

Prevalence of Aerobic Bacterial Vaginosis and *Trichomonas vaginalis* Associated with Socioeconomic Factors among Women in Misan Governorate

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Abstract

Vagina is an ecosystem balance, human vagina is dominated by *Lactobacillus spp* which creates a vaginal acidity environment (3.8-4.5) to protect vagina from Vaginitis pathogen. Vaginitis is an inflammation of vagina caused by bacterial vaginosis and *Trichomonas vaginalis*. The aims of this study was investigated aerobic bacterial vaginosis, *Lactobacillus spp.* and T.V in women with vaginitis. Study the clinical feature and demographic factors with vaginitis. 345 samples were collected from women with vaginitis. Study a demographic variables such as age, education level, socioeconomic state, residence, parity, in pregnant and non-pregnant women.

Keywords: Aerobic bacterial vaginosis, Vaginitis, *Trichomonas vaginalis*, Socio demographic, *Lactobacillus spp*

Introduction

Aerobic bacterial vaginosis is abnormal vaginal bacteria derived from bacterial vaginosis types. It is caused by replacement *Lactobacillus spp*, with *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*. Aerobic bacterial vaginosis caused to increase in Vaginal pH > 4.5 and inflammation with WBCs infiltration⁽¹⁾. Bacterial vaginosis causes abnormal vaginal secretions⁽²⁾. *Trichomonas vaginalis* is an extracellular single cell flagellated parasite of different in shape, with active motility⁽³⁾.

Materials and Method

Patients: Three hundred and forty five sample were obtain from females with different age (15-45) year attending to gynecology Out-patients department in Al-Sadder hospital (Missan city).

Measurement of pH: For pH measurement, vaginal secretion be occurred by placed on pH paper. The pH paper color after that compared to the pH value on a standard chart⁽⁴⁾.

Whiff test: Examination a whiff test is positive when a (10%)KOH is added to discharge and release a bad odor as a positive indicator⁽⁵⁾.

Microscopic examination: Which include a wet amount technique for vaginal discharge to detected *Trichomonas vaginalis*, bacteria, clue cells, and WBCs. Clue cells is a large epithelial vaginal cells adhesion coated with bacilli bacteria⁽⁶⁾. Gram stain used for examining aerobic bacterial vaginosis⁽⁷⁾.

Cultivation of vaginal specimens: All vaginal swabs were cultured immediately on Blood, MacConKey Chocolate agar for bacterial isolates and MRS agar as a selective media for *Lactobacillus spp.* about three vaginal swabs from each infected woman, first swabs for cultivation laboratory media and second swabs for cultivation on MRS agar with or without (3-5)% CO₂ for *Lactobacillus spp* grow. Third swabs for wet amount technique and gram stain.

Biochemical tests: Some biochemical test was used for diagnosing bacterial vaginosis such as Vitex-2, Catalase test and Oxidase test⁽⁸⁻⁹⁾.

Identification of *Trichomonas vaginalis*: To identify of *Trichomonas vaginalis* in swabs by taking vaginal discharge from women with vaginosis. Then take one drop from PBS and place it on glass slide and put a cover slide on it this technique called wet a mounting technique and giemsa stain to determinate a flagellated

parasite positive results was a motility of parasite ⁽¹⁰⁾.

Results

Socio Demographic characteristics of population:

In table(1) high percentage was age groups F range from (40-45) year were 68(80%) . The second variable was education level and related with vaginal infection is

an important part due to education may help to decreased a diseases . The socio-economic level was taken the high percentage was in women with a middle social level of 114(76%) , in this study rural was recorded a high percentage 157(79.3%). As for the parity of the study, the high ratio with multiparity 158 (77.1%).The pregnancy state effect on women with vaginosis but highest ratio was documented in non-pregnant women 277 (75.9%) .

Table (1):Socio -demographic characteristics of respondents in vaginosis women

Variable factors	No. of cases	Frequency	Positive cases%	Negative cases %	χ^2	P -value R ²
Age groups)years)						
GA: 15-19	49/345	14.2%	33(67.3%)	16(32.7%)	5.16	P=0.135 R=0.071
GB: 20-24	56/345	16.2%	46(82.1%)	10(17.9%)		
GC: 25-29	59/345	17.1%	41(69.5%)	18(30.5%)		
GD: 30-34	60/345	17.4%	42 (70%)	18 (30%)		
GE :35-39	36/345	10.4%	30(83.3%)	6(16.7%)		
GF: 40-45	85/345	24.6%	68 (80%)	17(20%)		
Education levels						
Illiterate	97/345	28.1%	73(75.3%)	24(24.7%)	6.044*	P=0.05* R=0.017
Primary	130/345	37.7%	100(76.9%)	30(23.1%)		
Secondary	93/345	27%	64(68.8%)	29(31.2%)		
Higher	25/345	7.2%	23(92%)	2(8.0%)		
Socioeconomic classes						
Low	145/345	42%	104(71.7%)	41(28.3%)	3.07	P=0.10 R=0.092
Middle	150/345	43.5%	114(76%)	36(24%)		
High	50/345	14.5%	42(84%)	8(16%)		
Residence						
Rural	198/345	57.4%	157(79.3%)	41(20.7%)	3.86*	P=0.025* R=0.106
Urban	147/345	42.6%	103(70.1%)	44(29.9%)		
Parity						

Cont... Table (1):Socio -demographic characteristics of respondents in vaginosis women

Nullipara	46/345	13.3%	30 (65.2%)	16(34.8%)	2.95	P=0.11 R=0.07
Unipara	94/345	27.2%	72(76.6%)	22(23.4%)		
Multipara	205/345	59.4%	158(77.1%)	47(22.9%)		
Woman state						
Pregnant	46/345	13.3%	33(71.7%)	13(28.3%)	0.37	P=0.25 R=0.03
Non Pregnant	299/345	86.7%	277(75.9%)	72(24.1%)		
P≤0.05)* (p≤0.01)**)						

Clinical manifestations of vaginal infection : Vaginal discharge was the most common sign in Vaginitis women . The highest percentage of discharge was recorded 247(76%), Than the lower percentage of pelvic inflammatory was documented 38(77.6%) as in table (2)

Table(2): A Clinical manifestations of vaginitis women

Symptoms and signs	No. of cases N=345 %	No. of positive cases* N=260	No. of negative cases	χ^2	P- value	R ²
Vaginal discharge	325(94.2%)	247(76%)	78(24%)	1.228	0.134	0.060
Profuse itching	317(91.9%)	239(75.4%)	78(24.6%)	0.002	0.48	0.002
Malodor	298(86.4%)	226(75.8%)	72(24.2%)	0.268	0.30	0.028
Abdominal pain	173(50.1%)	131(75.7%)	42 (24.3%)	0.024	0.43	0.008
Pelvic inflammatory	49(14.2%)	38(77.6%)	11(22.4%)	0.147	0.35	0.021

* (In present trichomonas vaginalis ,aerobic bacterial and *lactobacillus spp*)

Effect of ph measurement with vaginosis pathogen:

Change in ph help to encourages the growth of pathogenic microorganisms which prefer alkaline ph . This study showed the effect of ph with *lactobacillus*

spp when ph was acidity.In *Trichomonas vaginalis*, the highest percentage was observed 62(78.5%).The high percentage of aerobic bacterial when ph was 156(60%) as in table (3) and figure (1)show amoeboid shape of *Trichomonas vaginalis* and *lactobacillus spp* .

Table(3): A correlation ph level with vaginosis pathogen

Microorganism ≤ 4.5		Vaginal pH		Total	P-value
		>4.5			
<i>Lactobacillus spp</i>	No.	187	113	300	0.014*
	%	62.3%	37.7%	100%	
<i>T. vaginalis</i>	No.	17	62	79	NS
	%	21.5%	78.5%	100%	
Aerobic bacterial vaginosis	No.	104	156	260	0.001**
	%	40.0%	60.0%	100%	

*(P ≤ 0.01), ** (P 0.001), NS = no significance

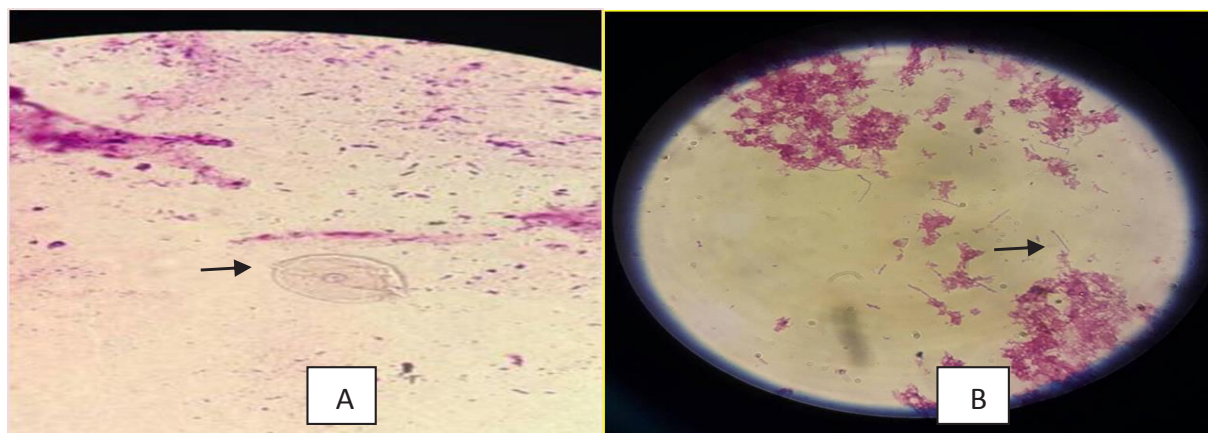


Figure (1):A- Amoeboid shape for *Trichomonas vaginalis* staining giemsa stain

B- Microscopic examination of *lactobacillus spp* by gram stain under 1000X magnification

Types of isolates of aerobic bacteria which causes vaginosis:

Different types of aerobic bacterial had been identified. The bacteria were diagnosed by vitex-2 and some biochemical test. The following results were shown in table (4) high incidence of bacteria vaginosis was recorded *Escherichia coli* 36(13.8%) .

Table(4):Types of isolates aerobic bacterial vaginosis

No.	Aerobic bacterial vaginosis	Frequency	%
1-	<i>Escherichia coli</i>	36	13.8%
-2	<i>Staphylococcus aureus</i>	27	10.4%
-3	<i>Enterobacter spp</i>	25	9.6%
4-	<i>Streptococcus spp</i>	23	8.8%
5-	<i>Klebsiella spp</i>	20	7.7%
6-	<i>Proteus spp</i>	17	6.5%

Cont... Table(4):Types of isolates aerobic bacterial vaginosis

7-	Staphylococcus epidermidis	14	5.4%
8-	Acrobacter spp	14	5.4%
9-	Enterococcus faecium	12	4.6%
10-	Pseudomonas spp	12	4.6%
11-	Leuconostoc menseteriods	12	4.6%
12-	Staphylococcus albus	9	3.5%
13-	Serratia spp	8	3.1%
14-	Acinetobacter spp	6	2.3%
15-	Kocuria spp	4	1.5%
16-	Pantoea spp	4	1.5%
17-	Streptococcus pneumonia	4	1.5%
18-	Citrobacter spp	4	1.5%
19-	Peptostreptococcus spp.	4	1.5%
20-	Providencia spp	2	0.8%
21-	Lactococcus gravies	2	0.8%
22-	Staphylococcus haemolyticus	1	0.4%
	Total	260	100%

Discussion

Socio-Demographic characteristics of population

Women in age groups F (40-45)years as a higher rate 68(80%). These results was disagree with Bhalla and Chawla.,2007 were recorded a highest percentage among age groups (25-49) year was 57(32.5%) which had a high sexual activity and high hormones activity with bacterial vaginosis ⁽¹¹⁾. In Sulaimania (Kadir and Fattah .,2010) were documented a high percentage between age group (26-35)year was 5(2.48%) these disagree with this results⁽¹²⁾. However, the age groups F (40-45)years more effect than young age . This age groups may be relation with low activity of hormones as estrogen hormone and low secretion of glycogen ,elevated ph to alkaline when advance in age which courage pathogens . Primary education was recorded a highest rate 100(76.9%)and this results in line with report in Nigeria ⁽¹³⁾. This results agree with Singh and Kanti .,2016 ⁽¹⁴⁾.In Iraq were documented that illiterates women were more effected ⁽¹⁵⁾.The most common causes related to neglect their health ,lack of education, bad body hygiene and lack of health are women's awareness programs ⁽¹⁶⁾. In socio-economic level a

highest percentage in middle state 114(76%). This study also agrees with Yasmeen and Mohamoud.,2011 ⁽¹⁷⁾.The main reasons for the role of socioeconomic stranded to infected by many women below the average in level of nutrition and health neglect ⁽¹⁸⁾.A current study reported a high percentage in rural residence157(79.3%).This results disagree with Mahmoud .,2017 due to lack of awareness of health neglect and low education and poor life style ⁽¹⁹⁾. In parity observed that multipara had a higher rate of 158(77.1%).(Singh.,2015) study a parity as parameter with *Trichomonas vaginalis* was recorded a high percentage in multipara 31(26.7%) ⁽²⁰⁾. These results related to abortion , vaginal delivery , douching uses and having oral contraceptive ,intrauterine device use and sexual activity those similar to Victor.,2016⁽²¹⁾. This related to low education, poor feeding, bad .Current study showed vaginitis based on women states with non-pregnant women state this agree with Yasmeen.,2011 ⁽¹⁷⁾.

Clinical Manifestations of vaginal infection :

Vaginal discharge recorded a high percentage 247(76%) . The percentage of vaginal discharge was 247(76%) with aerobic bacterial and *Trichomonas vaginalis*. Others studies had shown a percentage of

discharge with bacterial vaginosis 11(34.4%) and *Trichomonas vaginalis* 130(13.1%)⁽²²⁾. An abundant itching was documented 239(75.4%) and this results different with Mateus.,2016 were documented a vaginal discharge 56(0.18%)⁽²³⁾. Also Ranjit.,2018 in her studies was reported ,itching as a vaginal signs 13(23.2%) and no vaginal itching 78(75%)⁽²⁴⁾. (Nzyomo.,2013) was reported a high rate abdominal pain⁽²⁵⁾,his study agree with this results . Bacterial vaginosis had a special bad odor.This study had another clinical manifestations as a malodor that was reported 226(75.8%) and agreement with Ranjit.,2018 was study a foul odor as one of a clinical manifestations was 31(29.9%) in non- present⁽²⁴⁾. A bad odor with *Trichomonas vaginalis* in this study similar to Al-Samarraie.,2002 was documented a bad odor (88.09%) and discharge (11.09%) this causes may be related to metabolic by products of aerobic bacterial⁽²⁶⁾Fishy odor may related to volatilization of amine such as putrescine and cadaverine which produce by bacterial⁽²⁷⁾. Abdominal pain in this study was reported 131(75.7%) ,this agreement with Bhallo.,2012⁽²⁸⁾. Ranjit .,2018 was study abdominal pain as symptoms of vaginosis was a 22(26.6%) agree with this results⁽²⁴⁾. Pelvic inflammatory, was recorded 38(77.6%),this study agrees with (Yasmeen.,2011) observed a pelvic inflammatory and reported 53(74.6%) and its similar with this data⁽¹⁷⁾this result may be related to douching use, that enhance to growth bacteria⁽²⁹⁾.

Correlation of ph levels of Vagina with vaginosis pathogen :

PH is complementary in diagnosis vaginitis as an evidence of inflammation . *Lactobacillus spp* was recorded a higher ph level at less than 4.5 was 187(62.3%). The study was observed and agreement with Tachedjian and others.,2017. Estrogen hormone also rise during puberty leading to increase glycogen deposition in vaginal epithelial cells as well as increased colonization of *lactobacillus spp* and pathogenic microorganisms .*Lactobacillus spp* breakdown glycogen into glucose and maltose ,then convert to lactic acid and led to decrease ph⁽³⁰⁾. *Trichomonas vaginalis* and bacterial vaginosis, which were preferable to alkaline ph at expense of *lactobacillus spp*. Valadkhani ,2004 showed the role of ph in adhesion and colonization of trichomonas with *lactobacillus spp* at 47% and 35%. *Trichomonas vaginalis*, was recorded a highest proportion with alkaline ph 62(78.5%)⁽³¹⁾. Also this study documented a high ph in alkaline ph 156(60%) with aerobic bacterial vaginitis and this identical with

Liston had shown a suitable ph is an important with development of *Trichomonas vaginalis* . Some factors that help to elevate ph level like antibiotics treatment , seminal fluid and intercourse. The vaginal acidity related to increase estrogen hormone and lead to increase a thick of stratified epithelium during sexually maturity which help *lactobacillus spp* to colonize epithelium and increase of glycogen.

Distribution of Aerobic bacterial vaginosis in vaginitis women:

This study documented a high rate of aerobic bacterial vaginosis was *E.coli* 36(13.8%). Adane was studied some bacterial Vaginitis like *Escherichia coli* was a high rate 43(28.5%) , therefore his data agree with our data which reported *Escherichia coli* a high rate⁽³²⁾. In McDonald work, on a vaginal discharge and associated with aerobic bacterial and recorded it ,he founded *Streptococcus spp* group , *Staphylococcus aureus* and *Escherichia coli*. were important aerobic bacterial vaginosis related with pregnancy. *Escherichia coli* is a causative agent of vaginosis because *Escherichia coli* is a normal flora in GI and may be transferred to genital tract and causes bacterial vaginosis . Also washing under wear with strong detergent poor body hygiene.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

- 1- Donder GGG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B. Defination of a type of abnormal vaginal vaginosis .Aerobic Vaginitis .Br.J.Obstet Gynecol 2002., (109):34-43.
- 2- Donali L, Di Vico A, Nucci M,. Vaginal Microbial flora and outcome of pregnancy .Arch Gynecology .Obstetric 2010., 281(14):589-600.
- 3- Ryan K .J, Ray C.G, Ahmad N et al . Sherri's Medical Microbiology : flagellates . 5Th ed .Mc Graw. Hill,2010., 813-35.
- 4- Valadkhani, Z. Role of ph on adhesion of and cervical epithelial change. Annals of *Trichomonas*

- vaginalis* isolated from symptomatic and asymptomatic women to vaginal epithelial cells *in vitro*. Iran. J. Med. Scie,2004., 29: 134-139.
- 5- Briselden, A . and Hillier, S. Evaluation of affirm VP Microbiol identification test for Gardnerella and *Trichomonas vaginalis* . J. Clin.Microbiol. 1994 ,(32): 148–152
 - 6- Donders G. , Vereecken A. , Dekeersmaecker A. , Van Bulck B. , and Spitz B. Wet mount microscopy reflects functional vaginal lactobacillary flora better than Gram stain J Clin Pathol. ., 2000;53(4): 308–313.
 - 7- Collins CH, Lyne PM and Grange JM:Microbiological methods.8th edition .Butterworth-Heinemann,London.,2004.
 - 8- Cruickshank k.R.,Dujiuid J.P, Marmoin B.Pand Swain R.H.A:Medical Microbiology 12th edition Vol.2.Churill Livingston ., Edingburing,London and new york., 1975.
 - 9- Crowan ST and Steel KH: Manual for identification of identification of medical bacteria., 1974 ;175,180,169,177,146.169,177,146.
 - 10- Sobel JD: Bacterial vaginosis.Annu.Rew. Medical.,2000,51:349-56.
 - 11- Bahalla P and Chawla R: Prevalence of bacterial vaginosis among women in Delhi, Indian J Med Res.,2007; 125, 167-172 .
 - 12- Machado, A., Almeida, C., Azevedo, N., Carvalho, L., Vieira, M.J., Cerca, N. *etal. Lactobacillus spp.* identification in mixed samples by PNA FISH. Presented in: Eurobiofilmes Conference, Copenhagen,2011.
 - 13- Singh A , Kanti V, Dayal S, Kumar S Shukla, Mishra N: Prevalence and risk factors of bacterial vaginosis among women of reproductive age attending rural Tertiary case institute of West rann uttar pradesh . J. Evolution Med. Dent. .,2016 Sci./ eISSN- 2278-4802, pISSN- 2278-4748/ Vol. 5/ Issue 43.
 - 15- Xu, F, Markowitz L E, Gottlieb S L and Berman S M; Seroprevalence of herpes simplex virus type 1 and 2 in pregnant women in the United State Am J Perinat. Med.,2007. 36: 206-12.
 - 16- Glenville, M: Vaginal infection , understanding vaginal infection e-book everything you need to know about vaginal infection from symptoms terms to solution the natural health practice.,2012.2-19.
 - 17- Yasmeen H, Mohammed AF, Asad R and Tamer E:Detection of trichomonas vaginalis in vaginal swab clinical samples from Palestinian women by culture. International scholar Research Net Work. ISRN .Micrbiology . 2011., Volum 2011,Article ID 872358,4 page.
 - 18- Shira CS, Frank JS:Viability of *Trichomonas vaginalis* in urine :Epidemiologic and clinical implication of clinical Microbiology .,2006.44:3787-3789.
 - 19- Waseeqa Nigeen, Abdus Sami Bhat, Khushboo Gulzar, Shehaaz Taing: Correlation of bacterial vaginosis with preterm labor :a care control study . international Journal of reproduction Contraception ,Obsterstetrics and Gynecology.,2015 ;ISSN 2320-1770 .e LSSN 2320-1789.
 - 20- Singh H. O., Singh A., Dhole T. N., and Nain S:“Factor Associated to Bacterial Vaginosis in Non-pregnant Women of North Indian Population, Journal of Biotechnology Biomaterials, ., 2015. 5, 195-200.
 - 21- Victor DA, Habilo M S, Abdullahi R and Banmgboye MA: Bacterial Vaginosis is a common vaginal infection among first .time Antenatal Clinical Attendees : Evidence from a tertiary health facility in North –west Nigeria .Journal of prevention and infection control., 2016, ISSN 2471-9668.Vol,2,NO.2 :14.
 - 22- Amsel R,Totten PA , Spiegel GA,Chen CCG,Eschenbach D,Holmes KK.:nonspecific vaginosis,1983:74:14-22.
 - 23- Maeus De ,Paul AG, Lana C,Hian D., Eleuza R.M: Prevalence of *Trichomonas vaginalis* and *Candida albicans* among Brazilian women of reproduction age .Journal of clinical and diagnostics Research , .,2016;Vol.10(11):LC24-LC27.
 - 24- Ranjit E ,Bijendra R. R ,Smirity Maskey and pramila P:Prevalence of bacterial vaginosis and its association with risk factors among no pregnant women :AHospital based study .International Journals of Microbiology ., 2018;Volume 2018,Article ID 8349601,9 pages .
 - 25- Nzomo J., P.Waiyaki, and R.Waihenya: Bacterial Vaginosis and Correlates in Women of Reproductive Age in Thika, Kenya, Advances in Microbiology ., 2013; 3 (03), 249–254.
 - 26- Al-Samarra’ie H.F: Comparative study of *Trichomonas vagina/is* and bacteria coexistence

- in vaginal infection in pregnant and no pregnant women. MSC thesis, College of Medicine, Baghdad University, 2002
- 27-Eschenbach D.A.: The lower genital tract infections, in Galask R.P. and Lareson B. Infectious diseases in the female patients. Springer -Verlag. New York, P: ,1986; 136-186.
- 28- Bhalla p ,Robit chavla several searchers observed the correlation of *Trichomonas vaginalis* with other bacterial vaginosis; Identification of *Trichomonas vaginalis* using molecular methods in Iraqi infected women. Iraqi J Biotechnol. , 2012 11(1):51–64.
- 29- Cottrell B.H: Vaginal douching. J Obstet Gynecol Neonatal Nurs., 2003: 12-18.
- 30- Tachedjian G ,Muriel Aldunate ,Catronia S. Bradshaw and Richard A. cone: The role of lactic acid production by production by Probiotics *Lactobacillus spp* in vaginal health .Research in Microbiology in microbiology., 2017; 168,782-792.
- 31- Valadkhani Z, Sharma S, Harjai K, Gupta L and Malla N: In vitro Comparative kinetics of adhesive and hemolytic potential of *Trichomonas vaginalis* isolates from systemic and asymptomatic female .India .Journal of pathological Microbiology., 2003; 46:693-699.
- 32- Adane B, Yehiwork A, Delayehu ,and Amete M: Prevalence of bacterial vaginosis and associated risk factors among women complaining of genital tract infection .International .J. of Microbiology., 2017; ID 4919404, 8 Pages.