Bacterial vaginosis in pregnancy

Afr. J. Clin. Exper. Microbiol. 2022; 23 (3): 311-317

Short Communication   Open Access

Prevalence of bacterial vaginosis in pregnant women attending Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria using the complete Amsel's diagnostic criteria

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Abstract:

Background: Bacterial vaginosis (BV) in pregnant women remains a cause for clinical concern among clinicians and health care professionals. BV has been linked to prenatal, antenatal and postnatal challenges in pregnant women. Information on prevalence of BV across trimesters of pregnancy is expected to give better clinical insight into the pathophysiology of this polymicrobial disorder. This study was conducted to determine the prevalence of BV in pregnant women attending the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria.
Methodology: This was a cross-sectional study of 120 pregnant women (40 in each trimester of pregnancy) who had symptoms suggestive of BV, selected by systematic random sampling from among the women attending the Obstetrics and Gynaecology (O & G) clinic of NAUTH, Nnewi. Each subject participant was examined by the attending clinician, and high vaginal swab (HVS) sample was collected for diagnostic analysis of BV using with complete Amsel's clinical criteria, which consists of three of the four criteria; (i) adherent and homogenous vaginal discharge, (ii) vaginal pH > 4.5, (iii) detection of clue cells on saline wet mount, and (iv) amine odor after the addition of potassium hydroxide (positive Whiff test).
Results: The mean age of the 120 selected participants was 27.25±6.09 years. The age groups 25-29 (36.7%) and 20-24 years (33.3%) constituted the largest proportion, while age groups <20 (5.0%) and 40-45 years (5.0%) constituted the least. Of the 120 participants, 26 (21.7%) were positive for BV by the Amsel's criteria. Pregnant women in age group <20 years had the highest prevalence of BV (100%, 6/6), followed by those in the age groups 20-24 (27.5%), 40-45 (16.7%), 25-29 (15.9%), 30-34 (9.1%) and 35-39 years (0%) (X^2=28.063, p=0.0001). Prevalence of BV was significantly higher in single (unmarried) pregnant women (45.5%, X^2=4.038, p=0.045), women with primary school education level (66.7%, X^2=14.530, p=0.001), unemployed women (36.1%, X^2=13.278, p=0.0013), and nulliparous women [36.4%, X^2 (for trend) = 4.805, p=0.0274), while there was no significant difference in the prevalence of BV with relation to trimester of pregnancy (X^2=2.750, p=0.253).
Conclusion: This study reveals a relatively high prevalence of BV and significant association with factors such as age group, education and occupational status among pregnant women attending NAUTH Nnewi. Regular screening of women for BV prenatally may enable appropriate interventions to prevent adverse pregnancy outcomes.

Keywords: Bacterial vaginosis, pregnancy, trimesters, Amsel's criteria, polymicrobial, Nnewi, Nigeria

Prévalence de la vaginose bactérienne chez les femmes enceintes fréquentant l'hôpital universitaire Nnamdi Azikiwe, Nnewi, Nigeria en utilisant les critères de diagnostic complets d'Amsel

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Résumé:

Contexte: La vaginose bactérienne (VB) chez les femmes enceintes demeure une cause de préoccupation clinique chez les cliniciens et les professionnels de la santé. La VB a été associée à des problèmes prénatals, prénatals et postnatals chez les femmes enceintes. Les informations sur la prévalence de la VB au cours des trimestres de la grossesse devraient donner un meilleur aperçu clinique de la physiopathologie de ce trouble polymicrobien. Cette étude a été menée pour déterminer la prévalence de la VB chez les femmes enceintes fréquentant le Université Nnamdi Azikiwe, Nnewi, État d'Anambra, Nigéria

Méthodologie: Il s'agissait d'une étude transversale de 120 femmes enceintes (40 à chaque trimestre de grossesse) qui présentaient des symptômes évocateurs de VB, sélectionnées par échantillonnage aléatoire systématique parmi les femmes fréquentant la clinique d'obstétrique et de gynécologie (O & G) de NAUTH, Nnewi. Chaque sujet participant a été examiné par le clinicien traitant et un échantillon d'écouvillonnage vaginal élevé (HVS) a été prélevé pour une analyse diagnostique de la VB en utilisant les critères cliniques complets d'Amsel, qui se composent de trois des quatre critères ; (i) pertes vaginales adhérentes et homogènes, (ii) pH vaginal > 4,5, (iii) détection de cellules indices sur montage humide salin et (iv) odeur d'amine après l'ajout d'hydroxyde de potassium (test Whiff positif).

Résultats: L'âge moyen des 120 participants sélectionnés était de 27,25±6,09 ans. Les tranches d'âge 25-29 (36,7%) et 20-24 ans (33,3%) constituaient la plus grande proportion, tandis que les tranches d'âge <20 (5,0%) et 40-45 ans (5,0%) constituaient la plus faible. Sur les 120 participants, 26 (21,7%) étaient positifs pour la VB selon les critères d'Amsel. Les femmes enceintes du groupe d'âge < 20 ans présentaient la prévalence la plus élevée de VB (100%, 6/6), suivies de celles du groupe d'âge 20-24 (27,5%), 40-45 (16,7%), 25-29 (15,9%), 30-34 (9,1%) et 35-39 ans (0%) (X^2=28,063, *p*=0,0001). La prévalence de la VB était significativement plus élevée chez les femmes enceintes célibataires (45,5%, X^2=4,038, *p*=0,045), les femmes ayant un niveau d'études primaires (66,7%, X^2=14,530, *p*=0,001), les femmes sans emploi (36,1%, X^2=13,278, *p*=0,0013), et femmes nullipares [36,4%, X^2 (pour tendance) = 4,805, *p*=0,0274], alors qu'il n'y avait pas de différence significative dans la prévalence de la VB en fonction du trimestre de grossesse (X^2=2,750, *p*=0,253).

Conclusion: Cette étude révèle une prévalence relativement élevée de VB et une association significative avec des facteurs tels que le groupe d'âge, l'éducation et le statut professionnel chez les femmes enceintes fréquentant NAUTH Nnewi. Le dépistage régulier de la VB chez les femmes avant la naissance peut permettre des interventions appropriées pour prévenir les issues défavorables de la grossesse.

Mots-clés: Vaginose bactérienne, grossesse, trimestres, critères d'Amsel, polymicrobien, Nnewi, Nigeria

Introduction:

The vaginal microbial milieu undergoes significant structural changes at various stages during the life of woman that may be linked to several factors such as normal flora colonization, vaginal PH, hormonal modulation among other factors (1). The vaginal milieu is a dynamic ecosystem usually inhabited by bacteria which may be aerobic or anaerobic, with *Lactobacillus* species making up a significant population of the vaginal microbiota (2). Bacterial vaginosis (BV) is a polymicrobial disorder with accession of various fastidious bacteria, concurrent with a decrease of Lactobacilli, the dominant bacteria in the normal vaginal flora of women of reproductive age (3). It is also charaterized by the overgrowth of anaerobes such as *Gardnerella*, *Bacteroides* and *Prevotella* species, which may counteract the growth of Lactobacilli and other commensals (4). Among other factors, genital mycoplasmas have also been implicated in various adverse conditions concerning pregnant women and neonates (5).

Although, a previous Nigerian study (6) did not demonstrate significant adverse effects of BV on pregnancy outcome, subsequent longitudinal study by Afolabi et al., (7) showed significant association between BV in pregnant women and preterm delivery, low birth weight (LBW) and preterm rupture of membranes (PROM) in pregnant women resident in southwestern Nigeria. In East Africa, findings by Mengistie et al., (8) also inferred that prevalence of BV is high among pregnant women, which was linked with adverse situations such as preterm birth. Similar findings have also been established in Europe by Donati et al., (9). The Amsel's criteria have been shown to have a positive predictive value of 90% and proven to be convenient (10), reliable and inexpensive for clinical diagnosis (4,11), especially in developing countries with weak health system.

There is a dearth of recent and similar studies examining association between socio-demographics and BV in pregnant women resident in southeastern Nigeria and specifically in

Anambra State. The objective of this study therefore is to determine the prevalence of BV in pregnant women attending the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria

Materials and method:

Study setting and design

The study setting was the Obstetrics and Gynaecology (O & G) of Nnamdi Azikiwe University Teaching (NAUTH), Nnewi, Anambra State, Nigeria. NAUTH is a tertiary health institution established in 1991 primarily to provide specialized clinical service, teaching and research. This was a cross-sectional study of pregnant women with symptoms suggestive of BV attending the ANC clinic of the hospital between June and September, 2021.

Study population, sample size calculation and subject selection/recruitment

The study population comprise pregnant women with complaints of vaginal symptoms suggestive of BV attending the O & G clinic of the hospital. The sample size was determined using Daniel's sample size determination formula (12); N= $Z^2PQ/$ D2, where N = minimum sample size, Z=level of statistical significance of the expected result (in this case 1.96 for 95% confidence interval), P = proportion in the target population, Q = 1-P, and D= maximum allowable error, which is normally put at 0.05. Using the prevalence rate of BV in pregnant women of 7.5% from a previous study (13), the sample size was calculated as 114, which was adjusted to 120 participants.

Eligible participants were pregnant women at any trimester, who had symptoms suggestive of BV or complained of symptoms resembling BV. Women with vaginal bleeding, previously diagnosed with human immunodeficiency virus (HIV) infection or were on antibiotics were excluded from the study. Written informed consent was obtained from each participant.

Ethical approval

This study is a part of a wider research conducted in NAUTH, with ethical approval granted by Nnamdi Azikiwe University Teaching Hospital Health Research Ethics Committee (NAUTHHREC) with reference number NAUTH/CS/66/VOL.14/VER 3/121/2021/039.

Sampling technique

Participants were recruited using systematic random sampling until the sample size was obtained. Participation of subjects in the study was voluntary. The subjects were pro-perly informed on the nature, merits and aim of the study before sample collection. At recruitment, subjects were enrolled only after signing the informed consent form.

Clinical examination and specimen collection

Each subject was clinically examined by the attending clinicians in the O & G clinic for the presence or absence of discharge. The consistency, homogeneity, color and odor of the discharge were recorded. Sterile cotton-tipped swabs were labelled with the participants' study identification numbers, and were used to obtain vaginal samples from each consenting subject with the aid of a Cusco speculum for the purpose of diagnostic analysis with complete Amsel's clinical criteria and laboratory analysis

Amsel's diagnostic criteria:

Clinical diagnosis for bacterial vaginosis requires that at least three of the following four criteria are met; an adherent and homogenous vaginal discharge, vaginal pH greater than 4.5, detection of clue cells (vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders are obscured) on saline wet mount, and an amine odor after the addition of potassium hydroxide (positive whiff test) (11).

Demographic and clinical data collection

Data collection was done with aid of a well-structured questionnaire which took into consideration demographic information and factors that could influence prevalence of BV intended for evaluation. Essentially, information on age, marital status, trimester, educational status, occupational status, parity, use of antibiotics and poly-herbals were collected. Participants of antibiotics and poly-herbals were not excluded from the study.

Laboratory analysis of specimens

Laboratory analysis of specimen was done in the medical microbiology unit by of the laboratory complex of NAUTH. Test for vaginal pH, wet preparations and Whiff's tests were conducted as recommended

Test for pH

Vagina pH was measured by rolling the swab over a pH strip immediately after swabbing and matched with the provided color range to record the pH of the subject.

Wet film preparation for clue cell

Normal saline was added into one of the swabs containers and mixed gently several times. A drop was then be placed on a glass slide and gently covered with a cover slip. The

wet mount was examined using the 40x objective of a compound light microscope for the presence of clue cells (11).

Whiff's test

A drop of 10% potassium hydroxide (KOH) was added on a glass slide, followed by rolling of the swab on it. It was then whiffed for the liberation of a fishy odor indicative of the presence of volatile amines such as trimethylamine. The whiff result as recorded as either positive or negative on the Amsel record sheet.

Data analysis

Data analysis was conducted using SPSS version 20. Data obtained from the subjects were presented in tables, percentages and mean ± SD.

Results:

A total of 120 pregnant women (40 each in first, second and third trimester) were recruited as participants in this study. The mean age of the participants was 27.25±6.09 years. The age groups 25-29 (36.7%) and 20-24 years (33.3%) constituted the largest percentage of the pregnant women, while age groups <20 (5.0%) and 40-45 years (5.0%) constituted the least. Majority of the pregnant women were married (90.8%), multiparous (48.3%), self-employed (50.8%) and had secondary level education (74.2%). (Table 1).

Of the 120 participants, 26 (21.7%) were positive for BV by the Amsel's criteria, while 94 (78.3%) were negative. Pregnant women in the age group <20 years had the highest prevalence of BV (100%, 6/6), followed by those in the age group 20-24 (27.5%), 40-45 (16.7%), 25-29 (15.9%), 30-34 (9.1%) and 35-39 years (0%), which shows significant association (X^2=28.063, p=0.0001) (Table 2). Prevalence of BV was significantly higher in single (unmarried) pregnant women (45.5%, X^2=4.038, p=0.045), women with primary school education level (66.7%, X^2=14.530, p=0.001), unemployed women (36.1%, X^2=13.278, p=0.0013), and nulliparous women [36.4%, X^2 (for trend) = 4.805, p=0.0274), while there was no significant difference in the prevalence of BV with relation to trimester of pregnancy (X^2=2.750, p=0.253).

Discussion:

Demographic data of pregnant women in our study such as age group, marital, educational and occupational status are similar to those reported in other research studies, and the mean age of 28.06±4.91 years among women of reproductive age group attending NAUTH Nnewi, reported by Okoli et al., (4) is similar to the mean age of 27.25±6.09 years among the women in our current study. Our study reported a BV prevalence of 21.7% for pregnant women, which is similar to the rate reported by Afolabi et al., (7), and also falls within the range of 20.0-49.0% reported in African countries among women of reproductive age (14,15). The cumulative relationship between a positive whiff test, presence of clue cells and a vaginal pH >4.5 in the evaluation of BV have been shown to be statistically significant and reliable as a diagnostic test (16), and these are implemented in the complete Amsel's criteria for diagnosis of BV and vaginitis (11).

Findings from this study show significant association between prevalence of BV and age groups of pregnant women, with high prevalence in those in age groups < 20 (100%) and 20-24 years (27.5%). A probable explanation for this may be that younger women are more sexually adventurous and are prone to multiple sexual relationships, compared to older women, as previously reported by Ranjit et al., (17). Another explanation may be that older women are more experienced with regards to pregnancy and are more likely to pay attention to antenatal care and personal health during pregnancy compared to younger women. Our study also reported a significant association between prevalence of BV and educational levels, with high prevalence in women who had primary school education level. It is generally understood that education play a vital role in behavioral modification and this may have a direct bearing on health and hygiene habits of participants in our study. Our finding agrees with Ness et al., (15), who reported a significant association between prevalence of BV and low levels of education. There was also a significant association between occupational status and BV prevalence in our study, with the unemployed pregnant participants having the

Table 1: Socio-demographic characteristics of pregnant women recruited for the study at NAUTH, Nnewi, Nigeria

Characteristics	Frequency (%)
Age group (years)	
<20	6 (5.0)
20-24	40 (33.3)
25-29	44 (36.7)
30-34	11 (9.2)
35-39	13 (10.8)
40-45	6 (5.0)
Mean age (mean ± SD)	27.25±6.09
Marital status	
Married	109 (90.8)
Single	11 (9.2)
Trimester of pregnancy	
First	40 (33.3)
Second	40 (33.3)
Third	40 (33.3)
Educational status	
Primary	9 (7.5)
Secondary	89 (74.2)
Tertiary	22 (18.3)
Occupational status	
Government employed	23 (19.2)
Self-employed	61 (50.8)
Unemployed	36 (30.0)
Parity	
Nulliparous	33 (27.5)
Primiparous	29 (24.2)
Multiparous	58 (48.3)

Table 2: Association of socio-demographic characteristics and other factors with prevalence of BV in the participants

Variables	Bacterial vaginosis		Total (n=120)	X^2	p value
	Positive (%) (n=26)	Negative (%) (n=94)			
Age group (years)					
<20	6 (100)	0	6	28.063	<0.0001*
20-24	11 (27.5)	29 (72.5)	40		
25-29	7 (15.9)	37 (84.1)	44		
30-34	1 (9.1)	10 (90.9)	11		
35-39	0	13 (100)	13		
40-45	1 (16.7)	5 (83.3)	6		
Marital status					
Married	21 (19.3)	88 (80.7)	109	4.038	0.045*
Single	5 (45.5)	6 (54.5)	11		
Trimester of pregnancy					
First	12 (30)	28 (70)	40	2.750	0.253
Second	8 (20)	32 (80)	40		
Third	6 (15)	34 (85)	40		
Educational status					
Primary	6 (66.7)	3 (33.3)	9	14.530	0.001*
Secondary	19 (21.3)	70 (78.7)	89		
Tertiary	1 (4.5)	21 (95.5)	22		
Occupational status					
Government employed	8 (34.8)	15 (65.2)	23	13.278	0.001*
Self-employed	5 (8.2)	56 (91.8)	61		
Unemployed	13 (36.1)	23 (63.9)	36		
Parity					
Nulliparous	12 (36.4)	21 (63.6)	33	4.805[+]	0.0274*
Primiparous	5 (17.2)	24 (82.8)	29		
Multiparous	9 (15.5)	49 (84.5)	58		

* = statistically significant; X^2 = Chi square; [+] = Chi square for trend

highest prevalence, and self-employed women with lowest prevalence. This is similar to the findings of Ranjit et al., (17), who reported a lower BV prevalence in women who were engaged in business ventures, compared to unemployed housewives and farmers. Furthermore, single (unmarried) women had significantly higher prevalence of BV (45.5%) than married women (19.3%) (p=0.045) in our study, which may be attributed to the tendency of single women to have multiple sexual relationships, that may enhance sexual transmission of BV. Similarly, nulliparous women in our study had significantly higher prevalence of BV (36.4%) compared to multiparous (15.5%) and primiparous women (17.2%) (X^2 for trend 4.0805, p=0.0274). A previous study (6) showed that BV was significantly commoner (p= 0.0341) in primigravida than multigravida woman but the significance of these findings is not known. Although, Nelson et al., (18) postulated that BV in the first trimester may proceed to cause preterm complications in pregnant women in the second trimester, there was no significant association between prevalence of BV and trimesters of pregnancy in our study.

Although adverse pregnancy outcomes have not been conclusively linked to BV in pregnancy (6), the possibility of negative pregnancy outcomes was noted in a longitudinal study conducted by Afolabi et al., (7), and therefore, significant association between BV, and education and socio-economic factors reported in our study should not be de-emphasized. It is understood that the more educated and economically empowered a woman is, the more open she is to health and education interventions and willingness to engage in antenatal care during pregnancy, which promotes positive pregnancy outcomes (19). Conversely, less educated and economically challenged women may perhaps be open to patronizing cheaper means of alternative medical and antenatal care provided by untrained persons (quacks and charlatans), who may sometimes administer unregistered polyherbal regimens, some of which have been proven to be unsafe for human consumption (20). To prevent the possibility of negative pregnancy outcomes arising from BV, propagation of health jingles and sensitization programs in local languages are recommended, and subsidized antenatal care is also advocated.

Limitations of study

The study design is hospital-based and therefore exclude pregnant women in the community who do not visit the teaching hospital but traditional birth attendants for antenatal care. This will limit the generalization of our findings in this study.

Contributions of authors:

CVU and NRA conceived and designed the study, CVU wrote the first draft of the manuscript (MS) and reviewed literature along with NRA, LNC and SIO. CBO collected clinical samples from subjects. CVU performed laboratory analysis on the samples collected, analyzed data, performed statistical analysis and revised updated latest version of the manuscript (MS). All authors read and approved the final manuscript.

Source of funding:

No funding was received for the study

Conflict of interest:

Authors declare no conflict of interest

References:

1. Farage, M., and Maibach, H. Lifetime changes in the vulva and vagina. Arch Gynaecol Obstetr. 2006; 273: 195-202.
2. Hyman, R. W., Fukushima, M., Diamond, L., Kumm, J., Giudice, L. C., and Davis, R. W. Microbes of human vaginal epithelium. PNAS. 2005; 102: 7952-7957.
3. Ling, Z., Kong, J., Liu, F., et al. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. BMC Genomics. 2010; 11: 488-504.
4. Okoli, A. C., Agbakoba, N. R., Ezeanya, C. C., Oguejiofor, C, B., and Anukam, K. C. Comparative Abundance and Functional Biomarker of Vaginal and Gut Microbiome of Nigerian women with Bacterial Vaginosis: A study with 16S rRNA Metagenomics. J Med Lab Sci. 2019; 29:1-26.
5. Agbakoba, N. R., Adetosoye, A. I., and Adewole, I. F. Presence of *Mycoplasma* and *Ureaplasma* species in the vagina of women of reproductive age. West Afr J Med. 2007; 6 (1): 28-31.
6. Adesiji, Y. O., Taiwo, S. S., Adekanle, D. A., Oboro V. O, Fayemiwo. S. A., and Opaleye, O. O. Bacterial vaginosis and pregnancy outcome in Osogbo, Nigeria. Res J Med Sci. 2007; 1 (4): 195-198.
7. Afolabi, B. B., Moses, E. O., and Oduyebo, O. O. Bacterial vaginosis and pregnancy outcome in Lagos, Nigeria. Op F Inf Dis. 2016; 3 (1): 30.
8. Mengistie, Z., Woldeamanuel, Y., Asrat, D., and Adera, A. Prevalence of bacterial vaginosis among pregnant women attending antenatal care in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. BMC Res Notes. 2014; 7: 822.
9. Donati, L., Di Vico, A., Nucci, M., et al. Vaginal microbial flora and outcome of pregnancy. Arch Gynaecol Obstetr. 2010; 281 (4): 589-600.
10. Bansal, R., Garg, P., and Garg, A. Comparison of Amsel's criteria and Nugent criteria for diagnosis of BV in tertiary care centre. Int. J Reprod Contr Obstetr Gynaecol. 2019; 8 (1): 297.

11.   Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C. S., Eschenbach, D. A., and Holmes, K. K. Nonspecific vaginitis: diagnostic criteria and epidemiologic associations. Am J Med. 1983; 74: 14-22.

12.   Daniel, W. Biostatistics: A Foundation for Analysis in the Health Sciences. 7th edition. New York: John Wiley & Sons. 1999: 658-736.

13.   Achdiat, A. P., Rowawi, S. R., Fakhrosa, et al. Bacterial vaginosis in pregnant women and child hospital, Java, Indonesia: High prevalence in asymptomatic females. Ser J Der Ven. 2019; 11 (2): 53-59.

14.   Ayenalem, S., Yusuf, L., and Ashenafi, M. Lactic Acid Bacterial Vaginosis among Outpatients in Addis Ababa. J Hlth Dev. 2010; 24 (3): 198-204.

15.   Ness, R. B., Hillier, S., Ritchter, H. E., et al. Can known risk factors explain racial differences in the occurrence of bacterial vaginosis? J Nat Med Ass. 2003; 95 (3): 201-212.

16.   Anukam, K. C., Idemoh, C., and Olise, N. A. Evaluation of bacterial vaginosis using Nugent scoring system. J Biol Sci. 2014; 13 (1): 25-32.

17.   Ranjit, E., Raghubanshi, R., Maskey, S., and Parajuli, P. Prevalence of bacterial vaginosis and its association with risk factors among non-pregnant women: A hospital-based study. Int J Microbiol. 2018; Article ID 8349601

18.   Nelson, D. B., Bellamy, S., Nachamkim, I., Ness, R. B., Macones, G. A., and Allen-Taylor, L. First trimester bacterial vaginosis, individual micro-organism levels and risk of second trimester pregnancy loss among urban women. Fertil Steril. 2007; 88 (5): 13966-1403.

19.   Jubril, U. N., Saleh, G. N, Badaki, O., Anyebe, E. E., Umar, A., and Kamal, A. Health Education Intervention on knowledge and accessibility of pregnant women to antenatal care services in Edu, Kwara state, Nigeria. Int J Women Hlth Reprod. 2018; 6(2): 154-160.

20.   Udeogu, C. V., Agbakoba, N. R., and Chukwuma, G. O. Comparative distribution of bacterial contaminants of packaged and unpackaged polyherbal products sold in Nnewi, Nigeria. Afr J Clin Exp Microbiol. 2020; 21 (4): 354-359. https://doi.org/10.4314/ajcem.v21i4.13