

The Microbiological Study of Bacterial Vaginosis in Women with Recurrent Spontaneous Abortion in Babylon Province

SumayaYahya Abed Obais and ShaimaaJassimAlsultany

Department of Biology, College of Science, University of Babylon, Iraq

sumiyahya1987@yahoo.com

Abstracts

The presents study included investigation of bacterial vaginosis in women with recurrent abortion , 56 sample that are collected and the identification of bacterial vaginosis by Amsels criteria, thin homogenous discharge represented (88%), with specificity of 13%, then the second sensitive parameter was clue cell which was 82% with specificity 18% and third sensitive parameter was pH which was 79% with specificity 22% while the lowest sensitive test, was the swiff test which was 55% with high specific parameter 45%. But the most specific parameter was the clue cell test, with (81%), When a combination (in series) was done among pH measurement, Swiff test, clue cells and thin homogenous discharge, this results in increasing specificity to become 24%, and sensitivity was 76%.

50 sample with recurrent abortion women 50(89.28%) While the percentage with non growth was 6(10.71%) and its divided according to age group, the age group 26-36 years was represented 51.78% and followed by 15-25year (28.57%) and then 37-57year 17.85% and finally ≥ 48 year 1.78%

Identification of isolates of bacterial vaginosis in recurrent abortion women by VITEK, shown identified only one isolate from Gram negative bacteria *E.coli* and its percentage was reached to 29 (58%) While, the isolates of Gram positive bacteria, the *Staphylococcus haemolyticus* isolate percentage was 18%(9) While, *Staphylococcus hominis* and *Staphylococcus lentus* isolates had been reached to 8% (4) for both isolate, Whereas each isolate (*Enterococcus faecalis*, *Staphylococcus pseudintermedius*, *Streptococcus pluranimalium* and *Staphylococcus saprophyticus*) were recorded 1 (2%)

the highest percentage of abortion was recorded in 3rd abortion and reached to 27 (54%), followed by 4th abortion was reached to 14 (28%) Whereas, 5th abortion was 4 (8%) While, 6th abortion was reached to 3(6%) and lowest percentage of abortion was recorded in 7th abortion and was reached to 2 (4%)

Keyword : Bacterial vaginosis, Amsel's criteria and recurrent abortion

Introduction

Recurrent spontaneous abortion is the most common complication of pregnancy and considered as one of the most important reproductive problems in the world, abortion is defined as termination of pregnancy that had been associated with multiple causes, A fetus of less than

twenty weeks gestation or a fetus weighing less than 500 gm is considered an abortus, recurrent abortion or recurrent pregnancy loss (medically termed habitual abortion) is defined as the loss of three or more consecutive pregnancies prior to 20th week gestation and affects 1% of couples (Laxmi *et al.*,2012 and Javed *et al.*,2019). Epidemiological studies demonstrated the risk subsequent pregnancy loss is approximately 24% after two clinical pregnancy losses , 30% after three , and 40% after four consecutive spontaneous abortion (Contini *et al.*,2018)

Numerous factors play essential a role in abortion, some of these include bacterial and viral infection, chromosomal abnormalities , genetic endocrine ,anatomical factors , thrombophilia disorders , environmental factors and immunological disorders (Cohain et al., 2017).

Bacterial vaginitis had been associated microorganisms in amniotic fluid and the placenta coming from cervicovaginal mucosa were found to associate with abortion and preterm labor (Fukuta et al., 2020), Bacterial vaginitis that are responsible for three fold risk of recurrent spontaneous abortion in the first trimester (Giakoumelou et al., 2016) whereas increased four fold of recurrent spontaneous abortion in late trimester (Donders et al.,2000 and Fukuta et al., 2020).

Amsel et al. (1983) introduced a criterion for diagnosing BV in 1983. They stated that the presence of three of the following four criteria shows diagnosis of BV , Increased homogeneous thin vaginal discharge , pH of the secretion greater than 4.5; Amine odor when potassium hydroxide 10% solution is added to a drop of vaginal secretions , Presence of clue cells in wet preparations (Amsel et al., 1983). Taj et al. examined Amsel's criteria and other microbiological methods to diagnose BV and showed that Amsel's criteria are acceptable for diagnosing BV (Taj et al., 2012) While Rangari et al. (2013) in their study of 2013 reported had a higher sensitivity in diagnosing BV while Amsel's criteria had less sensitivity and higher specificity .

Material and Methods

Collection samples

In the presents study, the collection sample of bacterial vaginosis that are collected from recurrent abortion women in Al-Hilla city that are attended to departments of obstetric and gynaecology at maternity and child teaching hospital, and some private clinics in Al-Hilla city, from period 1/12/2019 to 1/6/2020, this study involved (56) samples,50 sample which were collected from women by high vaginal swab with recurrent spontaneous abortion and 6

samples which are appeared with non growth, All subjects were within 15-45 years of age, tests of vaginal secretions that are used to evaluate of bacterial vaginosis, the amsel criteria appeared positive if three test of amsel criteria as positive that are:-

- Detection of clue cells by direct microscopic examination of vaginal swabs.
- Vaginal pH measurements : by using whatman pH paper dipped into the vaginal discharge.
- Thin homogenous discharge
- Swiff test: by adding a drop of 10% potassium hydroxide to the vaginal discharge with the release of a fishy amine odor and detection of the shapes and color of secretions (Amsel *et al.*, 1983 and Mahdi and Al-Hamdani, 1998).

Isolation and Identification of bacterial vaginosis by VITEK compacts-2 systems

A culturing of vaginal swabs of women with trimester recurrent spontaneous abortion on blood agar , MacConkey , and Chocolate agar medium and incubated at 37 C° and for 48 h under aerobic and anaerobic condition and it was identified depending on its morphology.

The vitec 2 device was used to identification of bacterial vaginosis, which was carried out in compliance with the manufacturer's instructions. This machine consists of personal device, reader incubator, which consisted of several internal components including: card filling process, card cassette, c loading process, bar code scanner, card sealer, cassette carousel and incubator. Besides optical transmission, waste processing, electronic control instruments and firm ware.

The system has been equipped with an extended identification database for all routine identification tests that provide an enhanced microbial diagnostic efficiency that reduces the need for additional testing to improve safety for both the test and the user.

The following steps are planned according to the directions of the manufacturers. Three ml of normal saline are placed in the plane test tube and inoculated with an isolated colony full of loop. The colony must be 24 hours of age, the test tube inserted into the colony standardization adens testing system to Macfarlands standard solution (1.510 β 8 cell / ml). the standardization of colony inoculums are placed into. Cassette and sample identification number by barcode in the device applications. The vitek2 card is then attached to the sample ID number. The cassette then

positions all the following measures performed by the instrument in the filler box, the instrument manages the incubation temperature, the card's optical reading and constantly tracks and moves the test data to the device for review.

1. standardization

The handling of the sample inoculum preparation, standardization and dilution step is minimized after primary isolation. The standardization inoculum is placed in the cassette, and barcode is used to interfere with the sample identification number in the computer software.

2. Traceability

The form of vitek2 card is then read from the barcode put on the card during fabrication, and the card is attached to the sample ID. This is a simple barcode reading move for manufacturers to connect the card to patient details.

3- load and go

In the filler module position the tape. The cassette is transferred to the reader / incubator module when the card is filled in. The instrument shall handle all subsequent steps

Statistical Analysis

Statistical analysis was carried out using statistical package for social science SPSS statistical software for windows version 24 to find means, Standard deviation, Least Significant differences by Duncan and Correlation by ANOVA

Results

Among 56 pregnant women who attended the departments of obstetric and gynaecology at maternity and child teaching hospital, and some private clinics in Al-Hilla city with recurrent abortion women, that age were ranged between (15-45) years from period 1/12/2019 to 1/6/2020 that are collected from vaginal of recurrent abortion women,

Identification of bacterial vaginosis by Amsel's criteria

The presents study in table (1-1) was the identification of bacterial vaginosis by Amsel's criteria, It was found that the most sensitive parameter, in identifying bacterial vaginosis was thin homogenous discharge measurement which was (88%), with specificity of 13%, then the second sensitive parameter was clue cell which was 82% with specificity 18% and third sensitive

parameter was pH which was 79% with specificity 22% while the lowest sensitive test, was the swift test which was 55% with high specific parameter 45%. But the most specific parameter was the clue cell test, with (81%), When a combination (in series) was done among pH measurement, Swift test, clue cells and thin homogenous discharge, this results in increasing specificity to become 24%, and sensitivity was 76%.

Table (1-1) The characteristics of Amsels criteria as test in screening of bacterial vaginosis

Amsels Criteria	Bacterial Vaginosis		Total	Sensitivity	Specificity
	Positive case	Negative case			
pH \geq 4.5	78.57% (44)	21.42% (12)	56	79%	22%
Swift test	55.35% (31)	44.64% (25)	56	55%	45%
Clue Cell	82.14% (46)	17.85% (10)	56	82%	18%
Thin homogenous discharge	87.5% (49)	25% (7)	56	88%	13%

the number of collection specimen were 56 sample, 50 isolate were belong to bacterial vaginosis and represent the percentage 50 (89.28)% While, the percentage of Non growth was 6 (10.71)% table (1-2). All samples were 50 specimen that are cultured on blood agar that are obtained positive growth on blood agar at percentage 86% (43), while the cultured on chocolate agar the percentage of growth was reached 100% (50) and MacConky agar that are cultured and the percentage of growth on this media was 100% (50)

Table (1-2) Shown the percentage of microorganisms that are isolated from vaginal of recurrent abortion women

Type of microorganisms	Number of organism	Percentage %
Bacterial vaginosis	50	89.28
Non growth	6	10.71
Total number of specimen	56	100%

The recurrent abortion women was studied according to age group , number of recurrent abortion and bacterial vaginosis, the age group that are mostly liable to have bacterial vaginosis in present study was recorded at 26-36 years and which was reached to 51.78% and followed by (15-25)

years that are reached to 28.57% and then 37-47 years and which shown the rate 17.85% and finally , the low percentage rates was recorded among female in age group at ≥ 48 years and which was reached to 1.78% figure (1-1).

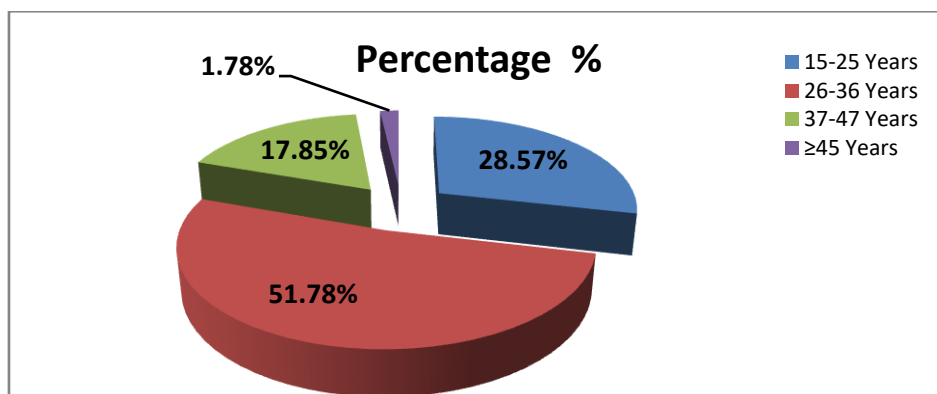


Figure (1-1) Distribution of women with recurrent abortion according to age

The percentage of Gram positive bacteria isolates were 21 (42%) and percentage of Gram negative bacteria isolates were 29 (58%) figure (1-2).

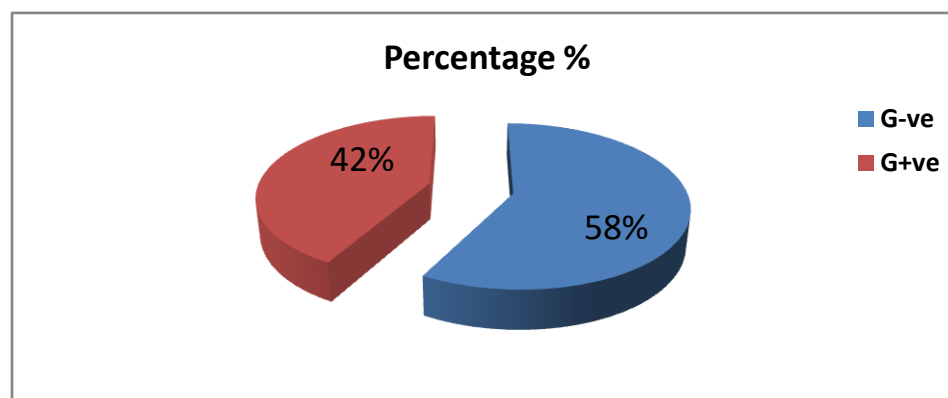


Figure (1-2) Gram negative bacteria and Gram positive bacteria isolation from vaginal with recurrent abortion women.

Identification of Bacterial vaginosis and number of Recurrent abortion in women with abortion

In this study we were used VITEK system to *Identification of* isolates of bacterial vaginosis in recurrent abortion women, the results of presents study shown identified only one isolate from Gram negative bacteria *E.coli*, its percentage was reached to 29 (58%) and While, the isolates of

Gram positive bacteria, the *Staphylococcus haemolyticus* isolate percentage was 18%(9) While, *Staphylococcus hominis* and *Staphylococcus lentus* isolates had been reached to 8% (4) for both isolate, Whereas each isolate (*Enterococcus faecalis*, *Staphylococcus pseudintermedius*, *Streptococcus pluranimalium* and *Staphylococcus saprophyticus*) were recorded 1 (2%) Table (1-4).

Table (1-4) the percentage of bacterial vaginosis in women with recurrent abortion

Gram positive vaginosis bacteria	Number	Percentage %
<i>Staphylococcus haemolyticus</i>	9	18%
<i>Staphylococcus hominis spp</i>	4	8%
<i>Staphylococcus lentus</i>	4	8%
<i>Enterococcus faecalis</i>	1	2%
<i>Staphylococcus pseudintermedius</i>	1	2%
<i>Streptococcus pluranimalium</i>	1	2%
<i>Staphylococcus saprophyticus</i>	1	2%
Total number of bacterial vaginosis	21	
Gram negative of bacterial vaginosis		
<i>E.coli</i>	29	58%

Table (1-5) explained the number of recurrent abortion in women with bacterial vaginosis, the highest percentage of abortion was recorded in 3rd abortion and reached to 27 (54%), followed by 4th abortion was reached to 14 (28%) Whereas, 5th abortion was 4 (8%) While, 6th abortion was reached to 3(6%) and lowest percentage of abortion was recorded in 7th abortion and was reached to 2 (4%).

Table (1-5) the Number of recurrent abortion in women with bacterial vaginosis

No. of recurrent abortion	No. of patient with BV	%
3	27	54%
4	14	28%
5	4	8%
6	3	6%
7	2	4%
Total	50	100%

Discussion

Bacterial vaginosis is associated in women with recurrent abortion (Xia *et al.*, 2015), the results of presents study that are the percentage of bacterial vaginosis and represent was 50 (89.28)% While, the percentage of other organism (non-bacterial vaginosis) was 6 (10.71)% in table (1-1). and the age group that are mostly liable to have bacterial vaginosis in present study was recorded at 26-36 years and which was reached to 51.78% figure (1-1) , this results agreements with results that performed by Isik *et al.*, (2017) that are founded the association between bacterial vaginosis with recurrent spontaneous abortion, the 56 (91.8%) of the 61 women were detected as having the history of a recurrent spontaneous abortion, the women in the study group were also evaluated in view of the abortion whether it has happened in the first or second trimester of gestation. 47 (77%) of the 61 women had the first trimester miscarriage (≤ 12 weeks), and the remaining 14 (23%) of 61 women had the second trimester miscarriage (> 12 weeks). While the age group that are correlated with bacterial vaginosis was not agreed with the results of Isik *et al.*, (2017) that age group was 14-49 years with percentage 29%.

The results of presents study, the percentage of Gram positive bacteria isolate were 21 (42%) and percentage of Gram negative bacteria isolate were 29 (58%) that are disagreement with results of study to identification of bacterial vaginosis in women with recurrent spontaneous abortion , Llahf-Camp *et al.*(2017) reported on a significantly higher frequency of bacterial vaginosis of gram negative bacteria among women with a history of recurrent miscarriage (21%) Whereas Ralph *et al.*(2009) also reported that bacterial vaginosis had a significantly higher prevalence ($p = 0.001$) in women with a history of at least one late miscarriage (21%).

Bacterial vaginosis (BV) is one of the most prevalent causes of vaginal secretions during reproductive ages. One-third of women with vaginosis have BV(Bohbot *et al.*, 2012).The prevalence of BV differs depending on geographical location, socio-economic status and ethnicity, from 8% to 51% (Haltas *et al.*, 2012). CDC has reported BV as 29.2% in American women aged 14–49 years old, and 25% among pregnant women (CDC, 2010). This infection is asymptomatic in 50%-75% of cases and the symptomatic cases present at homogeneous grayish white smelly secretions, fishy smell after intercourse or during mense (Decherney, *et al.*, 2013).

In the results of presents study in table (1-3) was shown the identification of bacterial vaginosis by Amsels criteria, It was found that the most sensitive parameter, in identifying bacterial vaginosis was thin homogenous discharge measurement which was (88%), with specificity of 13%, then the second sensitive parameter was clue cell which was 82% with specificity 18% and third sensitive parameter was pH which was 79% with specificity 22% while the lowest sensitive test, was the swiff test which was 55% with high specific parameter 45%. But the most specific parameter was the clue cell test, with (81%), When a combination (in series) was done among pH measurement, Swiff test, clue cells and thin homogenous discharge, this results in increasing specificity to become 24%, and sensitivity was 76%. While *the presence* of clue cells in vaginal wet mount and whiff tests had the greatest sensitivity (97.6%) and specificity (85.7%), respectively, Ling et al.(2010) observed grayish white homogeneous vaginal discharge in 87% of women with BV, Simbar et al. (2008) reported the prevalence of grayish white homogeneous vaginal discharge as 100% .

The whiff test that are released of a fishy odor upon adding one drop of 10% potassium hydroxide is another criterion for BV diagnosis with a sensitivity of 33.9% and a specificity of 86.9% also They found positive whiff test results in 95% of the patients (Hallen *et al.*, 1987). Similarly, we determined the sensitivity and specificity of the test as 54.0% and 85.7%,

Vaginal $\text{pH} \geq 4.5$ is also an Amsel's criterion with very high sensitivity (97%), but low specificity (26%) (Simoes et al., 2006). While vaginal pH normally falls between 3.8 and 4.2, it can change based on the activity of vaginal microflora (Ma et al., 2012). In addition to BV, trichomoniasis, cervical secretions, contact with semen, and application of lubricant gels can increase vaginal pH. Therefore, combining pH tests with other symptoms can enhance the accuracy of the test in diagnosis of various infectious conditions and vaginal pH to have the lowest specificity (46.6%), Various factors such as simultaneous infections of vagina and cervical mucus might have influenced this criterion (Gutman et al., 2005).

The bacterial isolate was appearing as Gram positive and Gram negative, so the biochemical test and Vitek 2 compact system was confirmed the final diagnosis, ,the result of blood culture and biochemical tests for isolated bacteria from women with spontaneous abortion were revealed that total bacterial isolate 8, the most popular bacteria, *Escherichia coli* is 29 (58%) Afterwards accompanied by *Staphylococcus haemolyticus* 9 (18%) , *Staphylococcus*

hominis spp and *Staphylococcus lentus* 4(8%) and the each of *Enterococcus faecalis* , *Staphylococcus pseudintermedius* , *Streptococcus pluranimalium* and *Staphylococcus saprophyticus* 1 (2%) As Show In (1-3).other local study also studying bacterial vaginosis and isolating types of bacteria (Alsultany,2012).some of these bacteria like *Staphylococcus* has virulence factor make it more pathogenic.(AlSultany ,2016).Also these bacteria pathogenic some type of pathogenic bacteria has ability to produce nanoparticles.(Hathal et al.,2020)

E coli is the most common bacteria that are appeared in women with recurrent abortion , because it has some virulence factor like LPS (AlSultany and Jassim,2016), which helps in pathogenesis. particularly adhesive molecules which have been acknowledged to be the most important determinants of pathogenicity. Pili responsible for adherence in the vagina (Etefia & Ben, 2020) However ,it is appeared that *Staphylococcus haemolyticus* 9 (18%) was important causative agent that cause recurrent abortion in this study ,because it is represented in (18%) and in addition *Staphylococcus hominis spp* and *Staphylococcus lentus* was important causative agent that cause in this study because it is represent (8%), so that these results were differ from the results that are obtained by epidemiologic studies had reported that *Staphylococcus aureus* (46%) . In another study *S.aureus* was isolated at the rate of 36% While *E.coli* (25%), *Klebsiella* (16%), and *Proteus specie* (2%). In other study, *E.coli* has been reported in 5%- 25% of vaginal cultures while *Klebsiella* and *Proteus* have been found in less than 10% of vaginal swabs in a study from USA.(Onanuga et al., 2005; Shabbir et al., 2015).other study in Iraq also studied immunological parameters to bacterial vaginosis to show their role in pathogenicity(Abid and Mohemeed,2013).

References

1. Abid,F.G;Mohemeed,S.J(2013).Humoral immunity response against *Gardnerellavaginalis* in rabbit.magazin of alkufa university for biology VOL.5 NO.2 .
2. . AlSultany,Sh.J.(2016) Studying Some Activities of Crude Alpha-Hemolysin Extracted from *Staphylococcus aureus*, international Journal of Advanced Research (2016), Volume 4, Issue 3, 1078-1081.
3. Alsultany; S JM.(2012).Study of Virulence Factors of *Gardnerellavaginalis* Isolated from Women Infected by Bacterial Vaginosis. Thesis,college of science, university of Babylon.

4. AlSultany, Sh.J. and Jassim, Y.A. (2016). Physiological and immunological effect of lipopolysaccharide of *Escherichia coli* was extracted by hot phenol-water in rabbits. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 7 (3) Page No. 1530.
5. Amsel R, Totten PA, Spiegel CA. (1983). Non specific vaginitis, diagnostic criteria and antimicrobial and epidemiologic accusations. *American Journal of Medicine*. 74:14-22.
6. Bohbot, J. M., Sednaoui, P., Verriere, F., & Achhammer, I. (2012). The etiologic diversity of vaginitis. *Gynecologie, Obstetrique & Fertilité*, 40(10), 578-581.
7. Brooks, G. F., Butel, J. S., Carroll, K. C. and Morse, S. A. 2010. *Medical microbiology*. 24th ed. Lange Medical books.
8. Centers for Disease Control and Prevention. (2010). Bacterial vaginosis statistics. Retrieved from Sep 14th, 2010.
9. Cohain, J.S.; Buxbaum, R.E.; Mankuta, D. Spontaneous first trimester miscarriage rates per woman among parous women with 1 or more pregnancies of 24 weeks or more. *BMC Pregnancy Childbirth* 2017, 17, 473.
10. Contini, C.; Rotondo, J.C.; Magagnoli, F.; Maritati, M.; Seraceni, S.; Graziano, A.; Poggi, A.; Capucci, R.; Vesce, F.; Tognon, M.; et al. Investigation on silent bacterial infections in specimens from pregnant women affected by spontaneous miscarriage. *J. Cell. Physiol.* 2018, 234, 100–107.
11. Decherney, A. H., Nathan, L., Laufer, N., & Roman, A. S. (2013). Bacterial vaginosis current diagnosis & treatment: *Obstetrics & Gynecology* (11th ed., p. 398). McGraw-Hill.
12. Donders, G.G.; Van Bulck, B.; Caudron, J.; Londers, L.; Vereecken, A.; Spitz, B. Relationship of bacterial vaginosis and mycoplasmas to the risk of spontaneous abortion. *Am. J. Obstet. Gynecol.* 2000, 183, 431–437.
13. Etefia, E., & Ben, S. (2020). Virulence markers, phylogenetic evolution, and molecular techniques of uropathogenic *Escherichia coli*. *Journal of Nature and Science of Medicine*, Vol. 3, p. 13
14. Fukuta, K.; Yoneda, S.; Yoneda, N.; Shiozaki, A.; Nakashima, A.; Minamisaka, T.; Imura, J.; Saito, S. Risk factors for spontaneous miscarriage above 12 weeks or premature delivery in patients undergoing cervical polypectomy during pregnancy. *BMC Pregnancy Childbirth* 2020, 20, 27.

15. Giakoumelou, S.; Wheelhouse, N.; Cuschieri, K.; Entrican, G.; Howie, S.E.M.; Horne, A.W. The role of infection in miscarriage. *Hum. Reprod. Update* 2016, 22, 116–133.
16. Gutman, R. E., Peipert, J. F., Weitzen, S., & Blume, J. (2005). Evaluation of clinical methods for diagnosing bacterial vaginosis. *Obstetrics & Gynecology*, 105(3), 551-556.
17. Hallen, A., Pahlson, C., & Forsum, U. (1987). Bacterial vaginosis in women attending STD clinic: Diagnostic criteria and prevalence of mobiluncus. *Genitourinary Medicine*, 63(6), 386-389.
18. Haltas, H., Bayrak, R., & Yenidunya, S. (2012). To determine of the prevalence of bacterial vaginosis, candida, mixed infections (bacterial vaginosis and candida), trichomonasvaginalis, actinomyces in Turkish women from Ankara, Turkey. *GinekologiaPolska*, 83(10), 744-748.
19. Hathal, W.A., Alsultany, S.J. and Abd, F.G. Synthesis of silver nanoparticles from *Streptococcus pyogenes* and antimicrobial activity, 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* **928** 062015.
20. Javed, A., Parvaiz, F., & Manzoor, S. (2019). Bacterial vaginosis: An insight into the prevalence, alternative treatments regimen and its associated resistance patterns. *Microbial pathogenesis*, 127, 21-30.
21. Laxmi, U., Agrawal, S., Raghunandan, C., Randhawa, V., & Saili, A. (2012). Association of bacterial vaginosis with adverse fetomaternal outcome in women with spontaneous preterm labor: a prospective cohort study. *The Journal of Maternal-Fetal & Neonatal Medicine*, 25(1), 64-67.
22. Ling, Z., Kong, J., Liu, F., Zhu, H., Chen, X., Wang, Y. and Xiang, Ch. (2010). Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. *Biomedical Central Genomics*, 11(1), 488.
23. Llahf-Camp JM, Rai R, Ison C, Regan L, Taylor-Robinson D. (2017) Association of bacterial vaginosis with a history of second trimester miscarriage. *Hum Reprod*;11:1575–1578.
24. Ma, B., Forney, L. J., & Ravel, J. (2012). Vaginal microbiome: rethinking health and disease. *Annual review of microbiology*, 66, 371-389.
25. Mahdi NK, Al-Hamdani MM.(1998). Sexually transmitted disease among women with habitual abortion. *Eastern Mediterranean Health Journal* 4(1):161- 163.

26. Onanuga A, Oyi A, Onaolapo J.(2005) Prevalence and susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. *Afr J Biotechnol.*4:1321–4. Shabbir I, Akhtar R, Nasreen F.(2015). Bacterial Vaginosis in women with vaginal discharge. *Mother and Child.* 37:127–32.
27. Ralph SG, Rutherford AJ, Wilson JD. (2009).Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *BMJ*;319:220–223.
28. RangariAmit, A., Parmjit, S., & Sharma, V. (2013). Comparison of the amsel's composite clinical criteria and nugent's criteria for diagnosis of BV: A step towards preventing misdiagnosis. *Journal of Advance Researches in Biological Sciences*, 5(1), 37-44.
29. Simbar, M., Azarbad, Z., Mojab, F., &AlaviMajd, H. A. M. I. D. (2008). A comparative study of the therapeutic effects of the *Zataria multiflora* vaginal cream and metronidazole vaginal gel on bacterial vaginosis. *Phytomedicine*, 15(12), 1025-1031.
30. Simoes, J. A., Discacciati, M. G., Brolazo, E. M., Portugal, P. M., Dini, D. V., & Dantas, M. C. M. (2006). Clinical diagnosis of bacterial vaginosis.*International Journal of Gynecology & Obstetrics*, 94(1), 28-32.
31. Taj, Y., Nasir, D., Kahkashan, N., & Anjum, A. (2012). Sensitivity and specificity of rapid clinical diagnostic test for BV and its analytical value. *J Dow Uni Health Sci*, 6(3), 91-94.
32. Xia H, Li X, Li X, Liang H, Xu H. The clinical management and outcome of term premature rupture of membrane in East China: Results from a retrospective multicenter study. *Int J Clin Exp Med* 2015;8:6212-7.