. This study therefore was aimed at the determination of the prevalence of BV among female students of the University of Calabar with a view of making recommendation on modifiable risk factors based on administered questionnaires.

2. MATERIALS AND METHODS

2.1 Study Population and Administration of Questionnaire

The study was conducted at the University of Calabar medical centre and Department of Microbiology both in the University of Calabar,

Calabar. After approval was sought and obtained from the University of Calabar Ethical committee, 150 female students from the University of Calabar, Calabar aged between 16-35 years of were recruited for this study following their informed consents. Socio-demographic and predisposing factors were obtained from participants using open ended questionnaires.

2.2 Sample Collection

For convenience and non- approval of other hostels, the simple random sampling was carried out to collect the samples from the only approved hostel. Sample size determination were based on previous studies [3,20]. Collection was done following procedures previously described [3]. One hundred and fifty (150) labelled sterile swabs were used to collect samples of vaginal fluid from participants. The participants were trained just before sample collection on how to self-collect vaginal swab by inserting the swab approximately 1-2 inches into their vagina and rolled in cycles inside the vagina, ensuring that they collect samples from all sides of their vagina and then kept inside the vagina briefly for 20 seconds. Afterwards, the swabs were allowed to air dry and then transported at 2-4^{0}C immediately to the microbiology laboratory for analysis using Giostyle box with ice.

2.3 Amsel's Criteria

Amsel's criteria of parameters including vaginal pH, whiff test for the presence of clue cells and presence of abnormal discharge were carried out following the standards of Amsel et al. [5].

pH: This was done following procedures previously [18]. Briefly, pH indicator strips were used to measure the pH of the vaginal fluid contained on the swab stick. Each swab was respectively rotated on the pH test paper to deposit some vaginal fluid and the colour reaction compared with a scale to determine vaginal pH.

Whiff test: This was done following procedures previously described by Munjoma [19]. Briefly, one drop of physiological saline was placed on the surface of a clean glass slide and the swab stick was then immersed in the saline to deposit some vaginal fluid. Afterwards, a drop of 10% potassium hydroxide (KOH) was added directly to the emulsion and the slide held up and fanned to determine the presence of fishy odour.

Wet preparation: This was performed following procedures previously described by Kuraga [18]. Briefly, a drop of normal saline was placed on a clean grease-free slide and the swab containing the sample rotated on the drop of saline to deposit some discharge. Afterwards, a cover slip was then placed on the suspension and the slide viewed for the presence or absence of clue cells.

Presence of abnormal discharge and itching: This was based on self-report by the participant. They were asked if they had observed any unusual discharge from their vagina. The response was recorded as either positive (+) or negative (-).

Microbiological analysis (Nugent's Criteria): This was done following procedures described previously [17-18]. Swabs were each rinsed in 9mls of distilled water to make a 10^{-1} dilution. Afterwards, a five step tenfold serial dilution was carried out. Then 1ml aliquots from 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions were each inoculated unto plates containing freshly prepared blood agar, nutrient agar, MacConkey agar and Sabouraud dextrose agar. Inoculated plates were then incubated at 37^{0}C for 24-48hrs. Discrete colonies were then characterized using biochemical reactions including Gram's reaction, motility, catalase, indole, urease, oxidase, citrate utilization, methyl red and Voges Proskauer tests for bacteria while yeasts were characterized using Gram's reaction.

3. RESULTS

The results of the study are presented in Figure 1 and the Tables 1 to 7. The results from Table 1 indicate that the age range of the participants ranged from 16 to 35 years and this was stratified into 16-20, 21-25, 26-30 and 31-35 years with a mean age of 25.5 years. Thirty-five of the women studied had BV giving an overall prevalence of 23.3%. The highest percentage occurrence of 16(45.7%) was found in the 21-25 age range, with the lowest 2(5.7%) was observed in the age range of 16-20years as presented in Table 1.

The result of the microscopic examination of vaginal swabs from women studied is reported in Table 2. The results revealed that 92(61.3%) of samples contained pus, 89(59.3%) contained significant epithelial cells while 17(11.3%) contained yeast cells. The results of prevalence of bacterial vaginosis according to sociodemographic and predisposing factors are as presented in Table 3. A total of 90 females out

of 150 douched with water, 104 used nylon pants while 49 douched with antiseptics including soaps. Out of the 54 females examined within the age group of 21-25 years, 14 had no sex partners, 35 had one sex partner while 5 had multiple sexual partners.

Table 4 shows the clinical indications for bacterial vaginosis and the results indicate that a large number of females 139 (92.7%) presented one form of symptoms or another. A total of 28 % of participants presented with yellowish vaginal discharge, 54 % with itching and 57% with odour.

Table 5 shows the sensitivity, specificity and predictive values of symptoms associated with bacterial vaginosis using vaginal discharge as a gold standard.

The distribution of microbial load from the samples is presented in Table 6. A total of 57 samples had significant bacterial growth of 105 x 10^5cfu/ml while 61 samples had scanty growth of 0.08 x 10^5cfu/ml on the average.

Table 7 shows the prevalence of bacterial isolates from vaginal swab samples. A total of 32 (28.1%) of *Candida species* was isolated out of the 114 isolates. *Escherichia coli* recorded 38(33.3%) while *Staphylococcus aureus* recorded 44 (38.6%).

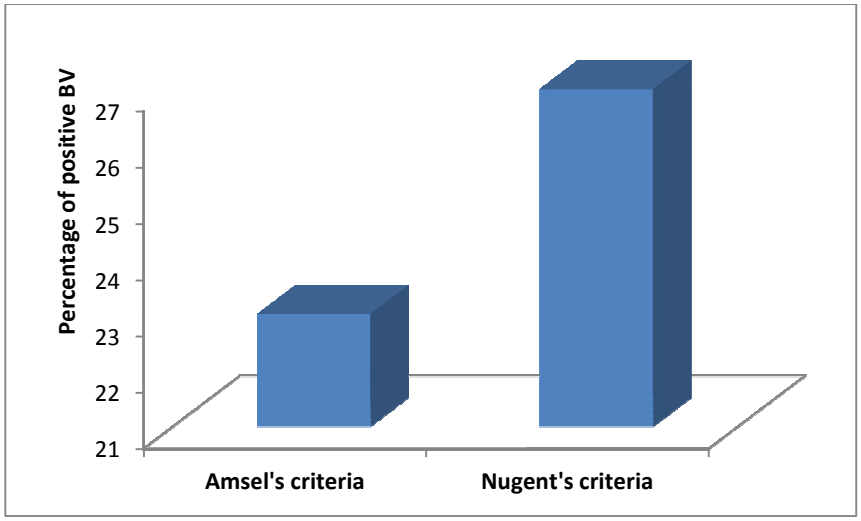

Figure 1. Percentage of positive BV based on Amsel's and Nugent's criteria

Table 1. Age distribution between participants with bacterial vaginosis

Age group	BV (%)	I (%)	N (%)
16-20	2 (5.7)	6 (12.2)	10 (15.1)
21-25	16(45.7)	22(44.9)	36 (54.5)
26-30	13(37.1	10(20.4)	14(21.2)
31-35	4(11.4)	11(22.4)	6(9.1)
Total	35(100)	49(100)	66(100)

Key: Bacteria vaginosis (BV) score of 7-10, Intermediate (I) 4-6 and Normal, score 0-3

Table 2. Microscopic examination of high vaginal swab sample

Observations	Significant sample	Scanty sample	No sample
Epithelial cells (%)	89(59.3)	43(28.7)	18(12.0)
Pus cells (%)	92(61.3)	34(22.7)	24(16.0)
Yeasts (%)	17(11.3)	14(9.3)	119(79.3)

Key: Significant sample = ≥ 5Hpf (High power field), Scanty sample = ≤ 5Hpf

Table 3. Prevalence according to socio-demographic and predisposing factors

Age range	No of vaginal swabs examined	No of sex partners			Douching with water (%)	Use of nylon pants (%)	Douching with antiseptics/soaps (%)
		None	1	2			
16-20	38	18	11	9	28(73.7)	16(42.1)	21(55.3)
21-25	54	14	35	5	33(61.1)	42(77.8)	18(33.3)
26-30	33	4	18	11	14(42.4)	26(78.8)	9(27.3)
31-35	25	5	12	8	15(60.0)	20(80.0)	1(4.0)
Total	150	41	76	33	90	104	49

Table 4. Indications for bacterial vaginosis

Indications	No with symptoms	Use of nylon pants (%)	No with multiple partners (%)	Douching with water (%)	Douching with antiseptics (%)
No symptoms	-	1	0	10	0
Itching	54	24(44.4)	15(27.8)	4(7.4)	11(20.4)
Yellow discharge	28	5(17.9)	11(39.3)	10(35.7)	2(7.1)
Odour	57	13(22.8)	15(26.3)	21(36.8)	8(14.0)

Table 5. Sensitivity, specificity and predictive value of symptoms associated with bacterial vaginosis

		Vaginal discharge (Gold standard)			Sensitivity (%)	Specificity (%)	PPV	NPV
		Diseased (+ve)	Non-diseased (–ve)	Total				
Test result	Positive	28 (TP)	15 (FP)	43	80	87	65	93
	Negative	7 (FN)	100 (TN)	107				
	Total	35	115	150				

Key: TP- true positive, FP- False positive, TN- True negative, FN-False negative, PPV- Positive predictive value, NPV-Negative predictive value

Table 6. Distribution of microorganisms isolated from HVS according to age

Age group	No of vaginal swabs examined	No of samples with significant bacterial growth	No with scanty growth	No of samples with yeast growth (%)
16-20	38	13	18	11(35.5%)
21-25	54	21	17	8(25.8%)
26-30	33	12	18	7(22.6%)
31-35	25	11	8	5(16.1%)
Total	**150**	**57**	**61**	**31**

Key: Scanty growth: = < 10^5 cfu/ml, significant growth: = > 10^5cfu/ml

Table 7. Prevalence of microorganisms isolated from vaginal swab samples

Organisms	Frequency (%)
Staphylococcus aureus	44(38.6)
Escherichia coli	38(33.3)
Candida species	32(28.1)
Total	**114**

4. DISCUSSION

Bacterial vaginosis (BV) is one of the most common vaginal diseases among women of reproductive age globally [17,21]. It is caused by an imbalance in vaginal flora composition associated with behavioral factors such as vaginal douching, menstrual hygiene practices, to mention a few [20]. Hormonal factors and sexual activity could also contribute to the development of BV amongst other factors [21-22]. In this study, a total of 150 high vaginal swab samples were collected from female students of the University of Calabar following informed consent and processed using Amsel's and Nugent's criteria for bacterial vaginosis. A total of 35(23%) were diagnosed as BV-positive using

Amsel's Criteria while 41(27%) were BV-positive using Nugent criteria [21]. This indicates that Nugent's criteria is more sensitive than Amsel's criteria in the diagnosis of bacterial vaginosis. This is in concordance with Awoniyi et al. [17], who reported that Nugent's scoring system was more sensitive and specific than Amsel's criteria. Our prevalence was higher than that previously reported 17.8% [23] but extremely lower than the 78% reported by Lawrence et al. [3] and 48.50% in India [24] These disparities in rates as previously noted could be due to systemic differences in the various populations as well as geographical distribution [22]. The contrasting prevalence rates observed could also be due to factors including socio-demographic, educational status and diagnostic methods employed. Reports from Allsworth et al. [12] revealed that the prevalence of bacterial vaginosis increases with age and this was in line with the high prevalence observed within the age range 21-25 years. Furthermore, Bhalla et al. [25] found no association between bacterial vaginosis and age > 25years and this was also consistent with our findings. As seen in our report, several authors have revealed that bacterial vaginosis is loosely associated with several sexual characteristics including age, life time number of sex partners, current history of multiple sex partners as well as new partners [26,27].

The high prevalence of odour, itching and vaginal discharge among study subjects 139 (92.7%) observed in this study is consistent with the report of Shobeiri et al. [28] who revealed that these factors gravely indicate bacterial vaginosis. Larsson et al. [29] revealed a relationship between bacterial vaginosis and associated risk behaviours such as multiple sex partners and sexually transmitted infections.

Roberta et al. [30] reported that bacterial vaginosis was significantly increased in patients who admitted to the practice of regular douching. This is in accordance with results from this study where participants douched with either water or antiseptics/soaps to ease the symptoms associated with bacterial vaginosis. However, studies revealed that even in the absence of symptoms, douching remain significantly associated with bacterial vaginosis [31,32]. As observed in this study, the douching method had a considerable effect on the symptoms associated with bacterial vaginosis and this is consistent with report of Roberta *et al*., (2002) who revealed a significant relationship among symptoms of bacterial vaginosis, douching and racial factors where they established that symptoms were more frequent among black women who douched.

The high negative predictive values and low positive predictive values with high specificity value using vaginal discharge as a goal standard observed in this study is consistent with the report of [3,23]. This implies that PPV rises with increase in prevalence whereas NPV is high in areas of low prevalence as observed in this study.

The results of this study revealed that *Staphylococcus aureus* is one of the microorganisms commonly found in most cases of bacterial vaginosis and is consistent with previous reports [32-34]. The 38.6% of *Staphylococcus aureus* reported in this study is slightly higher than the 25% reported by Olusanya and Olutiola (1984) [35] and the 24% recorded by Lawrence et al. [3].

The microbial flora of the vagina has been reported by Lawrence et al. [3] to include members of the *Lactobacilli* family who function by preventing the overgrowth of possible pathogens as well as maintain the pH of the vagina.

5. STUDY LIMITATIONS

The conclusions we have drawn from this study may not be representative enough given the limitations of the study in terms of sample size and sampling technique. For convenience and non approval of other hostels, simple random sampling was used in sample collection in just one hostel. Thus, this informed the sample size of one hundred and fifty employed in this study.

6. CONCLUSION

In conclusion, this study shows that prevalence of BV amongst female students of the University of Calabar is relatively high and this study could provide important epidemiologic data on BV for future population-based studies.

7. RECOMMENDATION

It is therefore recommended that comprehensive healthcare education should be given to the students. This should include the use of barrier methods, and regular check up of their vaginal health to reduce complications that may arise as a result of BV infection.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hill GB. The microbiology of bacterial vaginosis. American Journal of Obstetrics and Gynecology. 1993;169:682-692.
2. Cohen S. Keynote presentation at the eight international congress of behaviour medicine: The Pittsburgh common cold studies: Psychosocial predictors of susceptible to respiratory infectious illness. International Journal of Behaviours Medicine. 2005;12:123-131.
3. Lawrence UC, Achi OK, Ifeanyi OE, Queen E. Prevalence of bacterial vaginosis among female students of Michael Okpara University Of Agriculture, Umudike, Abia State, Nigeria. Journal of Pharmacy and Biological Sciences. 2014;39-52.
4. van de Wijgert JHHM, Mason PR, Gwanzura L, Mbizvo TM, Chirenje ZM, Iliff V, Shoboski S, Padian NS. Intravaginal practices, vaginal flora disturbances, and acquisition of sexually transmitted diseases in Zimbabwean women. J. Infect. Dis. 2000;181:587-594.
5. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med. 1983;74(1):14-22.
6. Klebanoff MA, Schwebke JA, Zhang Y, Nandel TR, Yu KF, Andrews WW. Vulvo vaginal symptoms in women with bacterial vaginosis. Obstetrics and Gynecology. 2004;104:267-272.
7. Cohen CR, Duerr A, Pruithithada N, Rugpao S, Hillier S, Garcia P, Nelson K. Bacterial vaginosis and HIV seroprevalence among female commercial sex workers in Chiang Mai, Thailand. AIDS. 1995;9:1093–7.
8. Embree J, Caliando JJ, McCormack WM. Nonspecific vaginitis among women attending a sexually transmitted diseases clinic. Sexually Transmitted Disease. 1984; 11:81–4.
9. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: A systematic review. American Journal of Obstetrics and Gynecology. 2013;209: 505–23.
10. Ness R, Kie KR, Hillier SL, Soper DE, Stamm CA, Sweet RL. Aclester analysis of bacterial vaginosis associated microflora and pelvic inflammatory disease. American Journal of Epidemiology. 2005;162;85-590.
11. Bump RC, Buesching W. Bacterial vaginosis in vaginal and sexually active adolescent females: Evidence against exclusive sexual transmission. American Journal of Obstetrics and Gynecology. 1988;158:935-939.
12. Allsworth JE, Peipert JF. Prevalence of bacterial vaginosis; 2001- 2004 National Health of Nutrition Examination survey data. Obstetrics and Gynecology. 2007; 109:114-120.
13. Culhane JF, Rauh V, Mccollum KF, Elo II, Hogan V. Exposure to chronic stress and ethnic differences in rate of bacterial vaginosis among pregnant women. American Journal of Obstetrics and Gynecology. 2002;187:1272-1276.
14. Cohen S, Karmack T, Mermelstein R. A global measure of perceived stress. Journal of Health and Social Behaviour. 1983;24:385-396.
15. Lin L, Song Y, Kimber N, Shott S, Tangora J, Aroutcheva A. The role of bacterial vaginosis in infection after major gynaecologic surgery. Infectious Diseases in Obstetrics and Gynecology. 1999;7: 169-774.
16. Watts DH, Fazzari M, Minkoff H, Hillier SL, Sha B, Glesby M, Levine AM, Burk R. et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high- risk HIV- 1-uninfected women. J Infect Dis. 2005;191:1129–1139.
17. Awoniyi AO, Komolafe OI, Bifarin O, Olaniran O. Bacterial vaginosis among pregnant women attending a primary health care center in Ile-Ife, Nigeria. Global Advanced Research Journal of Medicine and Medical Science. 2015;4(1):057-060.

18. Kuruga MW. Bacterial vaginosis: Prevalence and value of different diagnostic tests among prenatal women at Kenyatta National Hospital. University of Kenya. PhD Thesis; 2012.
19. Munjoma MW. Simple method for the detection of Bacterial vaginois in pregnant women (Unpublished Master's Thesis). University of Oslo. 2004;55-70.
20. Mbim EN, Mboto CI, George UE, Umego CF, Edet UO, Orajiaka NA. Prevalence of vaginal candidiasis among female students of a hostel in the University of Calabar, Calabar. Journal of Applied Life Science International. 2017;13(3):1-7.
21. Bahram A, Hamid B, Zohre T. Prevalence of bacterial vaginosis and impact of genital hygiene practices in non-pregnant women in Zaiyam, Iran. Oman Medical Journal. 2009;24:288-293.
22. Pendhakar S. The prevalence and risk factors for the most frequent lower genital tract infections among adolescents and young females (Unpublished Doctorate Thesis). Doctoral school of Semmelweiss University, Budapest. 2013;55-60.
23. Muvunyi CM, Hernandez TC. Prevalence of bacterial vaginosis in women with vaginal symptoms in south province, Rwanda. African Journal of Clinical and Experimental Microbiology. 2009;10(3): 156-163.
24. Aggarwal AK, Kumar R, Gupta V, Sharma M. Community based study of reproductive tract infections among ever married women of reproductive age in rural area of Haryana, Indian. J Commun. Dis.1999;31: 223-228.
25. Bhalla P, Chawla R, Garg S, Singh MM, Raina U, Bhalla R, et al. Prevalence of bacterial vaginosis among women in Delhi, India. Indian J Med Res. 2007;125(2):167-72.
26. Ugwumadu, A, Hay P, Taylor-Robinson D. HIV-1infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet. 1997;350:1251-1252.
27. Bradshaw CS, Morton AN, Hocking J, Garland SM, Morris MB, Moss LM, Horvath LB, Kuzevska I, Fairley CK. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J Infect Dis. 2006;193(11):1478-86.
28. Shobeiri F, Nazari M. A prospective study of genital infections in Hamedan, Iran. Southeast Asian Journal Trop Medicine Public Health. 2006;37:174–177.
29. Larsson PG, Bergström M, Forsum U, Jacobsson B, Strand A, Wölner-Hanssen P. Bacterial vaginosis. Transmission, role in genital tract infection and pregnancy outcome: An enigma. APMIS. 2005; 113(4):233-45.
30. Roberta BN, Sharon LH, Holly ER, David ES. Douching in relation to bacterial vaginosis, lactobacilli, and Facultative bacteria in the vagina. Obstet Gynecol. 2002;100:765–72.
31. Holzman C, Leventhal JM, Qiu H, Jones NM, Wang Y, Group BVS. Factors linked to bacterial vaginosis on non-pregnant women. American Journal of Public Health. 2001;91:1664-1670.
32. Ness R, Hillier S, Richter H. Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. Obstet Gynaec. 2002;100:765-772.
33. Akerele JP, Abhulimen, Okonofua F. Prevalence of asymptomatic genital infection among pregnant women in Benin City, Nigeria. J. Obstetr Gynaecol. 2002; 21:141-144.
34. Anh PT, Mai TP, Phuong HT. Prevalence of lower genital tract infections among Vietnamese women attending a maternal and child Health Centre in Hanoi, Vietnam. Southeast Asian J. Trop. Med. Public Health. 1996;27:193–195.
35. Olusanya B, Olutiola PO. Studies of bacteriuria in patients and students in Ile–Ife, Nigeria. West Africa Journal of Medicine. 1984;3(3):177–183.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22186